

# XIII<sup>th</sup> International Symposium on Amyloidosis

## From Misfolded Proteins to Well-Designed Treatment



**ISA** INTERNATIONAL SOCIETY OF AMYLOIDOSIS



Groningen Unit for Amyloidosis  
Research & Development (GUARD)

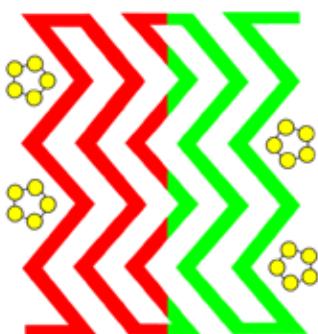
May 6 - 10, 2012

Groningen, The Netherlands

University Medical Center Groningen

# **From Misfolded Proteins to Well-Designed Treatment**

**XIIIth International Symposium on Amyloidosis  
Groningen, The Netherlands, May 6-10, 2012**



## Table of Contents

|  |     |
|--|-----|
| ACKNOWLEDGEMENTS.....                                | 5   |
| WELCOME ADDRESS.....                                 | 6   |
| ORGANIZING COMMITTEE AND FACULTY<br>INFORMATION..... | 7   |
|  | 8   |
| <br>PROGRAM.....                                     | 15  |
| <br><br><br>KEYNOTE LECTURE.....                     | 23  |
| <br><br><br>MONDAY, MAY 7 .....                      | 25  |
| PLENARY SESSION 1 .....                              | 25  |
| PLENARY SESSION 2 .....                              | 29  |
| PLENARY SESSION 3 .....                              | 33  |
| <br><br><br>TUESDAY, MAY 8.....                      | 39  |
| PLENARY SESSION 5.....                               | 39  |
| PLENARY SESSION 6.....                               | 44  |
| PLENARY SESSION 7.....                               | 49  |
| <br><br><br>WEDNESDAY, MAY 9.....                    | 59  |
| PLENARY SESSION 8.....                               | 59  |
| PLENARY SESSION 9.....                               | 63  |
| PLENARY SESSION 10.....                              | 66  |
| PLENARY SESSION 11 .....                             | 70  |
| <br><br><br>THURSDAY, MAY 10.....                    | 75  |
| PLENARY SESSION 12.....                              | 75  |
| <br><br><br>POSTERS MONDAY PA 01 – 65.....           | 83  |
| <br><br><br>POSTERS TUESDAY PB 01 – 66.....          | 127 |
| <br><br><br>POSTERS WEDNESDAY PC 01 – 66 .....       | 171 |
| <br><br><br>PRESENTING AUTHOR INDEX .....            | 221 |

## **Acknowledgements**

The Organizing Committee of the XIIIth International Symposium on Amyloidosis is grateful to the following sponsors whose generous contributions made this Symposium possible:

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## Welcome address

Dear Friends and Colleagues,

On behalf of the Organizing Committee, I would like to welcome you in Groningen at our XIIIth International Symposium on Amyloidosis. In 1967, Professor Enno Mandema hosted here the first International Symposium on Amyloidosis. At his retirement in 1986, an International Course on Amyloidosis was also held in Groningen. Continuous progress of amyloidosis research was reported in Helsinki (1974), Povoa de Varzim (1979), New York (1984), Hakone (1987), Oslo (1990), Kingston (1993), Rochester (1998), Budapest (2001), Tours (2004), Woods Hole (2006), and Rome (2010). The ash clouds of the volcano in Iceland intervened unexpectedly and prevented some of us, including me, to visit Rome. However, this did not affect the spirit and enthusiasm of the amyloidosis community. We will now, only two years later, continue our search for better understanding of protein misfolding, fibril formation and amyloidogenesis, for improving early diagnosis and monitoring disease severity and - in the end - for finding new, effective, and safe ways to cure our patients from these serious diseases.

The number and quality of the submitted abstracts was very high and it was difficult to make choices. It was a pleasure to collaborate with many of you in preparing the talks and the programme. Generous sponsoring made it possible to keep the costs for the participants at a reasonable level. I would especially thank the Board of Directors of the International Society of Amyloidosis for the continuous and huge help I received to overcome obstacles. The personal friendship and support I experienced are heartwarming.

To conclude, I hope this symposium will be a success for all of us and will fulfill our expectations. I also hope that our young researchers and colleagues will become fascinated by the challenges and opportunities of fundamental and clinical research in this interesting area of protein misfolding diseases. If this Symposium in Groningen attracts a new generation of dedicated amyloid researchers, we can safely conclude that it was a success.

I wish you a pleasant time with us in Groningen.

Bouke Pier C. Hazenberg,  
President of the Symposium



# Organizing Committee and Faculty

## International Members

Bouke P.C. Hazenberg, Host XIIIIth International Symposium on Amyloidosis, Groningen, The Netherlands

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Giampaolo Merlini, Immediate Past President ISA, Pavia, Italy

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Monique C. Minnema, Utrecht

Elisabeth B. Haagsma, Groningen

Edo Vellenga, Groningen

Philip M. Kluin, Groningen

## Guest Speakers

### Keynote speaker:

Jan Münch, Ulm, Germany

### Enno Mandema Memorial Lecturer:

Sir Mark B. Pepys, London, UK

# Information

## Scientific Presentation Instructions

### Invited speakers and oral presentations:

- All speakers and presenters are requested to stay within time.
- Oral presentations are limited to 12 minutes (approximately 9 minutes for presentation and 3 minutes for discussion).
- PowerPoint 2003 and up will be acceptable.
- All speakers and presenters are urged to bring their presentation stored on a USB or CD-ROM the day before the session (on Monday at least 1 hour before the beginning of the session).
- NB. Please include the abstract number and the presenter's name in the PowerPoint file name.
- We will handle all data responsibly and delete them after the presentation.

### Poster presentations:

- The panel size of the poster board is 120 cm wide and 120 cm high. Please choose the sizes of your poster to fit into this format, so it can be mounted on the board without hampering the posters of your neighbors. Pins will be present to mount the posters.
- The presenter is invited to be near his/her board at the hours of the poster sessions.
- Posters are numbered PA (Monday), PB (Tuesday) or PC (Wednesday) followed by the number of the board (1-70).
- Please mount your poster on the board preferably before the program starts at 8.00 and no later than 15 minutes before the start of the poster session.

NB. The poster should be removed at the end of the day after the last poster session. Posters not removed at the end of the day will be removed by the organizers.

- The first poster session of the day will enable participants to view and discuss posters with the presenters individually.
- During the second poster session of the day, each poster will be presented and discussed in five groups of 12-14 posters each. Poster presentations are limited to 5 minutes for both presentation and discussion (approximately 3 minutes for presentation and 2 minutes for discussion).
- A small group of posters (about 5 per day) is selected to be presented in a short talk each of 6 minutes (4 minutes talk in 3-5 slides, followed by 2 minutes discussion) in the last half hour of the poster session in the 'Blauwe Zaal'.

## **General Information**

### Venue

The congress will be held at the University Medical Center Groningen.

Address: Hanzeplein 1, 9713 GZ Groningen, The Netherlands.

Virtual tour: <http://virtueel.umcg.nl/index.php?projectid=umcg>

Conference room: Blauwe Zaal, ground floor.

Reception and registration is at the Fonteinpatio, ground floor.

Posters are located at the Blauwe Patio, first floor; route will be indicated by signposts.

### Certificate

Participants will receive a certificate of attendance at the end of the Symposium.

### Insurance

The meeting organizer cannot accept any liability for personal injuries, loss or damage to properties belonging to participants, either during or as a result of the meeting. Participants are advised to take out their own personal travel insurance.

### Accreditation

- Accreditation is granted by the European Accreditation Council for Continuing Medical Education (EACCME) with 24 European CME credits (ECMEC).
- Accreditation has already been received from three Dutch Societies:
  - NIV (Internal Medicine) and NVvC (Cardiology) with 23 points: May 6: 1 point; May 7, 8 and 9 (each day): 6 points; May 10: 4 points.
  - NVR (Reumatology) with 15 points: May 6: 1 point; May 7: 5 points; May 8 and 9 (each day): 4 points; May 10: 1 point
- Accreditation is requested at the Dutch Societies of Gastroenterology, Neurology and Pathology.
- The participants need to register daily for all accreditations at the desk.

### Language

The official language during the Symposium is English. No simultaneous translation will be provided.

### Social program

All participants are invited to join the social program. For the welcome party on Sunday evening a voucher – on behalf of the University of Groningen, the Municipality of Groningen and the Province of Groningen – will be provided to all participants at registration. On Monday, Tuesday, and Wednesday evening, a visible symposium badge will be sufficient.

## **The Netherlands**

### Language

Dutch is the predominant language in the Netherlands but English is very popular and is spoken almost everywhere.

### Currency and Banks

The currency is Euro (€). Other currencies can be changed at banks and hotels. Banking times are 09.00-17.00 every day except Saturday and Sunday.

### Shopping

Most shops and department stores are open from 09.00-18.00 from Tuesday to Friday, Monday from 13.00-18.00, and Saturday from 9.00-17.00. Sunday all shops are mostly closed. Major credit cards are widely accepted.

### Weather

The climate in the Netherlands is a sea-climate with temperatures between 15-18°C in May. Please note that there is always a chance of showers, due to variable weather patterns.

### Time

Summertime in The Netherlands is equal to Central European Time (CET), one hour ahead on Greenwich Mean Time (GMT), and six hours ahead of U.S. Eastern Time.

### Power

Electricity sockets take two pin round plugs. The electrical current is 220 volts.

## **Travel to and in Groningen**

Groningen is situated in the north of the Netherlands and is easily accessible from all parts of Europe.

### By air

The city airport, Groningen Airport Eelde, has direct scheduled services from and to Aberdeen. Check [www.flybmi.com](http://www.flybmi.com) for more information. Aberdeen has also connections with: Birmingham, London Gatwick, London Heathrow and Newcastle.

The main airport is Schiphol Amsterdam Airport, located in Amsterdam.

### By train

Train connections link the city with Schiphol International Airport every 30 minutes. Travel time by train is approximately 2.5 hours. The Central Station in Groningen is at 20 minutes walking distance from the UMCG. For timetables visit <http://www.ns.nl/en/travellers/home>.

Tickets can be bought in the hall of the railway station at Schiphol Airport. There are two transport classes. See for more information <http://www.ns.nl/en/travellers/arrange-and-buy>. A touch-screen ticket machine is the easiest way to obtain a ticket, see <http://www.ns.nl/en/travellers/arrange-and-buy/tickets-and-passes/purchasing-tickets/ns-self-service-ticket-machine.html>

### By bus in Groningen

Arriving at the Central Station in Groningen, several busses will take you to the city center. Bus 8 and 22 will take you directly to the University Medical Center Groningen. Both the city center (5 min) and the University Medical Center (10 min by bus) are also at walking distance from the Central Station.

If you are not travelling a lot by bus just buy a 1.50 euro ticket in the bus (just ask for 1.50 ticket at bus driver). This ticket can be used for an hour, check the stamp the bus driver put on it to see when it was given to you and thus when it cannot be used anymore (simply add an hour). Within this hour you can change from bus to bus. Just show the ticket to the bus driver. See for more information <http://noimnot.hubpages.com/hub/Travelling-by-bus-in-the-Netherlands-how-does-it-work>

### By car

Parking facilities are available in Parking garage Noord (paid parking, for directions visit [www.umcg.nl](http://www.umcg.nl)).

### Excursions

No group arrangements have been organized. Information about individual possibilities to visit Groningen town centre by foot or bike and the canals by boat will be available at the registration desk. See also [http://www.virtualtourist.com/travel/Europe/Netherlands/Provincie\\_Groningen/Groningen-461234/General\\_Tips-Groningen-TG-C-1.html](http://www.virtualtourist.com/travel/Europe/Netherlands/Provincie_Groningen/Groningen-461234/General_Tips-Groningen-TG-C-1.html)

## Wenckebach Instituut

For health care professionals



**umcg**

The Wenckebach Instituut is a department of  
the University Medical Center Groningen.

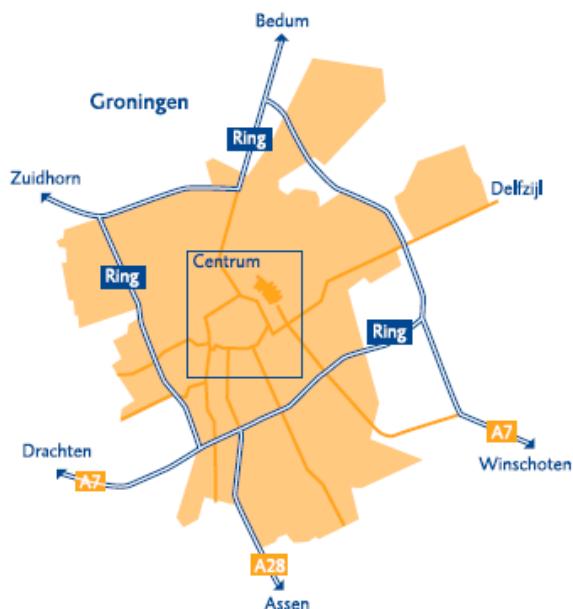


### Route by car

For visitors to congresses or other events the University Medical Center Groningen (UMCG) has a car parking at the north end of the terrain. To get there follow from the Groningen Ring the signs 'UMCG Noord'.

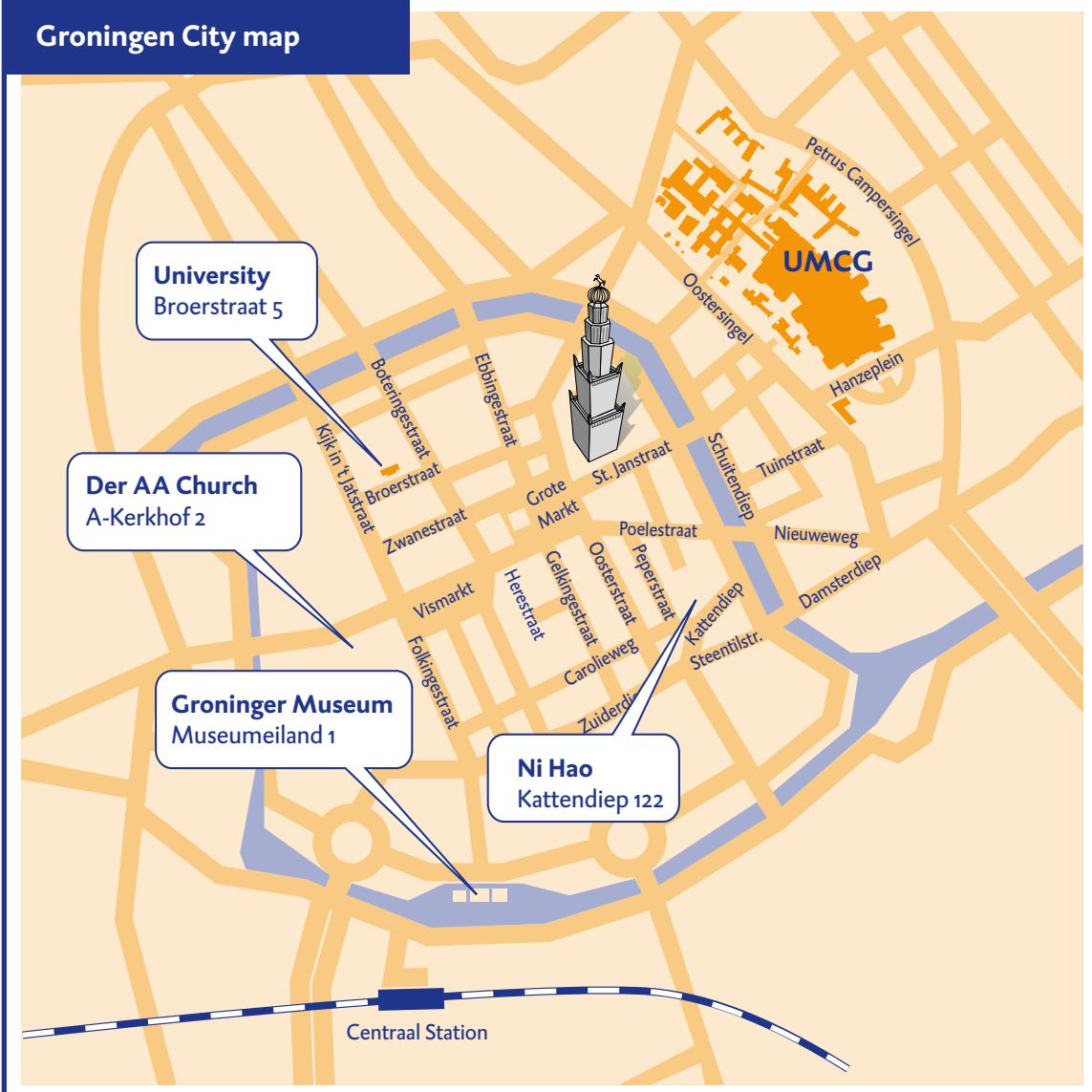
### Public transport

The UMCG is easily reached by public transport. Several bus lines departing from the Central Railway Station drive in about 10 minutes to the UMCG. Some buses stop at the head entrance of the hospital, others at the side and at the north end. You can also use the 'park and ride' city bus. Because the bus schedules vary often, we direct you for up to date information to the sites for public transport ([www.9292ov.nl](http://www.9292ov.nl) and [www.citybus.nl/English](http://www.citybus.nl/English)). Regretfully, the 9292ov.nl site is in Dutch. However, its homepage offers the possibility to make an inquiry that probably is understandable, even if you do not speak Dutch.



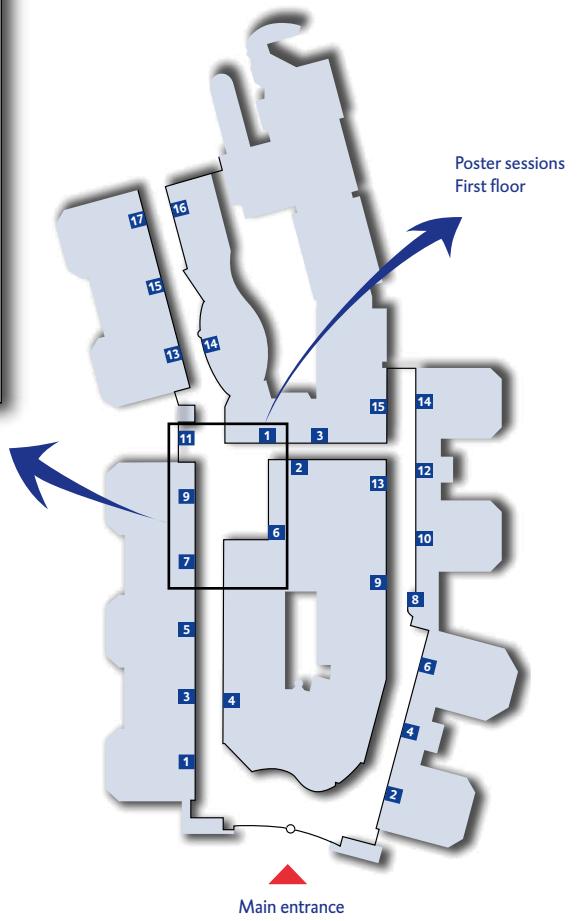
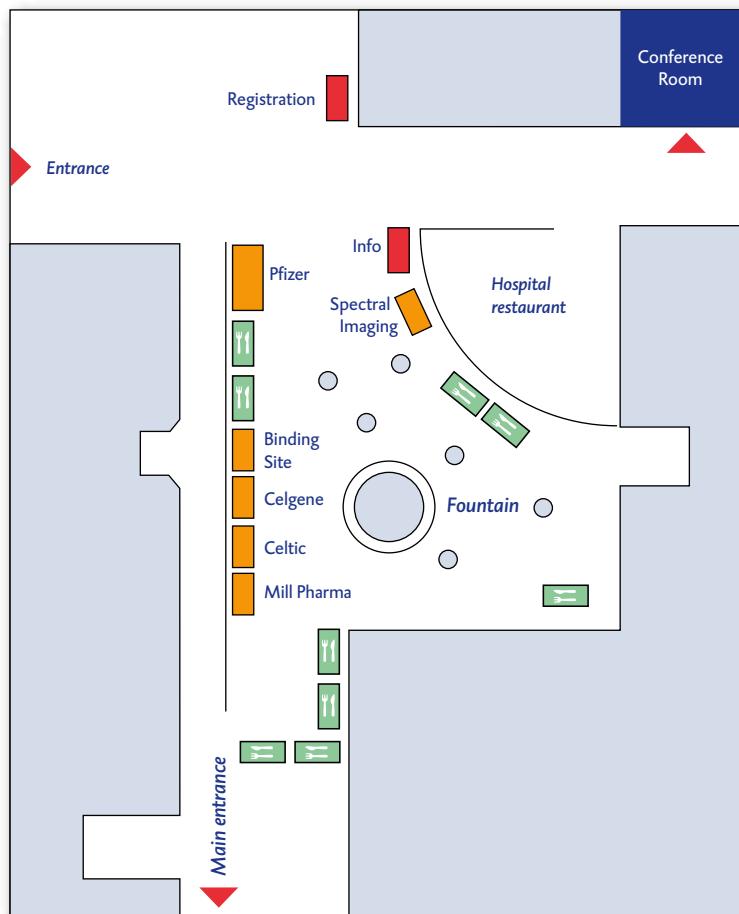
**Note:**  
Follow the signs to UMCG-Noord and not to the  
UMCG main entrance.

## Groningen City map



# Hospital map

## Ground floor



## Program

### Sunday, May 6

|                    |   |
|--------------------|---|
| 12.00              | Registration opens in UMCG ('Fonteinpatio')   |
| <b>15.30-16.00</b> | <b>Coffee</b>   |
| <b>16.00-16.30</b> | <b>Opening ceremony ('Blauwe Zaal')</b>   |
| 16.00-16.10        | Welcome address - Van Rijswijk  |
| 16.10-16.20        | Introduction to the symposium - Hazenberg   |
| 16.20-16.30        | A patient's experience and perspective - Magalhaes  |
| <b>16.30-17.00</b> | <b>Opening Lecture:</b> History of Amyloidosis - Kyle   |
| <b>17.00-17.30</b> | <b>Keynote Lecture:</b> Amyloid in semen boosts HIV-1 transmission - Münch  |
| 18.00-18.30        | Walk to the University Building   |
| <b>18.30-20.30</b> | <b>Welcome party;</b> offered to you by the University of Groningen, the Municipality of Groningen and the Province of Groningen ( <i>University Building</i> ) |
| 18.30-18.45        | Welcome address - Poppema   |
| 18.45-20.30        | Refreshments, party   |
| 20.00-21.30        | ISA Board Meeting ('Engelse Zaal' in <i>University Building</i> )   |

### Monday, May 7

|                    |   |
|--------------------|---|
| <b>7.30-8.00</b>   | <b>Coffee ('Fonteinpatio')</b>  |
| <b>8.00-8.30</b>   | <b>Enno Mandema Memorial Lecture ('Blauwe Zaal'):</b><br>Amyloidosis: 1979-2012-... - Sir Mark Pepys  |
| <b>8.30-10.20</b>  | <b>Plenary session 1: Fibril and Amyloid Formation.</b> Chairmen: Seldin and Limburg<br>State of the art - Fändrich                               |
| 8.35               | OP 01 Aggregates of the Alzheimer's Disease Amyloid Peptide A $\beta$ 40 Studied by Solid State NMR. Lopez del Amo (München, Germany)             |
| 8.50               | OP 02 Structural modulation of A $\beta$ fibrils by glycosaminoglycans. Madine (Liverpool, UK)  |
| 9.02               | OP 03 Structure and pathogenicity of mature fibrils and their structural precursors. Fändrich (Halle, Germany)                                    |
| 9.14               | OP 04 Towards a cure for Parkinson's Disease: inhibitors of $\alpha$ -synuclein aggregation. Lorenzen (Aarhus, Denmark)                           |
| 9.26               | OP 05 In vivo and in vitro anti-Amyloid functions of the systemic amyloid precursor Transthyretin (TTR). Buxbaum (San Diego, USA)                 |
| 9.38               | OP 06 Two types of fibril compositions in ATTR amyloidosis and their correlation to clinical phenotype. Ihse (Upsala, Sweden)                     |
| 9.50               | Perspectives - Bellotti   |
| <b>10.20-10.45</b> | <b>Coffee break ('Fonteinpatio')</b>  |
| <b>10.45-12.30</b> | <b>Plenary session 2: Cell and tissue targeting and toxicity ('Blauwe Zaal')</b><br>Chairmen: Grateau and Luiten<br>State of the art - Kisilevsky |
| 10.45              | OP 07 Biochemical properties of highly virulent prion strains. Sigurdson (San Diego, USA)   |
| 11.00              | OP 08 Detection of endogenous amyloid fibrils in human semen. Usmani (Ulm, Germany)   |

|                    |       |   |
|--------------------|-------|---|
| 11.24              | OP 09 | A proteomic approach to unravel the cytotoxic responses to different aggregation-prone proteins. Picotti (Zürich, Switzerland)                                      |
| 11.36              | OP 10 | Investigating vulnerability to proteasome inhibition in primary light chain amyloidosis Oliva (Milano, Italy)   |
| 11.48              | OP 11 | Unique features that characterize urinary exosomes in light chain amyloidosis (AL). Ramirez-Alvarado (Rochester, USA)   |
| 12.00              | OP 12 | Fighting familial amyloidosis using an impaired ER-stress response yeast strain. Rodrigues (Lisboa, Portugal)   |
| 12.12              |       | Perspectives - Buxbaum  |
| <b>12.30-14.00</b> |       | <b>Lunch</b> (Sponsor Spectral Imaging) in the ' <i>Fonteinpatio</i> '  |
| <b>13.00-14.00</b> |       | <b>Poster viewing</b> individually (' <i>Blauwe Patio</i> ')  |
| <b>14.00-16.00</b> |       | <b>Plenary session 3: Animal models and cell culture systems ('Blauwe Zaal')</b>  |
|                    |       | Chairpersons: Gruys and G. Westermark   |
| 14.00              |       | State of the art - Saraiva  |
| 14.15              | OP 13 | Heparin/Chitosan nanoparticle delivery system for in vivo evaluation of glycosaminoglycans influence on transthyretin deposition. Gonçalves (Porto, Portugal)       |
| 14.27              | OP 14 | Multi-system disease-specific induced pluripotent stem cell modeling of familial amyloidosis. Leung (Boston, USA)   |
| 14.39              | OP 15 | Anti-amyloid drug development using Drosophila as a model. Segal (Tel Aviv, Israel)   |
| 14.51              | OP 16 | <i>C. elegans</i> expressing human β2-m recapitulates the molecular mechanisms underlying dialysis-related amyloidosis. Soria (Pavia, Italy)                        |
| 15.03              | OP 17 | Doxycycline Interaction with Amyloidogenic Proteins and Activity in Mouse Models. Ward (Boston, USA)  |
| 15.15              | OP 18 | An all human cell culture model of AA amyloid formation: human serum amyloid A (SAA) and human peripheral blood mononuclear cells (PBMC). Ishii (Indianapolis, USA) |
| 15.27              | OP 19 | Macrophage-mediated natural clearance of Amyloid A is not impaired in the absence of immunoglobulins or central complement factors. Sponarova (Zürich, Switzerland) |
| 15.40              |       | Perspectives - Kluge-Beckerman  |
| <b>16.00-16.30</b> |       | <b>Tea break ('Fonteinpatio')</b>   |
| <b>16.30-17.30</b> |       | <b>Poster viewing (PA 1-65) in 5 groups ('Blauwe Patio')</b>  |
| <b>17.30-18.00</b> |       | <b>Selected poster presentations</b> PA 15, PA 22, PA 51, PA 53, and PA 54 ('Blauwe Zaal')  |
|                    |       | Chairmen: Limburg and Gruys   |
| 17.00-18.00        |       | Meetings: Nomenclature Committee; other working groups and committees ('Lokaal 10, 15')   |
| <b>18.00-19.30</b> |       | <b>Buffet in UMCG ('Fonteinpatio')</b>  |
| <b>19.30-22.00</b> |       | <b>Plenary session 4: Looking for consensus ('Blauwe Zaal')</b>   |
| 19.30              |       | Early detection of amyloid; reporting - Picken, P. Westermark, Hazenberg  |
| 20.20              |       | Organ involvement and response criteria in AL - Gertz, Wechalekar, Palladini  |
| 21.10              |       | Organ involvement and response criteria in non-AL - Suhr, Obici, Lachmann, Merkies  |
| 22.00              |       | Closing   |

#### Tuesday, May 8

|                   |   |
|-------------------|---|
| <b>7.30-8.00</b>  | <b>Coffee ('Fonteinpatio')</b>  |
| <b>8.00-10.05</b> | <b>Plenary session 5: Diagnosis and typing: Histochemistry and proteomics ('Blauwe Zaal')</b> |

Chairmen: Linke and Kluin

|                    |   |
|--------------------|---|
| 8.00               | State of the art - Röcken   |
| 8.15               | OP 20 Amyloid fibrils possess characteristic electronegative fingerprints that can be distinguished by poly-basic peptides. Wall (Knoxville, USA)   |
| 8.27               | OP 21 Luminescent conjugated oligothiophenes: two new dyes for amyloid screening diagnostics. Sjölander (Linköping, Sweden)   |
| 8.39               | OP 22 Accuracy of routine amyloid typing using immunohistochemistry on a large number of consecutive patients: preconditions for its success and validation. Linke (Martinsried, Germany) |
| 8.51               | OP 23 An indirect ELISA for transthyretin quantification in fat tissue of patients with ATTR Amyloidosis. Hazenberg (Groningen, The Netherlands)  |
| 9.03               | OP 24 A native human monoclonal IgG with pan-amyloid binding specificity. Dessain (Wynnewood, USA)  |
| 9.15               | OP 25 Diagnosis of Amyloidosis Subtype by Laser-Capture Microdissection (LCM) and Tandem Mass Spectrometry (MS) Proteomic Analysis. Mollee (Brisbane, Australia)                          |
| 9.27               | OP 26 Mass spectrometry based proteomics for classification of amyloidosis: Mayo Clinic Experience. Dogan (Rochester, USA)  |
| 9.39               | OP 27 A comparison of immunohistochemistry and mass spectrometry for determining the amyloid fibril protein from formalin fixed biopsy tissue. Gilbertson (London, UK)                    |
| 9.51               | Perspectives - Dogan  |
| <b>10.05-10.30</b> | <b>Coffee break ('Fonteinpatio')</b>  |
| <b>10.30-12.30</b> | <b>Plenary session 6: Imaging in amyloidosis ('Blauwe Zaal')</b>  |
|                    | Chairmen: Van Rijswijk and Glaudemans   |
| 10.30              | State of the art - Hawkins  |
| 10.45              | OP 28 Patterns of late gadolinium enhancement and survival in cardiac amyloidosis: a systematic review of 95 patients with AL or ATTR type. Dungu (London, UK)                            |
| 10.57              | OP 29 $^{99m}$ Tc-3,3-Diphosphono-1,2-Propanodicarboxylic Acid ( $^{99m}$ Tc DPD) scintigraphy in 171 patients with suspected systemic amyloidosis. Hutt (London, UK)                     |
| 11.09              | OP 30 Accuracy of $^{99m}$ Tc-HMPD myocardial scintigraphy for the diagnosis of cardiac involvement in patients with familial amyloid polyneuropathie. Algalarondo (Clamart, France)      |
| 11.21              | OP 31 Iodine-123 metaiodobenzylguanidine for the evaluation of cardiac sympathetic denervation in early stage amyloidosis. Noordzij (Groningen, The Netherlands)                          |
| 11.33              | OP 32 The added value of SPECT(-CT) compared with planar $^{123}$ I-SAP scintigraphy in patients with systemic amyloidosis. Van Rheenen (Groningen, The Netherlands)                      |
| 11.45              | OP 33 AL amyloid imaging and therapy with an amyloid specific monoclonal antibody. Wall (Knoxville, USA)  |
| 11.57              | OP 34 Radioimmunoimaging of Patients with AL Amyloidosis. Wells (Knoxville, USA)  |
| 12.10              | Perspectives - Wall   |
| <b>12.30-14.00</b> | <b>Lunch (Sponsor Alnylam) in the 'Fonteinpatio'</b>  |
| <b>13.00-14.00</b> | <b>Poster viewing individually ('Blauwe Patio')</b>   |
| <b>14.00-16.30</b> | <b>Plenary session 7: Biology, clinics and prognosis in AL amyloidosis ('Blauwe Zaal')</b>  |
|                    | Chairpersons: Comenzo and Croockewit  |
| 14.00              | State of the art - Merlini  |

|                    |       |  |
|--------------------|-------|--|
| 14.15              | OP 35 | Diagnostic performance of the novel monoclonal assay for the measurement of circulating free light chain (FLC) in 220 consecutive newly-diagnosed patients with AL amyloidosis. Palladini (Pavia, Italy) |
| 14.27              | OP 36 | A Revised Prognostic Staging System for Light Chain Amyloidosis (AL) Incorporating Cardiac Biomarkers and Serum Free Light Chain Measurements. Kumar (Rochester, USA)                                    |
| 14.39              | OP 37 | Coronary Microvascular Function in Cardiac Amyloidosis. Dorbala (Boston, USA)  |
| 14.51              | OP 38 | Depressed midwall fractional shortening is a powerful prognostic determinant in cardiac AL amyloidosis. Perlini (Pavia, Italy)   |
| 15.03              | OP 39 | ALchemy - A Large Prospective 'Real World' Study of Chemotherapy in AL Amyloidosis. Gillmore (London, UK)  |
| 15.15              | OP 40 | Prognostic significance of cytogenetic aberrations in light chain amyloidosis patients treated with melphalan / dexamethasone as first-line therapy. Schönland (Heidelberg, Germany)                     |
| 15.27              | OP 41 | A Framework for Clinical Research in Systemic Light-chain (AL) Amyloidosis: Consensus Report of the First Amyloidosis Foundation Roundtable. Comenzo (Boston, USA)                                       |
| 15.39              | OP 42 | An Italian single center prospective study on outcomes in AL amyloidosis. Palladini (Pavia, Italy)   |
| 15.51              | OP 43 | Outcomes and Treatment of Relapsed Systemic Amyloidosis. Warsame (Rochester, USA)  |
| 16.03              | OP 44 | Solid organ transplantation in AL amyloidosis and monoclonal immunoglobulin deposition disease: the French experience. Desport (Poitiers, France)  |
| 16.15              |       | Perspectives - Gertz   |
| <b>14.00-16.30</b> |       | <b>Satellite Workshop (Sponsor Spectral Imaging): Amyloid Diagnosis and Research Workshop ('Lokaal 16')</b>  |
| 14.00              |       | Welcome and Aim of the Workshop - Hammarström  |
| 14.30              |       | The use of LCP probes for classification of amyloid deposits - Nilsson   |
| 15.30              |       | Hyperspectral-imaging for direct analysis of LCP-coupled amyloid deposits - Sluszny  |
| <b>16.30-17.00</b> |       | <b>Tea break ('Fonteinpatio')</b>  |
| <b>17.00-18.00</b> |       | <b>Symposium (Sponsor Pfizer): Advancing the understanding and management of TTR-FAP ('Blauwe Zaal')</b>   |
|                    |       | Chair: Hazenberg   |
| 17.00              |       | Welcome - Hazenberg  |
| 17.05              |       | Unravelling the TTR misfolding hypothesis - Kelly  |
| 17.30              |       | New approaches to the management of TTR-FAP – Coelho   |
| 17.55              |       | Conclusions - Hazenberg  |
| <b>18.00-19.30</b> |       | <b>Drinks and snacks (Sponsor Proteotech) in the 'Fonteinpatio'</b>  |
| <b>18.00-19.00</b> |       | <b>Poster viewing (PB 1-66) in 5 groups ('Blauwe Patio')</b>   |
| <b>19.00-19.30</b> |       | <b>Selected poster presentations PB 12, PB 41, PB 45, PB 47, and PB 66 ('Blauwe Zaal')</b>   |
|                    |       | Chairpersons: Croockewit and Van Rijswijk  |
| 18.30-19.30        |       | Committee Meetings ('Lokaal 15 and W2270')   |
| 19.30-20.00        |       | Walk to the restaurant   |
| <b>20.00-22.30</b> |       | <b>Dinner in Ni Hao wok restaurant</b>   |

**Wednesday, May 9**

|                    |  |
|--------------------|--|
| <b>7.30-8.00</b>   | <b>Coffee ('Fonteinpatio')</b>   |
| <b>8.00-9.50</b>   | <b>Plenary session 8: Biology, clinics and prognosis in ATTR amyloidosis ('Blauwe Zaal')</b>   |
|                    | Chairmen: Suhr and Van den Berg  |
| 8.00               | State of the art - Ikeda   |
| 8.15               | OP 45 First online registry of mutations in hereditary amyloidosis: amyloidosismutations.com.<br>Rowczenio (London, UK)  |
| 8.27               | OP 46 The Prevalence of Holter Abnormalities in ATTR Cardiac Amyloidosis. Maurer (New York, USA)   |
| 8.39               | OP 47 Circadian rhythm of blood pressure reflects the severity of cardiac impairment in Familial Amyloid Polyneuropathy. Algalarrondo (Clamart, France)  |
| 8.51               | OP 48 Senile Systemic Amyloidosis: a large cohort study detailing clinical features, laboratory results, and survival. Connors (Boston, USA)   |
| 9.03               | OP 49 Systematic review of 1142 admissions with acute heart failure reveals high frequency of transthyretin V122I cardiac amyloidosis in Afro-Caribbean patients. Dungu (London, UK)   |
| 9.15               | OP 50 Comparison of V122I and Senile Cardiac Amyloidosis: Differences in Clinical Features and Outcomes. Maurer (New York, USA)  |
| 9.30               | Perspectives - Berk  |
| <b>9.50-11.05</b>  | <b>Plenary session 9: Biology, clinics and prognosis in AA (and other types of) amyloidosis</b>  |
|                    | Chairpersons: Lachmann and Van Gameren   |
| 9.50               | State of the art - Livneh  |
| 10.05              | OP 51 A single centre 20 year case series of AA amyloidosis – changing epidemiology. Lachmann (London, UK)   |
| 10.17              | OP 52 Long term effectiveness of surgery in localized laryngeal amyloidosis. Hazenberg (Groningen, The Netherlands)  |
| 10.29              | OP 53 First liver and kidney transplant for leukocyte chemotactic factor 2-amyloidosis presenting with acute liver failure. Fix (San Francisco, USA)   |
| 10.41              | OP 54 The role of liver transplantation in the hereditary amyloidoses; the U.K experience. Stangou (Birmingham, UK)  |
| 10.53              | Perspectives - Skinner   |
| <b>11.05-11.30</b> | <b>Coffee break (Sponsor GSK) in the 'Fonteinpatio'</b>  |
| <b>11.30-13.00</b> | <b>Plenary session 10: Therapy of ATTR amyloidosis ('Blauwe Zaal')</b>   |
|                    | Chairpersons: Haagsma and Kuks   |
| 11.30              | State of the art - Ando  |
| 11.45              | OP 55 Familial Dynamics, Attachment and Psychopathology in FAP Patients. Lopes (Porto, Portugal)   |
| 11.57              | OP 56 Tolerability of diflunisal therapy in patients with transthyretin amyloidosis. Whelan (London, UK)   |
| 12.09              | OP 57 Can development of post liver transplant cardiomyopathy be predicted from amyloid fibril composition? Suhr (Umea, Sweden)  |
| 12.21              | OP 58 Long term Effect of Liver transplantation on FAP on the neuropathy: Risk factors for progression of the walking disability. The 18 years French experience: a monocentric study in 200 patients. Adams (Paris, France) |

Program

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|--------------------|--|
| 12.33              | OP 59 Treatment of Advanced Heart Failure in Cardiac Amyloidosis with Left Ventricular Assist Device Therapy. Swiecicki (Rochester, USA)   |
| 12.45              | Perspectives - Zeldenrust  |
| <b>13.00-14.30</b> | <b>Lunch ('Fonteinpatio')</b>  |
| <b>13.30-14.30</b> | <b>Poster viewing individually ('Blauwe Patio')</b>  |
| 13.00-14.30        | Business meeting of the ISA ('Lokaal 16')  |
| <b>14.30-16.15</b> | <b>Plenary session 11: Chemotherapy in AL amyloidosis ('Blauwe Zaal')</b>  |
|                    | Chairmen: Palladini and Vellenga   |
| 14.30              | State of the art - Dispenzieri   |
| 14.45              | OP 60 Evaluation of an early switch to second line chemotherapy in AL amyloidosis among patients who fail to achieve a very good partial response to frontline treatment. Wechalekar (London, UK)  |
| 14.57              | OP 61 A phase I / II study of Lenalidomide with low dose oral cyclophosphamide and low dose dexamethasone (RdC) in AL amyloidosis. Kastritis (Athens, Greece)                                      |
| 15.09              | OP 62 Efficacy of Bortezomib/Cyclophosphamide/Dexamethasone (VCD) Chemotherapy In Naive Patients with High Risk Cardiac Light Chain Amyloidosis (Mayo Clinic stage III). Jaccard (Limoges, France) |
| 15.21              | OP 63 Salvage therapy with bendamustine and prednisone (BeP) in AL amyloidosis: a pilot study. Milani (Pavia, Italy)   |
| 15.33              | OP 64 The Activity of Pomalidomide in Patients with Immunoglobulin Light Chain Amyloidosis. Dispenzieri (Rochester, USA)   |
| 15.45              | OP 65 Phase I study of MLN9708, a novel, investigational oral proteasome inhibitor, in patients with relapsed or refractory light-chain amyloidosis (AL). Sanchorawala (Boston, USA)               |
| 16.00              | Perspectives - Schönland   |
| <b>16.15-16.30</b> | <b>ISA information: AMYLOID Journal – P. Westermark</b>  |
| <b>16.30-17.00</b> | <b>Tea break (Sponsor GSK) in the 'Fonteinpatio'</b>   |
| <b>16.30-17.15</b> | <b>Poster viewing (PC 1-66) in 5 groups ('Blauwe Patio')</b>   |
| <b>17.15-17.45</b> | <b>Selected Poster presentations PC 08, PC 28, PC 45, PC 50, and PC 61 ('Blauwe Zaal')</b>   |
|                    | Chairpersons: Minnema and Kuks   |
| 17.45-18.00        | Walk to the Groninger Museum   |
| <b>18.00-20.00</b> | <b>Visit Groninger Museum</b>  |
| 20.00-20.30        | Walk to the Der Aa Church  |
| <b>20.30-23.30</b> | <b>Congress Dinner and awards presentation ('Der Aa Church')</b>   |

**Thursday, May 10**

|                   |  |
|-------------------|--|
| <b>7.30-8.00</b>  | <b>Coffee ('Fonteinpatio')</b>   |
| <b>8.00-10.30</b> | <b>Plenary session 12: Design of targeted molecules and innovative drugs ('Blauwe Zaal')</b>   |
|                   | Chairmen: Ando and Hazenberg   |
| 8.00              | State of the art - Seldin  |
| 8.15              | OP 66 Intrinsic Apoptosis Can Occur Promptly After Silencing Lambda Light Chain Genes in Human Clonal Plasma Cells. Zhou (Boston, USA) |

|                    |       |   |
|--------------------|-------|---|
| 8.27               | OP 67 | Marked Removal and Clearance of AA Amyloid Deposits in Target Organs (Kidney, Liver and Spleen) by the Small Molecule Systebryl™ Following Oral Administration: A Potential Break-through Drug for the Treatment of Systemic AA Amyloidosis. Snow (Kirkland, USA) |
| 8.39               | OP 68 | A clinical Phase 3 confirmatory trial of eprodisate in the treatment of AA amyloidosis patients. Garceau (Laval, Canada)  |
| 8.51               | OP 69 | The Diflunisal Trial: Demographics, baseline neurologic staging, and adverse events. Berk (Boston, USA)   |
| 9.03               | OP 70 | Curcumin as a novel natural compound acting as TTR amyloidosis inhibitor <i>in vivo</i> . Ferreira (Porto, Portugal)  |
| 9.15               | OP 71 | Safety and efficacy of doxycycline plus taurooursodeoxycholic acid in transthyretin amyloidosis. Obici (Pavia, Italy)   |
| 9.27               | OP 72 | Antibody therapy against amyloid forms of transthyretin for familial amyloidotic polyneuropathy. Su (Kumamoto, Japan)   |
| 9.39               | OP 73 | Clinical Development of an Antisense Therapy for the Treatment of Hereditary Transthyretin Amyloidosis. Ackermann (Carlsbad, USA)   |
| 9.51               | OP 74 | Final Phase I safety, pharmacokinetic and pharmacodynamic results for ALN-TTR01, a novel RNAi therapeutic for the treatment of transthyretin amyloidosis. Coelho (Porto, Portugal)  |
| 10.03              | OP 75 | RNAi therapy using cholesterol-conjugated siRNA for TTR-related ocular amyloidosis. Tasaki (Kumamoto, Japan)  |
| 10.15              |       | Perspectives - Benson   |
| <b>10.30-11.00</b> |       | <b>Coffee break ('Fonteinpatio')</b>  |
| <b>11.00-12.00</b> |       | <b>Closing session: Conclusions and prospects ('Blauwe Zaal')</b>   |
|                    |       | Chair: Van Rijswijk   |
| 11.00              |       | Monday: Basic research - P. Westermark  |
| 11.15              |       | Tuesday/Wednesday: Clinical challenges - Skinner  |
| 11.30              |       | Wednesday/Thursday: Therapeutic prospects - Merlini   |
| 11.45              |       | Closing remarks - Hazenberg   |
| <b>12.00-13.00</b> |       | <b>Lunch ('Fonteinpatio')</b>   |
| 12.00-13.00        |       | Certificates of attendance ('Fonteinpatio')   |

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**14.00-18.00 PATIENTS' AFTERNOON: LIVING WITH AMYLOID**

|                    |  |
|--------------------|--|
| <b>13.30-14.00</b> | <b>Registration and coffee ('Fonteinpatio')</b>                                      |
| <b>14.00-15.20</b> | <b>Plenary session 1: Information about Amyloidosis, the disease ('Blauwe Zaal')</b> |
|                    | Chair: Hazenberg   |
| 14.00              | Welcome, introduction - Hazenberg  |
| 14.05              | Amyloid, typing, disease manifestations - Hazenberg                                  |
| 14.20              | Neuropathy as example of ATTR amyloidosis; treatment principles - Vrancken           |
| 14.35              | Cardiomyopathy and nephropathy as examples of AL: treatment principles - Minnema     |
| 14.50              | New developments in diagnosis; an overview - Skinner                                 |
| 15.05              | New developments in treatment; an overview - Merlini                                 |

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| <b>15.20-15.40</b> | <b>Tea break, collection of questions ('Fonteinpatio')</b>                                |
| <b>15.40-17.00</b> | <b>Plenary session 2: Do we need a patients' organization? ('Blauwe Zaal')</b>            |
| 15.40              | Amyloidosis in Australia - Neely  |
| 15.50              | Participation of patients and their organizations in research, cure and care - Oosterwijk |
| 16.05              | My experiences in establishing a patient's organization for a rare disease - Smit         |
| 16.20              | Panel discussion  |
| 16.50              | Summary of the afternoon; prospects and plans; closing part - Hazenberg                   |
| <b>17.00-17.45</b> | <b>Refreshing drinks and informal discussion ('Fonteinpatio')</b>                         |

Sunday, May 6. 17.00-17.30

## Keynote Lecture

### Amyloid in semen boosts HIV-1 transmission

**Jan Münch**

*Institute of Molecular Virology, Ulm University Medical Centre, Germany*

The great majority of all HIV-1 transmissions results from unprotected sexual intercourse and virus contaminated semen represents the major vector for the spread of the AIDS pandemic. We sought to identify factors in semen that modulate HIV-1 infection and found that semen contains small fragments of the prostatic acid phosphatase that spontaneously form amyloid structures termed SEVI<sup>1</sup>. These fibrils facilitate virion attachment to target cells and drastically enhance viral infectivity<sup>1</sup>. Thus, SEVI amyloid in semen may play a role in sexual HIV-1 transmission. In agreement with this hypothesis we showed that semen itself also boosts virus infection, and that the magnitude of the virus enhancing effect of semen correlates with the levels of SEVI<sup>2</sup>. More recently, we identified additional amyloid structures in semen with potent HIV enhancing activity<sup>3,4</sup>. Compounds that inhibit amyloid formation in semen or block the ability of fibrils to promote virus infection may represent a novel class of agents inhibiting sexual HIV-1 transmission<sup>5</sup>. Indeed, we and others identified and developed first "amyloid inhibitors" with different mechanisms of action that also abrogate semen mediated infectivity enhancement<sup>6-10</sup>. In addition, we show that SEVI also potently increases other virus infection<sup>11</sup>. Furthermore, our unpublished results suggest that a variety of natural amyloids also boost virus infection suggesting that this is an intrinsic amyloid property. In sum, our "seminal" finding that amyloid interacts with viruses and increases their infectivity opened an exciting new and interdisciplinary field of research with consequences not only for basic research in virology but eventually also for therapy and prevention of viral diseases.

#### References:

1. Münch J, et al., Cell. 2007; 131:1059-71.
2. Kim KA, et al., Retrovirology. 2010 Jun 23;7:55.
3. Arnold F, et al., J Virol. 2011 Nov 16.
4. Roan NR, et al., Cell Host Microbe. 2011. 10:541-50.
5. Kirchhoff and Münch. Future Virology. 2011, 6: 183-186
6. Hauber I, et al., Proc Natl Acad Sci U S A. 2009; 106:9033-8.
7. Roan NR, et al., J Virol. 2009; 83:73-80.
8. Roan NR, et al., J Biol Chem. 2010; 285:1861-9.
9. Olsen JS, et al., J Biol Chem. 2010; 285:35488-96.
10. Sievers SA, et al., Nature. 2011; 475:96-100
11. Hong S, et al., J Virol. 2009; 83:6995-7003



## Monday, May 7

- 7.30-8.00 Coffee ('Fonteinpatio')
- 8.00-8.30 Enno Mandema Memorial Lecture ('Blauwe Zaal'): Amyloidosis: 1979-2012-... - Sir Mark Pepys

## Plenary session 1

- 8.30-10.20 Fibril and Amyloid Formation ('Blauwe Zaal')  
Chairmen: Seldin and Limburg

- 8.35 State of the art - Fändrich

8.50 - OP 01

### Aggregates of the Alzheimer's Disease Amyloid Peptide A<sub>β</sub>40 Studied by Solid State NMR

Juan Miguel Lopez del Amo<sup>1</sup>, Uwe Fink<sup>2</sup>, Matthias Schmidt<sup>3</sup>, Gerlinde Grelle<sup>4</sup>, Jan Bieschke<sup>4</sup>, Marcus Fändrich<sup>3</sup>, Erich E. Wanker<sup>4</sup>, Bernd Reif<sup>5</sup>,

<sup>1</sup>Helmholtz German Research Center for Environmental Health

<sup>2</sup>Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)

<sup>3</sup>Max-Planck-Forschungsstelle für Enzymologie der Proteinfaltung

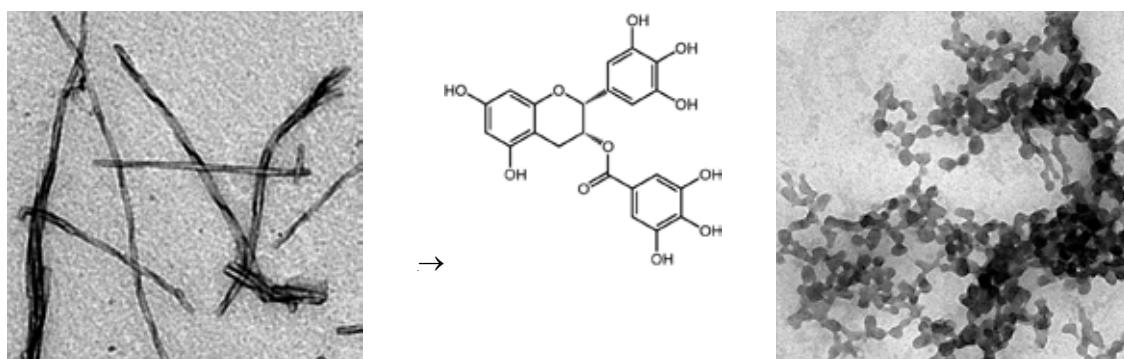
<sup>4</sup>Max Delbrück Center for Molecular Medicine (MDC)

<sup>5</sup>Technische Universität München (TUM), Germany

**Objectives:** The objectives of the present work are the investigation of the Alzheimer's disease responsible Amyloid-β (A<sub>β</sub>) peptide, the characterization of the A<sub>β</sub> fibrils present on the patients affected by the Alzheimer's disease, and the understanding at a molecular level the mechanism by which some natural compounds can eliminate the toxicity of the A<sub>β</sub> peptide.

**Results:** Depending on the preparation conditions, A<sub>β</sub> fibrils can adopt different morphologies and quarternary assemblies. In the past, a two-fold and a three-fold symmetric structural model for A<sub>β</sub>(1-40) fibrils have been suggested. We show that twisted fibrils formed by recombinantly produced A<sub>β</sub>(1-40) yield well dispersed solid-state NMR spectra. By NMR, we observe two sets of resonances which correspond to residues 12-40 and residues 21-38 in the A<sub>β</sub> primary sequence. Statistical analysis of the fibril morphology by electron microscopy (EM) reveals a single A<sub>β</sub> polymorph, indicating that the building unit in our A<sub>β</sub> amyloid fibril consists of an asymmetric dimer.

The green tea compound epigallocatechin-gallate (EGCG) inhibits Alzheimer's disease β-amyloid peptide (A<sub>β</sub>) neurotoxicity. We show that EGCG induced A<sub>β</sub> oligomers adopt a well defined structure. We find that EGCG interferes with the aromatic hydrophobic core of A<sub>β</sub>. The C-terminal part of the A<sub>β</sub> peptide (residues 22–39) adopts a β-sheet conformation, whereas the N-terminus (residues 1–20) is unstructured. The characteristic salt bridge involving residues D23 and K28 is present as well in the structure of these oligomeric A<sub>β</sub> aggregates. The structural analysis of small molecule induced amyloid aggregates will open new perspectives for Alzheimer's disease drug development.



**9.02 - OP 02****Structural modulation of A $\beta$  fibrils by glycosaminoglycans**

**J Madine<sup>1</sup>, M Pandya<sup>2</sup>, S Radford<sup>2</sup>, D Middleton<sup>1</sup>**

<sup>1</sup>*Institute of Integrative Biology, University of Liverpool,*

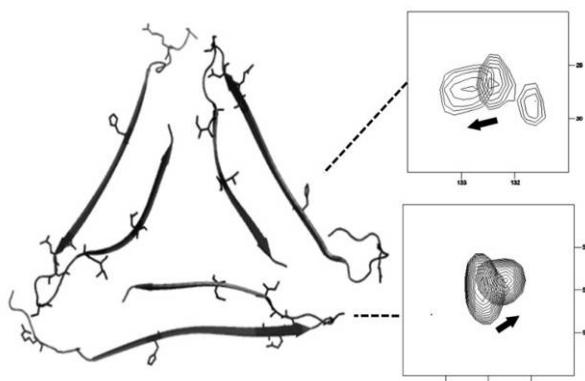
<sup>2</sup>*Astbury Centre for Structural Molecular Biology, University of Leeds, UK*

**Background:** The structural features of amyloid may be linked to cytotoxicity and is consequently a subject of intensive research. Completely unexplored at a structural level, is how fibril assemblies are modulated by factors ubiquitously associated with amyloid *in vivo*, including glycosaminoglycans (GAGs). Here, we are using solid-state nuclear magnetic resonance (SSNMR) methods to examine the structure of A $\beta_{1-40}$  fibrils formed in the presence of GAGs.

**Objective:** To provide insight into the role of the biological environment in influencing amyloid architecture.

**Methods:** Biophysical techniques (Thioflavin T, electron microscopy and GAG-binding assays) have been used to detect the interactions between A $\beta_{1-40}$  and a variety of GAGs. A range of SSNMR measuring chemical shifts and intermolecular dipolar couplings experiments are being employed to monitor the effect of GAGs on A $\beta_{1-40}$  fibrillar and oligomeric structure and to explore the GAG-protein interaction in greater detail (Madine, 2009a).

**Results:** SSNMR has identified key areas of sequence that are involved in the interaction of heparin with A $\beta_{1-40}$ . Initial results indicate that heparin binds to A $\beta_{1-40}$  during the fibril assembly process and also to the mature fibrils.



**Figure:** Proposed model for A $\beta_{1-40}$  3Q fibril architecture (Paravastu, 2008) with side-chains shown for residues with identified chemical shift changes upon heparin presence.

**Discussion:** In the light of these structural insights we now aim to test the effects of our recently discovered class of amyloid inhibitors (Madine, 2009b) on A $\beta_{1-40}$  and A $\beta_{1-42}$  aggregation and cytotoxicity in the presence of GAGs.

**Conclusion:** This data can be exploited to establish a platform to guide the structure-inspired design of compounds that target GAG-polypeptide interactions for therapeutic gain.

**References:** Madine et al. 2009a, Organic & Biomolecular Chemistry 7:2414-2420; Paravastu et al. 2008, PNAS 105:18349; Madine et al. 2009b, ChemBioChem 10:1982-1987.

**9.14 - OP 03****Structure and pathogenicity of mature fibrils and their structural precursors**

**Marcus Fändrich**

*Max-Planck-Forschungsstelle für Enzymologie der Proteinfaltung & Martin-Luther Universität Halle-Wittenberg, Weinbergweg 22, D-06120 Halle (Saale), Germany.*

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**Background:** The understanding of the mechanism of amyloid formation and pathogenicity is hampered by extremely limited data regarding the structural details of amyloid fibrils and their structural precursors. This lack of knowledge mainly reflects the heterogeneity of aggregate samples and their intrinsic inaccessibility to traditional techniques of high-resolution protein structure analysis.

**Objectives:** We have employed a battery of biophysical techniques to study certain amyloid states and to correlate structure with possible pathogenic properties.

**Methods:** Electron cryo microscopy (cryo EM), NMR and other biophysical techniques, various biological assays. Most analyses were done by using Alzheimer's A $\beta$  peptide.

**Results and Discussion:** Cryo EM enabled us to reconstruct the three-dimensional architecture of full-size fibrils at resolutions of better than 10 Å. These data revealed the protofilament subunit structure and packing of the cross- $\beta$  sheet within mature fibrils. We also identified commonalities between different fibril morphologies and analyzed the significant bending rigidity of mature filaments, which is thought to underlie their pathogenicity. However, this pathogenic mechanism differs from the neurotoxicity associated with oligomeric or protofibrillar intermediates. We have characterized their activity with long term potentiation measurements and other techniques. In addition, we mapped out the secondary structural elements of these states by NMR spectroscopy. Taken together, these analyses led to the development of a novel inhibitory peptide, targeting oligomer-dependent dysfunctions.

**Conclusions:** Currently available techniques enable detailed structural analyses of the mechanism of fibril formation, but solving the atomic structures of amyloid states remains challenging. However, developing methodologies hold the promise to more routinely enable determination of atomic resolution structures in the near future.

**References:** Angew. Chem. Int. Edt. 2012, DOI: 10.1002/anie.201105638; Angew. Chem. Int. Edt. 2011, 50, 2837 –2840; TiBS, 2011, 36, 338-345; Angew. Chem. Int. Edt. 2010, 49, 1321 –1323; PNAS 2009, 106, 19813–19818; PNAS 2008, 105, 7462–7466

## 9.26 - OP 04

### Towards a cure for Parkinson's Disease: inhibitors of $\alpha$ -synuclein aggregation

**Nikolai Lorenzen**<sup>1</sup>, Lise Giehm<sup>1</sup>, Søren B. Nielsen<sup>1</sup>, Wojciech Paslawski<sup>1</sup>, Jørn D. Kaspersen<sup>1</sup>, Brian V. Stougaard<sup>1</sup>, Jan S. Pedersen<sup>1</sup>, Warren D. Hirst<sup>2</sup>, Daniel E. Otzen<sup>1</sup>

<sup>1</sup>Interdisciplinary Nanoscience Research Centre (iNANO), Aarhus University, Denmark

<sup>2</sup>Pfizer Ltd. Neuroscience Research Unit, Pfizer Incorporated, Groton, Connecticut, United States of America

Aggregation of  $\alpha$ -synuclein ( $\alpha$ SN) is a central event in the development of Parkinson's Disease (PD). A key component is an oligomer that accumulates in the fibrillation process which has been shown *in vitro* to have membrane-permeabilizing properties and to be cytotoxic *in vivo*. Thus, inhibition of the formation or function of this oligomer is of potential therapeutic benefit for PD. Using small-angle X-ray scattering, we have determined the low-resolution structure of the oligomer accumulating during the fibrillation of  $\alpha$ SN [1]. Our results reveal a wreath-shaped structure containing around 16 monomers, with a central channel that could possibly function as a membrane pore and with dimensions corresponding to the width of the mature fibril. Developing compounds that can inhibit fibril formation and oligomer toxicity is challenged by the fact that  $\alpha$ SN is natively unfolded, making it essentially impossible to target the monomer, and furthermore aggregation is notoriously erratic. However, we have developed a high throughput assay in which we exploited the ability of SDS to induce reproducible aggregation behavior [2,3]. The reproducibility resides in the fact that at suitable protein: SDS molar ratios, SDS induces the formation of shared micelles in which 4 protein molecules convene to "pool" bound SDS. This presumably allows the unbound part of the proteins to engage with other such micellar complexes in amyloid bridging complexes, bypassing the rate-limiting and stochastic nucleation step. This assay has allowed our collaboration partners at Pfizer to screen 746,000 compounds. The best 60 of these compounds have been analyzed for their effect on  $\alpha$ SN aggregation and have a remarkable ability to inhibit oligomer activity towards membranes. I will discuss progress in our understanding of how these compounds modulate  $\alpha$ SN aggregation behavior.

1. Giehm *et al.* PNAS 2011
2. Giehm *et al.* JMB 2010
3. Giehm & Otzen AnalBiochem 2010

### 9.38 - OP 05

#### In vivo and in vitro anti-Amyloid functions of the systemic amyloid precursor Transthyretin (TTR)

**Joel N. Buxbaum**

*Departments of Molecular and Experimental Medicine and Molecular Integrative Neuroscience, The Scripps Research Institute, USA*

**Background:** Several laboratories (including our own) have shown increased TTR expression in human AD neurons and the neurons of transgenic AD model mice. We have also shown that over-expression of wild type human TTR suppresses the neuropathologic and behavioral manifestations of AD in the APP23 transgenic model of the human disease.

**Objective:** To examine the mechanism of the in vivo effect

**Methods:** Immunohistochemistry, immunologic identification and purification; in vitro fibril forming assays, transmission EM, AFM, dynamic light scattering, surface Plasmon resonance, primary neuronal culture

**Results:** Human and murine transthyretins inhibit fibril formation by a variety of amyloid precursors including A $\beta$ , Curli, HypF-N. The inhibitory capacity is inversely related to the stability of the molecular species of TTR. Complexes of TTR and A $\beta$  can be isolated from the hippocampi and cerebral cortex of APP23 AD model mice and some human AD brains. TTR and 6E10 positive material co-localize in cultured primary neurons obtained from APP23 mice.

**Discussion:** The breadth of molecules with which TTR interacts suggests a common structure that allows interaction between and among various amyloid precursors. In at least one model, i.e. the APP23 AD mouse, the interaction is salutary in vivo. The full implication of these findings, with respect to protein homeostasis in vivo and clinical aspects of AD, remains to be elucidated.

**Conclusion:** TTR can inhibit fibril formation by a variety of amyloid precursors including A $\beta$ , IAPP and prokaryotic HypF-N and the functional amyloid precursor curli.

**References:** Buxbaum et al PNAS (USA) 105:2681, 2008; Li et al JNS 31:12483, 2011

### 9.50 - OP 06

#### Two types of fibril compositions in ATTR amyloidosis and their correlation to clinical phenotype

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**Introduction:** We have previously shown that two types of fibril compositions exist in ATTRV30M amyloidosis. The fibrils are either composed of a mixture of fragmented and full-length ATTR (type A), or only full-length ATTR (type B) [1]. The fibril type is correlated to different clinical phenotypes. In essence, type A is associated with a late disease onset and myocardial deposition causing cardiomyopathy, while type B is found in patients with early onset and cardiac amyloid mainly restricted to the subendocardium [2]. In an effort to see if this phenotype-fibril type correlation is a universal phenomenon in familial ATTR amyloidosis, we determined the fibril type in patients with other mutations than V30M and correlated it to their age of onset and presence of cardiomyopathy.

**Material/Method:** Cardiac or abdominal adipose tissue from 48 patients with 21 different TTR mutations was investigated. Fibril type was determined by western blot analysis. Interventricular septum thickness, determined by echocardiography, was used as a measurement for cardiomyopathy.

**Results:** Fragmented ATTR (type A fibrils) was found in all patients. Four patients had an interventricular septum thickness just within normal limits, while all other patients had an abnormally thickened septum. Sixteen patients had an age-of-onset below 50 years.

**Discussion:** The consistent observation of fibril type A in all patients, regardless of age, suggests that a fibril composition with fragmented ATTR is the standard fibril composition and that fibrils with only full-length ATTR are an exception, perhaps only found among young V30M patients. However, fibril type A seems to be strongly associated with cardiomyopathy, regardless of mutation. This could be one reason why progression of cardiac amyloidosis after liver transplantation in general is a larger problem among patients with other TTR mutations than V30M.

**Conclusion:** Our studies indicate that amyloid fibril composition can affect both clinicopathology and treatment outcome.

**References:**

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**10.02              Perspectives - Bellotti**

**10.20-10.45      Coffee break ('Fonteinpatio')**

## Plenary session 2

**10.45-12.30      Cell and tissue targeting and toxicity ('Blauwe Zaal')**

Chairmen: Grateau and Luiten

**10.45              State of the art - Kisilevsky**

**11.00 - OP 07**

### Biochemical properties of highly virulent prion strains

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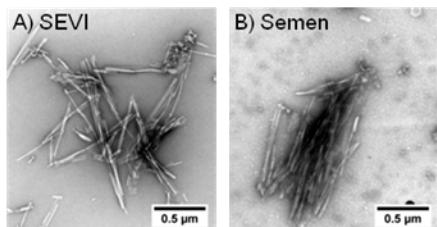
Prion diseases are fatal neurodegenerative diseases caused by the conformational conversion of the cellular prion protein, PrP<sup>C</sup>, into an aggregated, β-sheet rich isoform, PrP<sup>Sc</sup>. Prion pathogenesis can vary dramatically depending on the strain, thought to be conformational variants of PrP<sup>Sc</sup>, yet the structural basis for the differences in pathogenesis is unclear. Using an array of mouse-adapted prion strains, here we define the neuropathology and biochemical features of prion strains that efficiently or poorly invade the CNS from their peripheral entry site. In brain sections, we find that the highly neuroinvasive prion strains primarily form diffuse aggregates that do not bind to the amyloid binding dye, Congo red. Biochemically these aggregates are conformationally unstable in denaturing conditions. These neuroinvasive strains also efficiently generate PrP<sup>Sc</sup> over short incubation periods. In contrast, the poorly neuroinvasive strains formed dense, Congo red binding aggregates that were conformationally stable. Our findings indicate that the most neuroinvasive, efficiently spreading strains are also the least conformationally stable, and support the concept that the unstable nature of the most rapidly converting prions may be a feature linked to their efficient spread into the CNS.

**11.12 - OP 08****Detection of endogenous amyloid fibrils in human semen**

**Shariq M. Usmani, Onofrio Zirafi and Jan Münch**

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AIDS is a sexually transmitted disease and virus laden semen represents the main vehicle for the spread of HIV-1. We found that semen potently enhances HIV-1 infection of its cellular targets. By screening a semen-derived peptide library we previously identified fragments of the abundant semen protein PAP that self-assembles *in vitro* into amyloid fibrils termed SEVI (Semen derived enhancer of viral infection) (Münch et al. Cell. 2007). SEVI amyloid increase HIV attachment to and infection of target cells. Recently, we identified additional amyloid-forming peptides in semen that also enhance HIV-1 infection (Roan et al. 2011 Cell Host Microbe; Arnold et al. 2012 J. Virol.). Several lines of evidence support that semen contains amyloid with HIV enhancing activity: 1) Peptides isolated from semen form amyloid fibrils; 2) the HIV-1 enhancing activity in individual semen samples correlates with SEVI levels; and 3) amyloid-specific antibodies allow depletion of HIV-1 enhancing activity of semen. These data suggest that amyloid in semen plays an essential role in the spread of the AIDS pandemic. However, up to now the presence of amyloid fibrils in semen has not been unequivocally shown. Here, we asked whether human semen contains typical amyloid-like fibrils. We first stained individual semen samples with an amyloid-specific fluorescence-based dye. Using confocal microscopy we identified fluorescent aggregates which numbers and sizes varied from donor to donor. Next, we incubated these samples with GFP-tagged viruses and observed a highly efficient complex formation between stained aggregates and the virions, which closely resembled those of previously characterized SEVI/HIV-1 complexes. Finally, transmission electron microscopy allowed detection of typical amyloid fibrils in semen. The fibrils range from 0.5-5 µm in size and show twisted cross-β structure. To our best knowledge, this is the first report providing evidence for the presence of amyloid fibrils in human semen.



**Fig. 1:** Transmission electron micrographs depicting amyloid fibrils. (A) SEVI fibrils generated from synthetic PAP248-286. (B) Liquefied human semen

**11.24 - OP 09****A proteomic approach to unravel the cytotoxic responses to different aggregation-prone proteins**

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Neurodegenerative disorders are frequently associated with the deposition of specific aggregation-prone proteins which are critically linked to neuronal dysfunction. Characterizing the cellular responses to these proteinopathies is of crucial importance to understanding disease pathogenesis and developing novel therapeutics. Over the last decade, yeast models using heterologous expression of proteins involved in Parkinson's disease (PD; α-synuclein)<sup>1</sup>, Alzheimer's disease (AD; Aβ<sub>1-42</sub>)<sup>2</sup> and frontotemporal lobar dementia/amyotrophic lateral sclerosis (FTLD/ALS; TDP-43)<sup>3</sup> have revealed intriguing novel insights into the pathobiology underlying these disorders.

We have now applied to a set of such yeast models a novel proteomic strategy, based on unbiased screens and targeted mass spectrometry assays<sup>4,5</sup>, to identify the proteome-wide responses of cells to

the different disease-associated proteins. Yeast cells expressing human  $\alpha$ -synuclein, A $\beta_{1-42}$ , or TDP-43, at different levels of toxicity were examined first using integrated shotgun proteomic and phosphoproteomic measurements. The analyses highlighted functional modules and their signaling states deregulated by each protein. Comparison of the different disease models allows discrimination between generic cellular responses and those that are specific to each proteinopathy. Such measurements were correlated at each time point to the amount of heterologous protein in its soluble and insoluble form. Next, we selected a set of ~100 protein and phosphoprotein markers of toxicity which responded at the level of different functional modules. An assay based on selected reaction monitoring (SRM) mass spectrometry was designed<sup>6</sup> to allow measurement of the ~100 markers in about 1 hour of instrument time, from whole yeast extracts. The assay will now be used to probe the effects of a set of known genetic and chemical modulators and to determine which affected cellular responses they restore. Such assay can probe multiple pathological features at the same time, thus constituting a new tool for drug screening and the elucidation of enigmatic mechanisms of action.

### References:

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### 11.36 - OP 10

#### Investigating vulnerability to proteasome inhibition in primary light chain amyloidosis

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Systemic light chain amyloidosis (AL) is a plasma cell (PC) dyscrasia caused by a small bone marrow PC clone producing an unstable immunoglobulin (Ig) light chain that aggregates and accumulates systemically into amyloid fibrils, impairing organ function.

Originating from a clonal PC expansion, AL is clinically treated with the same chemotherapy exploited against multiple myeloma (MM). Among different treatments, the proteasome inhibitor (PI) bortezomib proved very effective, yielding very high response rates, nearing 90% complete remission when associated with alkylators.

Studies on primary MM PCs implicated an unfavorable balance between the degradative load on proteasomes, likely deriving from Ig production, and overall proteasome capacity as a crucial intrinsic determinant of cell sensitivity to bortezomib.

We thus hypothesized that amyloidogenic chain production generates additional cellular stress as compared to non amyloidogenic chains, possibly underlying the exquisite PI sensitivity of AL cells, and offering a framework for identifying new molecular targets.

To test our hypothesis, we assessed ex vivo apoptotic responses to bortezomib in bone marrow-purified PCs from AL and MM, the prototypical PI-sensitive cancer.

PCs from AL patients proved very sensitive to bortezomib, and interestingly, twice more than MM PCs (EC50:  $6.3 \pm 1.2$  nM vs.  $12.1 \pm 3.3$  nM). Unexpectedly, though, the higher bortezomib sensitivity observed was not associated with heavier proteotoxic stress (as assessed by immunofluorescence of accumulated ubiquitin-conjugates), nor with reduced degradative capacity (as assessed by overall beta-chymotryptic activity), as compared to MM PCs.

Altogether, our data reveal, for the first time, an intrinsic vulnerability of primary AL PCs under pharmacologic proteasome stress, despite efficient basal management of proteostasis, possibly owing to the higher degradative demand posed by amyloidogenic chains. Thus, further dissection of the intracellular fate of amyloidogenic light chains holds promise towards the identification of new therapeutic targets against AL.

Conflicts of interest: G.P.: Honoraria from Janssen-Cilag and Celgene

#### 11.48 - OP 11

#### Unique features that characterize urinary exosomes in light chain amyloidosis (AL)

**Marina Ramirez-Alvarado**, Christopher J. Ward, Bing Q. Huang, Xun Gong, Marie C. Hogan, Benjamin J. Madden, Cristine Charlesworth, Nelson Leung

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Recent studies have shown urinary exosomes containing kidney-derived membrane and cytosolic proteins that can be used to understand the cellular status in health and disease within the urinary system from the glomerulus to the bladder in a non-invasive manner. This is particularly relevant for light chain (AL) amyloidosis as the most common organ involved is the kidney. We have previously observed the presence of oligomeric light chains in the urinary exosomes from AL amyloidosis patients. These oligomeric light chains were found in glomerular exosome populations, the main site of amyloid deposition in the kidney. Urinary exosomes from multiple myeloma (MM), monoclonal gammopathy of undetermined significance (MGUS) and non-paraproteinemia related kidney disease controls only present monomeric light chains followed by western blot. In this study, we have performed additional analysis of urinary exosomes from patients with AL amyloidosis that have monoclonal lambda or kappa protein. High molecular weight light chain species are also found in exosomes from AL amyloidosis patient with monoclonal lambda or kappa, but the amount of oligomeric species is reduced with respect to AL amyloidosis patients with monoclonal immunoglobulin (Ig) presents. The glomerular exosomal population in all AL amyloidosis patients' samples has increased its density with regards to normal controls for both lambda and kappa and Ig exosomes. This is probably due to the presence of large oligomeric light chain species as observed by electron microscopy. Proteomic analysis of exosome samples from AL amyloidosis patients and controls demonstrate unique protein profile for pathogenic exosomes. Our results showed that urinary exosomes may have tremendous potential in furthering our understanding of the pathophysiology and diagnosis of plasma cell dyscrasia related kidney diseases.

#### 12.00 - OP 12

#### Fighting familial amyloidosis using an impaired ER-stress response yeast strain

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Amyloidosis is a group of diseases caused by the deposition of insoluble and toxic proteins in tissues. Hereditary amyloidosis, also designated transthyretin-mediated amyloidosis, are rare and lethal disorders mostly caused by mutations in transthyretin (TTR), a plasma protein associated with an autosomal dominant neurodegenerative disorder called Familial Amyloidotic Polyneuropathy (FAP). Currently, over 100 different TTR mutations have been identified. V30M mutation is associated with the Portuguese form of FAP and L12P mutation is associated with CNS deposition. It is known that induction of endoplasmatic reticulum (ER) stress due to expression and accumulation of TTR variants contributes to the pathogenesis of amyloidosis, although the precise mechanism is still unknown. The

aim of this study was to develop an ER-stress response TTR dependent disease model in yeast. Cell viability assays were performed in both solid and liquid media. Interestingly, it was observed that although expression of TTR variants in a wild type yeast strain did not affect cell growth, TTR L12P expression in  $\Delta hac1$  and  $\Delta ire1$  knock-out strains, caused total growth impairment. Surprisingly immunoblotting analysis revealed that this TTR variant was the less expressed, suggesting a toxic effect of TTR L12P even at low protein levels. To validate the sensitivity of  $\Delta hac1$  and  $\Delta ire1$  to ER stress response, a reporter vector expressing GFP under the control of the yeast ER stress promoter *KAR2* was used. As expected, GFP signal was decreased in  $\Delta hac1$  and  $\Delta ire1$  strains as compared to a wild type strain, indicating that ER stress response machinery in the knock-out strains susceptible to TTR L12P expression is impaired, mechanistically validating our hypothesis. These results show that expression of mutant TTR in ER-stress impaired yeast can model toxicity associated to the human pathology and provide the basis of a screening tool for the identification of compounds able to modulate ER stress response pathway in FAP.

### **References:**

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**12.12 Perspectives - Buxbaum**

**12.30-14.00 Lunch (Spectral Imaging) in the 'Fonteinpatio'**

**13.00-14.00 Poster viewing individually ('Blauwe Patio')**

## **Plenary session 3**

**14.00-16.00 Animal models and cell culture systems ('Blauwe Zaal')**  
Chairpersons: Gruys and G. Westermark

**14.00 State of the art - Saraiva**

**14.15 - OP 13**  
**Heparin/Chitosan nanoparticle delivery system for *in vivo* evaluation of glycosaminoglycans influence on transthyretin deposition**

**Nádia Pereira Gonçalves<sup>1,2</sup>, Ana Paula Pêgo<sup>3</sup>, Maria João Saraiva<sup>1,2</sup>**

*1. Molecular Neurobiology Unit, IBMC – Instituto de Biologia Molecular e Celular, Portugal*

*2. Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Portugal*

*3. New therapies Group, INEB - Instituto de Engenharia Biomédica, Portugal*

**Background:** Familial amyloidotic polyneuropathy (FAP) is a human autosomal dominant neurodegenerative disorder characterized by extracellular deposition of mutant transthyretin (TTR) aggregates and amyloid fibrils, particularly in the peripheral nervous system. Recent studies indicate the potential involvement of heparan sulfate proteoglycan with amyloidogenic proteins and in modulation of TTR deposition.

**Objective:** In the present work, nerve biopsies of human asymptomatic carriers and FAP patients were analyzed for heparan sulfate and although mainly associated with amyloid fibrils, this component is also present in earlier stages of disease in association with non-fibrillrogenic aggregates. Therefore, to study *in vivo* the role of glycosaminoglycans in TTR deposition, we created a novel local delivery system into mice sciatic nerve using heparin/chitosan (CH) nanoparticles.

**Methods and Results:** To address drug release and diffusion throughout nerve tissue, fluorescently labeled CH and heparin were used in fluorescence microscopic analysis. Our results show maximal absorption throughout the nerve extracellular matrix and no major inflammatory response activation. Using this optimized system, heparin/CH nanoparticles were locally injected into sciatic nerve of transgenic mice carrying the most prevalent TTR mutation – V30M, in a TTR null background and lacking the heat shock factor 1 (HSF1), having in average 14 months of age. Contralateral nerve receiving uncoupled CH particles was used as control. 15 days after nanoparticles application, both heparan sulfate and TTR deposition significantly increased in treated nerves, although no amyloid was detected, by Congo Red staining.

**Discussion and Conclusion:** Our work demonstrates that changes in proteoglycan type and distribution could possibly account for alteration of the physical properties of tissues, increasing TTR deposition *in vivo*. Moreover, this new and versatile nanoparticle delivery system opens novel avenues in the field of neuropathology and probably in the design of therapeutic strategies targeting directly nerve tissue.

**Reference:** Gonçalves NP et al, Nanomedicine, *In Press*

#### 14.27 - OP 14

#### Multi-system disease-specific induced pluripotent stem cell modeling of familial amyloidosis

**Amy Leung**<sup>1,5</sup>, Shirley Nah<sup>1,5</sup>, Clarissa Koch<sup>2</sup>, Stefano Monti<sup>2</sup>, John Berk<sup>2</sup>, Lawreen Connors<sup>2</sup>, David Seldin<sup>1,2</sup>, Darrell Kotton<sup>3,5</sup>, Gustavo Mostoslavsky<sup>4,5</sup>, George Murphy<sup>1,5</sup>.

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5. Center for Regenerative Medicine (CReM), \*All sections affiliated with the Department of Medicine, Boston University School of Medicine, Boston, Massachusetts, USA

The recent discovery that pluripotent cells similar to embryonic stem cells (ESC) can be generated by somatic cell reprogramming provides an unprecedented opportunity to model and to develop regenerative therapies for human genetic diseases. Unlike ESC, induced pluripotent stem cells (iPSC) are genetically identical to the individual they are derived from, allowing for disease modeling and the development of novel therapeutics in the exact genetic context of the patient.

Familial TTR Amyloidosis (ATTR) is an autosomal-dominant, multisystemic genetic disease that is characterised by the misfolding and accumulation of mutant serum-protein transthyretin (TTR). While aberrant TTR is produced and secreted by the liver, disease-characteristic cellular damage is seen peripherally, primarily in the peripheral nervous system and cardiac tissue. There is currently a need to understand the molecular and cellular basis of the disease and to develop effective therapeutics to prevent/slow disease progression.

Using our highly efficient stem cell cassette (STEMCCA) reprogramming system, we have generated the first known disease-specific iPSC lines from hereditary amyloidosis patients. Harnessing the flexibility of a pluripotent stem cell-based system, we can demonstrate the successful modeling of this multi-systemic disease through the directed differentiation of disease-specific iPSC into both the effector cells (hepatocytes) that produce mutant TTR protein and the peripheral target cells (cardiomyocytes, neurons) that are damaged by exposure to the aberrant protein.

In our model, we demonstrate that ATTR-iPSC derived neuronal and cardiac cells display oxidative stress and an increased level of apoptosis and cell death when exposed to aberrant TTR protein produced in the supernatant of ATTR-iPSC derived hepatocytes, thus recapitulating aspects of the disease in an *in vitro*, genetically tractable setting. Furthermore, the deleterious effects of ATTR hepatic supernatants were negated in the presence of known small molecule stabilizers of TTR such as diflunisal, thus validating the iPSC-based system as a suitable platform for small molecule drug testing.

**14.39 - OP 15****Anti-amyloid drug development using Drosophila as a model**

**Daniel Segal**<sup>1</sup>, Roni Scherzer-Attali<sup>1</sup>, Sivan Peled<sup>1</sup>, Ronit-Shaltiel-Karyo<sup>1</sup>, Moran Frenkel-Pinter<sup>1</sup>, Dorit Farfara<sup>2</sup>, Dan Frenkel<sup>2</sup>, Ehud Gazit<sup>1</sup>

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Drosophila offers an attractive model for drug development which can reduce the use of vertebrates and cut costs. Our recent work on developing inhibitors of amyloid assembly exemplifies this strategy. We have previously identified a key role of aromatic residues in the molecular recognition and self-assembly leading to the formation of various amyloid assemblies. Aromatic interactions provide selectivity as well as stability to the interacting molecules. Our strategy is to use small aromatic molecules that would bind the aromatic residues of the beta-amyloid (A $\beta$ ) monomers in Alzheimer's disease (AD) thereby inhibit the early steps of the molecular recognition and structural transition of the monomers which lead to the formation of the toxic amyloid species. We have synthesized a series of N-linked tryptophan-modified quinones and screened them for anti-A $\beta$  activity. Two compounds, NQTrp and Cl-NQTrp, were most effective. They inhibit A $\beta$  oligomerization and fibrillization in vitro and reduce the cytotoxic effect of A $\beta$  oligomers towards cultured cells. NMR spectroscopy and molecular dynamics simulations provide a mechanistic basis for the activity of these compounds. When fed to Drosophila expressing A $\beta$  in their nervous system, these compounds alleviated their AD-related symptoms while having no effect on control flies. When injected intraperitoneally to 5xFAD transgenic acute AD mice they led to specific and significant improvement of their cognitive behavior, dramatic reduction in the level of both soluble and insoluble A $\beta$  in their brain extracts and marked decrease in A $\beta$  deposition in their brains. The compounds can cross the blood-brain-barrier and have no adverse effects. Initial results indicate that they are effective also against other amyloidogenic proteins. We also showed that  $\beta$ -synuclein-derived peptidomimetics designed to inhibit the toxic amyloid assembly of  $\alpha$ -synuclein in Parkinson disease (PD) have marked remedial effect in Drosophila expressing  $\alpha$ -synuclein in their brain which serve as an established model for PD.

**14.51 - OP 16****C. elegans expressing human  $\beta$ 2-m recapitulates the molecular mechanisms underlying dialysis-related amyloidosis**

**Cristina Soria**<sup>2</sup>, Luisa Diomedè<sup>1</sup>, Sofia Giorgetti<sup>2</sup>, Margherita Romeo<sup>1</sup>, Patrizia Mangione<sup>2,3</sup>, Riccardo Porcari<sup>2</sup>, Sara Raimondi<sup>2</sup>, Loredana Marchese<sup>2</sup>, Irene Zorzoli<sup>2</sup>, Mario Salmona<sup>1</sup>, Monica Stoppini<sup>2</sup>, Vittorio Bellotti<sup>2,3</sup>

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$\beta$ 2-microglobulin ( $\beta$ 2-m) is the light chain of MHC I and is the causative protein of the amyloidosis associated to long-term haemodialysis where a form of this protein, free from the heavy chain, circulates in plasma at high concentration. In the absence of any well-established mammalian model of dialysis-related amyloidosis, the nematode *C. elegans*, lacking of the heavy chain of MHC I, represents an attractive and powerful *in vivo* animal model to investigate the pathophysiology basis of this disease, because all the expressed  $\beta$ 2-m is in the free form.

We have generated two new transgenic strains of *C. elegans* constitutively expressing, in the body-wall muscle, the human wild type  $\beta$ 2-m and the more *in vitro* amyloidogenic variant P32G  $\beta$ 2-m, that well reproduce the non-native folding intermediate I<sub>T</sub>.

$\beta$ 2-m expression in transgenic worms causes a significant slowdown in larval development and a reduction in lifespan, in comparison with the control strain, as well as behavioral defects which are worsened in P32G expressing worms. The worsened disease phenotypes of mutated worms, specifically correlates with the higher propensity of the P32G protein to form oligomeric assemblies. Despite the differences among nematodes and vertebrates, the availability of these new transgenic *C. elegans* strains recapitulates *in vivo* the molecular mechanisms of protein misfolding and aggregation underlying dialysis-related amyloidosis. These models may be an ideal platform to develop and test innovative amyloid inhibitors.

### 15.03 - OP 17

#### Doxycycline Interaction with Amyloidogenic Proteins and Activity in Mouse Models

**Jennifer Ellis Ward**<sup>1,2</sup>, Elena Klimtchuk<sup>1</sup>, Ruiyi Ren<sup>1,3</sup>, Varuna Shibad<sup>1,2</sup>, Carl O'Hara<sup>1,4</sup>, Vickery Trinkaus-Randall<sup>1,3</sup>, John Berk<sup>1,2</sup>, Jeffery W. Kelly<sup>5</sup>, Lawreen H. Connors<sup>1,3</sup> and David C. Seldin<sup>1,2</sup>  
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**Background:** Doxycycline (DOX) and other tetracycline (TET) analogs have been found to interfere with protein aggregation in a variety of diseases.

**Objective:** To assess the ability of DOX to bind to amyloidogenic proteins, disrupt fibrils *in vitro*, and block or reverse amyloid deposition *in vivo*.

**Methods:** Interaction of DOX with LC was assessed by surface plasmon resonance (SPR). Fibrils extracted from patient tissue and recombinant LC were incubated *in vitro* with DOX and examined by negative stain electron microscopy. Amyloid deposition was assessed in mouse models of AL (CMV-λ6 transgenics<sup>1</sup>) and AF (D187N-gelsolin transgenics<sup>2</sup>) by Congo red staining visualized by polarized light or fluorescence microscopy. Matrix metalloproteinase (MMP) activity was assessed by zymography.

**Results:** As assessed by SPR, DOX interacted directly with recombinant amyloidogenic LC. Incubation of LC with DOX reduced fibril formation *in vitro*, and disrupted fibrils purified from tissues *ex vivo*, producing disordered aggregates. In the CMV-λ6 light chain transgenic mice, DOX markedly reduced development of fibrils when administered throughout adulthood, and MMP levels were affected. Deposits and MMP activity in D187-gelsolin transgenic mice is also being investigated.

**Conclusion:** DOX interacts directly with amyloidogenic LC, disrupts LC fibrils *in vitro*, and can prevent amyloid formation *in vivo*. In addition to directly interacting with amyloidogenic proteins, DOX may also affect the proteolytic milieu in tissues, altering precursor protein processing or the tissue microenvironment.

#### References:

1. Ward JE et al., Doxycycline reduces fibril formation in a transgenic mouse model of AL amyloidosis. *Blood*. 2011;118(25):6610-6617.
2. Page LJ et al., Secretion of amyloidogenic gelsolin progressively compromises protein homeostasis leading to the intracellular aggregation of proteins *PNAS* 2009; 106 (27) 11125-1113.

Supported by the Gruss and Wildflower Foundations, and NIH Grants DK090696 (DCS) and AG18917 (JWK).

### 15.15 - OP 18

#### An all human cell culture model of AA amyloid formation: human serum amyloid A (SAA) and human peripheral blood mononuclear cells (PBMC)

**Wataru Ishii**<sup>1</sup>, Toshiyuki Yamada<sup>3</sup>, Juris Liepnieks<sup>1</sup>, Merrill D. Benson<sup>1, 2</sup>, and Barbara Kluve-Beckerman<sup>1</sup>

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**Background:** Mouse SAA1.1 was previously shown to undergo amyloid formation in cultures of human peripheral blood mononuclear cells (PBMC) in medium containing fetal calf serum (FCS). Human SAA in this system did not form amyloid.

**Objective:** To develop cell culture system that supports amyloid formation from human SAA.

**Methods:** PBMC from 8 donors were cultured in serum-free medium (SFM) containing human SAA. Amyloid-enhancing factor was not used. SAA was characterized by SDS-PAGE, N-terminal sequencing, western blotting, and molecular sieve chromatography. Amyloid was identified by Congo red staining.

**Results and Discussion:** Human SAA-derived amyloid formation has been achieved in PBMC cultures through use of SFM, PBMC from certain donors, and SAA1.3. Rapid and extensive amyloid formation occurred in SFM versus FCS medium and correlated with greater amount of SAA transitioning from medium to cells. SAA-cell association was essential but not sufficient for amyloid formation. Cultures established from all PBMC donors demonstrated cellular accumulation of SAA (n = 8), while conversion into amyloid occurred only in cultures from certain donors (n = 4). Amyloid (-) and amyloid (+) cultures exhibited distinct differences possibly reflecting amyloid-protective or permissive factors. The most obvious feature of amyloid (-) cultures was aggressive reorganization of monocytes into dense 3-dimensional aggregates. Some cells within aggregates died exposing gritty, SAA immunoreactive, non-birefringent material. Amyloid (+) cultures lacked dense cell aggregates and instead contained strongly Congophilic, birefringent deposits overlaying or intermeshed amongst cells that had grouped into loose clusters. Cells of dendritic or fibrocytic morphology were prominent and often in networks encompassing amyloid. These features suggest factors affecting cell migration, e.g., chemokines and matrix proteins, may be differentially expressed in amyloid (+) and amyloid (-) cultures. While all 3 human isoforms, SAA1.1, 1.3, and 1.5, formed amyloid, SAA1.3-derived deposits were most extensive, consistent with SAA1.3 being the most prevalent in Japanese subjects with AA amyloidosis.

#### 15.27 - OP 19

#### Macrophage-mediated natural clearance of Amyloid A is not impaired in the absence of immunoglobulins or central complement factors

**Sponarova J<sup>1</sup>, Nuvolone M<sup>2</sup>, Whicher C<sup>1</sup>, Westerman GT<sup>3</sup> and Aguzzi A<sup>1</sup>**

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AA amyloid deposits regress upon the reduction of SAA concentration and this proves that AA amyloid can be naturally cleared. Here, we have used genetically modified mouse strains to investigate the importance of immunoglobulins and complement components, common constituents of AA deposits, on the natural clearance of amyloid. AA amyloidosis was induced in wild-type, B-cell deficient ( $J^{H-/-}$ ) and complement factor three and four deficient ( $C3C4^{-/-}$ ) mice. The resolution of amyloid was studied after staining with amyloid-specific dyes (Congo Red and pFTAA) in spleen and liver collected between 2–20 weeks post-induction. Resolution of spleen amyloid started in wild-type and  $C3C4^{-/-}$  animals 4 weeks after discontinuation of the inflammatory stimuli and only after 9 weeks in  $J^{H-/-}$  mice. Amyloid clearance subsequently intensified, and approximately 40% of the original amyloid mass remained at time point 20 weeks post-induction. A much faster clearance was observed in liver and 13 weeks post-induction only traces of amyloid surrounding central veins were detected. The amyloid removal was accompanied, in both spleen and liver, by an up-regulation of CD68, a phagosome lysosome marker, on the infiltrated F4/80+ macrophages, and at later time points these cells contained intracellular amyloid. Western blot analysis of amyloid with AA/SAA specific antibodies showed that amyloid resolution occurred through a gradual cleavage of AA fragments. In newly formed amyloid, we identified four distinct AA proteins with molecular sizes between 6–11 kDa, and the 11 kDa fragment was the predominant finding. In contrast, at 20 weeks post-induction, the remaining amyloid was predominantly composed of ~6kDa AA fragments. Interestingly, this material showed partial resistance to protease K treatment. In conclusion, natural clearance of amyloid progressed similarly in wild-type, agammaglobulinemic and complement-deficient mice. This unexpected finding suggests the existence of a powerful innate-immune system mechanism for amyloid removal.

#### 15.40 Perspectives - Kluwe-Beckerman

#### 16.00-16.30 Tea break ('Fonteinpatio')

#### 16.30-17.30 Poster viewing (PA 1-65) in 5 groups ('Blauwe Patio')

|                    |   |
|--------------------|---|
| <b>17.30-18.00</b> | <b>Selected poster presentations ('Blauwe Zaal')</b>  |
|                    | Chairmen: Limburg and Gruys   |
| 17.30              | PA 15 High-resolution crystal structure of the C-terminal truncated human apoA-I sheds new light on the amyloid formation by the N-terminal fragment – Gursky |
| 17.36              | PA 22 Molecular Mechanisms of $\beta$ 2-Microglobulin Amyloid Fibril Formation – Ozawa  |
| 17.42              | PA 51 Novel fluorescent probes for the spectral assignment of a plethora of protein aggregates – Klingstedt   |
| 17.48              | PA 53 Subtyping of amyloidosis by direct proteomic analysis of fixed biopsy samples – Liuu  |
| 17.54              | PA 54 The amyloidophilic peptide p5 binds rapidly and stably to visceral amyloid <i>in vivo</i> : A potential radiotracer for PET/CT imaging – Martin         |
| <b>17.00-18.00</b> | <b>Meetings: Nomenclature Committee; other working groups and committees ('Lokaal 10, 15')</b>  |
| <b>18.00-19.30</b> | <b>Buffet in UMCG ('Fonteinpatio')</b>  |
| <b>19.30-22.00</b> | <b>Plenary session 4: Looking for consensus ('Blauwe Zaal')</b>   |
| 19.30              | Early detection of amyloid; reporting - Picken, P. Westermark, Hazenberg  |
| 20.20              | Organ involvement and response criteria in AL - Gertz, Wechalekar, Palladini  |
| 21.10              | Organ involvement and response criteria in non-AL - Suhr, Obici, Lachmann, Merkies  |

## Tuesday, May 8

7.30-8.00      Coffee ('Fonteinpatio')

### Plenary session 5

8.00-10.05    Diagnosis and typing: Histochemistry and proteomics ('Blauwe Zaal')  
Chairmen: Linke and Kluin

8.00           State of the art - Röcken

8.15 - OP 20

Amyloid fibrils possess characteristic electronegative fingerprints that can be distinguished by poly-basic peptides

**Jonathan S. Wall**<sup>1,2</sup>, Angela Williams<sup>1</sup>, Ying Huang<sup>2</sup>, and Stephen J. Kennel<sup>1,2</sup>

Departments of <sup>1</sup>Medicine or <sup>2</sup>Radiology, University of Tennessee Graduate School of Medicine, Knoxville, TN

**Background:** Amyloid deposits are heterogeneous matrices composed of protein fibrils, amyloid P component, and heparan sulfate proteoglycans. During our search for novel reagents that bind amyloid, we observed that certain poly-basic peptides bound synthetic amyloid fibrils in the absence of HSPG.

**Objective:** Examine the electrostatic nature of the interaction of peptides with amyloid extracts and synthetic fibrils.

**Methods:** Synthetic poly-basic peptides were synthesized by Fmoc chemistry, purified by RP-HPLC and the sequence integrity confirmed by mass spectrometry. The reactivity of peptides with synthetic rV<sub>1</sub>6Wil, A<sub>β</sub>(1-40) and IAPP fibrils was assessed by using solid phase ELISA and SPR. The reactivity of <sup>125</sup>I-labeled peptides with synthetic fibrils as well as human or murine amyloid extracts was assessed in a tissue binding assay.

**Results:** The interaction of peptides with synthetic rV<sub>1</sub>6Wil fibrils did not correlate with net peptide charge or the affinity for heparin. Notably, the peptides designated p5Lys and p5Arg bound with nanomolar affinity to AL-derived fibrils. In contrast, p5Glu and p5Leu were non-reactive. Peptides p5Lys and p5Arg also bound to synthetic A<sub>β</sub>(1-40) peptides. The interaction with fibrils was inhibited by increasing NaCl concentrations and was not enhanced by chaotropic anions. Analyses of over 10 different peptides suggested that the charge distribution displayed on the folded peptide was critical for fibril reactivity.

**Discussion:** We have found that poly-basic peptides can bind certain amyloid fibrils due to the presence of an electronegative "fingerprint" on the fibril. It has been shown that the reactivity of the camelid Ab B10 with some fibrils (but notably not AL) resulted mainly from electrostatic interactions. We posit that many ligands with an appropriate poly-basic motif will bind amyloid fibrils and that there may be an optimal charge distribution for each fibril type. Custom peptides could be optimized for fibril reactivity based on the aforementioned determinants.

SK and JW are co-founders of Solex LLC that has intellectual property associated with the p5 peptide  
This work was supported a PHS grant R01DK079984 from the NIDDK

8.27 - OP 21

Luminescent conjugated oligothiophenes: two new dyes for amyloid screening diagnostics

**Daniel Sjölander**<sup>1</sup>, Gunilla T. Westermark<sup>2</sup>, Per Westermark<sup>3</sup>, Per Hammarström<sup>1</sup>, Peter Nilsson<sup>1</sup>

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<sup>3</sup>Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

**Background:** Presently Congo red staining is the golden standard for screening and diagnosis of amyloidoses. Under ideal conditions amyloid stained with Congo red, viewed with linearly polarized light between crossed polarizers, will display a green (anomalous) color. However, due to inherent drawbacks of both the dye and the microscope technique; a laborious staining process and following microscopy diagnosis of amyloid calls for a highly skilled and experienced pathologist.

Previously luminescent conjugated polythiophenes/oligothiophenes (LCPs/LCOs) have successfully been utilized for the detection and typing of systemic amyloidoses as well as localized amyloidoses (Nilsson, 2006; 2010). The LCOs constitute a new set of fluorophores that preferentially binds to amyloid deposits and upon doing so changes its spectral signature. Staining with LCOs is very simple and these dyes might be utilized as an additional screening method for amyloid deposits.

**Objective:** The purpose of the study was to evaluate the luminescent conjugated oligothiophenes p-FTAA and h-FTAA performance of detecting a diverse set of systemic amyloidoses (40 cases); and to compare this result with Congo red stained amyloid examined both with fluorescence and bright-field polarization microscopy.

**Methods:** In this study we employed wide-field fluorescence and bright-field polarization microscope for detection of amyloid deposits in immunohistochemically confirmed systemic amyloidoses cases. Consecutive tissue sections of the systemic amyloidosis cases were stained with Congo Red according to the modified Puchtler method (Westerman, 1999), p-FTAA or h-FTAA (diluted 1:500 from a 1g/L solution in PBS for 30 min) for evaluation of the detecting ability towards amyloid deposits.

**Results:** Luminescent conjugated oligothiophenes (LCOs) p-FTAA and h-FTAA can detect amyloid deposits with similar degree as Congo red as determined by fluorescence. However Congo red also showed unspecific binding towards connective tissue in fluorescence mode which was not the case for the LCOs. In all cases bright-field polarization microscopy of Congo red was the least sensitive method.

**Conclusion:** This study concludes that the LCO fluorophores p-FTAA and h-FTAA is a better first screen alternative for suspected amyloidosis than Congo red due to ease of use, sensitivity and selectivity.

#### References:

1. Westerman GT, et al. Staining methods for identification of amyloid in tissue. Methods Enzymol.; 1999;309:3-25.
2. Nilsson KP, et al. Conjugated polyelectrolytes--conformation-sensitive optical probes for staining and characterization of amyloid deposits. ChemBioChem; 2006; 7(7):1096-1104.
3. Nilsson KP, et al. Structural Typing of Systemic Amyloidoses by Luminescent-Conjugated Polymer Spectroscopy. Am J Pathol., 2010; 176(2); 563-574.

#### 8.39 - OP 22

#### Accuracy of routine amyloid typing using immunohistochemistry on a large number of consecutive patients: preconditions for its success and validation

Heidi Maier-Boetzel and **Reinhold P. Linke**

Reference Center of Amyloid Diseases, Martinsried, Germany

During the last decades a plethora of pathologic amyloid diseases have been described resulting in the description of more than 27 different amyloid diseases with each presenting as various syndromes which ultimately amount in more than 500 individual diseases. Since therapies have been developed that can either prevent, arrest or even improve, to some extent, the systemic and most fatal diseases, their exact diagnosis affords, as a first step, Congo red or tissue sections in every patient. The second important diagnostic step is the identification of the amyloidogenic protein of each of the patients. This can be done in all institutes that use immunohistochemistry. (Both methods have been validated by an international comparison, see Ring study I). The immunohistochemical classification of amyloid, however, needs to be properly performed. The accuracy is based on the competence of the investigator, the experience of the laboratory and the use of appropriate antibodies applied with a suitable technique. We have prepared polyclonal rabbit and some murine monoclonal antibodies that fulfill these preconditions on formalin-fixed paraffin sections. With this panel of antibodies directed against all major hereditary amyloids (ATTR, AApoAI, AFib, ALys, AGel, ACys, Abeta, APrion, AA) and sporadic forms of amyloid (AA, ALlambda, ALkappa, ATTR, Abeta<sub>2</sub>M, Abeta, APrion), as well as other more organ-limited and local forms, we have demonstrated reliable diagnoses of the amyloid class in 97.9 % in 581 consecutive patients and all (100%) of the samples from the 119 prototype

amyloids. This approach is easy, fast and can be performed in every Institute of Pathology carrying out immunohistochemistry after training. Its various internal controls and the expert evaluation are crucial for the accuracy. This diagnosis represents an independent marker without the determination of additional risk factors. Its value has been confirmed by clinical, chemical and genetic data as well by mass spectrometry.

R.P.L. is owner of amYmed ([www.amymed.net](http://www.amymed.net))

### 8.51 - OP 23

#### An indirect ELISA for transthyretin quantification in fat tissue of patients with ATTR amyloidosis

Johan Bijzet<sup>1</sup>, Lammie de Boer<sup>1</sup>, Elizabeth B. Haagsma<sup>2</sup>, and Bouke P. Hazenberg<sup>1</sup>

Departments of <sup>1</sup>Rheumatology & Clinical Immunology and <sup>2</sup>Gastroenterology & Hepatology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

**Background:** The diagnostic performance was studied of an indirect transthyretin (TTR) ELISA for detection and characterization of transthyretin-derived (ATTR) amyloid in subcutaneous fat tissue.

**Methods:** Fat tissue specimens were analyzed of 49 consecutive patients with ATTR amyloidosis, 204 controls (21 AA, 46 AL, 22 localized, and 115 non-amyloidosis controls), and 20 carriers of a TTR mutation. The amount of amyloid was graded semi-quantitatively in Congo red-stained specimens (0-4+). Amyloid was extracted from tissue in guanidine and the TTR concentration was measured using a newly developed indirect TTR-ELISA.

**Results:** Mean TTR concentration in controls was 0.10 ng/mg fat tissue with a 98% interval (mean ± 2.33 SD) ranging from 0.002 to 4.0 ng/mg fat tissue. The TTR concentration of patients with ATTR amyloidosis (mean 16.2 ng/mg fat tissue; 95% interval 0.037 - 7180 ng/mg fat tissue) was higher than controls ( $p < 0.0001$ ). The TTR concentration of 4.0 ng/mg fat tissue was chosen as cut-off value (upper limit of 99% of the controls) and 36 of all 49 ATTR patients were identified resulting in overall sensitivity for finding patients with ATTR amyloidosis of 73% (95% CI, 59-85%). If the six ATTR patients without any amyloid detected in fat aspirates were excluded, 36 of 43 ATTR patients having amyloid in fat tissue were identified resulting in sensitivity for this group of 84% (95% CI, 69-93%). All but one of the 204 controls had TTR values below the cut-off value resulting in specificity 99% (95% CI, 97-100%). All 20 carriers had values below the cut-off value. ANOVA showed a linear trend between TTR and amyloid grades.

**Conclusions:** The TTR concentration in fat tissue is useful for detecting ATTR patients. In patients with 3+ or 4+ amyloid in fat tissue, the TTR concentration of fat tissue is highly sensitive for characterizing the amyloid as ATTR-type.

### 9.03 - OP 24

#### A native human monoclonal IgG with pan-amyloid binding specificity

Sharad P. Adekar<sup>1</sup>, Brian O'Nuallain<sup>2</sup>, Yona Levites<sup>3</sup>, Sally Macy<sup>4</sup>, Alan Solomon<sup>4</sup>, Todd E. Golde<sup>3</sup>, Scott K. Duggan<sup>1</sup>

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<sup>3</sup>Center for Translational Research in Neurodegenerative Disease, University of Florida, Gainesville, FL 32610 USA

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**Background:** Healthy humans express a population of IgGs that are cross-reactive against conformational epitopes on amyloid aggregates and can dissociate amyloid *in vivo* [1]. These may have therapeutic potential in amyloid diseases.

**Objective:** To obtain a native human monoclonal antibody specific for a pan-amyloid epitope.

**Methods:** We used a hybridoma method to immortalize splenic B-cells from a healthy young person [2]. We screened the hybridoma supernatants for human IgG immunoreactive with a recombinant, amyloidogenic lambda immunoglobulin (JTO) and aggregated amyloid beta (A $\beta$ ). We performed a series of *in vitro* binding experiments with A $\beta$  monomers, oligomers, and fibrils, as well as other

amyloidogenic molecules. We also performed immunohistochemistry with human tissues containing pathologically deposited amyloid.

**Results:** We identified a human IgG, 3H3, which preferentially binds to oligomeric and fibrillar A $\beta$ , as well as other amyloid proteins *in vitro*. 3H3 binds to A $\beta$  plaques in the brains of AD patients, as well as to pathological deposits in tissue of kappa immunoglobulin, lambda immunoglobulin, and transthyretin. DNA sequence analysis indicates that the 3H3 antibody has undergone affinity maturation, indicating that it is a post-germinal center antibody.

**Discussion:** 3H3 is a human antibody obtained from a healthy person which is able to specifically recognize a binding epitope common to diverse amyloid species.

**Conclusion:** Native human IgG antibodies with pan-amyloid-specific binding activities can be cloned using hybridoma methods. They may have utility as diagnostics or therapeutics for amyloid diseases.

#### References:

1. O'Nuallain B, Hrncic R, Wall JS, Weiss DT, Solomon A (2006) Diagnostic and therapeutic potential of amyloid-reactive IgG antibodies contained in human sera. *J Immunol* 176: 7071-7078.
2. Adekar SP, Jones RM, Elias MD, Al-Saleem FH, Root MJ, et al. (2008) Hybridoma populations enriched for affinity-matured human IgGs yield high-affinity antibodies specific for botulinum neurotoxins. *J Immunol Methods* 333: 156-166.

#### 9.15 - OP 25

#### Diagnosis of Amyloidosis Subtype by Laser-Capture Microdissection (LCM) and Tandem Mass Spectrometry (MS) Proteomic Analysis

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<sup>3</sup>The University of Queensland Diamantina Institute, Brisbane, Australia

**Aim:** Correct identification of the protein that is causing amyloidosis is crucial for clinical management. Current diagnostic methods have limited ability to detect the full range of amyloid forming proteins. We assessed combining specific sampling of amyloid deposits by LCM and analysis of tryptic digests by tandem MS proteomic analysis.

**Methods:** We studied 13 cases of well characterised amyloid deposition and three cases in which the amyloid subtype was unable to be diagnosed with confidence. For all specimens, 10 $\mu$ m sections of formalin-fixed paraffin embedded tissue were stained with Congo Red using a standard technique. LCM was performed using an Arcturus XT instrument with an infrared capture laser. Proteins were digested with trypsin and peptides were analysed by nano-liquid chromatography-coupled tandem mass spectrometry using a Chip CUBE-QTOF. Database searching was performed using Spectrum Mill (Agilent) with the NCBI human protein database.

**Results:** The amyloid subtype was able to be determined in all 16 cases analysed. Proteins identified included immunoglobulin light chain (localised amyloid n=2, systemic AL n=4), transthyretin (senile amyloid n=4, hereditary ATTR n=2), serum amyloid A2 (AA n=2), TGF $\square$  (corneal lattice amyloid n=1) and semenogelin (seminal vesicle amyloid n=1). One of the diagnostically challenging cases had: extensive gastrointestinal amyloidosis and no evidence of clonal light chain disease; negative kappa, lambda, SAA and transthyretin immunohistochemistry; and negative genetic studies. Tandem MS revealed immunoglobulin lambda light chain type. The second diagnostically challenging case had: isolated renal amyloidosis with a positive AA stain and kappa restricted serum free light chains. Tandem MS revealed serum amyloid A2 protein. The third case had: cardiac, neurological and gastrointestinal involvement; and equivocal immunohistochemistry. Tandem MS demonstrated transthyretin and genetic studies showed a A97S ATTR mutation.

**Conclusion:** LCM and tandem MS allows correct typing of amyloid deposits in clinical biopsy samples.

#### 9.27 - OP 26

#### Mass spectrometry based proteomics for classification of amyloidosis: Mayo Clinic Experience

Ahmet Dogan, Jason D Theis, Julie A. Vrana

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**Background:** Accurate classification of amyloidosis is essential for management of amyloidosis. In 2008, we developed and clinically validated a mass spectrometry based proteomic assay (MSPA) to classify amyloidosis. (1)

**Objective:** The aim of this study was to review the clinical utility of the MSPA for classification of amyloidosis since implementation in March 2008.

**Methods:** The laboratory database was interrogated to identify cases of amyloidosis submitted for MSPA. The clinically reported result for each case was included in the analysis. MSPA was performed and reported as previously described (1)

**Results:** Between March 2008-December 2011, 2798 cases were submitted for classification of amyloidosis by MSPA. MSPA was diagnostic in 93% of the cases, highly suggestive of a specific amyloid type in 62 cases (2%) and inconclusive in 60 cases (2%). In remaining 95 cases (3%), the biopsy specimens were deemed to be insufficient for analysis. By far the most common types of amyloidoses were immunoglobulin (Ig)-associated amyloidoses; 1707 cases (61%). Of these, AL-lambda accounted for 1048 cases, AL-kappa for 575, and the remaining represented AH or mixed AH/AL amyloidosis. The other common amyloid types were ATTR, 582 cases (21%); ALECT2, 87 cases (3%); and AA, 59 cases (2%). Additionally MSPA identified 16 other amyloid types successfully.

**Discussion:** This study shows that MSPA can be applied with efficiency in a routine clinical setting with a low failure rate. Virtually all causes of amyloidosis can be identified and accurately classified by a single test requiring minimal tissue. Our results suggest a change in the epidemiology of amyloidosis; ALECT2 emerging as the third most common amyloid.

**Conclusion:** MSPA is a powerful tool for classification of amyloidosis in routine clinical setting.

**References:** Vrana et al. Blood 2009

### 9.39 - OP 27

#### A comparison of immunohistochemistry and mass spectrometry for determining the amyloid fibril protein from formalin fixed biopsy tissue

<sup>1</sup>Gilbertson, Janet A., <sup>2</sup>Theis, Jason D., <sup>1</sup>Hunt, Toby., <sup>2</sup>Vrana, Julie A., <sup>1</sup>Hawkins, Philip N., <sup>2</sup>Dogan, Ahmet, <sup>1</sup>Gillmore, Julian D.

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<sup>2</sup>Dept of Laboratory Medicine & Pathology, Mayo Clinic, Rochester, MN 55905. USA

**Background and Objectives:** There are many types of amyloid, which are classified according to the protein precursor from which the fibrils are derived. Accurate identification of amyloid type is critical in every case since therapy is type-specific. At the UK National Amyloidosis Centre (NAC), all biopsy specimens containing amyloid, the vast majority of which are formalin fixed, are routinely stained with a panel of 11 antibodies to determine the amyloid fibril protein. In ~20-25% cases however, immunohistochemistry (IHC) fails to prove the amyloid type and further tests are required. Laser capture microdissection and mass spectrometry (LDMS) is a powerful tool for identifying proteins from formalin-fixed, paraffin embedded tissues. We undertook a blinded comparison of IHC (NAC) and LDMS (Mayo Clinic) in 142 consecutive biopsy specimens from 39 different tissue types.

**Methods and Results:** Renal tissue was the most common biopsy sent for analysis (30%). There was 100% concordance with respect to amyloid fibril type between IHC and LDMS in 108 biopsies that stained positively by IHC. Thirty-four of 142 (24%) cases did not stain immunospecifically such that the amyloid type was not confirmed by IHC alone. LDMS was diagnostic in 25/34 (74%) such cases, confirming AL (lambda) and AL (kappa) type amyloid in 7 and 10 cases respectively, heavy chain (AH) in 2 cases, apolipoprotein A4 in 3 cases and atrial natriuretic factor (ANF) amyloid in 2 cases. LDMS failed for technical reasons in 1 case, there was no remaining amyloid in the specimens in 2 cases, and was inconclusive in 7 cases, although a fibril protein was suggested in 3 of these 7.

**Conclusions:** Running both IHC and LDMS simultaneously resulted in positive identification of the amyloid fibril protein in ~94% of biopsies compared to 76% by IHC alone. LDMS however, is labour-intensive, expensive and requires considerable expertise.

### 9.51 Perspectives - Dogan

### 10.05-10.30 Coffee break ('Fonteinpatio')

## Plenary session 6

**10.30-12.30 Imaging in amyloidosis ('Blauwe Zaal')**  
 Chairmen: Van Rijswijk and Glaudemans

**10.30 State of the art - Hawkins**

**10.45 - OP 28**

**Patterns of late gadolinium enhancement and survival in cardiac amyloidosis: a systematic review of 95 patients with AL or ATTR type**

**Jason Dungu**<sup>1,2</sup>, Carol J Whelan<sup>1</sup>, Simon DJ Gibbs<sup>1</sup>, Jennifer H Pinney<sup>1</sup>, Sanjay M Banypersad<sup>1</sup>, Christopher P Venner<sup>1</sup>, Helen J Lachmann<sup>1</sup>, Ashutosh Wechalekar<sup>1</sup>, Julian D Gillmore<sup>1</sup>, Philip N Hawkins<sup>1</sup>, Lisa J Anderson<sup>2</sup>

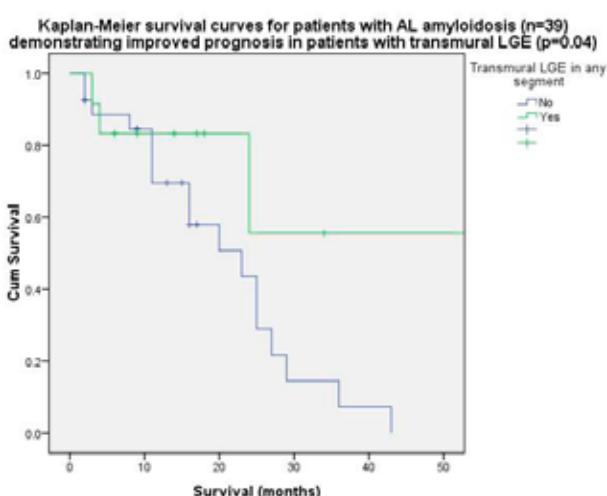
<sup>1</sup>Centre for Amyloidosis and Acute Phase Proteins, University College London Medical School, UK

<sup>2</sup>St George's University of London, UK

**Background:** Cardiac MRI (CMR) is increasingly used to investigate patients with suspected amyloidosis. Circumferential subendocardial late gadolinium enhancement (LGE) has been reported to be typical in AL amyloidosis, and by contrast, transmural LGE associated with transthyretin amyloidosis (ATTR).

**Methods:** We studied the CMR LGE pattern in 95 patients who had been referred to the UK National Amyloidosis Centre (NAC) between 2007 and 2011.

**Results:** The mean age was  $69.2 \pm 11.3$  years with male predominance (73%). The amyloid type was AL in 39 patients (41%) and ATTR in 56 patients (59%). LGE was evident in all ATTR patients and 36 AL patients (92%,  $p < 0.01$ ). Fifty-three ATTR patients (95%) demonstrated  $\geq 1$  transmural segment, compared to 12 AL patients (31%,  $p < 0.01$ ). Circumferential transmural LGE was specific to ATTR (6 patients (10.7%),  $p < 0.01$ ). Right ventricular (RV) LGE was present in all ATTR patients but only 26 AL patients (67%,  $p < 0.01$ ). Circumferential subendocardial LGE was present in only 8 AL patients (21%) and 2 ATTR patients (3%,  $p < 0.01$ ). Median survival was significantly reduced in AL patients (24 vs 36 months,  $p < 0.01$ ). However, the presence of transmural LGE in any segment was associated with improved survival in AL amyloidosis ( $p = 0.04$ ).



**Conclusion:** A transmural pattern of LGE is more suggestive of ATTR but only the absence of RV LGE in one-third of AL patients reliably distinguished the two types of amyloid. Transmural LGE in any segment was associated with prolonged survival in patients with AL amyloid.

**10.57 - OP 29**

**$^{99m}$ Tc-3,3-Diphosphono-1,2-Propanodicarboxylic Acid ( $^{99m}$ Tc DPD) scintigraphy in 171 patients with suspected systemic amyloidosis**

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**Background:** Cardiac involvement is a major clinical feature and determinant of outcome in AL and ATTR amyloidosis, for which better diagnostic methods are required.

**Methods:** We report <sup>99m</sup>Tc-3,3-Diphosphono-1,2-Propanodicarboxylic Acid (<sup>99m</sup>Tc DPD) scintigraphy in 171 patients with suspected cardiac amyloidosis. Patients received 700 MBq <sup>99m</sup>Tc DPD, and planar whole body images were acquired after 5 minutes and 3 hours, along with cardiac SPECT-CT. Myocardial uptake was scored as grade 0-3 based on the amount of cardiac uptake and proportionate reduction in bone signal. Final diagnosis was supported by biopsy histology, proteomics, SAP scintigraphy, cardiac MRI, and DNA analysis.

**Results:** Amyloidosis was ultimately excluded in 31 patients, all of whom had normal DPD scans. Cardiac uptake occurred in all 76 patients with ATTR amyloidosis who fulfilled consensus criteria for cardiac involvement, but in only 16 (55%) of those with AL ( $p<0.0001$ ); myocardial uptake was also present in eight other ATTR patients (one wild-type) who did not meet the consensus criteria. SPECT-CT showed cardiac uptake that was not evident on planar scans in 6 patients. Myocardial DPD uptake according to grade 1/2/3 was associated with median LV wall thickness of 14/16.5/17 mm, LV ejection fraction of 56/49.5/46%, and NT pro-BNP of 295/295/354 pMol/L respectively. One patient with cardiac AA and 3 with cardiac apolipoprotein AI amyloidosis had myocardial DPD uptake. Abnormal DPD uptake into the liver and spleen each occurred in only 4% of cases, in contrast to the high frequency of visceral amyloidosis demonstrated on SAP scintigraphy.

**Conclusions:** This study confirms the utility of <sup>99m</sup>Tc DPD scintigraphy in cardiac amyloid imaging, most specifically for amyloid of ATTR type. Its evident potential for early diagnosis, screening and quantitative monitoring of cardiac ATTR amyloidosis is under evaluation, as is a SPECT-CT based algorithm for differentiating AL from ATTR cardiac amyloidosis.

Free radiopharmaceutical kits provided by IBA Molecular UK Ltd.

### 11.09 - OP 30

#### Accuracy of <sup>99m</sup>Tc-HMPD myocardial scintigraphy for the diagnosis of cardiac involvement in patients with familial amyloid polyneuropathy

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<sup>2</sup>*CHU. Bichat Claude Bernard, Nuclear Medicine (Paris, France)*

<sup>3</sup>*Neurology, CHU de Bicêtre, APHP, (Kremlin Bicêtre, France)*

All authors belong to the French reference center for FAP (NNERF)

**Background:** Familial amyloid polyneuropathy (FAP) is a hereditary form of amyloidosis, due to deposits of a genetic variant transthyretin (TTR) produced by the liver. Cardiac involvement is of major prognostic value. Diphosphonate scintigraphy has been suggested as a diagnostic tool for TTR-related cardiac amyloidosis with cardiac wall thickening.

**Objective:** Our aim was to evaluate the accuracy of <sup>99m</sup>Tc-hydroxymethylene diphosphonate (<sup>99m</sup>Tc-HMPD) scintigraphy in FAP patients with various stages of cardiac involvement.

**Methods and results:** We evaluated 24 patients with proven TTR-related amyloidosis (17 due to Val30Met mutation, 13 females, age 42±12 years). Cardiac involvement was evaluated by EKG, echocardiography and cardiac MRI, and was classified as absent (n=3), moderate (n=8) or manifest (n=13). Acquisitions were performed 3h (planar and SPECT) after i.v. injection of 740 MBq of <sup>99m</sup>Tc-HMPD. Myocardial uptake was visually semi-quantitatively assessed on planar images (absent, moderate, intense compared to bone uptake), and a quantification was performed on tomographic slices to avoid bone uptake (myocardial to noise ratio) in case of uptake.

**Results:** Nine patients over 24 presented a myocardial uptake: 8 intense, 1 moderate, all with severe cardiac involvement. Patients with positive uptake had significantly higher cardiac walls thickening, lower LVEF and higher E/E' ratio. Myocardial to noise ratio ranged from 2.6 to 8.6 (mean 5.4 ± 2), and

was correlated to relative wall thickness ( $r=0.6$ ,  $p=0.05$ ). Among the 16 patients with negative scans, 4 presented with manifest and 8 with moderate cardiac amyloidosis.

**Conclusion:**  $^{99m}\text{Tc}$ -HMPD scintigraphy displayed cardiac uptake in 43% patients with FAP, all of which occurred in patients with manifest cardiac amyloidosis. Cardiac amyloidosis was more serious in patients with positive scan. Thus,  $^{99m}\text{Tc}$ -HMPD scintigraphy does not represent a sensitive diagnostic tool in early stages of FAP but mostly a marker of severity.

### 11.21 - OP 31

#### Iodine-123 metaiodobenzylguanidine for the evaluation of cardiac sympathetic denervation in early stage amyloidosis

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<sup>1</sup>Departments of Nuclear Medicine & Molecular Imaging, <sup>2</sup>Rheumatology & Clinical Immunology, and

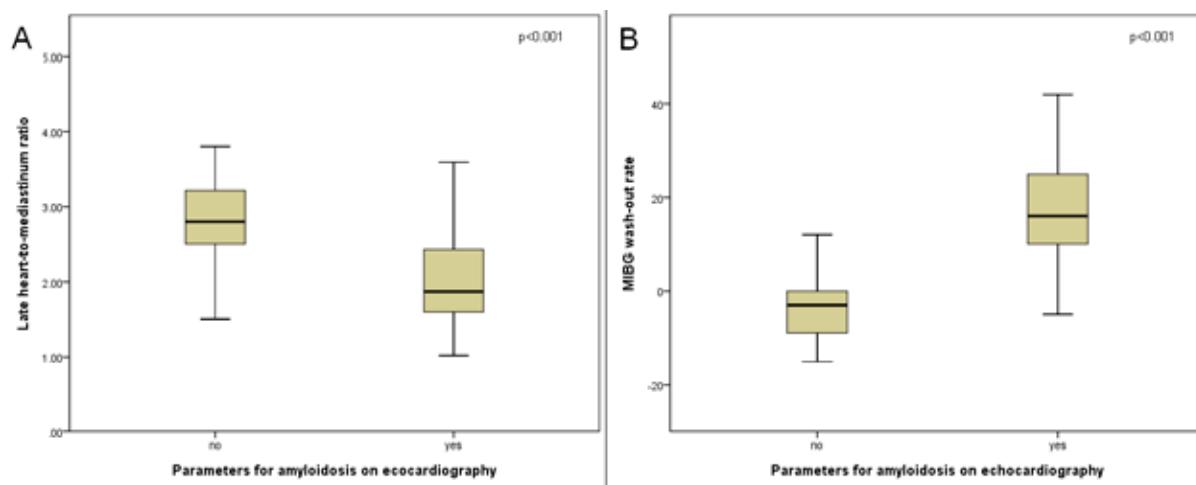
<sup>3</sup>Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

**Background:** cardiac amyloidosis is a rare disorder; however it may lead to potentially life-threatening restrictive cardiomyopathy. It frequently occurs in primary (AL) and familial (ATTR) amyloidosis, but is uncommon in secondary (AA) amyloidosis. Echocardiography is the method of choice for assessing cardiac amyloidosis. Amyloid depositions impair the function of sympathetic nerve endings. Disturbance of myocardial sympathetic innervations may play an important role in the remodelling process. Iodine-123 metaiodobenzylguanidine ( $^{123}\text{I}$ -MIBG) can detect these innervation changes.

**Methods:** 61 patients (30 women and 31 men) with biopsy proven amyloidosis underwent general work-up, echocardiography and  $^{123}\text{I}$ -MIBG scintigraphy. Left ventricular (LV) internal dimensions and LV wall thickness were measured, and highly refractile cardiac echoes (sparkling) were analysed. Early (15 min) and late (4 hrs) heart to mediastinum ratio (HMR) and wash-out rate were determined after administration of MIBG.

**Results:** 61 patients (mean age 62 years, three subgroups (AL (n=39), AA (n=11), ATTR (n=11)) were included in this study. Echocardiographic parameters were not significantly different between the subgroups. Sparkling was present in 72% of ATTR patients, in 54% of AL and in 45% of AA patients. Median late HMR of all patients was 2.4 (range 1.0-4.4), median wash-out rate was 7% (0 – 42%), both not different between the subgroups. Late HMR was significantly lower in patients with echocardiographic parameters for amyloidosis (1.9 (1.0-4.4) versus mean  $2.8 \pm 0.6$ ,  $p<0.001$ ). Wash-out rates were significantly higher in these patients ( $-3.3 \pm 9.9\%$  vs  $17 \pm 10\%$ ,  $p<0.001$ ). In ATTR patients without echocardiographic parameters of amyloidosis, HMR was lower than those patients with other types ( $2.0 \pm 0.59$  vs  $2.9 \pm 0.50$ ,  $p=0.007$ ).

**Conclusion:** MIBG HMR is lower and wash-out rate is higher in patients with echocardiographic signs of amyloidosis. Also,  $^{123}\text{I}$ -MIBG scintigraphy can detect cardiac denervation in ATTR type patients before signs of amyloidosis are evident on echocardiography.



### 11.33 - OP 32

#### The added value of SPECT(-CT) compared with planar $^{123}\text{I}$ -SAP scintigraphy in patients with systemic amyloidosis

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<sup>1</sup>Departments of Nuclear Medicine & Molecular Imaging and <sup>2</sup>Rheumatology & Clinical Immunology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

**Introduction:**  $^{123}\text{I}$ -Serum Amyloid P (SAP)-scintigraphy is used to image the extent and distribution of amyloid deposition in patients with systemic AA, AL and ATTR amyloidosis. Due to physiological uptake of iodine metabolites in the stomach and high  $^{123}\text{I}$ -SAP uptake in an enlarged liver or spleen, visualization of adjacent abdominal organs is hampered. The aim of this study is to evaluate if SPECT (-CT) provides additional information about amyloid involvement of these adjacent abdominal organs.

**Methods:** In total 99  $^{123}\text{I}$ -SAP-scans - including planar and SPECT(-CT) images – in 87 patients were retrospectively evaluated. The patients were divided in five groups: AL type (n=44), AA type (8), ATTR type (13), AL local type (13) and a control group without amyloidosis (9). Sensitivity, specificity, interpretation change and inter-observer agreement were analysed.

**Results:** SPECT had an overall high specificity, 81% - 100%, and changes in interpretation were observed in 11% of the cases for the liver, 28% for the kidneys and 22% for the spleen.

In particular, kidneys often changed from a positive scan to a negative scan and for the spleen the opposite was observed. In 70% of the scans intense stomach uptake on planar views was observed, that was bypassed on the SPECT(-CT) imaging.

For both planar and SPECT images there was a general low sensitivity for all organs, 33% to 88%, with an also low inter-observer agreement.

**Conclusion:** SPECT(-CT) images were found to have a high specificity value. The added value of the SPECT was most often observed in the evaluation of spleen and kidney, and often resulted in a change in diagnosis of amyloid involvement in those organs. Finally, the overall low sensitivity rates and low inter-observer agreement indicate further optimisation of amyloid burden quantification.

#### References:

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2. Hachulla E, Maulin L, Deveaux M, et al. Prospective and serial study of primary amyloidosis with serum amyloid P component scintigraphy: From diagnosis to prognosis. Am J Med 1996;101:77-87.
3. Hazenberg BP, van Rijswijk MH, Piers DA, et al. Diagnostic performance of  $^{123}\text{I}$ -labeled serum amyloid P component scintigraphy in patients with amyloidosis. Am J Med 2006;119:355.e15,355.e24.

### 11.45 - OP 33

#### AL amyloid imaging and therapy with an amyloid specific monoclonal antibody

Jonathan S. Wall<sup>1,2</sup>, Stephen J. Kennel<sup>1,2</sup>, Angela Williams<sup>1</sup>, Tina Richey<sup>1</sup>, Alan Stuckey<sup>2</sup>, Ying Huang<sup>1</sup>, Robert Donnell<sup>3</sup>, Robin Barbour<sup>4</sup>, Peter Seubert<sup>4</sup>, Alan Solomon<sup>1</sup>, and Dale Schenk<sup>4</sup>

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**Background:** A monoclonal antibody, 2A4, was developed with reactivity directed to a small peptide, derived from the cleavage site of sAA and dependent upon the presence of -Glu-Asp- amino acids. The majority of immunoglobulin light chain germline genes encode an invariant -Glu-Asp- amino acid pair at approximately position 81 in the variable domain. The goal of this study was therefore to determine whether the 2A4 mAb could bind AL amyloid fibrils.

**Methods:** The binding of murine 2A4 mAb with synthetic rV $\lambda$ 6 Wil fibrils and human immunoglobulin light chain (AL) amyloid extract was assessed by using surface plasmon resonance and europium-linked immunosorbent assay (EuLISA), respectively. Co-localization of mAb 2A4 with human AL amyloid was shown by immunohistochemistry and by SPECT/CT imaging of radiolabeled mAb in mice with sc amyloidomas. Finally, this murine model was also used to show the therapeutic efficacy of 2A4.

**Results:** EuLISA demonstrated mAb binding to synthetic rV $\lambda$ 6 Wil fibrils and human AL amyloid extracts with high affinity even in the presence of soluble light chain proteins. Immunohistochemistry with biotinylated mAb specifically stained AL $\kappa$  and AL $\lambda$  human amyloid deposits in various organs. Surface plasmon resonance analyses revealed binding to rV $\lambda$ 6 Wil fibrils with a KD of ~ 10 nM. Binding was inhibited in the presence of the -Glu-Asp- containing immunogen peptide. Radiolabeled 2A4 mAb successfully localized with human amyloid extracts implanted in mice as evidenced by SPECT imaging and was confirmed by measuring the biodistribution of radiolabeled mAb and by micro-autoradiography. When treated with mAb 2A4, AL $\kappa$  amyloidomas regressed faster than those treated with a non-reactive mAb. The regression was shown to be mediated by the action of macrophages and neutrophils.

**Conclusion:** These data indicate that the 2A4 mAb cross-reacts with AL amyloid and may be clinically beneficial for imaging and immunotherapy in patients.

RB, PS, and DS are employees of Neotope Biosciences, a division of Elan Pharmaceuticals. This work was supported by a collaborative research grant from Neotope Biosciences Limited to JSW and AS.

#### 11.57 - OP 34

#### Radioimmunoimaging of Patients with AL Amyloidosis

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**Background:** Heretofore, there has been no radiographic means in the US to visualize AL deposits. We have reported that our amyloidolytic fibril-specific mAb, 11-1F4, labeled with I-124, could image by PET/CT hepatic, splenic, nodal, or bone marrow deposits in 11 of 18 subjects.<sup>1</sup>

**Objectives:** To extend this study to include an additional 18 patients and determine if modifications in PET acquisition would increase the capability of <sup>124</sup>I-labeled-11-1F4 to image amyloid in the heart or kidney.

**Table 1: Results of Radioimmunoimaging using <sup>124</sup>I-mAb 11-1F4 and PET/CT in Patients with AL Amyloidosis**

| Light Chain Isotype | Organ Affected* | PET/CT* | Light Chain Isotype | Organ Affected* | PET/CT*    | Light Chain Isotype | Organ Affected* | PET/CT* |
|---------------------|-----------------|---------|---------------------|-----------------|------------|---------------------|-----------------|---------|
| K                   | K               | 0       | $\lambda$           | K               | L,S        | $\lambda$           | H,K             | S       |
| $\lambda$           | LN              | LN      | $\lambda$           | K,L,LN          | L,S,BM     | $\lambda$           | T,H,I?          | 0       |
| $\lambda$           | K               | 0       | $\lambda$           | H,K,L,S         | S          | $\lambda$           | L,K,H           | S       |
| $\lambda$           | Lu              | 0       | $\lambda$           | I               | 0          | $\lambda$           | K,H             | H       |
| $\lambda$           | K               | 0       | $\lambda$           | K               | S,BM       | $\lambda$           | K,H,S           | H,S     |
| $\lambda$           | F               | 0       | $\lambda$           | H,BM            | I          | $\lambda$           | K,H,S           | 0       |
| K                   | H               | I       | $\lambda$           | K               | 0          | K                   | SC,PV,S         | SC      |
| $\lambda$           | K               | 0       | $\lambda$           | H,L,S           | S,LN,V     | $\lambda$           | LN              | 0       |
| K                   | H,I,L,S         | L,S     | $\lambda$           | I               | 0          | K                   | H,T,SC,GI       | K       |
| $\lambda$           | T,L,S           | 0       | $\lambda$           | H,L,S           | 0          | $\lambda$           | H,GI,L          | L,S,LN  |
| $\lambda$           | L,K,BM          | L,S,BM  | K                   | I,L,S,H?        | L,S,I,BM,A | $\lambda$           | H,K             | K,S     |
| K                   | H,L,S           | L,S     | $\lambda$           | H,L,K,BM,I?     | L,S,BM,A   | $\lambda$           | H,S             | S,GI    |

\*A, adrenal; BM, bone marrow; F, fat; H, heart; I, intestine; K, kidney; L, liver; LN, lymph node; Lu, lung; PV, perivascular; SC, subcutaneous deposits; T, tongue; V, vascular

**Methods:** Methods are as described in Ref. 1. To increase the sensitivity of true count detection, the time of PET acquisition was extended by 35 min for the kidneys and heart (gated).

**Results:** Information from all 36 patients is provided in Table 1. Uptake of the radiolabeled antibody in organs/tissues occurred in 22 cases. For the last 13 subjects, who also had the 35-min scans, the results were positive in 4 of 11 who had kidney or heart involvement. Comparison of the imaging and

immunohistochemical data (biopsies were available from 33 cases) revealed that the unlabeled reagent immunostained the deposits in 17 (13 of whom had positive PET scans).

**Conclusions:** The fact that  $^{124}\text{I}$ -11-1F4 does not image AL amyloid in every case may indicate inaccessibility (or loss) of the epitope recognized by this antibody. Alternatively, the concentration of the immune target may be too low and therefore undetectable by PET imaging. Overall, our study suggests that AL deposits, which are not yet evident clinically, may be identified using this sensitive technology. Most importantly, because this antibody can effect amyloidolysis, positive imaging results could be used to identify AL patients as candidates for passive immunotherapy using the chimeric version of 11-1F4, now under production for a Phase I clinical trial.

**References:** 1. Wall JS, et al. Radioimmunodetection of amyloid deposits in patients with AL amyloidosis. Blood 2010; 116:2241-4.

**12.10 Perspectives - Wall**

**12.30-14.00 Lunch (Alnylam) ('Fonteinpatio')**

**13.00-14.00 Poster viewing individually ('Blauwe Patio')**

## Plenary session 7

**14.00-16.30 Biology, clinics and prognosis in AL amyloidosis ('Blauwe Zaal')**  
Chairpersons: Comenzo and Croockewit

**14.00 State of the art - Merlini**

**14.15 - OP 35**

**Diagnostic performance of the novel monoclonal assay for the measurement of circulating free light chain (FLC) in 220 consecutive newly-diagnosed patients with AL amyloidosis**

**Giovanni Palladini<sup>1</sup>, Tiziana Bosoni<sup>2</sup>, Francesca Lavatelli<sup>1</sup>, Laura Pirolini<sup>2</sup>, Andrea Foli<sup>1</sup>, Gabriele Sarais<sup>1,2</sup>, Paolo Milani<sup>1</sup>, Leda Roggeri<sup>1</sup>, Elena Cigalini<sup>1</sup>, Giovanbattista Vadacca<sup>2</sup>, Riccardo Albertini<sup>2</sup>, Giampaolo Merlini<sup>1,2</sup>**

<sup>1</sup>Amyloidosis Research and Treatment Center – “Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo” and Department of Molecular Medicine – University of Pavia, Pavia, Italy

<sup>2</sup>Clinical Chemistry Laboratory, “Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo”, Pavia, Italy

**Background:** In the last 10 years the availability of the polyclonal assay for the measurement of circulating FLC has dramatically improved diagnosis, prognostic stratification and follow-up of patients with AL amyloidosis. Our group and others showed that the identification of the amyloidogenic FLC requires the combination of serum and urine immunofixation electrophoresis (IFE) with nephelometric quantification of FLC in order to ensure best sensitivity. Recently, a novel method for FLC quantitation based on monoclonal antibodies has been developed.

**Methods:** We assessed the diagnostic sensitivity of serum and urine IFE and of the two assays for FLC quantification in 220 consecutive newly-diagnosed patients with AL amyloidosis, in whom the amyloidogenic light chain was identified by immuno-electron microscopy of the amyloid deposits. Semiautomated serum and urine IFE was performed with a commercial Hydragel 2IF/BJ(HR) kit on a Hydrasys apparatus (Sebia). Serum FLC concentration was measured by a polyclonal (Binding Site) and a monoclonal (Siemens) immunoassay on a Behring BNII nephelometer. Reference ranges are  $\kappa$ -FLC 3.3-19.4 mg/L,  $\lambda$ -FLC 5.7-26.3 mg/L,  $\kappa/\lambda$  ratio 0.26-1.65 for the Binding Site assay, and are  $\kappa$ -FLC 6.7-22.4 mg/L,  $\lambda$ -FLC 8.3-27.0 mg/L,  $\kappa/\lambda$  ratio 0.31-1.56 for the Siemens assay.

**Results:** Two patients were excluded from the calculation of the diagnostic sensitivity, because a biclonal gammopathy was detected by IFE. Both patients had  $\lambda$  amyloid deposits. In these subjects,

FLC  $\kappa/\lambda$  ratios were 0.42 and 0.08 by the Binding Site assay, classifying one patient as  $\lambda$ , and 0.30 and 0.08 by the Siemens assay, identifying the amyloidogenic clone in both. The diagnostic sensitivity of the tests is reported in Table 1 (see addendum Page 57).

**Conclusion:** The polyclonal and monoclonal assays for FLC measurement have comparable sensitivity. The combination of a nephelometric measurement of FLC with serum and urine IFE allows the identification of 99% of amyloidogenic clones.

#### 14.27 - OP 36

#### A Revised Prognostic Staging System for Light Chain Amyloidosis (AL) Incorporating Cardiac Biomarkers and Serum Free Light Chain Measurements

**Shaji Kumar**<sup>1</sup>, Angela Dispenzieri<sup>1</sup>, Martha Q. Lacy<sup>1</sup>, Suzanne R. Hayman<sup>1</sup>, Francis K. Buadi<sup>1</sup>, Colin Colby<sup>3</sup>, Kristina Laumann<sup>3</sup>, Steve R. Zeldenrust<sup>1</sup>, Nelson Leung<sup>1,2</sup>, David Dingli<sup>1</sup>, Philip R. Greipp<sup>1</sup>, John A. Lust<sup>1</sup>, Stephen J. Russell<sup>1</sup>, Robert A. Kyle<sup>1</sup>, S. Vincent Rajkumar<sup>1</sup>, Morie A. Gertz<sup>1</sup>

<sup>1</sup>Divisions of Hematology and <sup>2</sup>Nephrology and Internal Medicine, <sup>3</sup>Department of Biostatistics, Mayo Clinic, Rochester, MN, USA

**Background:** Cardiac involvement predicts poor prognosis in light-chain amyloidosis (AL) and the current prognostic classification is based on cardiac biomarkers troponin-T (cTnT) and N-terminal pro-B-type Natriuretic Peptide (NT-ProBNP). However, long-term outcome is dependent on the underlying plasma cell (PC) clone and incorporation of clonal characteristics may allow for better risk stratification.

**Patients:** We developed a prognostic model based on 810 patients with newly diagnosed AL; which was further validated in two other datasets; 303 patients undergoing stem cell transplant (SCT) and 103 patients enrolled in clinical trials.

**Results:** We examined the prognostic value of PC related characteristics (difference between involved and uninvolved light chain (FLC-diff), marrow PC%, circulating PCs, PC labeling index, and beta-2 microglobulin). In a multivariate model that included these characteristics and cTnT and NT-ProBNP, only FLC-diff, cTnT and NT-ProBNP were independently prognostic for overall survival (OS). Patients were assigned a score of 1 for each of FLC-diff  $\geq 18$  mg/dL, cTnT  $\geq 0.025$  ng/mL, and NT-ProBNP  $\geq 1800$  pg/mL; creating stages I to IV with scores of 0 to 3 points, respectively. The proportion of patients in stages I, II, III and IV were 189 (25%), 206 (27%), 186 (25%) and 177 (23%) and their median OS from diagnosis were 94.1, 40.3, 14, and 5.8 months, respectively ( $P < 0.001$ ). Among those undergoing SCT, the median OS was not reached, 96.5 months, 58.2 months and 22.2 months respectively for stages I, II, III and IV,  $P < 0.001$ . For the pts on trials, the median OS from study entry was not reached, 62.8, 16.8 and 5.8 months for patients in stage I, II, III, and IV respectively ( $P < 0.001$ ).

**Conclusion:** Incorporation of serum FLC-diff into the current staging system improves risk stratification for patients with AL and will help develop risk-adapted therapies for AL.

#### 14.39 - OP 37

#### Coronary Microvascular Function in Cardiac Amyloidosis

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**Background:** Amyloid deposits in the heart can compress the microvasculature, resulting in angina, even in the absence of epicardial coronary artery disease (CAD). We therefore sought to assess myocardial blood flow (MBF) and coronary vasodilator reserve using N-13 ammonia PET in subjects with cardiac amyloidosis.

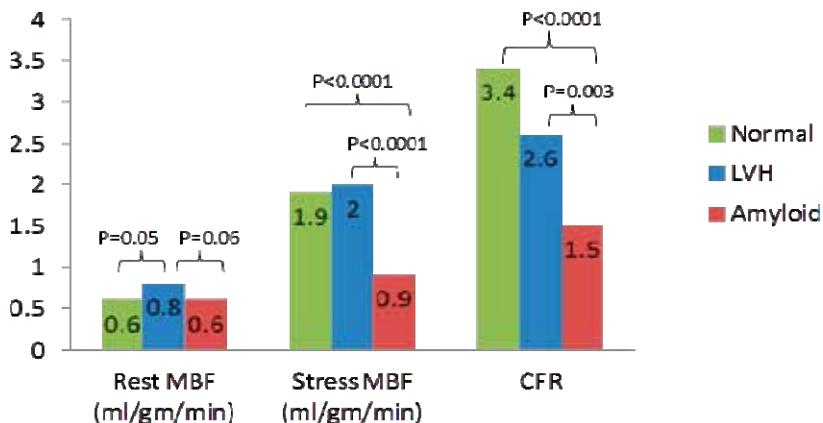
**Methods:** 13 subjects with cardiac amyloidosis (8 AL and 5 SSA) were compared to 10 normals and 5 patients with LV hypertrophy (LVH). None had obstructive epicardial CAD. Rest and vasodilator stress N-13 ammonia PET was performed and global LV MBF (ml/gm/min) was quantified at rest and at peak vasodilator stress. Coronary flow reserve (CFR) was computed as the ratio of the stress MBF to the rest MBF.

**Results:** The MBF at rest was similar among groups, but peak stress MBF was significantly lower in the amyloid (0.92) compared to normal (1.94) and LVH subjects (2.13),  $P < 0.0001$  for each

comparison (Figure). CFR was significantly lower in the amyloid (1.5) compared to normal (3.4, P <0.0001) and LVH subjects (2.7, P <0.003) (Figure).

**Conclusions:** Subjects with cardiac amyloidosis demonstrate significantly lower peak stress myocardial blood flow and vasodilator coronary flow reserve compared to normal volunteers and subjects with LVH. This could contribute to the poor cardiac function in patients with CA and the phenomenon of exertional syncope and sudden death. PET imaging with vasodilator stress may be a useful tool to evaluate patients prior to aggressive therapy for amyloidosis that might tax the compromised heart.

#### Rest MBF, Stress MBF, and CFR In Normal, LVH and Amyloid Subjects



Funding Sources: Amyloid foundation; American Society of Nuclear Cardiology; NHLBI K23HL092299

#### 14.51 - OP 38

#### Depressed midwall fractional shortening is a powerful prognostic determinant in cardiac AL amyloidosis

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**Background:** Cardiac amyloidosis represents an archetypal form of restrictive heart disease, characterized by profound diastolic dysfunction. Since ejection fraction is preserved until the late stage of the disease, the majority of patients with cardiac AL amyloidosis do fulfil the definition of diastolic heart failure, i.e. heart failure with preserved ejection fraction. In another clinical model of diastolic heart failure, i.e. pressure-overload left ventricular hypertrophy, depressed midwall fractional shortening (i.e. a marker of myocardial contractile dysfunction) has been shown to be a powerful prognostic factor.

**Aim:** The present study was aimed at assessing a potential prognostic role of midwall fractional shortening in cardiac AL amyloidosis patients.

**Methods:** We enrolled 221 consecutive untreated subjects, in whom a first diagnosis of cardiac AL amyloidosis was concluded between 2006 and 2009. Patients in whom cardiac involvement was excluded served as controls (n=121). All patients underwent complete echocardiographic evaluation as well as NT-proBNP determination at diagnosis. Patients with ejection fraction below 50% (n=28) were excluded. Prognosis was assessed after a median follow-up of 561 days.

**Results:** When compared with AL patients without myocardial involvement, cardiac AL was characterized by increased wall thickness (p<0.001) and reduced end-diastolic LV volumes (p<0.001). As expected, diastolic dysfunction was evident in all cardiac AL patients, as evident by increased E/E' ratio (p<0.001). Midwall fractional shortening was markedly depressed (11.2±4.3 vs 22.1±4.4%, p<0.001), despite preserved ejection fraction. At multivariable analysis, midwall fractional shortening

( $p=0.003$ ) and NT-proBNP ( $p=0.00002$ ) were the only significant prognostic determinants, whereas other indices of diastolic (E/E' ratio, transmitral and pulmonary vein flow velocities) and systolic function (tissue-Doppler systolic indices, ejection fraction) did not enter the model.

**Conclusions:** In cardiac AL amyloidosis with normal ejection fraction, depressed midwall fractional shortening, a marker of myocardial contractile dysfunction, is a powerful predictor of survival.

#### 15.03 - OP 39

#### ALchemy - A Large Prospective 'Real World' Study of Chemotherapy in AL Amyloidosis

**Julian D Gillmore**, Thirusha Lane, Lisa Rannigan, Darren Foard, Simon DJ Gibbs, Jennifer H Pinney, Christopher P Venner, Sanjay Banpersad, Helen J Lachmann, Ashutosh D Wechalekar, Philip N Hawkins

National Amyloidosis Centre, UCL Medical School, London, United Kingdom

**Background:** There have been no large prospective clinical trials in AL amyloidosis, and previous studies have excluded poor prognosis patients.

**Methods:** ALchemy is a prospective observational study of chemotherapy in AL amyloidosis, which opened at the UK National Amyloidosis Centre on 1st September 2009, for which all new patients requiring chemotherapy are eligible. Participants underwent a detailed clinical and biochemical evaluation at baseline, after completion of 3 cycles of chemotherapy (Cy3) and 6, 12, 18 and 24 months from baseline. Clonal disease assessments were performed monthly. Details of tolerability, dose and toxicity of chemotherapy, administered by local haematologists, were collected via a case record form.

**Results:** By January 1<sup>st</sup> 2012, 350 patients had enrolled and recruitment is planned to continue long-term. Herewith is data from 250 patients which will be updated in Groningen. At baseline, 20% patients had Mayo stage 1 disease, and 40% each were stage 2 and 3. Renal (50%) and cardiac (31%) presentations predominated. Upfront chemotherapy was CTD in 77% patients and alterations were usually to bortezomib. Intention to treat analysis of clonal response at Cy3 showed that 14 patients had not yet completed cycle 3, 12 had uninterpretable FLCs, and 4 patients were withdrawn; 171 of 220 (78%) remaining patients reached Cy3 and 49 (22%) died beforehand. Among 220 evaluable cases, 72 (33%) achieved CR/VGPR, 53 (24%) PR, and 46 (21%) NR. Approximately 50% patients were hospitalised for toxicity. CR/VGPR to cycle 1 overcame the poor prognosis associated with Mayo stage 3 disease. After median follow-up of 7 months, 29% patients had died and risk factors were identified.

**Conclusion:** ALchemy, the design of which will facilitate validation of clinical endpoints and should serve as a paradigm for studying rare diseases, is fast becoming the largest prospective study in AL amyloidosis. Inclusion of patients with all stages of disease indicates a persistently poor prognosis in a substantial proportion.

#### 15.15 - OP 40

#### Prognostic significance of cytogenetic aberrations in light chain amyloidosis patients treated with melphalan / dexamethasone as first-line therapy

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<sup>1</sup>Department of Internal Medicine, Division of Hematology/Oncology, University Hospital, Heidelberg;

<sup>2</sup>Division of Biostatistics, German Cancer Research Center, Heidelberg; <sup>3</sup>National Center for Tumor Diseases, Heidelberg; <sup>4</sup>Institute of Human Genetics, University Hospital Heidelberg

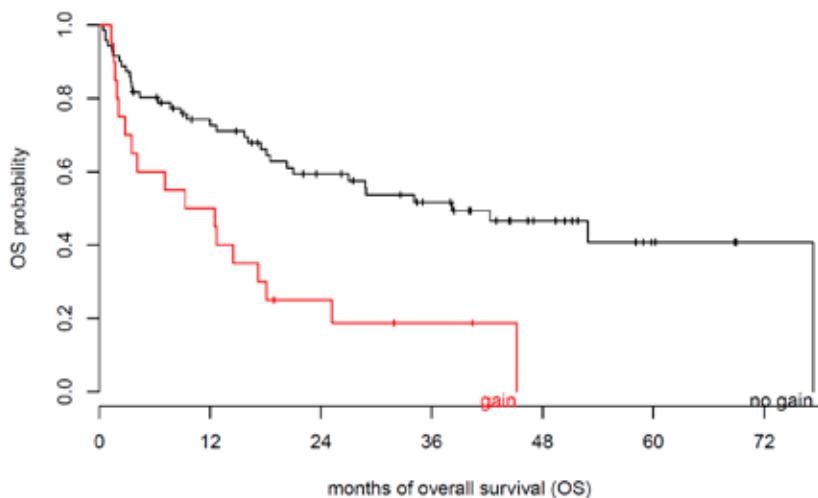
**Background and objective:** It has been previously shown that cytogenetic aberration patterns in light chain (AL) amyloidosis are reminiscent of those in multiple myeloma (MM) (ref 1). The objective of this study was to investigate whether iFISH cytogenetics also have prognostic significance in AL amyloidosis as in MM.

**Methods:** We retrospectively assessed a consecutive cohort of 93 newly diagnosed patients who received a standard chemotherapy with melphalan / dexamethasone (ref 2) for overall survival (OS) and event free survival (EFS), the latter being defined as time until hematologic relapse or progression, further therapy or death.

**Results:** Median OS of all patients was 23.5 months. Gain 1q21, which was detected in 22% of patients, was associated with an inferior median OS (9 vs. 38 months  $p<0.001$ , **figure**) and an inferior EFS ( $p=0.076$ ). On the contrary, translocation t(11;14) appeared to confer a slightly favorable OS (median 18 vs. 42 months,  $p=0.15$ ). IgH translocations with an unknown partner, deletion 13q14 and hyperdiploidy were prognostically neutral regarding OS and EFS. In a multivariate analysis (**table**), which tested four most prevalent cytogenetic aberrations in combination with the main clinical parameters, gain 1q21 emerged as an independent predictor of adverse prognosis ( $p=0.003$ ) along with age ( $p=0.047$ ) and NT-pro-BNP ( $p<0.001$ ). Interestingly, dFLC lost its prognostic value. Finally, gain 1q21 also retained its independent prognostic significance with respect to EFS ( $p=0.001$ ).

**Discussion and conclusion:** This study comprehensively analyzed the prognostic relevance of the major cytogenetic aberrations in AL amyloidosis patients treated with standard first-line chemotherapy for the first time. Gain 1q21, which is known as a progression related marker in MM (ref 3), emerged as an independent predictor of poor survival.

#### Figure:



**Table:** Multivariate analysis of prognostic factors

|                                | Overall survival<br>(OS)<br>p | Event free survival<br>(EF)<br>p |
|--------------------------------|-------------------------------|----------------------------------|
| <b>Cytogenetic aberrations</b> |                               |                                  |
| t (11;14)                      | 0.53                          | 0.83                             |
| deletion 13q14                 | 0.49                          | 0.43                             |
| hyperdiploidy                  | 0.14                          | 0.51                             |
| gain 1q21                      | <b>0.003</b>                  | <b>0.001</b>                     |
| <b>Clinical parameters</b>     |                               |                                  |
| age                            | <b>0.047</b>                  | 0.66                             |
| gender                         | 0.15                          | 0.08                             |
| Karnofsky index                | 0.07                          | 0.76                             |
| organ number                   | 0.67                          | 0.44                             |
| NT-proBNP                      | <b>&lt;0.001</b>              | <b>&lt;0.001</b>                 |
| <b>Hematologic parameters</b>  |                               |                                  |
| light chain restriction        | 0.48                          | 0.72                             |
| difference of FLC levels       | 0.59                          | 0.20                             |
| intact immunoglobulin          | 0.48                          | 0.90                             |

#### References:

- Bochtler T, Hegenbart U, Heiss C et al, Blood 2011 117(14):3809-15
- Palladini G, Perfetti V, Obici L et al, Blood 2004 103(8):2936-8
- Hanamura I, Stewart JP, Huang Y et al, Blood 2006 108(5):1724-32

This project was supported by an unrestricted grant from Celgene.

### 15.27 - OP 41

#### A Framework for Clinical Research in Systemic Light-chain (AL) Amyloidosis: Consensus Report of the First Amyloidosis Foundation Roundtable

**RL Comenzo**<sup>1</sup>, D Reece<sup>2</sup>, G Palladini<sup>3</sup>, D Seldin<sup>4</sup>, V Sanchorawala<sup>4</sup>, H Landau<sup>5</sup>, R Falk<sup>6</sup>, K Wells<sup>7</sup>, A Solomon<sup>7</sup>, A Wechalekar<sup>8</sup>, J Zonder<sup>9</sup>, A Dispenzieri<sup>10</sup>, M Gertz<sup>10</sup>, H Streicher<sup>11</sup>; M Skinner<sup>4</sup>, RA Kyle<sup>10</sup>, G Merlini<sup>3</sup>

<sup>1</sup>Tufts Medical Center, Boston, MA, USA; <sup>2</sup>Princess Margaret Hospital, Toronto, ONT, Canada;

<sup>3</sup>Fondazione IRCCS Policlinico San Matteo University of Pavia, Italy; <sup>4</sup>Boston University School of Medicine, Boston, MA, USA; <sup>5</sup>Memorial Sloan-Kettering Cancer Center, New York, NY, USA;

<sup>6</sup>Brigham and Women's Hospital, Boston, MA, USA; <sup>7</sup>University of Tennessee College of Medicine, Knoxville, TN, USA; <sup>8</sup>University College London, UK; <sup>9</sup>Barbara Ann Karmanos Cancer Institute, Detroit, MI, USA; <sup>10</sup>Mayo Clinic, Rochester, MN, USA; <sup>11</sup>National Cancer Institute, Washington, DC, USA

Given recent advances in diagnosis, evaluation and treatment of patients with AL, we worked as a group of investigators to develop a framework for clinical research that would encourage rapid testing of therapies and expedite new drug development. We defined clinically relevant endpoints, study populations, and other criteria for collaborative clinical research. Our seven key recommendations are: (1) that the XII<sup>th</sup> Amyloid Symposium consensus criteria for hematologic and cardiac responses, although validated only in newly diagnosed patients, be used in constructing end-points for clinical trials of all phases and study populations; (2) that AL patients comprise four specific study populations: newly diagnosed untreated patients either with or without advanced (stage III) cardiac involvement, relapsed/refractory patients, and responders to initial therapy; (3) that in phase III trials for newly diagnosed untreated patients without advanced cardiac involvement, hematologic response (HR) is a more appropriate primary endpoint than overall survival (OS), while in phase III trials for the newly diagnosed with advanced cardiac involvement OS is more appropriate; (4) that progression-free (PFS) and OS be routinely included as secondary endpoints in multicenter phase II trials in order to provide information for phase III trial design; (5) that phase I trials should be limited to patients with relapsed/refractory disease for whom no other standard therapy exists; (6) that both consolidation and maintenance be explored in multicenter clinical trials in responders to initial therapy and that in consolidation the primary endpoint be improvement in HR and in maintenance PFS; and (7) that in trials using anti-amyloid agents PFS and OS are appropriate primary endpoints but that a compelling and substantial rationale must exist to limit or not permit chemotherapy for the monoclonal disease. We hope that the strategy we outline provides a timely and effective platform that proves to be of benefit to patients.

### 15.39 - OP 42

#### An Italian single center prospective study on outcomes in AL amyloidosis

**Giovanni Palladini**, Paolo Milani, Andrea Foli, Laura Obici, Francesca Lavatelli, Mario Nuvolone, Stefano Perlini, Giampaolo Merlini

*Amyloidosis Research and Treatment Center – “Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo” and Department of Molecular Medicine – University of Pavia, Pavia, Italy*

**Background:** Prospective studies in AL amyloidosis are hampered by patient selection, small numbers and short follow-up, and retrospective series tend to underestimate early deaths.

**Methods:** Starting in 1994, we prospectively collected parameters of clonal and organ disease at baseline and every 3 months during treatment in all the 1399 patients diagnosed at our center. We report the outcome of 531 subjects enrolled after 2004, when systematic collection of cardiac biomarkers and FLC data started.

**Results:** Median age was 65 years. Involved organs were heart (74%), kidney (65%), liver (17%) and PNS (28%), ≥3 organs were involved in 28% of cases, NYHA class was ≥3 in 43%, Mayo stage was I in 18% of patients, II in 43% and III in 39%. Forty-nine percent of patients died (15% within 3 months). Median survival was 42 months. Mayo stage (HR 3.4, p<0.001) and dFLC >500 mg/L (HR 1.8, p<0.001) were independent prognostic determinants. In particular, dFLC >500 identified subgroups

with shorter survival among stage II (median 25 vs. 54 months,  $p=0.03$ ) and III (3 vs. 7 months,  $p=0.007$ ) patients. In stage III subjects, NT-proBNP  $>10,000$  ng/L was an additional independent prognostic factor: median survival was 2 months if both NT-proBNP and dFLC were above the cutoff (84% of these subjects died before being evaluable for response). Treatment was MDex in 55% of patients, thalidomide-based in 18%, bortezomib-based in 12%, ASCT in 3%. Severe AE occurred in 25% of patients, most common being fluid retention (10%) and cytopenia (9%). On an ITT basis, 12% of patients achieved CR, 16% VGPR and 31% PR. Hematologic response independently predicted prognosis ( $HR=0.25$ ,  $p<0.001$ ).

**Conclusion:** In an unselected population a significant proportion of patients die before having the chance to benefit from treatment. The addition of dFLC and a higher NT-proBNP cutoff can improve the staging system.

G.P.: honoraria from Janssen-Cilag and Celgene

### 15.51 - OP 43

#### Outcomes and Treatment of Relapsed Systemic Amyloidosis

**Rahma Warsame**, Soo Mee Bang, Shaji K. Kumar, Morie A Gertz, Martha Q. Lacy, Francis Buadi, David Dingli, Suzanne R. Hayman, John A. Lust, Stephen J. Russell, Thomas E. Witzig, Robert A. Kyle, Nelson Leung, Steven R. Zeldenrust, S. Vincent Rajkumar, Angela Dispenzieri

*From the Division of Hematology and Internal Medicine, Mayo Clinic Rochester, MN United States*

Systemic light chain amyloidosis is a condition in which misfolded insoluble immunoglobulin light chains are deposited in various organs. Chemotherapy and stem cell transplant when eligible is the standard treatment options for patients with systemic AL amyloidosis. Our group has reported that the 10 year survival rate for patients who received SCT was 25% and 53% for those achieving complete hematologic response. The outcomes of patients who relapse after SCT have not been well described. We performed a retrospective study to assess the outcomes and treatment regimens employed following relapse after SCT. Between 2005 and 2009, 193 patients received with SCT at the Mayo Clinic. Twenty-seven died prior to relapse, 87 were progression free at last follow-up, and 79 had documented relapse. These 79 patients are the subject of the present study. Of those who relapsed, median time to relapse was 14.4 months. Heme progression (68% of progressed patients) was more common than organ progression (37% of progressed patients). At relapse, 34 patients were treated with IMiDs, 16 with bortezomib, 9 with steroids, and 12 with alkylator; and the respective response rates were 56%, 56%, 50%, and 38%. The remaining eight relapsed patients received organ transplants, a second SCT, were too ill to be treated or not evaluable. With a median follow-up of surviving patients of 26 months from relapse, 1 and 3 year OS were 87% and 59%, respectively. (Figure 1) These data provide novel and important information about expected outcomes among patients relapsing after SCT.

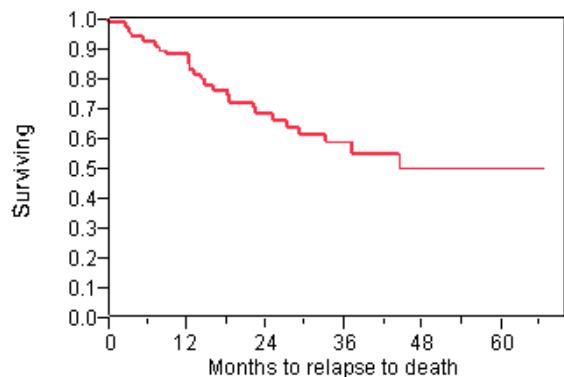


Figure 1. The overall survival at 1 and 3 years for relapsed patients

#### References:

- Cohen, A.D. and R.L. Comenzo, Systemic light-chain amyloidosis: advances in diagnosis, prognosis, and therapy. *Hematology / the Education Program of the American Society of Hematology*. American Society of Hematology. Education Program, 2010. 2010: p. 287-94.

2. Gertz, M.A., Immunoglobulin light chain amyloidosis: 2011 update on diagnosis, risk-stratification, and management. American journal of hematology, 2011. 86(2): p. 180-6.
3. Gertz, M.A., et al., Trends in day 100 and 2-year survival after auto-SCT for AL amyloidosis: outcomes before and after 2006. Bone marrow transplantation, 2011. 46(7): p. 970-5.

#### 16.03 - OP 44

#### Solid organ transplantation in AL amyloidosis and monoclonal immunoglobulin deposition disease: the French experience

François Pourreau<sup>1</sup>, Clotilde Muller<sup>2</sup>, Antoine Thierry<sup>1</sup>, Estelle Desport<sup>1</sup>, Jean Paul Fermand<sup>3</sup>, Guy Touchard<sup>1</sup>, Bruno Moulin<sup>2</sup>, Frank Bridoux<sup>1</sup>

<sup>1</sup>Departments of Nephrology, transplantation and dialysis CHU Poitiers, <sup>2</sup>Department of Nephrology and Transplantation CHU Strasbourg, <sup>3</sup>Department of Immunology and Haematology, CHU Saint Louis, Paris, France

AL amyloidosis (AL) and monoclonal immunoglobulin deposition disease (MIDD) are rare complications of plasma cell disorders characterized by systemic deposition of a monoclonal Ig. Renal (R) and cardiac (C) involvement are frequent and may progress to organ failure. Solid organ transplantation (Tx) is debated because of the risk of systemic progression and relapse on the allograft. However, due to improved survival, an increasing number of patients are candidates to solid organ Tx. In this retrospective study results of CTx and RTx in patients with biopsy-proven AL or MIDD who underwent CTx and/or RTx in France between 1991 and 2011 were evaluated.

Eighteen CTx and 21 RTx were performed in 37 patients (AL:28; MIDD:9). Mean age was 47±9 years and 28 patients had ≥ 2 organs involved. Two patients received combined C+R Tx and 1 received 2 RTx.

After a median follow-up of 19 months, 7/18 CTx recipients (AL:17; MIDD:1) had died, including 3 in the peri-operative period and 3 from disease progression. Median survival (perioperative death censored) was 4 years. Recurrence occurred in 5 patients after a median of 15 months resulting in 1 graft loss. One and 5-year survival was 64.3% and 40% respectively. When partial or complete haematologic response was obtained with chemotherapy, 1-year survival was 74% and 100%, respectively.

After a median follow-up of 26 months, 15/20 RTx recipients (AL:12; MIDD:8) had a functioning graft, and 2 had died of disease progression. Median patient survival was 8 years. Five grafts were lost, because of recurrence (n=2) or chronic rejection (n=3). No death or graft loss related to disease progression occurred in patients with haematological response.

Solid organ Tx in AL and MIDD may provide prolonged survival when performed in patients aged <60years without other severe organ involvement, and when haematological response is obtained before or after Tx.

#### 16.15 Perspectives - Gertz

##### 14.00-16.30 Sponsored Satellite Workshop (Spectral Imaging)

##### Amyloid Diagnosis and Research Workshop ('Lokaal 16')

|       |   |
|-------|---|
| 14.00 | Welcome and Aim of the Workshop - Hammarström                                       |
| 14.30 | The use of LCP probes for classification of amyloid deposits - Nilsson              |
| 15.30 | Hyperspectral-imaging for direct analysis of LCP-coupled amyloid deposits - Sluszny |

##### 16.30-17.00 Tea break ('Fonteinpatio')

##### 17.00-18.00 Sponsored Symposium (Pfizer)

##### Advancing the understanding and management of TTR-FAP ('Blauwe Zaal')

|  |
|--|
| Chair: Hazenberg   |
| 17.00 Welcome - Hazenberg                                  |
| 17.05 Unravelling the TTR misfolding hypothesis - Kelly    |
| 17.30 New approaches to the management of TTR-FAP – Coelho |
| 17.55 Conclusions - Hazenberg                              |

- 18.00-19.30 Refreshments (Proteotech) in the 'Fonteinpatio'**
- 18.00-19.00 Poster viewing (PB 1-66) in 5 groups ('Blauwe Patio')**
- 19.00-19.30 Selected poster presentations ('Blauwe Zaal')**  
 Chairpersons: Croockewit and Van Rijswijk
- |       |  |
|-------|--|
| 19.00 | PB 12 Neurological manifestations of senile systemic amyloidosis – Ikeda   |
| 19.06 | PB 41 Cardiac involvement in Cardiac AL Amyloidosis as measured by Equilibrium Contrast Cardiovascular Magnetic Resonance – Banypersad                               |
| 19.12 | PB 45 Amyloid Deposits in the Bone Marrow of Patients with AL Amyloidosis Do Not Impact Stem Cell Mobilization or Engraftment – Cowan                                |
| 19.18 | PB 47 Determinants of Cardiac Severity in Patients with Systemic Light Chains Amyloidosis: An echocardiography and cardiac magnetic resonance imaging study – Ettaif |
| 19.24 | PB 66 Functional proteomics investigation of the mechanisms of cardiac damage in AL amyloidosis – Lavatelli  |
- 18.30-19.30 Committee Meetings ('Lokaal 15 and W2270')**
- 19.30-20.00 Walk to the restaurant**
- 20.00-22.30 Dinner in Ni Hao wok restaurant**
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**Addendum to OP 35 (page 49 and 50 continued):**

**Table 1. Diagnostic sensitivity of IFE and FLC κ/λ ratio by Binding Site (BS) and Siemens (S) in 218 patients with systemic AL amyloidosis**

|   | Patients with κ clones (N=46) |              | Patients with λ clones (N=172) |              | Overall population (N=218) |             |
|---|-------------------------------|--------------|--------------------------------|--------------|----------------------------|-------------|
|   | N positive                    | % (95% CI)   | N positive                     | % (95% CI)   | N positive                 | % (95% CI)  |
| <b>Serum IFE</b>                              | 37                            | 80 (66-91)   | 162                            | 94 (90-97)   | 199                        | 91 (87-95)  |
| <b>Urine IFE</b>                              | 38                            | 83 (67-92)   | 153                            | 89 (83-93)   | 191                        | 88 (82-92)  |
| <b>Serum and urine IFE</b>                    | 43                            | 93 (82-99)   | 170                            | 99 (96-100)  | 213                        | 98 (95-99)  |
| <b>FLC κ/λ ratio BS</b>                       | 44                            | 96 (85-99)   | 137                            | 80 (73-85)   | 181                        | 83 (77-88)  |
| <b>FLC κ/λ ratio S</b>                        | 40                            | 87 (74-95)   | 138                            | 80 (73-86)   | 178                        | 82 (76-87)  |
| <b>Serum and urine IFE + FLC κ/λ ratio BS</b> | 46                            | 100 (92-100) | 171                            | 99 (97-100)  | 217                        | 99 (97-100) |
| <b>Serum and Urine IFE + FLC κ/λ ratio S</b>  | 44                            | 96 (85-99)   | 172                            | 100 (98-100) | 216                        | 99 (97-100) |

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Plenary sessions Tuesday May 8

## Wednesday, May 9

7.30-8.00      **Coffee ('Fonteinpatio')**

### Plenary session 8

8.00-9.50      **Biology, clinics and prognosis in ATTR amyloidosis ('Blauwe Zaal')**  
Chairmen: Suhr and Van den Berg

8.00      **State of the art - Ikeda**

**8.15 - OP 45**

**First online registry of mutations in hereditary amyloidosis: amyloidosismutations.com**

**Rowczenio, Dorota M.**; Noor, Islam; Gillmore, Julian D.; Hawkins, Philip N. and Wechalekar, Ashutosh D  
*Centre for Amyloidosis and Acute Phase Proteins, University College London Medical School, UK*

**Background:** Hereditary systemic amyloidosis is a group of rare autosomal dominant monogenic diseases that have been associated with mutations in the genes for at least seven proteins including transthyretin, apolipoprotein AI, apolipoprotein AII, lysozyme, fibrinogen alpha chain, cystatin C and gelsolin. Whilst the most commonly identified variants are well documented, information on others, sometimes including their amyloidogenic potential, can be difficult to obtain.

**Objective:** With support from the EU EURAMY project, we set out to create an online database to provide up to date information on the genes and mutations associated with hereditary amyloidosis, and their associated clinical phenotypes.

**Methods:** A comprehensive search of the published literature describing mutations in familial amyloidosis was performed, and the database was constructed to comprise: the cDNA sequence for each gene, showing the position of identified amyloidogenic and non-amyloidogenic variants, along with the change at the protein and DNA levels, the location of the variant, the type and number of bases altered, a short description of the phenotype, ethnic origin and references with links to published abstracts on PubMed.

**Results:** The database is now openly accessible at [www.amyloidosismutations.com](http://www.amyloidosismutations.com). By default, the website opens a page with a tabular list of eight buttons giving access to the genes associated with hereditary amyloidosis, and to a discussion forum. There are tabs for each gene showing the sequence and known variants, which are linked to a table of corresponding data and PubMed citations. The database search facility provides a quick and simple way to find specific data, and offers the choice to submit novel variants via a moderator.

The database currently describes 168 variants, including eight as yet otherwise unpublished cases identified in our centre.

**Discussion:** The database for hereditary amyloidosis has been designed to provide a useful and convenient tool for molecular biologists, researchers and clinicians. We encourage submission of novel variants to the database, and will ensure that providers of new information will be credited accordingly, including a link to publications when available.

**8.27 - OP46**

**The Prevalence of Holter Abnormalities in ATTR Cardiac Amyloidosis**

A. Reshad Garan<sup>1</sup>, Sheela Kolluri<sup>2</sup>, Ilise Lombardo<sup>2</sup>, and **Mathew S. Maurer<sup>1</sup>**

<sup>1</sup>*Columbia University Medical Center, New York, NY;* <sup>2</sup>*Pfizer, Inc, New York, NY, USA*

**Background:** Little is known about the prevalence of cardiac arrhythmias in patients with ATTR cardiac amyloidosis. Small studies have reported the prevalence of arrhythmias in patients with light chain amyloidosis but findings have varied<sup>1-2</sup>. Furthermore, there is little known about the prognostic importance of cardiac arrhythmias in this population.

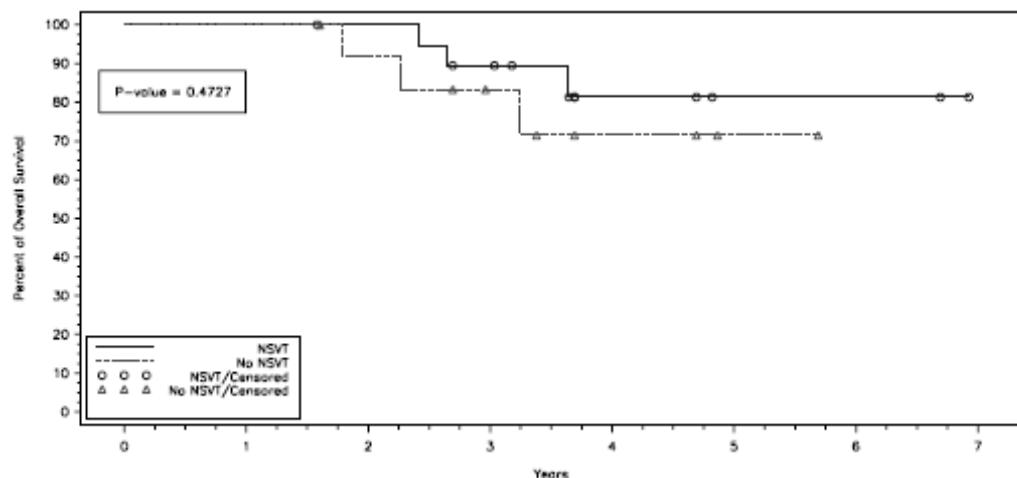
**Objective:** We sought to characterize cardiac arrhythmias in ATTR cardiac amyloidosis patients.

**Methods:** We analyzed 24 hour Holter data from 34 patients with ATTR cardiac amyloidosis in order to determine the prevalence of arrhythmias in this population.

**Results:** The mean age of patients was  $76.4 \pm 4.7$  years and 32 (91.4%) were men. Ninety-four percent were either NYHA class I or II. The mean heart rate was  $74.7 \pm 7.8$  beats per minute. Sixteen patients (45.7%) were taking a beta-blocker, eight (22.9%) were taking amiodarone, and two (5.7%) were taking another anti-arrhythmic medication. Non-sustained ventricular tachycardia (NSVT) was present in 20 patients (58.8%). No patients had sustained ventricular tachycardia (defined by > 30 seconds). There was no difference in overall survival between those with and without NSVT on the 24 hour Holter monitor (see figure).

**Discussion:** These findings underscore the magnitude of the burden of ventricular arrhythmias in the ATTR cardiac amyloid patient population. However, despite the significant burden of malignant arrhythmias, the presence of these arrhythmias is of limited prognostic importance.

**Conclusion:** In this sample, malignant ventricular arrhythmias were present in the majority of patients with ATTR cardiac amyloidosis, though the presence of NSVT on Holter monitor did not confer a worse survival.



**Figure 1:** Survival among ATTR cardiac amyloidosis patients with and without NSVT on 24 hour Holter monitor.

#### References:

- Palladini G, Malamani G, Co F, et al. Holter monitoring in AL amyloidosis: prognostic implications. *Pacing Clin Electrophysiol* 2001;24:1228-33.

This study was an analysis of the baseline Holters performed in the Fx1B-201 Study (NCT00694161) which was sponsored by FoldRx Pharmaceuticals, Inc, that is a wholly owned subsidiary of Pfizer, Inc.

#### 8.39 - OP 47

#### Circadian rhythm of blood pressure reflects the severity of cardiac impairment in Familial Amyloid Polyneuropathy

**Vincent Algalarrondo**<sup>1,2</sup>, Ludivine Eliahou<sup>1</sup>, Isabelle Thierry<sup>1</sup>, Abdeslam Bouzeman<sup>1</sup>, Claude Sebag<sup>1</sup>, Dominique Le Guludec<sup>3</sup>, Didier Samuel<sup>4</sup>, David Adams<sup>5</sup>, Sylvie Dinanian<sup>1</sup> and Michel S. Slama<sup>1</sup>

<sup>1</sup>: Cardiology Department, CHU Antoine Béclère, Université Paris Sud, AP-HP, Clamart.

<sup>2</sup>: Inserm UMR-S 769; Signalisation et Physiopathologie Cardiaque; LabEx LERMIT

<sup>3</sup>: Nuclear Medecine Department, CHU Xavier Bichat, Université ParisDiderot-Paris VII, AP-HP, Paris

<sup>4</sup>: Centre Hépato-Biliaire, CHU Paul Brousse, Université Paris Sud, AP-HP, Villejuif.

<sup>5</sup>: Neurology Department, CHU Kremlin Bicêtre, Université Paris Sud, AP-HP, Bicêtre.

All authors belong to the French reference center for FAP (NNERF)

**Background:** Cardiac amyloidosis due to familial amyloid polyneuropathy (FAP) includes restrictive cardiomyopathy, thickened cardiac walls, conduction disorders and cardiac denervation. Impaired blood pressure variability (BPV) has been documented in FAP related to the Val30Met mutation.

**Aims:** to document BPV in FAP patients with various mutation types and its relation to the severity of cardiac involvement.

**Methods:** BPV was analyzed in 49 consecutive FAP patients and was compared to a matched control population. Cardiac evaluation included echocardiography, right heart catheterization, electrophysiological study, Holter-ECG and MIBG scintigraphy.

**Results:** A non dipping pattern was found in 80% FAP patients and in 35% control patients ( $P<0.0001$ ). Non dipping pattern was observed more frequently in FAP patients with hemodynamic involvement (92% vs. 67%;  $P=0.04$ ) and with conduction disorders (95% vs. 68%;  $P=0.03$ ), but not with cardiac thickening, or cardiac denervation. The percentage of systolic blood pressure dipping was correlated to the E/Ea ratio ( $P=0.04$ ). Impaired BPV was more frequent and more pronounced in patients with severe cardiac amyloidosis.

**Conclusion:** Low BPV is common in cardiac amyloidosis due to FAP. A non dipping pattern was more frequently observed in FAP patients with hemodynamic impairment or conduction disorders. It is suggested that impairment of circadian rhythm of blood pressure reflects the severity of cardiac amyloidosis due to FAP.

#### 8.51 - OP 48

#### **Senile Systemic Amyloidosis: a large cohort study detailing clinical features, laboratory results, and survival**

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**Background/Objective:** Amyloid deposits of wild-type transthyretin (TTR) are the hallmark of senile systemic amyloidosis (SSA). We are conducting a cross-sectional and longitudinal study of patients with SSA to precisely define disease progression, identify biomarkers and prognostic factors, and understand mechanisms of disease.

**Methods:** From 1994-2011, 109 patients were diagnosed with SSA, based upon the presence of amyloid cardiomyopathy and lack of evidence of a TTR mutation or AL amyloidosis. Data was collected with approval of the BUMC IRB.

**Results/Conclusions:** Amyloid disease was established in 109 patients by biopsy positive Congo red staining; immunohistochemical/biochemical proof of TTR was obtained in 80/109. No amyloidogenic TTR mutations were detected; polymorphic TTR-G6S was present in 9% of patients. The group was predominantly male (105/109), and the median (range) ages at clinical presentation and histopathological diagnosis were 72.5 (54.4-86.6) and 74.7 (59.0-87.5) years. Average time from earliest involvement to diagnosis was  $1.9 \pm 2.0$  years. Cardiac involvement at presentation occurred in 96% and symptomatic heart failure in 86% of the group. Common clinical features at initial visit included dyspnea on exertion (84%) and peripheral edema (69%); history of hypertension (24%) and carpal tunnel syndrome (16%) were less likely. Arrhythmias were reported in 58/108 patients; 31 had pacemakers/defibrillators. From echocardiography, median values for left ventricular end diastolic and systolic dimensions were within normal limits, while interventricular septal thickness and left ventricular ejection fraction (LVEF) were 16 mm (range, 9-25) and 45% (range, 10-70). BNP (n=77) was  $461 \pm 311$  pg/mL; cTnI (n=54) was  $0.220 \pm 0.224$  pg/mL. Age-adjusted median survival time in 102 patients (excluding 4 females and 3 males treated with diflunisal) was 4.6 years [95% CI (3.5, 5.5) years]. In 24 patients, 1-year follow-up data indicated that cTnI, BNP, and LVEF were modestly increased (< 20%) from initial values. In this large SSA cohort, 96% of patients were male, 86% presented with congestive heart failure, and age-adjusted median survival was < 5 years.

*Supported by NIH RO1AG031804 and the Young Family Amyloid Research Fund.*

#### 9.03 - OP 49

#### **Systematic review of 1142 admissions with acute heart failure reveals high frequency of transthyretin V122I cardiac amyloidosis in Afro-Caribbean patients**

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<sup>1</sup>*Centre for Amyloidosis and Acute Phase Proteins, University College London Medical School, UK*

<sup>2</sup>*St George's University of London, UK*

**Background:** The mutation encoding transthyretin (TTR) isoleucine 122 (V122I) is present in ~4% of African-Americans and is associated with cardiac TTR amyloidosis, but penetrance is unknown. Little is known about the frequency or clinical phenotype of TTR V122I amyloidosis in the British Afro-Caribbean population.

**Methods:** We reviewed the diagnoses of 1142 patients admitted with heart failure between September 2005 and February 2011. Diagnosis was supported by echocardiography, cardiac MRI, and genetic testing; endomyocardial biopsies were performed in 68 patients (6%) in whom amyloidosis was suspected or uncertainty about diagnosis remained. Survival analysis was performed to August 2011.

**Results:** The median age was 72 years (range 18-98) with male (66.7%) and Caucasian (71.0%) predominance. Ischaemic cardiomyopathy (ICM) was the primary diagnosis in 428 patients (37%). There were 170 Afro-Caribbean patients (14.9%) among whom ICM was less common (22 patients (13%), p<0.01). Seventeen Afro-Caribbean patients (10%) were confirmed to have cardiac ATTR V122I amyloidosis. Survival of Afro-Caribbean patients with ICM and ATTR V122I amyloidosis was similar (45 vs 36 months, p=0.54), but overall survival (n=1142) was significantly inferior in cardiac amyloidosis compared to non-amyloid cardiomyopathy (34 vs 59 months, p<0.01).

| Black ethnicity data       | Afro-Caribbean patients (N = 170) | All other patients (N = 972) | P value         |
|----------------------------|-----------------------------------|------------------------------|-----------------|
| Age                        | 71 (53-77)                        | 73 (63-81)                   | <0.01           |
| Male gender                | 110 (64.7%)                       | 652 (67.1%)                  | <0.01           |
| Ischaemic cardiomyopathy   | 22 (12.9%)                        | 406 (41.8%)                  | <0.01           |
| Non-ischaemic (total)      | 148 (87.1%)                       | 566 (58.2%)                  | <0.01           |
| Amyloid (AL and TTR)       | 27 (15.9%)                        | 21 (2.2%)                    | <0.01           |
| <b>ATTR V122I</b>          | <b>17 (10%)</b>                   | <b>4 (0.4%)</b>              | <b>&lt;0.01</b> |
| Dilated cardiomyopathy     | 46 (27.1%)                        | 179 (18.4%)                  | <0.01           |
| Hypertensive heart disease | 32 (18.8%)                        | 68 (7.0%)                    | <0.01           |

**Conclusion:** ATTR V122I amyloidosis is an important cause of heart failure in the British Afro-Caribbean population, but it is commonly misdiagnosed as hypertensive cardiomyopathy. Diagnosis requires specialist multidisciplinary investigation including CMR, genetic testing and histology, enabling appropriate management in an era when novel treatments for amyloidosis are entering the clinic.

## 9.15 - OP 50

### Comparison of V122I and Senile Cardiac Amyloidosis: Differences in Clinical Features and Outcomes

Chris Russo, Phil Green and **Mathew S. Maurer**

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**Background:** In the United States, ATTR cardiac amyloidosis is most often caused by either a mutated variant of transthyretin (ATTRmt) in which isoleucine is substituted for valine at position 122 (V122I) or from wild-type variant (ATTRwt, SCA).

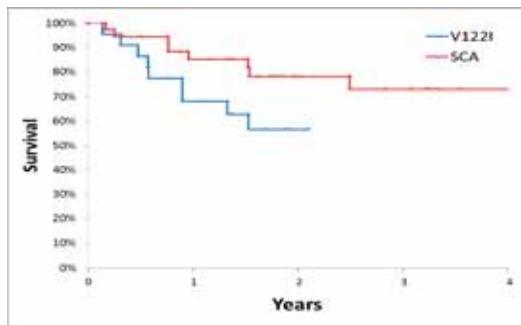
**Objective:** We compared these two forms of ATTR cardiac amyloidosis including clinical features and outcomes in order to determine if differences exist.

**Results:** Subjects with V122I were younger and all were of African American descent. B-type natriuretic peptide and pulmonary vascular resistance tended to be higher and EF lower among subjects with V122I than wild type disease (see table below). Survival curves indicating time to death or cardiac transplant show a median survival of 36 months for V122I subjects compared with 58 months for wild type subjects, p=0.026 by log rank.

**Discussion:** Despite the ability to identify subjects with V122I by DNA sequencing, these subjects present to our institution at a more advanced stage of disease than subjects with SCA for whom serologic testing is not available. Whether this is attributable to a different natural history or delayed diagnosis is unknown but warrants further investigation.

**Conclusion:** Outcomes of patients with ATTR cardiac amyloid in the United States differ between subjects with the V122I mutation and those with wild type disease.

| Parameter                             | SCA<br>(n=36) | V122I<br>(n=22) | P value |
|---------------------------------------|---------------|-----------------|---------|
| <b>Demographics</b>                   |               |                 |         |
| Age at Diagnosis (years)              | 77±6          | 70±8            | <0.01   |
| Gender (% male)                       | 97%           | 86%             | 0.148   |
| Race (% Black)                        | 3%            | 100%            | <0.0001 |
| <b>Electrocardiography (%)</b>        |               |                 |         |
| Atrial Fibrillation/Flutter           | 25%           | 36%             | 0.386   |
| Pacemaker                             | 28%           | 0%              | 0.0071  |
| <b>Laboratory</b>                     |               |                 |         |
| BNP (pg/ml)                           | 822±539       | 1191±727        | 0.0525  |
| Troponin I (ng/ml)                    | 0.19±0.44     | 0.31±0.6        | 0.4355  |
| eGFR (ml/min)                         | 49±22         | 53±23           | 0.5432  |
| <b>Echocardiogram</b>                 |               |                 |         |
| LVIDd (mm)                            | 43±7          | 44±5            | 0.708   |
| IVS (mm)                              | 19±4          | 18±4            | 0.1579  |
| EF (%)                                | 47±15         | 26±13           | <0.0001 |
| LV mass index (grams/m <sup>2</sup> ) | 225±87        | 217±69          | 0.7319  |
| LA size (mm)                          | 48±5          | 46±5            | 0.2828  |
| <b>Right Heart Catheterization</b>    |               |                 |         |
| RA pressure (mm Hg)                   | 10±6          | 10±8            | 0.8403  |
| PA Pressure (mm Hg)                   | 47±12         | 44±15           | 0.4964  |
| PCWP (mm Hg)                          | 19±7          | 16±8            | 0.3462  |
| PaSat (%)                             | 60±7          | 56±10           | 0.2162  |
| Cardiac Output (L/min)                | 3.5±0.8       | 3.1±0.9         | 0.0984  |
| PVR (dynes)                           | 240±105       | 398±219         | 0.0356  |



### 9.30 Perspectives - Berk

## Plenary session 9

**9.50-11.05 Biology, clinics and prognosis in AA (and other types of amyloidosis)**  
Chairpersons: Lachmann and Van Gameren

**9.50 State of the art - Livneh**

**10.05 - OP 51**  
**A single centre 20 year case series of AA amyloidosis – changing epidemiology**

**Lachmann, Helen J.**, Gillmore, Julian D., Wechalekar, Ashutosh D., Gibbs, Simon D.J., Pinney, Jennifer H., Rowczenio, Dorota M., Trojer, Hadija, Lane, T., Venner, Christopher P., Banypersad, Sanjay M., Gilbertson, Janet A., Hunt, F., Toby, Wassem, Nancy, Gopaul, Dorothea, Hutt, David F., Pepys, Mark B., Hawkins, Philip N.

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**Background:** AA amyloidosis is thought to be becoming less common in the developed world but there are few systematic data.

**Objective and Methods:** To analyze experience of AA amyloidosis over the past 20 years at a single national centre.

**Results:** 493 patients with confirmed AA amyloidosis were assessed between 1992 and 2011. The referral rate of new AA patients has been stable at ~30 per year during the past decade, in contrast with a three-fold increase among other types of amyloidosis. The commonest underlying diseases are: rheumatoid arthritis (RA) 28%, chronic sepsis 19% and seronegative arthritis 10%, but in 18% the underlying inflammatory disease was uncharacterized at diagnosis.

Comparing the cohort referred 1992-96 with the most recent 5 years, there has been a reduction in patients with RA from 33% to 19% ( $p = 0.038$ ), and juvenile idiopathic arthritis from 18% to 2% ( $p < 0.001$ ), but a rise in AA amyloidosis of unknown aetiology from 8% to 28% ( $p < 0.001$ ). Age at referral has risen from a median of 48 to 61 years ( $p < 0.001$ ), and the proportion referred with established end-stage renal failure (ESRF) has remained stable at 26%. Median time to ESRF is unchanged at 157 months. Median survival is 118 months with no significant difference between cohorts, but age at death has increased from a median of 60.6 years in the earliest cohort to 79 in the most recent ( $p < 0.001$ ).

**Discussion:** In contrast to a threefold increase in referrals to the NAC of AL and other types of amyloidosis during the past decade, referral rates for AA have not changed, perhaps reflecting a reduction in incidence among patients with inflammatory arthritis. A greater proportion of recent AA patients have uncharacterized underlying inflammatory disorders, posing challenges for clinical management. Age at both diagnosis and death have increased significantly over the last 20 years.

#### 10.17 - OP 52

#### Long term effectiveness of surgery in localized laryngeal amyloidosis

**Aldert J Hazenberg<sup>1</sup>, Bouke P Hazenberg<sup>1</sup> and Frederik G Dikkers<sup>2</sup>**

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**Objective:** To study long term effects of surgery in localized laryngeal amyloidosis.

**Methods:** This retrospective study comprised all consecutive patients with localized laryngeal amyloidosis who visited our tertiary referral center between 1994 and 2012 and who were treated with surgery. Systemic amyloidosis was made unlikely by thorough clinical evaluation [1]. Recurrence rate, number of revision surgery procedures, progression to systemic amyloidosis and changes in voice were monitored yearly.

**Results:** Fifteen patients (6 women) were included. Median age was 49 years (21-72 years) and median follow-up was 5.5 years (1-16 years). Amyloid was found subglottic (3), glottic (4), in false vocal folds (8) and in other supraglottic areas (3). Combinations of localizations occurred in contiguous regions. One patient had a small plasma cell clone in the bone marrow without systemic amyloidosis. C02 laser was used in supraglottic and subglottic amyloid, assisted with microdebrider in three cases. Cold steel excision was used in three of four cases with glottic amyloid.

Nine patients needed one revision and four of them needed a second revision, because of progression with dysphonia (9), dyspnea (3), dysphagia (2), exclusion of malignancy (1), and aphonia (1). Time until first revision surgery was median 19 months (6 months – 138 months). One patient had been treated with radiotherapy elsewhere nine years before she was seen with progression of amyloid necessitating revision surgery. No patient developed systemic amyloidosis during follow-up.

**Discussion:** Although local progression urges revision surgery in the first four years postoperatively, progression seems to slow down after four years. Late progression, however, remains possible as shown in two patients, nine and eleven years after radiotherapy and surgery, respectively. Localized laryngeal amyloidosis does not progress to systemic amyloidosis.

#### References:

1. Bartels H, et al. Ann Otol Rhinol Laryngol 2004;113:741-8.

#### 10.29 - OP 53

#### First liver and kidney transplant for leukocyte chemotactic factor 2-amyloidosis presenting with acute liver failure

**Oren K. Fix<sup>1</sup>, Christopher E. Freise<sup>2</sup>, Nathan J. Shores<sup>3</sup>, Lloyd E. Damon, Brian K. Lee<sup>5</sup>, Daniel A. Brenner<sup>6</sup>, Dana P. McGlothlin<sup>6</sup>, Linda D. Ferrell<sup>7</sup>, Jean L. Olson<sup>7</sup>, Juris J. Liepnieks<sup>8</sup>, Merrill D. Benson<sup>8</sup>**

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**Background:** Leukocyte chemotactic factor 2 (LECT2)-amyloidosis is a recently described amyloidosis that primarily affects the kidneys and may be the third most common form of renal amyloidosis after AL and AA.

**Results/Case:** A 66-year-old Mexican American man with ulcerative colitis presented with jaundice and pruritus, bilirubin 26.3 mg/dL, ALT 505 U/L, INR 1.9 and creatinine 0.76 mg/dL. Liver biopsy showed parenchyma replaced by globular amyloid that stained negative for serum amyloid A (SAA) and transthyretin, and was nonspecific for kappa and lambda light chains. He developed confusion and rapidly worsening renal function with creatinine 2.4 mg/dL. Urine protein excretion was 357 mg/day. Serum and urine electrophoresis, bone marrow biopsy, echocardiogram, and cardiac catheterization were normal. Colon biopsies showed chronic active colitis without amyloid. Endomyocardial biopsy revealed focal areas of amyloid infiltration that stained negative for light chains, SAA and transthyretin. He underwent a simultaneous liver and kidney transplant. Intraoperative native kidney biopsy showed interstitial amyloid. Immunohistochemical staining of liver and kidney was positive for LECT2, and amino acid N-terminal sequencing of isolated liver amyloid gave a sequence of LECT2. There was insufficient cardiac amyloid for characterization. Genomic DNA analysis demonstrated valine homozygosity at amino acid 58.

**Discussion:** A majority of the reported LECT2-amyloidosis (ALECT2) patients have been Mexican American and all who have had DNA genotyping carry the homozygous valine polymorphism described in this case. Almost all reported cases presented primarily with renal involvement. This is the first reported case of ALECT2 with primary liver involvement resulting in acute liver failure, and the first case to undergo liver and kidney transplantation. This is the first case with presumed cardiac involvement.

**Conclusion:** Physicians must consider ALECT2 in patients with unusual presentations of an otherwise uncharacterized amyloidosis and, in the appropriate clinical situation, life-saving liver and/or kidney transplantation should be considered.

#### 10.41 - OP 54

#### The role of liver transplantation in the hereditary amyloidoses; the U.K experience

**Arie J Stangou, Peter Ashcroft, Paolo Muijsen**

NHS Amyloid Transplant Programme, Liver Unit, Queen Elizabeth Hospital, University Hospitals Birmingham, United Kingdom

We evaluated the role of liver transplantation in the hereditary amyloidoses and present here the UK experience of LT for fibrinogen A- $\alpha$  chain (AFib), apolipoprotein apoAI (AApoAI) and lysozyme (ALys) amyloidosis.

Between 1993-2011, fifteen patients with hereditary amyloid forms received LT between Queen Elizabeth Hospital and King's College Hospital in the UK. Ten patients (median age 58 years) with E526V AFib amyloidosis and renal failure had combined liver and kidney transplant (LKT). At median follow-up of 76 months (range 9-172), 7 patients are alive (cumulative survival 70%). Three fatal outcomes occurred in long-term renal replacement therapy (RRT) cases, and were due to vascular events or biliary complications. All but one recipients maintain normal dual grafts with no amyloid progression, while two patients transplanted pre-emptively before RRT retain stable native kidney function at 8 and 7 years post-LKT. Three patients aged 50, 52 and 56 received combined LKT for AApoAI amyloidosis with renal and liver failure. All maintain normal dual graft function with no amyloid progression at 36, 109 and 152 months post-LKT. Two siblings with ALys (Asp67His) associated hepatic amyloidosis which caused spontaneous liver rupture or haematoma received emergency LT at 15 and 24 years of age respectively. The first patient died 12 years post-LT with massive GI

haemorrhage related to mucosal amyloidosis, while the second is alive and well with normal graft function at 7 years.

**Conclusions:** 1. Combined LKT for end-stage renal failure in AFib is curative, but incurs significant risks. Preemptive isolated LT at early stages of amyloid nephropathy merits prospective evaluation. 2. The addition of LT to kidney transplant may be curative in AApoAI amyloidosis, however, the indication of LT in AApoAI is reserved for liver failure. 3. LT as an emergency life-saving treatment is justifiable in patients with ALys, and posttransplant life expectancy is acceptable.

**10.53 Perspectives - Skinner**

**11.05-11.30 Coffee break (GSK) in the 'Fonteinpatio'**

## **Plenary session 10**

**11.30-13.00 Therapy of ATTR amyloidosis ('Blauwe Zaal')**  
Chairpersons: Haagsma and Kuks

**11.30 State of the art - Ando**

**11.45 - OP 55**

**Familial Dynamics, Attachment and Psychopathology in FAP Patients**

**Lopes A** <sup>1,2</sup>, Rodrigues C<sup>1</sup>, Sousa A<sup>2</sup>, Cunha Z<sup>2</sup>, Teixeira L<sup>3</sup>, Coelho T<sup>1</sup>

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<sup>3</sup>Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal

**Background:** Studies on familial dynamics and attachment in Familial Amyloidotic Polyneuropathy (FAP) patients are few. There isn't much information about psychopathology in these patients.

**Objective:** Evaluation of attachment and psychopathology in FAP patients.

**Methods:** In 31 FAP patients in consultation and control group (n=30) were applied: a socio-demographic and a familial description questionnaire, EVA (Adult Attachment Scale) and BSI (Brief Symptoms Questionnaire).

**Results:** Most of participants were female (clinical group 64.5% and control 53.3%). Mean age was 42.4 y for clinical group and 36.8 y for control. Most of participants were married and had one child. 61.30% of patients were retired and 35.5% had lost one of parents at childhood/adolescence.

We found significant statistically differences between groups for two dimensions of EVA (Closeness and Confidance, p<0.001); FAP females presented higher Anxiety (p<0.001).

In BSI, with exception of paranoid ideation, we found in clinical group significant statistically differences for all dimensions (p<0.05). Females of this group had higher and significant statistically values for depression, anxiety and obsessive compulsive dimensions (p<0.05).

**Discussion/ Conclusion:** FAP is a genetic disease with symptomatic expression in adult age. This implies that these patients are a special group with particular types of familial characteristics, with difficulties in attachment that may be responsible for psychopathology patterns. Females represent a more problematic group.

**References:**

1. Canavarro, M. C. (1999). Inventário de sintomas psicopatológicos – BSI. In M. R. Simões, M. Gonçalves, L. S. Almeida (Eds.), Testes e Provas Psicológicas em Portugal (II vol.). Braga: APPORT/SHO.
2. Coelho T, Sousa A, Lourenço E, Ramalheira J. (1994). A study of 159 Portuguese patients with familial amyloidotic polyneuropathy (FAP) whose parents were both unaffected. Journal of Medicine Genetics, 31, 293-299.
3. Collins, N. L., & Read, S. J. (1990). Adult attachment, working models, and relationship quality in dating couples. Journal of Personality and Social Psychology, 58(4), 644-663.

### 11.57 - OP 56

#### Tolerability of diflunisal therapy in patients with transthyretin amyloidosis

**Whelan CJ**, Sattianayagam P, Dungu J, Pinney J, Gibbs S, Banypersad S, Venner C, Lachmann HJ, Wechalekar AD, Gillmore JD and Hawkins PN

*Centre for Amyloidosis and Acute Phase Proteins, University College London Medical School, UK*

**Background:** In the absence of any therapy of proven value, many patients with senile systemic amyloidosis (SSA) and familial amyloid polyneuropathy (FAP) attending the National Amyloidosis Centre have elected to be receive speculative treatment with diflunisal, a non-steroidal anti-inflammatory agent (NSAID) that has been shown *in vitro* to stabilise the transthyretin tetramer. Adverse effects of diflunisal include renal dysfunction, peripheral oedema and gastrointestinal complications, including peptic ulceration. This approach is not pursued in patients with contraindications to NSAIDs, including those receiving anticoagulants. A proton pump inhibitor is routinely co-prescribed with diflunisal. We report here the tolerability of diflunisal in patients with transthyretin amyloidosis.

**Methods:** Patients receiving treatment with diflunisal were identified using the National Amyloidosis Centre database, and tolerability and adverse events were ascertained. Duration of treatment was also determined.

**Results:** Concurrent warfarin therapy was the commonest reason for not prescribing diflunisal, followed by patients' preference not to receive a treatment of undetermined value. Forty two patients in total were identified as having received diflunisal, each at the dose of 250 mg twice daily. Four patients had ATTR V122I amyloidosis, of whom three discontinued diflunisal due to adverse events comprising fluid retention requiring hospital admission, stroke and abdominal pain in one case each. The median duration of treatment was 0.4 years (0.25- 4.33). Thirty two patients had FAP, of whom 18 stopped therapy due to GI symptoms in nine, including peptic ulceration in one, fluid retention in three, renal dysfunction in two, and progressive disease in four. The median duration of treatment was 1.2 years (0.1-5.6). Among six patients with SSA, three discontinued diflunisal, due to abdominal pain, fluid retention and renal dysfunction in one case each. The median duration of diflunisal treatment was 1.75 years (0.3-4.4).

**Conclusion:** Twenty four (57%) out of forty-two patients discontinued diflunisal due to adverse effects. The results of ongoing trials to determine the efficacy of diflunisal in ATTR amyloidosis are eagerly awaited.

### 12.09 - OP 57

#### Can development of post liver transplant cardiomyopathy be predicted from amyloid fibril composition?

Sandra Gustafsson<sup>1</sup>, Elisabet Ihse<sup>2</sup>, Michael Y Henein<sup>1</sup>, Per Westermark<sup>2</sup>, Per Lindqvist<sup>1</sup>, **Ole B Suhr<sup>1</sup>**  
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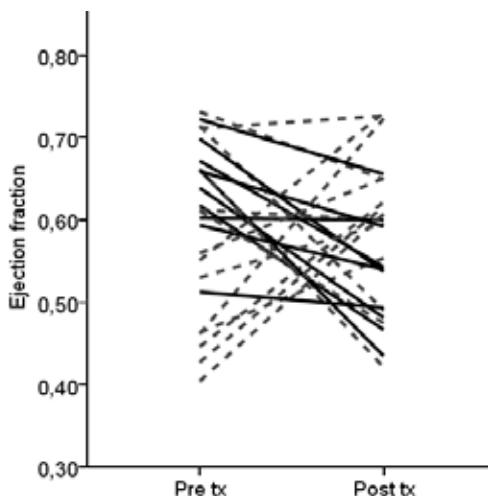
**Background:** Liver transplantation (LTx) is a favoured treatment for hereditary transthyretin (TTR) amyloidosis (ATTR). However, unforeseen heart complications, especially a rapid development of cardiomyopathy after LTx has compromised anticipated survival and clinical outcome. Recently, a relationship between ATTR-fibril composition and cardiomyopathy has been reported (1).

**Objective:** To investigate if development of cardiomyopathy and heart failure in LTx ATTR amyloid patients is related to amyloid fibril composition.

**Methods:** 24 patients with hereditary ATTR amyloidosis who had undergone liver transplantation and have had their amyloid fibril type tested were available for the study. They had been examined by echocardiography including tissue Doppler and speckle tracking echocardiography before and after LTx. Patients were divided into two groups according to fibril composition, 10 with type A fibrils (a mixture of truncated and full length TTR) and 14 with type B fibrils (isolated full length TTR fibrils only). There was no difference in time to the follow up echocardiography between the two groups.

**Results:** After LTx, type A fibrils patients developed symptoms of heart failure along with reduced systolic and diastolic ventricular function as shown by echocardiography (Fig), whereas no similar deterioration was noted in type B fibrils patients.

**Discussion and conclusion:** After LTx, patients with type A fibrils develop new manifestations or further deterioration of an already existing cardiomyopathy and heart failure in contrast to patients with type B fibrils, who are less likely to develop those complications, and where an improvement was noted for several individuals. These findings may have significant clinical implications in optimising ATTR amyloid patient selection for LTx.



*Straight line represents patients with type A fibrils, dotted lines type B fibrils. The decrease in ejection fraction was statistically significant only for patients with type A fibrils ( $P=0.005$ ) not in type B fibril*

**Reference:** Ihse E, Ybo A, Suhr O, Lindqvist P, Backman C, Westermark P. Amyloid fibril composition is related to the phenotype of hereditary transthyretin V30M amyloidosis. J Pathol 2008; 216 (2): 253.

## 12.21 - OP 58

**Long term Effect of Liver transplantation on FAP on the neuropathy: Risk factors for progression of the walking disability. The 18 years French experience: a monocentric study in 200 patients**

**Adams D**, Antonini T, Lozeron P, Mincheva Z, Theaudin M, Lacroix C, Ducot B, Algalarondo V, Dinanian S, Karam V, Blandin V, Misrahi, M, Azoulay D, Slama M, Castaing D, Samuel D  
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**Background:** Transthyretin (TTR)-FAPs are disabling, unremitting progressive sensory-motor polyneuropathy with autonomic dysfunction, lethal within 10 years. LT removes the main source of variant TTR.

**Objective:** To report the long term effect of LT on the neuropathy and to determine the independent prognostic factors for neuropathy progression.

**Methods:** Prospective and monocentric study with periodic clinical evaluation. Between 1993 and 2010, 200 patients with a symptomatic TTR-FAP underwent LT; 69% with met30TTR variant. The mean age at LT was 45.5 y. (23-69) with an early onset (< 50y.) in 62.5%. We used the Kaplan-Meier method to define median time to reach PND score 3 and the Cox proportional hazards regression model, to identify single and independent prognostic factors. PND scores: 1: only sensory disturbances; 2: impaired walking, able without stick or crutches; 3: walking aid: one stick or crutch; 4: 2 sticks or crutches; 5: wheelchair bound or bedridden.

**Results:** At time of LT, mean PND score: 1.59 (1-4); mean duration of the disease: 3.5 y.(1-15). 37% worsened after LT. 21.3% patients required walking aid (reach PND3 score) after LT. Independent risk factors to reach PND 3 score were: major weight loss (>20%) ( $p=0.0001$ ), PND2 score ( $p=0.0001$ ) and late onset ( $p=0.001$ ). 17/200 patients (8.5 %) reached PND5 stage after LT including 10 with PND 1 or 2 at LT time. The mean interval from LT to reach PND5 was 6.5 y. (1.4-17.2).

**Discussion:** LT is able to stabilize most of FAP patients. Worsening of the neuropathy after LT could be due to continued deposition of amyloid derived from wild-type TTR (Liepnieks et al, 2010).

**Conclusions:** LT stabilizes neuropathy in 63% of patients. Innovative drugs against variant and wild-type TTR should be recommended in FAP patients with late onset and preexisting walking disability.

**12.33 - OP 59**

**Treatment of Advanced Heart Failure in Cardiac Amyloidosis with Left Ventricular Assist Device Therapy**

**Paul L. Swiecicki<sup>1</sup>; Brooks Edwards<sup>2</sup>; Sudhir Kushwaha<sup>2</sup>; Angela Dispenzieri<sup>1</sup>; Soon Park<sup>3</sup>; Morie A. Gertz<sup>1</sup>**

<sup>1</sup>*Division of Hematology, Mayo Clinic, Rochester, MN, United States;* <sup>2</sup>*Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN, United States;* <sup>3</sup>*Division of Cardiovascular Surgery, Mayo Clinic, Rochester, MN, USA*

**Background:** Advanced cardiac involvement is an ominous manifestation of systemic amyloidosis for which there are no effective treatments outside of heart transplantation. Left ventricular assist devices (LVAD) are frequently used in heart failure secondary to dilated cardiomyopathy but no study has investigated their use in patients with cardiac amyloidosis.

**Objective:** We sought to analyze the long term outcomes in patients with cardiac amyloidosis treated with LVAD implantation.

**Methods:** Patients were selected via review of all patients who received continuous axial flow LVAD between December 2008 and October 2011 for either destination or bridge to transplant therapy.

**Results:** Seven patients with cardiac amyloidosis underwent LVAD implantation. Of these 5 had senile systemic amyloidosis, 1 had familial amyloidosis, and 1 had immunoglobulin light chain amyloidosis. All patients were NYHA Class IV and had a significantly decreased cardiac index (mean: 2.02 L/min/m<sup>2</sup> [1.66, 2.36]). All patients tolerated the LVAD procedure well, the most common post-operative complication being bleeding. Post-operatively, 1 patient developed right heart failure necessitating prolonged inotropic support. Mean ICU stay was 7 days and mean hospitalization was 24 days. None of the patients were re-hospitalized within 30 days. Only 1 patient has died since the procedure and all patients reported significantly improved NYHA Class (6 patients now Class III, 1 patient now Class II). One patient has undergone a heart and liver transplant.

**Discussion:** In patients with advanced heart failure secondary to cardiac amyloidosis, LVAD implantation may be an acceptable option for either destination or bridge to transplant therapy. This series shows a survival rate in patients with cardiac amyloidosis treated with LVAD implantation better than expected outcomes without intervention.

**Conclusion:** Firm conclusions cannot be drawn but these observations support a future prospective study into the use of LVAD implantation in patients with end stage cardiac amyloidosis.

Dr. Park has received funding from Thoratec in the past for education, research, and consulting. Otherwise, all other authors have no conflicts of interest.

**12.45              Perspectives - Zeldenrust**

**13.00-14.30      Lunch ('Fonteinpatio')**

**13.30-14.30      Poster viewing individually ('Blauwe Patio')**

**13.00-14.30      Business meeting of the ISA ('Lokaal 16')**

## Plenary session 11

**14.30-16.15 Chemotherapy in AL amyloidosis ('Blauwe Zaal')**  
Chairmen: Palladini and Vellenga

**14.30 State of the art - Dispenzieri**

### 14.45 - OP 60

**Evaluation of an early switch to second line chemotherapy in AL amyloidosis among patients who fail to achieve a very good partial response to frontline treatment**

**Ashutosh D Wechalekar**, Julian D Gillmore, Darren Foard, Lisa Rannigan, Thirusha Lane, Jennifer H Pinney, Simon DJ Gibbs, Christopher P Venner, Sanjay Banypersad, Carol J Whelan, Helen J Lachmann and Philip N Hawkins

*Centre for Amyloidosis and Acute Phase Proteins, University College London Medical School, UK*

**Background:** Studies in AL amyloidosis suggest that a very good partial response or better (VGPR) is associated with most favourable outcomes but is achieved in only ~30-40% of cases after frontline treatment. Uncertainty remains about the timing, choice and impact of second line chemotherapy for patients who do not achieve VGPR.

**Methods and Results:** We report outcomes of 91 patients assessed prospectively in the ALchemy study who did not achieve a VGPR after three cycles of chemotherapy, and according to protocol were assessed for an early change to a second line regimen. The disease was Mayo stage 1, 2 and 3 in 18%, 49% and 33% respectively, and 64 patients. At 3 cycles, 14 (15%) patients continued the frontline regime, and treatment was discontinued in 8 cases. 69 patients (75%) switched to a second line regimen comprising: bortezomib combinations in 58 (84%); CTdA or lenalidomide in 3 cases each; and melphalan or bendamustine combination in 2 cases each. Thirty six (52%) patients achieved an improvement in clonal response – 23 (33%) improved to a VGPR, and 13 (33%) from NR to PR. Of the remaining patients in PR, 20 (60%) achieved a further 18% median decrease in the dFLC to achieve a 77% median reduction over baseline. Six patients (7%) died during second line treatment and 2 (3%) discontinued due to toxicity. At 18 months, patients who achieved a VGPR, PR and NR after second line treatment had estimated survival of 90%, 75% and 61%, respectively, compared to 98% for patients achieving a VGPR with only frontline treatment (log rank p=0.53 when compared to VGPR after 2<sup>nd</sup> line).

**Conclusions:** An early switch to second line treatment improved clonal responses in half of patients not achieving VGPR upfront, including a 25% improvement in VGPR rate with associated improvement in outcomes.

### 14.57 - OP 61

**A phase I / II study of Lenalidomide with low dose oral cyclophosphamide and low dose dexamethasone (RdC) in AL amyloidosis**

**Efstathios Kastritis**, Maria Roussou , Evangelos Terpos, Maria Gavriatopoulou, Constantinos Pamboukas, Ioannis Boletis, Smaragda Marinaki, Theofanis Apostolou, Nikitas Nikitas, Georgios Gkortzolidis , Eurydiki Michalis, Sossana Delimpasi, Meletios A. Dimopoulos  
*Department of Clinical Therapeutics, University of Athens School of Medicine, Athens, Greece*

**Background:** Lenalidomide has significant activity in myeloma and may be active in patients with AL amyloidosis but is associated with significant toxicity at standard doses.

**Objective:** we explored the feasibility and the activity of an oral regimen of lenalidomide with low dose cyclophosphamide and low dose dexamethasone (RdC) in patients with AL amyloidosis

**Methods:** This phase I/II study included pretreated or previously untreated patients with AL amyloidosis. Patients received dexamethasone 20 mg (days 1-4), cyclophosphamide(days 1-10) and lenalidomide (days 1-21) every 28 days for up to 12 cycles in prespecified dose cohorts using a standard 3+3 design. Consensus criteria were used for the definition of organ involvement and assessment of hematologic and organ response.

**Results:** Thirteen patients were treated in phase I and 24 in phase II; 65% were previously untreated, most had renal and/or cardiac involvement and elevated cardiobiomarkers. Lenalidomide 15 mg/day

and cyclophosphamide 100 mg/day were further evaluated in the phase II. On intent to treat (ITT), 20(55%) patients achieved a hematologic response, including 3(8%) with CR. Hematologic responses were seen within all dose levels, and in 4 of 5 patients who had previously received bortezomib. An organ response was recorded in 28% of patients on ITT and in 40% of patients that survived  $\geq 6$  months. An increase in NTproBNP in discordance with FLC levels was observed in 69.5% of evaluable patients. These increases were more frequent in patients with cardiac involvement and were not associated with fluctuations of renal function. The median time to progression is 10 months and 2-year survival is 41%. Fatigue, non-neutropenic infections, and rash were the most common toxicities.

**Conclusion:** Oral RdC has activity in AL amyloidosis and may be an additional treatment option especially for patients with preserved organ function or for patients who cannot receive or who relapse after bortezomib. (clinicaltrials.govNCT00981708)

Celgene provided lenalidomide. M.A. Dimopoulos has received honoraria for Celgene and Orthobiotech.

### 15.09 - OP 62

#### Efficacy of Bortezomib/Cyclophosphamide/Dexamethasone (VCD) Chemotherapy In Naive Patients with High Risk Cardiac Light Chain Amyloidosis (Mayo Clinic stage III)

**Jaccard A<sup>1</sup>, Comenzo RL<sup>2</sup>, Wechalekar AD<sup>3</sup>, Hawkins PN<sup>3</sup>, Roussel M<sup>4</sup>, Morel P<sup>5</sup>, Macro M<sup>6</sup>, Longy-Boursier M<sup>7</sup>, Decaux O<sup>8</sup>, Bridoux F<sup>9</sup>, Venner CP<sup>3</sup>**

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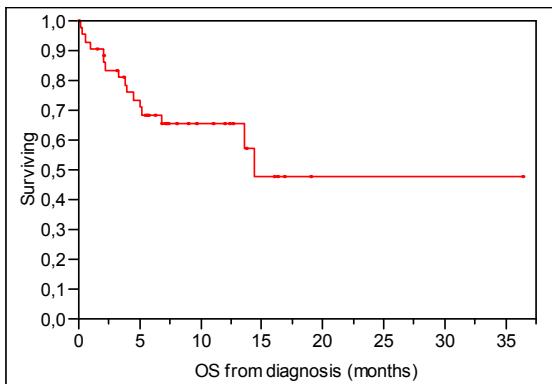
<sup>6</sup>Hematology Unit, CHU Caen, France; <sup>7</sup>Internal Medecine Unit, CHU Bordeaux, France; <sup>8</sup>Internal Medecine Unit, CHU Rennes, France; <sup>9</sup>Nephrology Unit, CHU Poitiers, France

**Background:** High-dose chemotherapy and M-Dex are not able to overcome the poor prognosis of patients with severe cardiac involvement in AL amyloidosis. Bortezomib is known to induce very rapid response in this disease. We used this drug combined with cyclophosphamide and dexamethasone (VCD regimen) for patients with high-risk cardiac amyloidosis as defined by Mayo Clinic stage III in London and Boston centres and among the French network for amyloidosis.

**Methods and Results:** We retrospectively evaluated 44 patients, 15 women and 29 men, with a median age of 64(44-83) classified as Mayo Clinic stage III cardiac amyloidosis. The median number of involved organ was 2 (1-5). Bortezomib (1.3 mg/m<sup>2</sup>) was usually administered once weekly on days 1, 8, 15 and 22 in a 5 week schedule with oral Dexamethasone (10 or 20 mg, 4 to 8 doses) and cyclophosphamide (300 mg/m<sup>2</sup> (max 500 mg) on days 1, 8 and 15. Median follow up of the entire cohort is 6.5 months (0-36), 16 patients died with a median time between first treatment and death of 3.5 months (0.2-14), estimated Kaplan-Meyer survival at 1 year is 65%. Based on free light chain (FLC) measurement in 34 patients after 1 to 6 cycles (median 3) hematological response was obtained in 30 (88% of evaluated patients and 68% of the whole cohort), 12 CRs, 10 PRs and 8 VGPR (dFLC below 40 mg). At diagnosis and evaluation respectively, the median dFLC of these 34 patients was 286 (51-6988) and 23 (0.6-1037), median NT-proBNP 4362 ng/l (500-22816) and 2118 (551-30000), median BNP 717 ng/l (277-12000) and 355 (65-4023).

Toxicity was acceptable with little haematological toxicity and 7 neuropathies (2 leading to treatment discontinuation).

Survival curve



**Conclusion:** These results compare favourably with results published in Mayo stage III patients and justify a prospective trial.

AJ receives honoraria and research funding from Janssen. RLC is a scientific advisor and also consults for Millenium Pharmaceuticals; MR receives honoraria from Janssen.

### **15.21 - OP 63**

#### **Salvage therapy with bendamustine and prednisone (BeP) in AL amyloidosis: a pilot study**

**Paolo Milani**, Giovanni Palladini, Andrea Foli, Laura Obici, Francesca Lavatelli, Mario Nuvolone, Stefano Perlini, Giampaolo Merlini

*Amyloidosis Research and Treatment Center – “Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo” and Department of Molecular Medicine – University of Pavia, Pavia, Italy*

**Background:** Several reports indicate that bendamustine is active in multiple myeloma and Waldenström macroglobulinemia.

**Methods:** We evaluated the safety and efficacy of 28-day cycles of bendamustine (100 mg/m<sup>2</sup> on days 1 and 2) and prednisone (100 mg on days 1-4) in 11 patients with relapsed/refractory AL amyloidosis, treated between March and September 2011. Response was evaluated every 2 courses. Bendamustine dosage was reduced to 60 mg/m<sup>2</sup> in 5 patients (45%) who had baseline cytopenia.

**Results:** Five patients (45%) were males and median age was 65 years (range 47-77 years). Involved organs were heart (5, 45%), kidney (4, 36%), soft tissues (3, 27%), liver (2, 18%) and lung (1, 9%), with 5 patients (45%) having >1 organ involved. Performance status (ECOG) was ≥2 in 8 cases (73%) and NYHA class was ≥III in 2 (18%). Cardiac stage was I in 5 patients (45%) and II in 6 (55%), and eGFR was ≥60 mL/min in 6 patients (55%) and 30-59 mL/min in 5 (45%). Two patients (18%) had IgM clones. Median bone marrow infiltrate was 15% (range 5-30%). Median dFLC was 140 mg/L (range 91-838 mg/L). The median number of previous therapies was 3 (range 1-6). All patients had been exposed to alkylators (ASCT in 3 cases), 10 (91%) to bortezomib, 8 (73%) to lenalidomide, 5 (45%) to thalidomide and the 2 IgM patients received prior rituximab. Four patients (36%) experienced severe adverse events, neutropenia in 3 cases and thrombocytopenia in 1. Six patients (55%) responded, with 1 VGPR and 5 PRs. Both the IgM patients responded. After a median follow-up of 8 months, 1 patient died due to progressive liver amyloidosis despite PR.

**Conclusion:** Treatment with BeP was associated with a promising response rate in heavily pretreated patients with AL amyloidosis, deserving further investigation.

G.P.: honoraria from Janssen-Cilag and Celgene.

### **15.33 - OP 64**

#### **The Activity of Pomalidomide in Patients with Immunoglobulin Light Chain Amyloidosis**

**A. Dispenzieri**, F. Buadi, K. Lauermann, B. LaPlant, S.R. Hayman, S.K. Kumar, S.R. Zeldenrust, N. Leung, J. Mikhael, R. Hall, C. Reeder, R. Fonseca, L. Bergsagel, A.K. Stewart, V. Roy, T.E. Witzig, J.A. Lust, S.V. Rajkumar, S.J. Russell, D. Dingi, M.A. Gertz, M.Q. Lacy

*Mayo Clinic, Rochester, USA*

Immunoglobulin light chain (AL) amyloidosis is a rare, incurable plasma cell disorder, whose drug armamentarium has been growing as it grows for multiple myeloma. Pomalidomide in combination with weekly dexamethasone (Pom/dex) has been shown to be active among patients with relapsed multiple myeloma. We set out to study the Pom/dex combination in patients with previously treated AL. Patients were eligible for this prospective phase 2 trial (registered at <http://ClinicalTrials.gov> as NCT00558896) if they had had at least one prior regimen and if they had reasonably preserved organ function. Patients were treated with pomalidomide 2 mg by mouth for 28 days (1 cycle) along with dexamethasone 40 mg by mouth weekly. Aspirin 325 mg daily was used as routine thromboprophylaxis. Between November 2008 and November 2010, 33 patients were enrolled. The data were frozen on January 3, 2012. The median age was 66 years. Median time from diagnosis to on-study was 37 months. Eighty-two percent had cardiac involvement. Forty-eight percent had prior high-dose chemotherapy with peripheral blood stem cell transplantation. The confirmed hematologic

response rate was 48%, with a median time to response of 1.9 months. Organ improvement was documented in 5 patients. With a median follow-up of surviving patients of 28 months (range 14 to 38), 17 patients have died, six of whom died on study. The median overall survival was 28 months with a 1-year OS rate of 76%. The median progression free survival was 14 months with a 1 year PFS of 59%. The most common grade 3-5 AEs, regardless of attribution neutropenia and fatigue. The study design goals were met for this study, again illustrating the remarkable activity of the Pom/dex combination. The results of this study indicate that pomalidomide will be a significant drug, covering an unmet clinical need in patients with previously treated AL.

A.D. receives honoraria and research funding from Celgene, and honoraria from Binding Site. M.Q.L. receives research funding from Celgene. M.A.G. receives honoraria from Celgene and Millennium, and is a member of an entity's Board of Directors or advisory committees for Millennium. S.K. is a consultant for, and receives research funding from, Celgene, Merck, and Genzyme; and receives research funding from Millennium, Novartis, and Cephalon. R.F. is a consultant for Genzyme, Medtronic, BMS, AMGEN, Otsuka, and Intellikine; receives research funding from Cylene; is a consultant for, and receives research funding from, Celgene; and receives research funding from, prognosticated on FISH probes in myeloma for, and receives patents and royalties from Onyx. P.L.B. is a consultant for Celgene, Centocor, Genentech, Amgen, and Novartis. A.K.S. receives honoraria from Celgene. The remaining authors declare no competing financial interests.

#### **15.45 - OP 65**

#### **Risk of second primary malignancies in patients with AL amyloidosis treated with lenalidomide**

**Vaishali Sanchorawala**, Anthony Shelton, Gheorghe Doros, David C. Seldin

*Amyloid Treatment and Research Program, Boston University School of Medicine, Boston, MA, USA*

An increase in second primary malignancies (SPM) has been reported in patients with myeloma treated with lenalidomide, particularly in association with melphalan. To determine the rate of second primary malignancies in AL amyloidosis patients treated with lenalidomide, we reviewed data on 82 patients treated on clinical trials at Boston Medical Center from 2004-2011. Data from lenalidomide and dexamethasone treatment in AL amyloidosis (ClinicalTrials.gov: NCT00091260) were analyzed in a post-hoc fashion for the incidence rate for SPM. The median age of these 82 patients was 62 years (range, 40-82); and 52 (63%) were male. There were 62 patients (76%) with lambda clonal plasma cell dyscrasia, and 43 (52%) had cardiac involvement by the consensus criteria from the Xth International Amyloidosis Symposium. Median duration for lenalidomide treatment was 9 cycles (range, 1-69); and 16 (20%) received lenalidomide for > 24 months. Of the 82 patients, 78 (95%) patients had prior therapies and 77 (94%) had prior alkylating agents based treatment. The median follow-up of the 82 patients was 28 months (range, 3-92). One patient, a smoker, treated with lenalidomide for 24 months, developed metastatic lung cancer. Additionally, there were 5 non-melanoma skin cancers. There was no case of hematologic malignancy or B-cell lymphoproliferative disorder in this group. This corresponds to an incidence rate of 0.44 per 100 patient-years. The annual incidence of invasive cancers is 2.1 per 100 patient-years for the general population in the US. In conclusion, lenalidomide based treatment in AL amyloidosis did not appear to increase the incidence rate of SPM compared to incidence rate for the invasive cancers in general population. Longer follow-up may be needed to better define the risk.

This study was supported by Celgene Corporation

#### **16.00 Perspectives - Schönland**

**16.15-16.30 ISA information: AMYLOID Journal – P. Westermark**

**16.30-17.00 Tea break (GSK) in the 'Fonteinpatio'**

**16.30-17.15 Poster viewing (PC 1-66) in 5 groups ('Blauwe Patio')**

**17.15-17.45 Selected Poster presentations ('Blauwe Zaal')**

|                    |   |
|--------------------|---|
|                    | Chairpersons: Minnema and Kuks  |
| 17.15              | PC 08 Quality of life as an outcome measure in AL amyloidosis following chemotherapy – Lane   |
| 17.21              | PC 28 25 Years of Amyloidosis in the UK– A Single Centre Experience of 5100 Patients – Wechalekar   |
| 17.27              | PC 45 Improved Hematologic Responses Following Risk Adapted Stem Cell Transplant (SCT) and Bortezomib Consolidation in Systemic Light-Chain Amyloidosis (AL) is Associated with Long Term Organ Improvement – Landau  |
| 17.33              | PC 50 Melphalan and dexamethasone (MDex) vs. bortezomib, melphalan and dexamethasone (BMDex) in AL amyloidosis: a matched case control study – Palladini  |
| 17.39              | PC 61 Autologous Stem Cell Transplant is an effective therapy for carefully selected patients with AL Amyloidosis: Experience of a single Institution – Jimenez-Zepeda  |
| 17.45-18.00        | Walk to the Groninger Museum  |
| <b>18.00-20.00</b> | <b>Visit Groninger Museum</b>   |
| 20.00-20.30        | Walk to the Der Aa Church   |
| <b>20.30-23.30</b> | <b>Congress Dinner and awards presentation ('Der Aa Church')</b><br>Award Committees:<br>Monday: Limburg (coordinator), Gruys, Fändrich, Luiten, Bellotti, Saraiva, Kluge<br>Tuesday: Croockewit (coordinator), Van Rijswijk, Wall, Gertz, Merlini, Comenzo, Hawkins<br>Wednesday: Minnema (coordinator), Kuks, Ando, Sanchorawala, Van Gameren, Dispenzieri, Obici |

## Thursday, May 10

7.30-8.00      Coffee ('Fonteinpatio')

## Plenary session 12

8.00-10.30      Design of targeted molecules and innovative drugs ('Blauwe Zaal')  
Chairmen: Ando and Hazenberg

8.00      State of the art - Seldin

8.15 - OP 66

**Intrinsic Apoptosis Can Occur Promptly After Silencing Lambda Light Chain Genes in Human Clonal Plasma Cells**

**Ping Zhou**, Xun Ma, Raymond Comenzo

*Division of Hematology-Oncology, Department of Medicine, Tufts Medical Center, Boston, MA, USA*

Treatment of systemic AL amyloidosis (AL) depends upon reduction of the pathologic light chain (LC) produced by clonal plasma cells in the bone marrow. Recently investigators have transfected murine Sp2.0 plasma cells with human AL Vκ1 LC genes and demonstrated that LC expression can be reduced with siRNAs targeting LC mRNA (Gene Therapy 2011;18:1150). We studied the effects of immunoglobulin gene silencing on human plasma cell behavior and sensitivity to bortezomib, using 3 human myeloma cell lines (ALMC1, ALMC2, EJM) that produce intact IgG lambda M-proteins. ALMC1 and ALMC2 cells were derived from an AL patient and produce Vλ6 LC that form amyloid (Blood 2008;112:1931), and EJM cells from a myelomatous effusion and produce Vλ1 LC. We silenced expression of the Ig light and heavy chains genes with optimized siRNA (Dharmacon) and streptolysin-O transfection and documented marked reductions in targeted proteins by flow cytometry and immunoblot. Silencing expression of heavy chain (si[lgGHC]) or intact IgGλ (si[lgGλ]) did not cause apoptosis, or alter viability or proliferation compared with scrambled (si[-]) control, by AnnexinV/PI staining, MTT and caspase3/7 bioluminescent assays. However, silencing the expression of the Vλ LC (si[VλLC]) caused significant apoptosis in all cell lines within 24 hours compared to controls. With si[lgGHC], apoptosis or reductions in viability or proliferation did not occur in ALMC1 or ALMC2 cells indicating that the free Vλ6 LC were not toxic to cells; by immunoblotting the levels of polyubiquitinated, lysine48- (K48-) and K63-ubiquitinated proteins were not altered and by bioluminescent activity assays proteasome activity was not changed, indicating that the ubiquitin-proteasome system was not perturbed by the increase in unmated Vλ6 light chains. Sensitivity to increasing doses of bortezomib was enhanced in all si[VλLC] cells compared to controls. These results provide the rationale for a novel approach to AL and other human plasma cell disorders.

8.27 - OP 67

**Marked Removal and Clearance of AA Amyloid Deposits in Target Organs (Kidney, Liver and Spleen) by the Small Molecule Systebryl™ Following Oral Administration: A Potential Breakthrough Drug for the Treatment of Systemic AA Amyloidosis**

**Alan D. Snow**, Thomas Lake, Luke Esposito, Kelsey Hanson, Qubai Hu, Judy Cam, Marisa-Claire Yadon, and Joel Cummings  
*ProteoTech Inc., Kirkland, WA, USA*

ProteoTech has developed a unique small molecule library that specifically targets different amyloid proteins. These include small molecules that specifically target the beta-amyloid protein of Alzheimer's disease (currently in clinical trials), the alpha-synuclein protein of Parkinson's disease (in late pre-clinical development and partnered with GlaxoSmithKline), and the IAPP amyloid of type 2 diabetes. ProteoTech has also developed a small molecule known as Systebryl™ that is a potent inhibitor of AA amyloid deposition and causes an impressive removal of pre-existing AA amyloid deposits. The

objective of this presentation is to review data that demonstrates the efficacy of Systebryl™ in different animal studies. In one study, 8-week CBA/J mice were first pre-treated orally with Systebryl™ for 14 days (100mg/kg/day in 20% PEG/PBS). AA amyloid was then induced with AEF and 2% silver nitrate. Animals were then treated with Systebryl™ for an additional 4 weeks. Using Thioflavin S fluorescence, Congo red staining, and AA amyloid antibody immunostaining (and image analysis and quantitation), Systebryl™ significantly ( $p<0.01$ ) prevented splenic AA amyloid deposition by ~54.5%, and hepatic AA amyloid deposition by >75%. In another study, AA amyloid deposition was first induced for 2-weeks in 8-week CBA/J mice with AEF + silver nitrate administration. The animals were then treated orally with either vehicle or Systebryl™ (in a SMEEDs oil-surfactant formulation) at 25mg/kg/day for 60 days. Systebryl™ treatment caused a marked ( $p<0.01$ ) 77-87% reduction of kidney AA amyloid deposits, a marked ( $p<0.01$ ) 73-79% reduction in liver AA amyloid, and a marked ( $p<0.01$ ) 84% reduction of splenic AA amyloid (demonstrated by Congo red fluorescence and AA amyloid immunostaining image analysis and quantitation). These studies demonstrate that Systebryl™ (currently in Phase 1 human clinical trials) may represent a future disease-modifying oral drug postulated to prevent deposition and cause the removal of AA amyloid deposits in patients with Systemic AA Amyloidosis.

Authors of this abstract are shareholders (either stock or stock options) in ProteoTech Inc.

### 8.39 - OP 68

#### A clinical Phase 3 confirmatory trial of eprodisate in the treatment of AA amyloidosis patients

**D. Garceau<sup>1</sup>**, H. Lachmann<sup>2</sup>, T. Sablinski<sup>3</sup>, L. Dember<sup>4</sup>

<sup>1</sup>Bellus Health, Laval, Canada; <sup>2</sup>Royal Free and University College Medical School, London, United Kingdom; <sup>3</sup>Celtic Therapeutic, New York, USA; <sup>4</sup>University of Pennsylvania, Philadelphia, USA

**Background:** AA amyloidosis occurs in chronic inflammatory conditions as a result of abnormal deposition of amyloid A protein tissues, predominantly the kidneys, leading to progressive organ dysfunction. Eprodisate (NC-503, Kiacta™) belongs to a new class of anti-amyloid compounds that inhibit interactions between glycosaminoglycans and amyloid protein thereby preventing amyloid fibril formation and deposition. In a first clinical Phase 2/3 study conducted in 183 AA patients, eprodisate administered for 2 years reduced the risk of renal function deterioration and death as compared to placebo (Cox Proportional Hazards Regression analysis: HR = 0.58; 95% CI (0.37, 0.93);  $p=0.025$ ). Eprodisate appeared to be safe and well tolerated in AA patients.

**Objectives and Methods:** To confirm the safety and efficacy of eprodisate for the treatment of AA amyloidosis, a second multicenter, international, randomized, placebo-controlled clinical Phase 3 trial has recently been initiated. A total of 230 patients with a biopsy confirmed diagnosis of AA amyloidosis and kidney involvement will be enrolled at 76 sites in 27 countries. The primary efficacy endpoint is a composite assessment of renal function deterioration defined as any of the following: persistent  $\geq 40\%$  decrease in CrCl, persistent  $\geq 80\%$  increase in SCr or progression to chronic ESRD/dialysis. This event-driven trial will end when a total of 120 primary efficacy events have occurred.

**Results and Conclusion:** Comparison of study design and statistical approaches will be made between the two eprodisate clinical Phase 3 studies and the rationale for differences will be discussed. An update on study progress of the confirmatory trial will be presented and a preliminary profile of the study population (i.e. demographics and baseline renal and inflammatory characteristics) will be compared to that of the first clinical Phase 2/3 trial.

Denis Garceau is an employee of Bellus Health and Consultant for Celtic Therapeutic, Sponsors of eprodisate trials; T. Sablinski is an employee of Celtic Therapeutic; Drs, Helen Lachmann and Laura Dember are Scientific Advisors and Principal Investigators for the eprodisate trials.

### 8.51 - OP 69

#### The Diflunisal Trial: Demographics, baseline neurologic staging, and adverse events

**Berk JL<sup>1</sup>**, Obici L<sup>2</sup>, Zeldenrust SR<sup>3</sup>, Sekijima Y<sup>4</sup>, Yamashita T<sup>5</sup>, Heneghan M<sup>6</sup>, Ikeda S-I<sup>4</sup>, Ando Y<sup>5</sup>, Gorevic P<sup>7</sup>, Merlini G<sup>2</sup>, Kelly JW<sup>8</sup>, Skinner M<sup>1</sup>, Bisbee AB<sup>1</sup>, Dyck PJ<sup>6</sup>, Suhr OB<sup>9</sup> and the Familial Amyloidosis Consortium

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**Background:** Familial transthyretin amyloidosis (ATTR) is a lethal autosomal dominant disorder that disrupts peripheral and autonomic nervous system functions. Amyloid results from misfolded transthyretin (TTR), a transport protein produced by the liver. Although liver transplantation effectively treats patients with certain ATTR mutations, not all patients are eligible and some progress post-operatively. Alternative treatments are needed. *In vitro*, thyroxine mimetics including diflunisal bind tetrameric TTR, prevent monomer dissociation, and suppress amyloid fibril formation.

**Methods:** To examine the effect of diflunisal on neurologic progression in ATTR, we designed a randomized, placebo-controlled, double blind, multicenter international study employing the diabetic polyneuropathy metric, Neurologic Impairment Score + 7 attributes (NIS+7®), as the primary endpoint. Entry criteria include all ATTR genotypes, biopsy-proven amyloid deposits, and peripheral or autonomic neuropathy. Alternate neuropathies, non-study NSAID use, severe heart or kidney dysfunction, or prior liver transplant were excluded. Study evaluations occurred at entry, 6, 12, and 24 months.

**Results:** We accrued 130 subjects. Enrollment by study site includes Boston 56, Umea 24, Pavia 21, Rochester 10, London 7, Shinshu 6, Kumamoto 3, New York 3. The cohort has 55% V30M and 45% non-V30M ATTR subjects; 67% men and 33% women, median age 63 years (range 24-76). Neurologic staging at baseline included 57% Stage I, 28% Stage II, and 15% Stage III disease. Study drug-related adverse events included GI (12%), renal (6%), and congestive heart failure (CHF) (3%). CHF (2 subjects) and GI bleeding/dyspepsia (3 subjects) resulted in study drug discontinuation. Six disease-related deaths have occurred, all off study drug.

**Conclusions:** The study cohort has wide representation of TTR mutations and age. Diflunisal continues to be well tolerated by ATTR subjects. Data collection concludes December 2012 with final analysis available in early 2013.

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### 9.03 - OP 70

#### Curcumin as a novel natural compound acting as TTR amyloidosis inhibitor *in vivo*

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**Background:** Familial Amyloidotic Polyneuropathy (FAP) is fatal hereditary amyloidosis characterized by the systemic extracellular deposition of transthyretin (TTR) amyloid fibrils throughout the connective tissue, affecting predominantly the peripheral nervous system (PNS), autonomic and motor. Recently we reported that curcumin (diferuloylmethane), a yellow pigment present in turmeric, strongly suppresses TTR amyloid fibril formation *in vitro*, either by stabilization of TTR tetramer or by generating small “off-pathway” intermediates that are innocuous to cultured neuronal cells (Ferreira et al., 2011).

**Objective:** In the present study, we aim to assess the effect of curcumin on TTR amyloidogenesis *in vivo*, using a well characterized mouse model for FAP.

**Methods and Results:** Mice were given 2% (w/w) dietary curcumin or control diet for a six weeks period. After treatment the interaction of curcumin with TTR *in vivo* was confirmed by analysis of plasma, from treated and control mice, incubated with [<sup>125</sup>I]T<sub>4</sub> and protein separation by polyacrylamide gel electrophoresis. The results showed less [<sup>125</sup>I]T<sub>4</sub> binding to plasma TTR from treated mice, indicating selective binding of curcumin to TTR. The effect on plasma TTR stability was determined by isoelectric focusing (IEF) analysis under semi-denaturing conditions. Curcumin considerably increased plasma TTR stability. Most important, immunohistochemistry (IHC) analysis of mice tissues after treatment demonstrated that curcumin significantly reduced TTR deposition and associated biomarkers, namely endoplasmic reticulum (ER)-stress and protein oxidation markers.

**Discussion and Conclusion:** Our results indicate that curcumin directly modulates TTR fibrillogenesis although we do not disregard that both its potent anti-oxidant and anti-inflammatory activities might synergistically potentiate curcumin anti-amyloidogenic effect *in vivo*. In conclusion, the present study adds to the number of natural potent TTR amyloid inhibitors and points towards the potential use of curcumin as a lead molecule for the design of new TTR amyloid inhibitors.

**References:** Ferreira N, Saraiva MJ, Almeida MR. FEBS Lett. 2011, 585(15):2424-30.

### 9.15 - OP 71

#### Safety and efficacy of doxycycline plus taurooursodeoxycholic acid in transthyretin amyloidosis

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**Background:** Treatment with doxycycline and taurooursodeoxycholic acid (TUDCA) has a synergistic effect on removal of TTR deposits in the mouse model of FAP. The safety and tolerability profiles of doxycycline and TUDCA are well established and favourable. The possible impact of this treatment on ATTR progression warrants evaluation in a clinical trial.

**Methods:** We designed a phase II, open-label study to evaluate the efficacy, safety and pharmacokinetics of orally doxycycline and TUDCA administered for 12 months. Primary endpoint is response rate defined as: < 2 point increase in NIS-LL and less than 10% decrease in mBMI in patients with neuropathy and a mBMI reduction of less than 10% and an increase in serum NT-proBNP concentration of less than 30% or < 300 pg/mL in subjects with isolated cardiomyopathy.

Entry criteria include symptomatic disease due to hereditary or senile ATTR. Patients with other neuropathies and severe heart, liver or kidney dysfunction are excluded. Evaluations are scheduled at entry, 6 and 12 months.

**Results:** We enrolled 22 subjects (15 males, median age 68 years). 17 patients have hereditary ATTR, 4 patients SSA, one was domino-transplanted. Median follow-up is six months. 2 patients discontinued the treatment within a month due to poor doxycycline tolerance. Treatment was well tolerated in the others, except for mild skin redness. 7 patients completed 12-month treatment, with a stable cardiac disease in 4/7 and a stable neurologic disease in 5/6. In the 2/3 pts in whom NT-proBNP increased, echocardiography remained stable. One patient discontinued because of neurological progression at 6 months. All the other patients remain in study with substantially stable disease.

**Conclusions:** Preliminary data indicate that the association of doxycycline and TUDCA is feasible in patients with ATTR with an acceptable toxicity profile. As the follow-up matures, more evidence of the clinical efficacy will be available.

### 9.27 - OP 72

#### Antibody therapy against amyloid forms of transthyretin for familial amyloidotic polyneuropathy

**Yu Su<sup>1</sup>, Hirofumi Jono<sup>1</sup>, Masaharu Torikai<sup>2</sup>, Akihiko Hosoi<sup>2</sup>, Kenji Soejima<sup>2</sup>, Jianying Guo<sup>1</sup>, Masayoshi Tasaki<sup>1</sup>, Yohei Misumi<sup>3</sup>, Mitsuharu Ueda<sup>1</sup>, Satoru Shinriki<sup>1</sup>, Makoto Shono<sup>1</sup>, Konen Obayashi<sup>1</sup>, Toshihiro Nakashima<sup>2</sup>, Keishin Sugawara<sup>2</sup>, and Yukio Ando<sup>1</sup>**

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**Introduction:** Familial amyloidotic polyneuropathy (FAP) induced by amyloidogenic transthyretin (ATTR) is characterized by systemic accumulation of amyloid fibrils. Although liver transplantation has become a well-established therapy, this therapy has several problems, and no other essential

therapies have been practically established. In the mechanism of TTR amyloid formation, it is believed that altered conformations exposing cryptic regions in TTR are intermediary steps. Our studies showed that the polyclonal TTR antibody, which specifically reacts with surface epitopes only exposed in amyloid forms of TTR, significantly inhibited TTR deposition in the transgenic rats possessing a human ATTR V30M gene. In this study, to establish the antibody therapy for FAP, we generated a monoclonal TTR antibody, which specifically reacts with surface epitopes of TTR (MAb ATTR) and evaluated its binding affinity and specificity for TTR amyloid fibrils.

**Methods:** Serum samples were obtained from healthy volunteers and FAP V30M patients. Autopsied frozen heart, kidney and thyroid gland specimens from FAP ATTR V30M patients were subjected to perform immunohistochemical staining. Amyloid fibrils were extracted from those autopsied frozen specimens. To evaluate the binding affinity and specificity of MAb ATTR, Western blotting and ELISA assay were performed.

**Results:** MAb ATTR showed specific binding affinity for TTR amyloid fibrils, while no affinity for native form of TTR was observed. Amyloid fibrils extracted from tissue specimens of FAP patients were specifically recognized by MAb ATTR. Furthermore, by immunohistochemical staining, MAb ATTR indeed showed the high consistency with Congo red positive areas in heart, kidney and thyroid gland specimens from FAP patients. Collectively, these data indicate that MAb ATTR showed binding affinity and specificity for TTR amyloid fibrils *in vitro* and *in vivo*.

**Conclusion:** MAb ATTR may have a potential to suppress TTR amyloid formation and become a candidate for the antibody therapy for FAP.

### 9.39 - OP 73

#### Clinical Development of an Antisense Therapy for the Treatment of Hereditary Transthyretin Amyloidosis

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Hereditary Transthyretin Amyloidosis (ATTR) is caused by autosomal dominant mutations in the TTR protein for which over 80 are known. The mutations destabilize the tetrameric protein structure and facilitate formation of toxic TTR fibril deposits in multiple tissues including the peripheral nervous system, gastrointestinal tract, and heart. When the nerves are predominantly affected the disease is referred to as familial amyloid polyneuropathy (FAP) and when the heart is predominantly affected the disease is referred to as familial amyloid cardiomyopathy (FAC).

Using second generation antisense technology, we identified an antisense oligonucleotide (ASO) targeting TTR, ISIS-TTR<sub>Rx</sub>, for the treatment of ATTR. ISIS-TTR<sub>Rx</sub> targets wild-type TTR and all known TTR mutants. When tested in a human TTR transgenic mouse model (hTTR Ile84Ser), and in cynomolgus monkeys, ISIS-TTR<sub>Rx</sub> treatment produced ~80% reductions in circulating mutant TTR and wild-type TTR, respectively. ISIS-TTR<sub>Rx</sub> is currently under evaluation in a double-blind, placebo-controlled dose-escalation, Phase 1 study conducted in healthy volunteers. The objective of the study is to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of single and multiple doses of ISIS-TTR<sub>Rx</sub> given as subcutaneous doses ranging from 50 to 400 mg. The single dose cohorts include 4 subjects randomized 3:1, and the multiple dose cohorts include 10 patients randomized 4:1, active to placebo. In the multiple dose cohorts, subjects are given 3 doses during Week 1 and then once weekly doses during Weeks 2-4, for a total of 6 doses. Results showing dose-dependent reductions in plasma TTR levels after both single and multiple-doses will be presented.

### 9.51 - OP 74

#### Final Phase I safety, pharmacokinetic and pharmacodynamic results for ALN-TTR01, a novel RNAi therapeutic for the treatment of transthyretin amyloidosis

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Transthyretin amyloidosis (ATTR) is a fatal, autosomal dominant, multisystem disease caused by abnormal extracellular deposits of transthyretin (TTR) amyloid that lead to familial amyloidotic polyneuropathy (FAP) or familial amyloidotic cardiomyopathy (FAC). More than 100 TTR mutations have been reported. Both mutant and wild-type TTR are found in amyloid deposits and contribute to clinical progression. There is a high unmet need for new therapies, with liver transplantation and tafamidis being the only treatments for a subset of FAP patients. ALN-TTR01 is a systemically administered lipid nanoparticle formulation of a small interfering RNA (siRNA) targeting wild-type and all mutant forms of TTR. This formulation delivers the siRNA predominantly to the liver, thereby inhibiting TTR synthesis at the primary site of production. In transgenic mice expressing the human V30M transgene on a heat shock transcription factor 1 null background, ALN-TTR01 led to robust reduction of TTR mRNA levels in the liver and TTR protein levels in the circulation, and significant regression of TTR protein in tissues known to be affected in ATTR, including the peripheral nervous system and gut. These results demonstrate the potential therapeutic benefit of ALN-TTR01 for the treatment of ATTR. A Phase I randomized, placebo-controlled, single-ascending dose trial of ALN-TTR01 in patients with ATTR was conducted in Portugal, Sweden, France and the United Kingdom. The objectives were evaluation of safety and tolerability and characterization of both pharmacokinetics and clinical activity based on measurements of serum TTR levels. A total of 32 patients were enrolled across 7 dose levels ranging from 0.01 to 1.0 mg/kg. Preliminary data presented at the International Symposium on FAP in Kumamoto, Japan in November 2011 showed a favorable safety profile and evidence of TTR lowering at the highest dose. In this presentation, the final results of the Phase I trial of ALN-TTR01 will be discussed.

#### 10.03 - OP 75

#### RNAi therapy using cholesterol-conjugated siRNA for TTR-related ocular amyloidosis

**Masayoshi Tasaki<sup>1</sup>, Hirofumi Jono<sup>1</sup>, Mitsuharu Ueda<sup>1</sup>, Ryuhei Hara<sup>2</sup>, Konen Obayashi<sup>1</sup>, Takahiro Kawaji<sup>2</sup>, Dinah Sah<sup>3</sup>, Yupeng Fan<sup>3</sup>, Taro Yamashita<sup>4</sup>, Yukio Ando<sup>1</sup>**

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**Introduction:** Transthyretin (TTR)-related familial amyloidotic polyneuropathy (FAP) is characterized by systemic accumulation of amyloid fibrils caused by a point mutation in TTR gene. In FAP patients, ocular manifestations are commonly found and cause loss of visual acuity. It has been reported that amyloidogenic transthyretin synthesized by retinal pigment epithelium (RPE) plays important roles in the progression of ocular manifestations. Our previous study showed that the small interfering RNA (siRNA) directed against TTR significantly reduced the TTR expression in RPE both in vitro and in vivo. However, the effect of siRNA persisted for only a short time. In this study, we generated a cholesterol-conjugated TTR (Cho-TTR) siRNA designed for improving knockdown effect and evaluated its effect both in vitro and in vivo.

**Materials and Methods:** Cho-TTR and control siRNAs were kindly provided by Alnylam Pharmaceuticals, Inc. The effect of siRNA on TTR expression was evaluated by real-time quantitative PCR and ELISA. Human RPE (ARPE-19) cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen) in vitro. For in vivo examinations, siRNAs were administered by direct intravitreal injection in rat. After 14 and 21 days, the RPE and aqueous humor were isolated to evaluate the effect of siRNA on TTR expression. In addition, isolated ocular specimens were evaluated morphologically any potential effect of intravitreal siRNA administration on the retina.

**Results:** TTR mRNA expression in ARPE-19 cells was markedly reduced by Cho-TTR siRNA. Cho-TTR siRNA efficiently knocked down TTR mRNA expression in the RPE 14 days after intravitreal injection in vivo. Moreover, even at 21 days after cholesterol-conjugated TTR siRNA injection, TTR mRNA and protein levels were efficiently suppressed in the RPE and aqueous humor. No histological change in response to siRNA administration was observed in the retina.

**Conclusion:** Cho-TTR siRNA treatment may be a promising therapeutic strategy for TTR-related ocular amyloidosis.

- 10.15**      **Perspectives - Benson**
- 10.30-11.00**    **Coffee break ('Fonteinpatio')**
- 11.00-12.00**    **Closing session: Conclusions and prospects ('Blauwe Zaal')**  
Chair: Van Rijswijk
- 11.00**      **Monday: Basic research - P. Westermark**
- 11.15**      **Tuesday/Wednesday: Clinical challenges - Skinner**
- 11.30**      **Wednesday/Thursday: Therapeutic prospects - Merlini**
- 11.45**      **Closing remarks - Hazenberg**
- 12.00-13.00**    **Lunch ('Fonteinpatio')**
- 12.00-13.00     Certificates of attendance ('Fonteinpatio')



## Posters Monday PA 01 – 65

**13.00-14.00: Individual viewing and discussing of the posters ('Blauwe Patio')**

**16.30-17.30: Poster viewing and presenting in 5 groups ('Blauwe Patio')**

**17.30-18.00: Selected poster presentations PA 15, PA 22, PA 51, PA 53, and PA 54 ('Blauwe Zaal')**

Chairmen Limburg and Gruys

### Group 1 (Moderator Fändrich)

- PA 01 Linking the molecular structure of amyloid fibrils to their mechanical properties – VandenAkker
- PA 02 Seeding effects of alpha-synuclein oligomers – Bergström
- PA 03 The crystal structure of the calcium-free Serum Amyloid P-component decamer – Coker
- PA 04 Sensitive, Histological Staining of Different Polysaccharide Complexes Associated with Amyloid Fibrils – Makovitzky
- PA 05 Beyond genetic factors in familial amyloidotic polyneuropathy: protein glycation and the loss of fibrinogen's chaperone activity – Gonçalo da Costa
- PA 06 Medin at the molecular level: impact for drug design – Davies
- PA 07 Structure of Cystatin B Amyloid and Intermediate Oligomers – Davis
- PA 08 Identifying Fibrillogenic Regions of the  $\lambda$ 6 Light Chains by Limited Proteolysis – Del Pozo-Yauner
- PA 09 Tyrosine residues mediate crucial interactions in amyloid formation for immunoglobulin light Chains – DiCostanzo
- PA 10 Heparan sulfate: dual roles in transthyretin amyloidosis? – Digre
- PA 11 Fibrinogen Glycation in ATTR – Fonseca
- PA 12 Transthyretin binding to heparin sulfate proteoglycan: differences between a commercial transthyretin and an senile amyloidogenic transthyretin: a biochromatographic study - Geneste
- PA 13 Biomolecular Characterization of Transthyretin Oligomeric Interactions with the Molecular Chaperone Clusterin – Greene

### Group 2 (Moderator Luiten)

- PA 14 Turnover of Human-proAPP and Human-IAPP Via Autophagy in a *Drosophila melanogaster* Model – Gu
- PA 15 High-resolution crystal structure of the C-terminal truncated human apoA-I sheds new light on the amyloid formation by the N-terminal fragment – Gursky
- PA 16 Curcumin accelerates A-beta amyloid fibril conversion and thereby mitigates neurotoxicity – Jonson
- PA 17 The relationship between thermal stability and amyloid fibril formation kinetics of immunoglobulin light chain proteins derived from the patients with primary systemic amyloidosis – Katoh
- PA 18 Effect of Dimerization via Cys214 on Immunoglobulin Light Chain Thermal Stability and Aggregation in AL Amyloidosis: Comparison to Germline and Multiple Myeloma LCs – Klimtchuk
- PA 19 Biochemical Characterization of Leptomeningeal Amyloid in Hereditary Transthyretin Amyloidosis – Liepnieks
- PA 20 Interaction studies of amyloid- $\beta$  peptide with ionic and fluorinated amphiphiles – Loureiro
- PA 21 Polarization optical analysis of various animal amyloid deposits and ex vivo isolated amyloid fibrils – Makovitzky
- PA 22 Molecular Mechanisms of  $\beta$ 2-Microglobulin Amyloid Fibril Formation – Ozawa
- PA 23 Detection of autoantibodies against ATTR in patients with FAP ATTR V30M – Obayashi
- PA 24 Fragmentation of heparan sulfate chains reduce Islet Amyloid Polypeptide amyloid load in islets of Langerhans – Oskarsson
- PA 25 Functional bacterial amyloid: make good use of a dangerous fold – Otzen
- PA 26 Amyloidosis induced by variants of Human Apolipoprotein A-I: effect of cellular microenvironment – Ramella

**Group 3** (Moderator Bellotti)

- PA 27 Role of mutations in the cellular internalization of amyloidogenic light chains into cardiomyocytes – Ramirez-Alvarado
- PA 28 Atomic structure of a nanobody trapped domain swapped dimer of an amyloidogenic  $\beta$ 2-microglobulin variant – Riccardo
- PA 29 The C-terminal sequence of type F apolipoprotein A-II inhibits the polymerization of apolipoprotein A-II into amyloid fibrils in mice – Sawashita
- PA 30 Molecular Strategies to Prevent Corneal dystrophies – Stenvang
- PA 31 Amyloid fibrils associated with neurodegenerative disorders enhance HIV infection – Arnold
- PA 32 The ubiquitin-proteasome system and proteolytic content in ATTR – Da Costa
- PA 33 Mechanisms of aggregation and toxicity inhibition in amyloidogenic peptides by Molecular Tweezers: Molecular Dynamics and Quantum Mechanics/Molecular Mechanics studies – Sanchez-Garcia
- PA 34 Differences of histopathological features and amyloid components among various tissue sites of FAP patients after liver transplantation – Ohshima
- PA 35 A study of the mechanism of fibril formation in spinal bulbar muscular atrophy – Chiesa
- PA 36 Heparan sulfate/heparin-HDL interaction dissociates serum amyloid A (SAA) from HDL-SAA complex leading to SAA aggregation – Noborn
- PA 37 Heat shock factor 1 (Hsf1) plays a key role in AApoAII cardiac amyloidosis in mice – Sawashita & Higuchi
- PA 38 Serum-free medium supports amyloid formation from human serum amyloid A in peripheral blood mononuclear cell cultures – Ishii
- PA 39 Cardiotoxicity of pre-fibrillar transthyretin oligomers and attenuation by doxycycline – Koch

**Group 4** (Moderator Saraiva)

- PA 40 Potential induction of vaccine-associated amyloid A amyloidosis in white young hens – Murakami
- PA 41 Interaction of ataxin 3 oligomers with rat cerebellar granule cells results in dysregulation of calcium homeostasis through different responses – Pellistri
- PA 42 Transcriptional profiling of human cardiomyocytes in response to amyloidogenic transthyretin – Reixach
- PA 43 Investigation on the functional consequences of amyloidogenic light chains on *Caenorhabditis elegans* – Rognoni
- PA 44 An alternative fluorescence based systems for studying modulation of HTT amyloid-like aggregation in cells – Rojas-Puente
- PA 45 Characterization of ICCs in the GI tract of mouse model for Familial Amyloid Polyneuropathy – Saraiva
- PA 46 Transthyretin depositon in cultured cells – Ueda
- PA 47 The association of macrophages and amyloid deposits in the pathogenesis of familial amyloidotic polyneuropathy – Misumi
- PA 48 TTR V30M oligomeric aggregates inhibit proliferation of renal progenitor cells but maintain their capacity to differentiate into podocytes *in vitro* – Moreira
- PA 49 Predicting Amyloidogenic Propensity of Novel Transthyretin Variants – Campos
- PA 50 Molecular basis of amyloid fibril recognition by the conformation-sensitive B10 antibody fragment – Haupt
- PA 51 Novel fluorescent probes for the spectral assignment of a plethora of protein aggregates – Klingstedt
- PA 52 Comparison of immunohistochemistry and mass spectrometry in amyloid typing (Ringstudy I) – Linke

**Group 5** (Moderator Kluge-Beckerman)

- PA 53 Subtyping of amyloidosis by direct proteomic analysis of fixed biopsy samples – Liuu

- PA 54** The amyloidophilic peptide p5 binds rapidly and stably to visceral amyloid *in vivo*: A potential radiotracer for PET/CT imaging – Martin
- PA 55** Diagnosing and typing early amyloidosis using Congo red fluorescence and immunohistochemistry - Michels
- PA 56** Monitoring amyloid formation and maturation *in vitro* and *in vivo* using LCO fluorescence – Nyström
- PA 57** Digitally reinforced Hematoxylin-eosin slides: First clue in detection of amyloid depositions – Pehlivanoglu
- PA 58** Proteomics analysis of amyloid deposits with sequence/structure analysis of light chain proteins from AL patients – Ramirez-Alvarado
- PA 59** Digitally reinforced Toluidine blue can safely be used for detection of amyloid depositions – Sen
- PA 60** The novel polybasic peptide p5R can be used to identify murine and human amyloid deposits *in vitro* and *in vivo* – Wall
- PA 61** Tandem Mass Spectrometry Analysis of Protein Deposits in Human Subcutaneous Fat Tissues of a Patient with Immunoglobulin Light Chain Amyloidosis: *De novo* Sequencing and Post-translational Modifications – Lu
- PA 62** Amyloid-reactive peptides bind MelA<sup>+</sup> melanocytes and extracellular melanin in human, canine and murine melanoma tumors – Wall
- PA 63** *In vivo* biodistribution of the amyloid-reactive peptide, p5, correlates with *ex vivo* amyloid quantitation based on Congo red tissue staining – Wall
- PA 64** Antibodies specific to AA76, the common species of AAs – Yamada
- PA 65** Topo-optical histochemical analysis of amyloid deposits in Alzheimer, Morbus Down and Creutzfeldt–Jakob brain – Makovitzky

**PA 01****Linking the molecular structure of amyloid fibrils to their mechanical properties**

**Corianne C. vandenAkker,<sup>1</sup> Maarten F. M. Engel,<sup>2</sup> Krassimir P. Velikov,<sup>3</sup> Mischa Bonn,<sup>2,4</sup> Gijsje H. Koenderink<sup>1</sup>**

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Nearly all proteins and peptides have the ability to self-assemble into amyloid fibrils when they are denatured. These fibrils are remarkably ordered and stable and they exhibit unique mechanical properties. The flipside of this stability is that fibrils which form in the context of conformational diseases are difficult to remove. Our aim is to unravel the link between molecular conformation and intermolecular forces with the mechanical properties of amyloid fibrils using a biophysics approach. In the long run, we hope that this knowledge will guide the rational design of compounds which influence amyloid self-assembly or break down the formed fibrils. As model systems, we used the natively disordered Alzheimer Aβ(1-42) peptide and two denatured model proteins (hen egg white lysozyme (HEWL) and β-lactoglobulin (β-Ig)). We studied the secondary structure of the amyloid fibrils by vibrational sum frequency generation (VSFG) spectroscopy and tip-enhanced Raman spectroscopy (TERS), and measured the bending rigidity of the fibrils by fluorescence microscopy. Moreover, we measured the stiffness of networks of fibrils by rheology. Our results show that the bending rigidity of the amyloid fibrils and the stiffness of amyloid fibril networks are strongly dependent on the beta-sheet of the amyloid fibrils [1]. Moreover, we show that the beta-sheet content can be influenced by addition of the polyphenol drug EGCG.

**References:** 1. VandenAkker et al., J. Am. Chem. Soc., 2011, 133, 18030-18033.

**PA 02****Seeding effects of alpha-synuclein oligomers**

Therese Fagerqvist<sup>1</sup>, Thomas Näsström<sup>1</sup>, Charlotte Sahlin<sup>2</sup>, Stina Tucker<sup>2</sup>, Veronica Lindström<sup>1</sup>, Mikael Karlsson<sup>3</sup>, Fredrik Nikolajeff<sup>3</sup>, Heinrich Schell<sup>4</sup>, Tiago Outeiro<sup>5</sup>, Philipp Kahle<sup>4</sup>, Lars Lannfelt<sup>1</sup>, Martin Ingelsson<sup>1</sup>, **Joakim Bergström<sup>1</sup>**

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**Background:** Aggregated alpha-synuclein is the major component of Lewy bodies, intracellular deposits observed in affected brain areas in disorders such as Parkinson's disease and dementia with Lewy bodies. Recently, data has suggested that alpha-synuclein pathology could spread from one cell to another and thereby affect additional neurons. Although the exact molecular mechanisms are unknown, oligomers of alpha-synuclein could potentially be transmitted between cells and accelerate the aggregation process.

**Objective:** As oxidative stress has been implicated as a risk factor for both Parkinson's disease and dementia with Lewy bodies, we wanted to investigate *in vitro* and *in vivo* seeding effects of alpha-synuclein oligomers induced by the lipid peroxidation product 4-oxo-2-nonenal (4-ONE).

**Methods:** To investigate the *in vitro* seeding effect of the generated 4-ONE-induced alpha-synuclein oligomers on monomeric protein, a thioflavin T assay was performed. Next, 4-ONE-induced alpha-synuclein oligomers were added to H4 neuroglioma cells expressing alpha-synuclein fused to GFP and the seeding effect was quantified by immunofluorescence. Finally, 4-ONE-induced alpha-synuclein oligomers were stereotactically injected in the neocortex of alpha-synuclein transgenic mice and the seeding effect was assessed immunohistochemically.

**Results:** The lag phase of fibril formation was reduced for monomeric preparations seeded with 4-ONE-induced alpha-synuclein oligomers as compared to both fibrillar-seeded and non-seeded alpha-synuclein samples. Furthermore, extracellular added 4-ONE-induced alpha-synuclein oligomers were taken up and affected intracellular alpha-synuclein oligomerization in H4 neuroglioma cells. However, no seeding effect could be observed in the neocortex of alpha-synuclein transgenic mice injected with 4-ONE-induced alpha-synuclein oligomers.

**Conclusions:** Here, we demonstrate that 4-ONE-induced alpha-synuclein oligomers initiate aggregation of monomeric alpha-synuclein *in vitro*; however, such seeding effect was not observed in an alpha-synuclein transgenic mouse model. Further studies are needed to investigate this discrepancy and to discern the molecular nature of nucleating alpha-synuclein species that may cause prion-like propagation of Lewy body pathology *in vivo*.

## PA 03

### The crystal structure of the calcium-free Serum Amyloid P-component decamer

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**Background:** Human serum amyloid P component (SAP) is a plasma glycoprotein universally associated with amyloid deposits. It has potent molecular chaperone activity [1] and both promotes [3] and inhibits [2] amyloid fibrillogenesis, depending upon the presence or absence of calcium ions respectively. In the inescapable presence of calcium ions extracellularly *in vivo*, SAP is pentameric and probably binds to amyloid fibres via its calcium binding sites on the B (binding)-face. In the absence of calcium ions *in vitro* SAP forms a stable decamer composed of two pentamers interacting face-to-face [4,5].

**Objective and method:** We performed X-ray analysis of SAP crystals grown in the absence of calcium ions to provide a structural basis for these unexplained observations.

**Results:** The 3.5Å resolution structure shows that two SAP pentamers interact closely via their B faces on a common five-fold axis, and so extensively that this decamer is most likely the form observed in solution.

**Discussion and Conclusion:** Since the inhibition of fibre growth and the chaperone activity of SAP can be observed in calcium free conditions, these activities probably do not involve the fibre-binding B-

face which is buried in our crystallographic decamer. The calcium independent, *in vitro* activities may thus be effected by the A face. The two distinct chaperone activities of human SAP, classical refolding and assembly-chaperone effects, are thus probably expressed by the two different faces of this toroidal molecule.

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#### PA 04

#### Sensitive, Histohemical Staining of Different Polysaccharide Complexes Associated with Amyloid Fibrils

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**Background:** Insoluble fibrillar deposits in different tissues are thought to be a final consequence of systemic amyloidosis. The unstable monoclonal immunoglobulin light chains are responsible for the primary amyloidosis. Amyloid fibril deposits are associated with glucosaminoglycans (GaGs) and sulphated compounds. To examine the complex fibrillar structure of amyloid in human brain, bacterial cellulose, chitosan and alginic acid polysaccharide materials were selected and comparative investigated.

**Objectives:** Three sample polysaccharides were chosen as well-characterized controls for structural components of amyloid fibrils.

**Methods:** Topo-optical, histochemical staining with conformation-specific and sequence-specific stains was used to localize different GaGs in polysaccharides. The toluidine blue (or 1,9-dimethyl methylene blue) topo-optical reaction can stain GaGs selectively at various pH additional the chemically intensified basophilic reaction (CIBR) and rivanol were used and critical electrolyte concentration (CEC) methods.

**Results:** Three optical phenomena produced by the ordered and sequence-specific cationic dye aggregates formed on spatial conformity polyanions were shown in case of bacterial cellulose, chitosan and alginic acid. These are metachromasy, dichroism and birefringence. All three effects occur in the same picture in combination. The presented staining methods also indicate that amyloid fibrils are not homogeneous but heterogeneous, and have a highly ordered structure in oriented fashion: the center is formed by a glycoprotein core (AP), helical structures of chondroitine and heparin sulphate surround this core and a filament network of protein (AA or AL and others) constitute the surface of the fibril similar like the selected polysaccharide complexes.

**Conclusions:** These data indicate that the presented polysaccharide complexes, glycosaminoglycans and sulphate groups by histochemical methods can be used for further amyloid research on *in vitro* fibrils prepared with and without GaGs and on *ex vivo* fibrils. Glucosamine, which is part of the structure of the selected polysaccharides chitosan and chitin, may facilitate the process of amyloidosis, and/or provide neuroprotection in the Alzheimer disease brain.

#### PA 05

#### Beyond genetic factors in familial amyloidotic polyneuropathy: protein glycation and the loss of fibrinogen's chaperone activity

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Transthyretin related amyloidosis (ATTR) is a systemic conformational disease characterized by extracellular amyloid fibril formation from plasma transthyretin (TTR)<sup>[1]</sup>. This is a crippling, fatal disease for which liver transplantation is the only effective therapy. More than 80 TTR point mutations are associated with amyloidotic diseases and the most widely accepted disease model relates TTR tetramer instability with TTR point mutations. However, this model fails to explain two observations. First, native TTR also forms amyloid in systemic senile amyloidosis, a geriatric disease. Second, age at disease onset varies by decades for patients bearing the same mutation and some mutation carrier individuals are asymptomatic throughout their lives.

We believe that mutations only accelerate the process and non-genetic factors must play a key role in the molecular mechanisms of disease. One of these factors is protein glycation, previously associated with conformational diseases like Alzheimer's and Parkinson's. The glycation hypothesis in ATTR is supported by our previous discovery of methylglyoxal-derived glycation of amyloid fibrils in ATTR patients<sup>[2]</sup>.

Plasma samples were collected from healthy subjects (controls) and ATTR patients heterozygous for the V30M mutation. Analysis was made by two-dimensional gel electrophoresis, FTICR-MS<sup>[3]</sup> and western blot against transthyretin and fibrinogen.

Here we show that plasma proteins are differentially glycated by methylglyoxal in ATTR patients and that fibrinogen is the main glycation target. Moreover, we also found that fibrinogen interacts with TTR in plasma<sup>[4]</sup>. Fibrinogen has chaperone activity which is compromised upon glycation by methylglyoxal both *in vitro* and *in vivo*. Hence, we propose that methylglyoxal glycation hampers the chaperone activity of fibrinogen, rendering TTR more prone to aggregation, amyloid formation and ultimately, disease.

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#### PA 06

#### Medin at the molecular level – impact for drug design

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**Background:** Amyloid proteins that penetrate the cardiovascular system can cause a variety of pathologies including stimulation of cardiomyopathy, congestive heart failure and, in some cases, death. This project investigates the aggregation properties and structural elements of the polypeptide medin, the causative agent of aortic medial amyloid (AMA). The cardiovascular pathology of aortic amyloid is poorly defined and under-researched compared to neurotoxic amyloid; yet there is evidence that medin aggregates play a role in thoracic aneurysm and dissection (1). Interestingly these pathologies are associated with a reduction in amyloid plaque load, suggesting a role for toxic prefibrillar precursors of medin.

**Objective:** The aim of this work is to investigate the molecular structure and morphology of medin fibrils and prefibrillar precursors, and to determine how the structural features correlate with cytotoxicity. We aim to identify core amyloidogenic regions of the medin sequence – or “self-recognition elements” – as target sites for the design of aggregation inhibitors for use as research tools for AMA and possible therapies in the longer term.

**Methods:** Several complementary techniques have been employed to investigate medin amyloid *in vitro*; these include thioflavin T fluorescence to monitor the dynamics of aggregation, electron

microscopy to examine aggregate morphology and solid-state NMR to investigate fibril architecture at the molecular level.

**Results:** Initial results have identified several residues within the C-terminal region of medin that appear to play an important role in fibril formation and stabilisation (2). Molecular modelling has also highlighted a second central region that may have similar structural elements to other known amyloid proteins, principally amyloid-beta.

**Conclusions:** Aided by these results, novel aggregation inhibitors are now being tested for efficacy against medin aggregation and cellular toxicity.

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#### PA 07

#### Structure of Cystatin B Amyloid and ntermediate Oligomers

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The Staniforth group has produced a volume of data to determine human cystatin B amyloid structure which forms from the natively folded cysteine protease inhibitor. Limited proteolysis of the fibrils has been used to examine the core structure by removing extraneous regions, indicating the fibril is primarily composed of residues 27-80 out of 98. The first 27 residues, a native β-sheet and α-helix is readily cleaved whilst residues 80-98 are natively β-sheet and digested at a slower rate than the N-terminal region. This information alongside hydrogen-exchange nuclear magnetic resonance data supports the premise that the first 27 residues are unfolded. In comparison, the C-terminal region is folded with observed hydrogen bonding yet remains readily cleaved throughout the region. It is thereby accessible and external to the critical fibre core or undergoes substantial undetected structural rearrangement upon digestion. Mass per unit length electron microscopy measurements of fibrils are additionally being used to develop the structural model in combination with an array of single and double cysteine mutants in which disulphide bonding has been probed with X-ray Absorption Near Edge Structure Spectroscopy. Combining this data is constraining the structural models of amyloid fibrils from cystatin B, a natively folded protein, which develops our understanding of amyloid structure and formation. In addition, intriguing and diverse intermediate oligomeric structures have been observed by electron microscopy. The development of amyloid and intermediate oligomer structures with knowledge of the assembly process will aid drug development, enhancement and treatment for associated diseases.

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#### PA 08

#### Identifying Fibrillogenic Regions of the λ6 Light Chains by Limited Proteolysis

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The particular structural features that render λ6 light chains highly amyloidogenic remain unknown. [1] We propose that the capacity of this family of immunoglobulins to aggregate as amyloid is driven by discrete regions of the variable domain which are directly involved in events relevant to the

aggregation mechanism. To test this hypothesis we used limited proteolysis/MALDI-TOF analysis and site-directed mutagenesis to identify fibril-forming fragments of the recombinant protein 6aJL2, a  $\lambda 6$  variable domain with germline sequence. Proteolysis of 6aJL2 with trypsin and the subsequent overnight incubation at 37 °C with constant shaking ended up with the formation of aggregates having both, fibrillar morphology under the electron microscopy, and the spectroscopic characteristics of amyloids. Four fragments were isolated and identified from the aggregates. One of them was composed of two peptides -Thr18-Arg25 and Thr80-Lys103- linked by the disulfide bridge Cys23-Cys88. The other three components were peptides Ser26-Arg39, Ser26-Arg54 and Phe62-Lys79. Kinetic experiments revealed that the fastest aggregating components were peptides Ser26-Arg39 and Ser26-Arg54. Fragment Thr18-Arg25-C23-C88-Thr80-Lys103 readily formed fibrils when incubated alone, but not if previously reduced with dithiothreitol. None of the two peptides composing this fragment aggregated as fibrils when incubated separately. Peptides Ser26-Arg39 and Phe62-Lys79 did not form fibrils when incubated independently, but did aggregate as fibrils when co-incubated. In experiments performed with the mutant 6aJL2-R25G –where Arg25 is substituted by Gly– in the presence of dithiothreitol, the formation of fibrillar aggregates was detected, and peptide Thr18-Arg39 was identified as the most abundant component. Site-specific mutagenesis was used to identify positions relevant for the aggregation mechanism. We conclude that the *in vitro* fibrillogenesis of the  $\lambda 6$  light chain proteolytic fragments involve cooperative interaction among them that could be relevant for the aggregation mechanism of the intact molecule.

**Reference:** 1. del Pozo Yauner, L., et al. (2008) *Proteins* 72(2): 684-692.

## PA 09

### Tyrosine residues mediate crucial interactions in amyloid formation for immunoglobulin light chains

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Light Chain (AL) Amyloidosis is an incurable protein misfolding disease where monoclonal immunoglobulin light chains misfold and deposit as amyloid fibrils causing organ failure and death. Previously, our laboratory reported that the altered conformation of the dimer interface of highly amyloidogenic light chain AL-09 favors amyloid formation [1, 2]. We observed that AL-09 fibril formation is stochastic and delayed at high pH; moreover, fibril formation is inhibited at pH 10. We noted that there are three Tyrosine residues (32, 91, and 96) clustered in the dimer interface, and that Tyrosine has a pKa of 10. These Tyrosines may be ionized at pH 10, causing repulsion and inhibiting fibril formation. We decided to mutate these Tyrosines to Phenylalanines to retain the aromatic residue, but prevent ionization. Given that Tyrosine residues are prevalent and highly conserved among immunoglobulin light chains, we hypothesize that Tyrosines mediate interactions critical for the initiation of amyloid formation. Although studying fibril formation at pH 10 may not seem physiologically relevant; these studies have allowed us to elucidate the initial structural changes that must occur for immunoglobulin light chains to form fibrils under physiological conditions.

AL-09 Y to F mutant proteins were recombinantly expressed, extracted, purified, and characterized by CD and NMR spectroscopy as previously reported [1-3]. Proteins were ultracentrifuged to remove preformed aggregates before fibril formation and crystallization experiments.

AL-09 Y to F mutants maintain similar structure and thermodynamic stability to AL-09. X-ray crystallography and NMR spectra of AL-09 Y32F Y96F reveal minor structural differences compared to AL-09. AL-09 Y to F mutants form fibrils faster and more consistently at high pH, including pH 10. Tyrosine residues in light chains mediate crucial interactions that initiate amyloid fibril formation and can be targeted for future therapeutic strategies to directly inhibit fibril formation in hopes of creating more effective treatments.

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## PA 10

### Heparan sulfate – dual roles in transthyretin amyloidosis?

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**Background:** Transthyretin (TTR) is associated with several distinct clinical forms of amyloidosis. The pathogenesis is initiated by dissociation of TTR's homotetrameric structure, resulting in monomeric subunits that may become misfolded. The misfolded monomers can self-assemble into amyloid fibrils which deposit predominately in the myocardium and/or the peripheral nervous system, affecting the functions of these organs<sup>1</sup>. We have previously demonstrated that heparan sulfate (HS), a sulfated glycosaminoglycan, promotes TTR aggregation through interaction with the monomers of TTR<sup>2</sup>.

**Objective:** In this study, we investigated whether HS is involved in TTR internalization.

**Methods and Result:** We found that dissociated TTR was internalized by wild-type Chinese hamster ovary (CHO) cells, but not by HS-deficient CHO cells. The internalized TTR appears to be accumulated in nucleus as demonstrated by prominent perinuclear staining with an anti-TTR antibody. 3D-analysis using confocal scanning microscopy suggested that the TTR immunosignal was in fact embedded in the peripheral surface of the nucleus. We found that the aggregated TTR was not internalized by any of the cells tested, indicating that the protein conformation is essential for cellular uptake.

**Discussion/Conclusion:** In conclusion, our study demonstrates that HS on cell surface is crucial for internalization of the TTR monomer. This suggest that dissociation of the homotetrameric structure occurs prior of internalization, an event also associated with TTR fibrillization. Further studies are to illustrate the functional roles of HS in modulation of TTR internalization and aggregation.

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## PA 11

### Fibrinogen Glycation in ATTR

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ATTR is an autosomal inherited neurodegenerative disorder in which the neuropathological hallmark is the extracellular deposition of amyloid fibrils on the peripheral nervous system. Transthyretin (TTR), an extracellular tetrameric protein found in serum and cerebrospinal fluid, constitutes the major component of this amyloid fibers.<sup>1</sup>

TTR has several known ligands, but recently a few more were identified as interacting partners.<sup>3</sup> From these, fibrinogen arises with particular interest since it has been recently described as a chaperone.<sup>2</sup> Moreover, this protein was also identified as differentially glycated in AATR patients. It is well documented that chaperones are specific targets of this non-enzymatic modification, resulting in

their function and activity modulation.<sup>4</sup> Also, glycation was previously described as being involved in other conformational diseases, such as Alzheimer's and Parkinson's diseases.<sup>5</sup>

In this work, we developed a methodology to determine *in vitro* fibrinogen's glycation sites using MALDI-FTICR mass spectrometry. We combined several MALDI matrices, specific proteases and advanced microchromatography techniques to achieve the highest possible sequence coverage. This approach was further used to map fibrinogen's *in vivo* glycation sites obtained from ATTR and healthy control individual's plasma samples.

Also, the effect of fibrinogen glycation in its chaperone activity was studied by monitoring model proteins' aggregation process using spectroscopic methods and a diminished activity was measured. Our work enabled the identification of fibrinogen *in vivo* glycation and the observation of its chaperone activity regarding glycation effects. These observations lead us to the conclusion that fibrinogen is a likely player in the mechanism of ATTR pathogenesis.

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### PA 12

#### **Transthyretin binding to heparin sulfate proteoglycan: differences between a commercial transthyretin and an senile amyloidogenic transthyretin: a biochromatographic study**

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Transthyretin (TTR) is an amyloidogenic protein involved in TTR amyloidosis and senile systemic amyloidosis. The interaction between heparan sulfate proteoglycan (HSPG) and TTR is a key-phase for the development of amyloid deposits. Senile TTR is more amyloidogenic than standard wildtype TTR.

A novel biochromatographic approach was developed to measure the thermodynamical data for the binding of a commercial TTR (TTRc) and a senile TTR (TTRs) to HSPG in a wide temperature range and at different pH of the medium. TTRc was provided by Sigma and TTRs was obtained by extraction from cardiac tissue provided by an American patient with senile amyloid.

The TTRc (or TTRs)-HSPG binding was enthalpically driven and increased when pH increased. A change in the association mechanism was observed at pH=7,4 for TTRs. The negative values of thermodynamic data showed that van der Walls and hydrogen bonds were preponderant in the association mechanism. When pH increased, the thermodynamic values increased also; it suggested a major role of both electrostatic and hydrophobic interactions. At pH= 6, the strongest value obtained for TTRs ( $\Delta H_{TTRc} = - 13,1 \text{ kJ.mol}^{-1}$ ,  $\Delta H_{TTRs} = - 8,1 \text{ kJ.mol}^{-1}$ ) suggested a conformational change between TTRc and TTRs in the binding mechanism.

The conformational change, suggested by our work, may enhance more favorable electrostatic interactions between the sulfate groups of HSPG and the basic amino acids (histidine, lysine, arginine) of TTRs.

**PA 13****Biomolecular Characterization of Transthyretin Oligomeric Interactions with the Molecular Chaperone Clusterin**

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Clusterin (CLU) is an abundant, circulating chaperone co-localizing with extracellular amyloid deposits of transthyretin (TTR). The precise interaction of CLU with misfolded, amyloidogenic TTR intermediates has not been defined and is our focus. *In vitro* acid (pH 2.0) denaturation was used to unfold wild-type TTR over 96 h and aggregation was induced by addition of NaCl. Reaction samples at timepoints from 0-24 h were cross-linked with glutaraldehyde and analyzed by SDS-PAGE. Results showed a transition from tetramers to oligomers to aggregates of increasing size. Moreover, samples from 0.5 to 4.0 h showed increasing reactivity to the A11 oligomer-specific antibody (Invitrogen); no reactivity was observed in the t=24 h sample. Parallel studies using circular dichroism (CD) spectroscopy and simultaneous light scattering showed a loss of native TTR structure (unfolding) at earlier reaction times; an increase in beta-sheet content and aggregate formation was observed for samples from later reaction times. CD and A11 oligomer-specific antibody data were consistent. Using surface plasmon resonance (SPR), we investigated the interactions of CLU with native, unfolded, and oligomeric forms of TTR. While little or no binding of CLU to native or unfolded TTR was noted, a concentration-dependent interaction of later stage TTR oligomers to active CLU surfaces was observed. Kinetic analysis of real-time SPR sensograms showed faster association rates of later stage oligomeric TTR vs. unfolded TTR; likewise, a greater affinity of CLU for later stage TTR oligomers was measured by kinetic off-rate analysis (Table 1). Investigation of CLU interactions with misfolded and oligomeric TTR will provide mechanistic insight for the co-localization of this chaperone with TTR amyloid deposits in patients with SSA and ATTR amyloidoses.

**Table 1. SPR Sensograms kinetic analyses using Langmuir modeling.**

| TTR oligomerization time (h) | $k_a$ (1/M s)         | $k_d$ (1/s)           |
|------------------------------|-----------------------|-----------------------|
| 0.0                          | $1.52 \times 10^{-2}$ | $1.46 \times 10^{-3}$ |
| 0.5                          | $2.18 \times 10^{-3}$ | $5.14 \times 10^{-4}$ |
| 1.0                          | $4.61 \times 10^{-3}$ | $4.67 \times 10^{-4}$ |
| 2.0                          | $1.59 \times 10^{-3}$ | $4.44 \times 10^{-4}$ |

This work has been supported by the NIH grant RO1AG031804 (LHC), and the Walk for an Angel Fund.

**PA 14****Turnover of Human-prolAPP and Human-IAPP Via Autophagy in a *Drosophila melanogaster* Model**

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**Background:** Intracellular islet amyloid is a frequent finding and a possible cause for the  $\beta$ -cell loss detected in islets of Langerhans in type 2 diabetes. Despite many attempts the pathway for cell death remains to be clarified. We have used the recently described *Drosophila melanogaster* model (1), and studied how expression of human prolAPP or prolAPP affects the autophagy pathway.

**Material and Methods:** The Gal4-UAS system was used to drive the expression of human-prolAPP (hprolAPP), human-IAPP (hIAPP) or mouse-IAPP (mIAPP) simultaneously with UAS-nlsGFP to pdf cells of *D. melanogaster* in order to visualize the cells.

Immunofluorescence was used to detect ubiquitin and Ref(2)p proteins that bind to autophagosomes and the reporter genes mcherry-Atg8a and GFP-LAMP-1 were used for studies of autophagic flux.

**Results:** The numbers of pdf cells were counted in 1, 15, 30 days old flies and overexpression of hproIAPP and hIAPP resulted in a significant reduction of pdf cells over-time when compared to wt or mIAPP expressing flies.

Expression of hproIAPP and hIAPP led to accumulation of large aggregates while mIAPP expression resulted in accumulation of smaller aggregates, and all aggregates were recognized by antibodies against ubiquitin and Ref(2)P.

Expression mCherry-Atg8, a reporter for autophagic activity, formed red fluorescent spots upon hproIAPP or hIAPP co-expression, but these red spots were absent in flies co-expressing mIAPP.

GFP-LAMP-1 is a reporter for lysosomes and in preliminary results on co-expression of hIAPP and GFP-LAMP-1 an accumulation of significantly larger lysosomes were detected compared to lysosomal size detected in flies co-expressing mIAPP and GFP-LAMP1 ( $p<0.01$ ).

**Conclusion:** Taken together these findings support that expression of aggregation prone hproIAPP and hIAPP activates autophagy and that it can cause disturbances in the autophagic flux.

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## PA 15

### High-resolution crystal structure of the C-terminal truncated human apoA-I sheds new light on the amyloid formation by the N-terminal fragment

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Apolipoprotein A-I (apoA-I) is the main protein of plasma high-density lipoproteins (HDL) that remove cell cholesterol and protect from atherosclerosis. In hereditary amyloidosis, apoA-I mutations promote proteolysis and the deposition of the 9-11 kDa N-terminal fragments as fibrils in organs such as kidney, liver and heart, causing organ damage. No treatment is available for apoA-I amyloidosis. All known amyloidogenic mutations in human apoA-I are clustered in residue segments 26-107 and 154-178. The high-resolution X-ray crystal structure of the C-terminal truncated human protein,  $\Delta$ (185-243)apoA-I [1], provides the structural basis for understanding apoA-I destabilization in amyloidosis [2]. The sites of amyloidogenic mutations correspond to key positions within the largely helical four-segment bundle comprised of residues 1-120 and 144-184. Mutations in these positions disrupt the bundle structure and destabilize lipid-free apoA-I, thereby promoting its proteolysis. Moreover, many mutations place a hydrophilic or Pro group in the middle of the hydrophobic lipid-binding face of the amphipathic  $\alpha$ -helices, which will likely shift the population distribution from HDL-bound to lipid-poor/free apoA-I that is relatively unstable and labile to proteolysis. Notably, the crystal structure shows segment L44-S55 in an extended conformation consistent with the  $\beta$ -strand-like geometry. Exposure of this segment upon destabilization of the four-segment bundle probably initiates the  $\alpha$ -helix to  $\beta$ -sheet conversion in amyloidosis.

**Conclusion:** We propose that the amyloidogenic mutations promote apoA-I proteolysis by destabilizing the protein structure not only in lipid-free but also in HDL-bound form, with segment L44-S55 providing a likely template for the cross- $\square$ -sheet conformation.

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**PA 16**

**Curcumin accelerates A-beta amyloid fibril conversion and thereby mitigates neurotoxicity**

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For several years, curcumin has been proposed to mitigate the neurotoxic amyloid species formed by the A $\beta$  peptide related to Alzheimer's disease. We herein analyze whether curcumin can function as a generic protective compound against amyloid induced degeneration in transgenic *Drosophila melanogaster*. Neurological impairments were assayed by survival, climbing behavior and locomotor activity, while amyloid deposition was examined by histological analysis. Further experiments were performed to quantify the level of A $\beta$  produced in the flies and to study the effect of curcumin by *in vitro* fibrillation of recombinant A $\beta$ .

Curcumin treatment showed positive pharmacological effects for all genotypes, except the control flies, in the locomotor assay and for three of the five genotypes in the survival assay. These effects were directly dependent on genotype, rendering the strongest effect for flies exhibiting the worst phenotype. When analyzing the immunohistochemistry at different time-points using combined antibody staining and the amyloid specific pFTAA, a luminescent conjugated oligothiophene, structure dependent fluorescence spectra showed an accelerated fibrillation in flies treated with curcumin. However, analysis of aged flies (day 20 post eclosion) showed no change in amyloid deposition load upon curcumin treatment, despite the positive pharmacological effects.

These observations were confirmed by *in vitro* fibrillation of recombinant A $\beta_{1-42}$  in the absence or presence of curcumin. Quantification of A $\beta$  levels in the flies showed no significant difference between curcumin treated and untreated flies, indicating that the enhanced fibril formation is due to curcumin provoked conformational conversion rather than a difference in A $\beta$  concentrations.

The increased survival (up to 75 % enhancement) and locomotor activity together with the histological analysis indicate that curcumin function as a protective compound by enhancing the formation of amyloid fibrils while reducing soluble oligomeric species of A $\beta$ . Taken together this renders a reduced neurotoxicity in transgenic *Drosophila melanogaster*.

**PA 17**

**The relationship between thermal stability and amyloid fibril formation kinetics of immunoglobulin light chain proteins derived from the patients with primary systemic amyloidosis**

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Primary systemic (AL) amyloidosis is a devastating disease characterized by systemic deposition of amyloid derived from abnormal immunoglobulin light chain produced by monoclonal plasma cells. Current available treatments involve conventional chemotherapy and autologous stem cell transplant. No phase III clinical trial had been conducted in the US to compare response in AL patients treated with these two available treatments. We have recently concluded such phase III trial at Mayo Clinic. AL patients who achieved hematological complete response (CR) do not necessarily achieve organ response regardless of the treatment they received. Organ function improvement varies among AL patients as well as among affected organs. In order to investigate the possible correlation between amyloid formation kinetics and organ response, in this study we selected AL patients from the trial who had kidney involvement as the most dominant manifestation at the time of diagnosis and showed eventual CR after the treatment. 6 patients were selected and their pathogenic immunoglobulin light chain variable regions were characterized. The purified protein was analyzed by Circular Dichroism for secondary structure and thermal stability (denaturation midpoint temperature: Tm) and by Thioflavine T Fluorescence enhancement for amyloidogenicity (time to reach 50% maximum ThT fluorescent: t50). We were unable to determine a single identical (monoclonal) light chain sequence for one patient. The protein from another patient was not successfully purified in the folded state, due to a

putative glycosylation site acquired after somatic hypermutation. The remaining 4 proteins showed differences in their stability and their kinetics of amyloid formation. Significant positive relationship was detected between melting temperature( $T_m$ ) and  $t_{50}$  at pH 7.4, showing that thermally unstable proteins are more likely to form amyloid fibrils earlier in physiological condition. Relationship between protein kinetics and clinical outcome is going to be evaluated in the future.

#### **PA 18**

#### **Effect of Dimerization via Cys214 on Immunoglobulin Light Chain Thermal Stability and Aggregation in AL Amyloidosis: Comparison to Germline and Multiple Myeloma LCs**

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In light chain (LC) amyloidosis (AL), free immunoglobulin LCs are deposited as fibrils in vital organs and are frequently found in serum and urine. Mass spectrometric analyses of urine-derived amyloidogenic LCs from both kappa and lambda families detected monomers, as well as homodimers covalently linked via Cys214 at the C-terminus of the LC constant region ( $C_L$ ). Our preliminary clinical analysis on 21 AL patients with renal involvement demonstrated the association of dimeric urinary LC with higher levels of 24 hr proteinuria compared to monomeric LC (mean $\pm$ SD: 4049 $\pm$ 2059 mg vs 1962 $\pm$ 1740 mg,  $p=0.031$ ), yet the role of LC dimerization in the disease mechanism is unclear. Although the pathogenesis of AL amyloidosis is generally attributed to the LC variable region ( $V_L$ ), recently we demonstrated the importance of full-length LC and  $C_L$ - $V_L$  interactions in initiation of protein misfolding and fibrillogenesis (*Biochemistry* (2010) 49, 9848-9857). To test the effect of dimerization via Cys214 on structural stability and aggregating propensity of full-length LC, we compared heat-induced unfolding and aggregation of recombinant dimeric full-length kappa-1 LCs to recombinant monomeric analogues containing a Cys214Ser substitution which prevents LC dimerization. Slow kinetically controlled thermal unfolding accompanied by irreversible aggregation was a universal property of all full-length LCs analyzed, including AL, non-amyloidogenic multiple myeloma (MM), and germline LC proteins in both monomeric and dimeric forms. Dimerization had a stabilizing effect on the MM and germline LCs (as evident from the increase in the apparent transition temperature by  $\Delta T_{1/2} \sim 6-8^\circ\text{C}$ ), but did not alter the thermal stability of the AL protein ( $\Delta T_{1/2} < 1^\circ\text{C}$ ). These data suggest that dimerization via the C-terminal region of  $C_L$  is peripheral to the interactions that are key to LC destabilization and misfolding in AL amyloidosis.

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#### **PA 19**

#### **Biochemical Characterization of Leptomeningeal Amyloid in Hereditary Transthyretin Amyloidosis**

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**Background:** The majority of the greater than 100 mutations in the transthyretin (TTR) gene associated with hereditary TTR amyloidosis result in cardiac and peripheral nervous system involvement. About a dozen TTR gene mutations, however, result in amyloid deposition in the leptomeninges with little, if any, in visceral organs. What determines the differences in organ targeting by the various TTR mutations is unknown. TTR is synthesized by the liver, choroid plexus, and retinal pigment epithelium. Plasma TTR synthesized by the liver is the source for cardiac and peripheral nerve TTR amyloid, and TTR synthesized by the choroid plexus is the source for leptomeningeal amyloid.

**Objective:** Biochemically characterize the amyloid protein in leptomeninges of TTR patients to determine if differences exist in the leptomeningeal TTR deposits compared to other organs such as heart and nerve.

**Methods:** Brain tissues were obtained at autopsy from patients heterozygous for Val30Gly, Gly53Arg, and Tyr114Cys TTR and stored at -80° C. Amyloid fibrils were isolated from leptomeninges dissected

from brain tissue by repeated homogenization in citrate-saline and centrifugation. Amyloid protein was solubilized from fibrils with guanidine hydrochloride and fractionated by molecular sieve chromatography. Pooled fractions were digested with trypsin and the resulting peptides were fractionated by reverse-phase HPLC. Relative amounts of normal and variant TTR in pooled fractions were estimated from recovered amounts of tryptic peptides containing the normal or variant residue as determined by Edman degradation analysis.

**Results:** In all three cases, the vast majority, if not all, of the leptomeningeal amyloid TTR was derived from the variant TTR. In contrast, cardiac and peripheral nerve TTR amyloid usually contain 50 – 70% variant and 30 – 50% normal TTR.

**Conclusion:** These results suggest that differences may exist in the processing pathway of TTR to amyloid deposits in heart and in leptomeninges.

## PA 20

### Interaction studies of amyloid- $\beta$ peptide with ionic and fluorinated amphiphiles

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Amyloid-beta peptide ( $A\beta$ ) is the major constitute of Alzheimer's disease plaques. The aggregates deposit in the brain extracellularly (beta-amyloid) or inside the cells (aggregates of hyperphosphorylated Tau protein) resulting in neuronal loss [1]. The studies of  $A\beta$  interaction with different amphiphile molecules are important to better understand the alterations and aggregation process of  $A\beta$ . They will also contribute to screen for molecules that can inhibit the amyloid fibril formation. Fluoroorganic compounds are of big interest in medical applications due to the unique properties of the fluorine atom [2,3]. According to recent studies, fluorinated nanoparticles inhibit  $A\beta$  fibril formation [4,5]. In this study  $A\beta$  was incubated at 37°C with perfluorooctanoic acid (PFOA), an anionic fluorinated amphiphile, and cetyltrimethylammonium chloride (CTAC), a cationic amphiphile. The results demonstrated that the concentration of amphiphiles can significantly influence the aggregation of  $A\beta(1-42)$  into amyloid fibrils and their morphologies. Micelles of CTAC inhibit the formation of fibrils by  $A\beta(1-42)$  whereas PFOA promote its aggregation.

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## PA 21

### Polarization optical analysis of various animal amyloid deposits and ex vivo isolated amyloid fibrils

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**Background:** Results on human amyloid deposits and isolated amyloid fibrils from human tissues (Makovitzky 2003, 2009, Makovitzky et al., 2009, Appel et al., 2003 and 2005) did show a glycoprotein core (AP) with helical structures of chondroitin and heparan sulphate surrounding this core and a filament network of protein (AA or AL and others) and lipid constituting the surface of the fibrils.

**Objectives:** Topo-optical staining reactions offer the ability to visualize components in pathological changed tissues more precisely (Romhányi 1963).

The aim of this study was to compare the occurrence of different chemical components in a variety of animal amyloid deposits.

**Methods:** Seven different species (formalin-fixed paraffin-embedded sections (2-5 µm) were investigated with various topo-optical staining reactions: Congo-red, eosin, pinacyanol, pinacyanolchloride, N,N'-dietylpseudoisocyanine-chloride (PSI) toluidine-blue, 1,9-dimethyl-methylene-blue and rivanol.

We have demonstrated sugar moieties in all amyloid deposits selectively with the ABT-reaction (anisotropic PAS-r). Sialic-acid and O-acetylsialic acid were demonstrated with a specific sialic- and O-acetylsialic acid specific reaction. The glycoaminoglycan components have been visualized in the amyloid deposits by the chemically intensified basophilic-reaction (CIBR) and by the critical electrolyte concentration (CEC) method.

**Results:** In tissues of dog, cat and cow origin amyloid deposits appeared to contain all four glycosaminoglycan components, however in goose and chicken amyloid samples heparin sulfate was absent based on this study (Kröger et al., 2009).

The amyloid deposits are from AA type and show sensitivity after potassium-permanganate + trypsin or - pronase digestion for 4-8 hours. The deposits and their birefringence are abolished. AL amyloid deposits and/or various immunohistochemical types of amyloids show a resistance with this investigation.

We have similar results after performic-acid + trypsin and/or - pronase digestions.

The linearly ordered OH groups of the sugar- and sialic acid /or O-acetylsialic acid components are perpendicularly ordered to the surface of amyloid fibrils.

The isolated amyloid fibrils from chicken and dog gave similar results with various topo-optical staining reactions and digestions to the human amyloid deposit (Makovitzky and Appel unpublished)

**Conclusions:** Based on our results: the animal amyloid has a structure similar to the human amyloid: having a highly ordered structure in an oriented fashion with protein, glycoprotein, glycosaminoglycan and lipid components.

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#### PA 22

#### Molecular Mechanisms of $\beta$ 2-Microglobulin Amyloid Fibril Formation

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**Background:** We developed the thioflavine-T (ThT) method<sup>1</sup> and established a nucleation-dependent polymerization model of amyloid fibril formation<sup>2</sup>. Then, we proposed that in  $\beta$ 2-microglobulin ( $\beta$ 2-m)-related amyloidosis, many amyloid-associated molecules including glycosaminoglycans, proteoglycans and lipids partially unfold  $\beta$ 2-m, catalyze its subsequent nucleus formation and stabilize the  $\beta$ 2-m fibrils formed<sup>2</sup>. Recently, the relationship between various amyloidoses and chaperones is gathering attention. In patients with dialysis-related amyloidosis,  $\alpha$ 2-macroglobulin ( $\alpha$ 2M), an extracellular chaperone commonly found in  $\beta$ 2-m amyloid deposits, forms a complex with  $\beta$ 2-m in the blood.

**Objective:** To elucidate the molecular mechanisms and biological implications of the  $\alpha$ 2M- $\beta$ 2-m complex formation.

**Methods:** Amyloid fibril formation was monitored by ThT method and EM.  $\alpha$ 2M- $\beta$ 2-m interaction was monitored by ELISA, analytical ultracentrifugation and Western blotting<sup>3</sup>.

**Results:** (i)  $\alpha$ 2M substoichiometrically inhibited the  $\beta$ 2-m fibril formation at neutral pH in the presence of oleate or SDS, a model for anionic lipids. (ii) The binding affinity between  $\alpha$ 2M and  $\beta$ 2-m in the presence of SDS was higher than that without SDS. (iii) SDS dissociated tetrameric  $\alpha$ 2M into dimers with increased surface hydrophobicity and both tetrameric and dimeric  $\alpha$ 2M interacted with SDS-denatured  $\beta$ 2-m. (iv) At physiologically relevant acidic pH and in the presence of heparin,  $\alpha$ 2M was also dissociated into dimers, and both tetrameric and dimeric  $\alpha$ 2M interacted with  $\beta$ 2-m, resulting in the fibril growth inhibition.

**Discussion:** Under conditions where native  $\beta$ 2-m is denatured, tetrameric  $\alpha$ 2M is also converted to dimers to favor the hydrophobic interaction with denatured  $\beta$ 2-m, thus dimeric (and tetrameric)  $\alpha$ 2M may play an important role in controlling  $\beta$ 2-m amyloid fibril formation.

**Conclusion:** Extracellular chaperones may bind to denatured  $\beta$ 2-m and inhibit  $\beta$ 2-m amyloid fibril formation in the extracellular space.

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#### PA 23

#### Detection of autoantibodies against ATTR in patients with FAP ATTR V30M

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**Background:** It has been well documented that conformational changes via instability of tetrameric form TTR occurs during the process of amyloid formation in FAP. Some cryptic epitopes expose on amyloidogenic TTR (ATTR) molecule surface during the process in sera of FAP patients. There is a possibility that de novo antibodies responded to cryptic epitopes of ATTR may be synthesized in serum of FAP patients. **Objective:** To examine whether a relationship exists between the incidence of autoantibodies against ATTR and the phenotype of FAP ATTR V30M.

**Methods:** Serum samples were collected from 25 Japanese (28-32 years old) and 8 Swedish (67-81 years old) FAP ATTR V30M patients, 4 Japanese gene carriers of ATTR V30M, and 24 Japanese healthy controls which had no genetic mutations of TTR. To detect the presence of autoantibodies against ATTR in these serum samples, enzyme-linked immunosorbent assay (ELISA) using recombinant ATTR V30M protein was immobilized on a polystyrene microtiter plates. Correlation between data of the ELISA and age of the subjects or duration of FAP was investigated.

**Results:** Three of 25 Japanese and 5 of 8 Swedish FAP ATTR V30M patients were found to have autoantibodies against ATTR, and these 8 patients were all late onset cases. Moreover, significant positive correlation between the presence of the antibody and age was observed in all the patients examined ( $r=0.77$ ,  $p < 0.05$ ). We are now trying to detect conformational epitope binding to the autoantibodies against ATTR V30M. Our preliminary data suggested the possibility that there were some epitopes at positions 24-35 or 105-115 of ATTRV30M.

**Conclusions:** Age-dependent increase in the incidence of autoantibodies was observed in patients with FAP ATTR V30M. This phenomenon may influence the clinicopathological differences between both early (<50 years old) and late (more than 50 years old) onset cases of the disease.

#### PA 24

#### Fragmentation of heparan sulfate chains reduce Islet Amyloid Polypeptide amyloid load in islets of Langerhans

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In 90 % of individuals with type 2 diabetes, Islet Amyloid Polypeptide (IAPP) derived amyloid deposits are found throughout the islets of Langerhans. In addition to IAPP, the deposits also contain the sulfated glycosaminoglycan heparan sulfate (HS) (1). Although studies show that HS chain length is critical in SAA amyloidosis (2), whether HS plays an active role in islet amyloidogenesis is not clear.

**Aim:** Investigate how fragmented HS chains and exogenously added short heparin fragments affect IAPP amyloid load in isolated islets.

**Methods:** Pancreatic islets were isolated from the following mouse strains; 1) transgenic mice overexpressing human IAPP and heparanase, 2) transgenic mice overexpressing hIAPP. Also, islets from human donors were analyzed (provided by the Nordic network for Clinical Transplantation). Islets were cultured in high glucose medium which stimulates IAPP secretion and subsequently amyloid deposition. After three weeks, the islets were fixed and amyloid load was visualized with thioflavin S staining.

**Results:** Islets isolated from mice overexpressing heparanase contained less amyloid than islets without heparanase overexpression. ( $p<0.0001$ ).

Addition of heparin fragments (12-mer) at a concentration of 150 nM in the culture of hIAPP overexpressing islets resulted in a significant reduction of IAPP amyloid load. This effect of heparin is size-dependent, as shorter fragments failed to reduce amyloid formation.

Preliminary results from human islets cultured in the presence of the heparin fragments also showed reduced amyloid load.

**Discussion:** Extensive degradation of HS by heparanase attenuated IAPP amyloidosis in islets. This indicates that IAPP's interaction with HS chains plays a role in aggregation of the peptides, where HS may function as scaffold for fibril formation. This function of HS can be attenuated by heparin fragments.

**Conclusion:** Fragmented HS chains and exogenously added heparin fragments reduce IAPP amyloid load in isolated islets of Langerhans.

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#### PA 25

#### Functional bacterial amyloid: make good use of a dangerous fold

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Amyloid fibrils are typically associated with misfolded proteins leading to neurodegenerative diseases such as Alzheimer's and Parkinson's. However, Nature makes good use of amyloid in many different tasks across the full range of life, from melanosome formation and peptide hormone storage in humans to insect cocoons and spore coating in fungi. We want to uncover the mechanisms for producing such controlled amyloid.

Amyloids are found in up to half of all bacterial species in different habitats (1, 2). *E. coli*'s curli, the best characterized bacterial amyloid system, reveal a remarkable chaperoning system for directing export and spatially controlled nucleation of the main curli protein (CsgA) onto the bacterial surface. We have discovered a new operon (Fap) coding for amyloid in *Pseudomonas* (3). Like the curli operon, it contains 6 proteins of which two are highly homologous and contain numerous internal repeats. Nevertheless, the proteins are not homologous with those of *E. coli* and show a different operon structure, and the internal repeats of the major amyloid protein FapC are separated by linker sequences of variable lengths. Expression of the Fap operon in *E. coli* leads to strongly enhanced biofilm formation. We have purified FapC and CsgA to study fibrillation *in vitro*. Neither protein forms cytotoxic oligomer during fibrillation, indicating an efficient and robust fibrillation pathway (4). Remarkably, a thioredoxin motif in FapC (absent in CsgA), has a major impact on fibril morphology

and may play a role in the extent of biofilm formation. This indicates that intrinsic sequence as well as chaperones regulate functional amyloid formation.

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#### **PA 26**

#### **Amyloidosis induced by variants of Human Apolipoprotein A-I: effect of cellular microenvironment**

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**Background:** Nonhereditary apolipoprotein A-I (apoA-I) amyloid is characterized by deposits of nonvariant protein in atherosclerotic arteries (1). In addition, mutant forms of apoA-I have been involved in familial amyloidosis (2). In order to understand the molecular events determining protein misfolding it is clue to study the complex scenario in which apoA-I circulates during its lifetime. Factors such as protein stability, chemical modifications pH or pro-inflammatory environment could be critical to shift protein structure into other conformation prone to aggregate or induce pathology (3).

**Objective:** We studied biophysical and biochemical events that could induce amyloidosis by Wild type apoA-I (Wt) and by two natural mutants detected in patients (Gly26Arg y Lys107-0).

**Methods:** Proteins were incubated under different conditions (low pH, in the presence of activated neutrophils, etc) and structural features associated to protein folding were analyzed by fluorescence spectroscopy and western blotting. Aggregates were characterized by Atomic Force Microscopy.

**Results:** Mildly acidic pH promoted misfolding, aggregation, and increased binding of apoA-I variants to extracellular matrix elements, thus favoring protein deposition as amyloid like-complexes. In addition, activated neutrophils and oxidative/proteolytic cleavage of the proteins gave rise to pro amyloidogenic products. Both pathological variants were less stable than Wt but only Lys107-0 showed higher tendency to aggregate.

**Discussion:** Drastic structural changes do not seem required in order to induce protein pro-amyloid processing, as weaker bonding at protein contacts could shift the equilibrium between native and pathological conformations.

**Conclusion:** Our results strongly suggest that different events taking place in chronic inflammatory hallmark, such as atherosclerosis conduct to a pro-amyloidogenic processing of apoA-I which in turn could impair vascular disease.

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#### **PA 27**

#### **Role of mutations in the cellular internalization of amyloidogenic light chains into cardiomyocytes**

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Light chain (AL) amyloidosis is characterized by the misfolding of monoclonal immunoglobulin light chains, accumulating as amyloid fibrils in vital organs. These amyloidogenic light chains have undergone somatic hypermutation. We are interested in the role that specific somatic mutations found in AL proteins play in AL pathophysiology. Multiple reports have indicated that amyloidogenic light

chains internalize into a variety of cell types, but these studies used urine-derived proteins without listing protein sequence information. As a result, the role of somatic mutations in amyloidogenic protein internalization has not been yet studied. Here, we characterize the internalization of recombinant full length light chain from an AL amyloidosis patient (AL-09) with cardiac involvement into cardiomyocytes. We also characterize the internalization of AL-09's corresponding germline protein (kl O18/O8), devoid of somatic mutations, and three AL-09 restorative mutations (I34N, Q42K, and H87Y) previously characterized for their role in light chain variable domain structure, stability, and amyloid formation kinetics. The five proteins adopt a  $\square$ -sheet structure as is expected for immunoglobulin light chains. All proteins share a common internalization pathway observed by confocal microscopy, although they internalize at different rates correlating with the rates of amyloid formation of their corresponding variable domain. Oregon green (OG) labeled AL-09 showed the most rapid internalization into cardiomyocytes, while OG-Q42K presented the slowest rate of internalization overall. The proteins caused different degrees of phenotypic transformations (lysosomal expansion). OG- $\square$ I O18/O8 exhibited a diverse profile of levels of lysosomal expansion within the same culture. The percent of cells with internalized protein after 24 hours correlates with the kinetics of amyloid formation of the corresponding variable domain, although the percent of cells with internalized protein does not follow a linear trend as the rate of amyloid formation does for those mutants.

#### PA 28

#### Atomic structure of a nanobody trapped domain swapped dimer of an amyloidogenic $\beta$ 2-microglobulin variant

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Atomic-level structural investigation of the key conformational intermediates of amyloidogenesis remains a challenge. Here we demonstrate the utility of nanobodies to trap and characterize intermediates of  $\beta$ 2-microglobulin ( $\beta$ 2m) amyloidogenesis by X-ray crystallography. For this purpose, we selected five single domain antibodies that block the fibrillogenesis of a proteolytic amyloidogenic fragment of  $\beta$ 2m ( $\Delta$ N6 $\beta$ 2m). The crystal structure of  $\Delta$ N6 $\beta$ 2m in complex with one of these nanobodies (Nb24) identifies domain swapping as a plausible mechanism of self-association of this amyloidogenic protein. In the swapped dimer, two extended hinge loops – corresponding to the heptapeptide NHVTLSQ that forms amyloid in isolation – are unmasked and fold into a new two-stranded antiparallel  $\beta$ -sheet. The  $\beta$ -strands of this sheet are prone to self-associate and stack perpendicular to the direction of the strands to build large intermolecular  $\beta$ -sheets that run parallel to the axis of growing oligomers, providing an elongation mechanism by self-templated growth.

#### PA 29

#### The C-terminal sequence of type F apolipoprotein A-II inhibits the polymerization of apolipoprotein A-II into amyloid fibrils in mice

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In murine senile amyloidosis, misfolded serum apolipoprotein A-II (apoA-II) deposits as amyloid fibrils (AApoAII), a process associated with aging. Some mouse strains having type C apoA-II (e.g., R1.P1-Apoa2<sup>C</sup>) exhibit a high incidence of severe systemic amyloidosis with aging, while other strains having type A apoA-II (e.g., C57BL/6J) exhibit a moderate incidence of amyloidosis. In our previous study, we showed that the N- and C-terminal amino acid sequences of apoA-II are critical for polymerization into amyloid fibrils *in vitro*, and both sequences are common between type A and C apoA-IIs (Sawashita *et al. Biochim Biophys Acta* 2009).

In this study, we hypothesized that some amino acid substitutions in these N- and C-terminal amyloidogenic sequences of apoA-II might inhibit the polymerization of apoA-II into amyloid fibrils. We have developed two strains of congenic mice that have type F apoA-II originating from *Mus Spretus* in the genetic backgrounds of R1.P1-Apoa2<sup>C</sup> and C57BL/6J. Type F apoA-II contained four amino acid substitutions in these amyloidogenic regions of type A and C apoA-II proteins. We found that those strains having type F apoA-II were absolutely resistant to amyloidosis even after the injection of type C AApoAII amyloid fibrils. We demonstrated *in vitro* that type F N- and C-terminal peptides did not polymerize into amyloid fibrils, and that type F C-terminal peptide was a strong inhibitor of their polymerization into amyloid fibrils. Thus, we have succeeded in suppressing amyloid deposition in amyloid-susceptible mice after the induction of amyloidosis by treatment with the C-terminal peptide of type F apoA-II.

We have shown that the C-terminal sequence of type F apoA-II plays an important role as an inhibitor of polymerization into amyloid fibrils *in vitro* and *in vivo*, and provide a new model system for investigating inhibitory mechanisms against amyloidosis *in vivo* and *in vitro*.

### PA 30

#### Molecular Strategies to Prevent Corneal dystrophies

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Corneal dystrophies (CDs) constitute a group of hereditary diseases afflicting both eyes, in which mutant forms of the protein TGFB1p (Transforming Growth Factor Beta Induced Protein) precipitate in the cornea, leading to loss of vision. TGFB1p consists of four different Fas domains, and most mutations are found in the C-terminal domain (Fas4). We have established that mutations in Fas4 alone recapitulate the aggregative behavior of full-length TGFB1p and that increased aggregation tendencies may be induced by mutations that reduce stability or alter surface electrostatics.

Our long-term goal is to find potential small molecular binders of Fas4 that reduce TGFB1p's aggregation tendency and halt the loss of vision. Our approach is to identify potential Fas4 binders which may stabilize it against unfolding or increase electrostatic repulsion between Fas4 monomers. Accordingly we have carried out a high-throughput virtual screening of 4 million compounds for their ability to bind to Fas4. Through progressively more accurate virtual docking, five compounds were identified as the most promising to reduce aggregation. When incubated with the aggregation-enhanced Fas4 R555Q mutant, two compounds changed the aggregation tendency slightly in a Thioflavin T-based plate reader assay. One compound increased the aggregation lag time while another decreased it. We are currently elucidating the molecular mechanisms underlying these effects.

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### PA 31

#### Amyloid fibrils associated with neurodegenerative disorders enhance HIV infection

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We have previously shown that fragments of the prostatic acid phosphatase form amyloid fibrils termed SEVI (Semen-derived Enhancer of Virus Infection). SEVI drastically enhances HIV attachment and infection and may play an important role in sexual transmission of the virus. Several studies reported that the quantity of amyloid deposits associated with neurodegenerative disorders, such as Amyloid- $\beta$  (A $\beta$ ) and  $\alpha$ -synuclein is increased in HIV patients and that amyloid accumulation might fuel the development of the AIDS dementia complex (ADC), a common neurological disorder associated with HIV infection. Furthermore, HIV induces inflammation of the brain and thus generates conditions triggering amyloid formation. Our study aims to clarify whether "brain amyloid fibrils" promote neuropathology of HIV infection with implications for the development of the ADC and Neuro-AIDS.

To examine whether the amyloid fibrils associated with neurodegenerative disorders enhance HIV infection we investigated their effects on viral entry and replication in several cell lines. We show that these fibrils dose-dependently enhance HIV infection of brain derived cells. Interestingly, zeta-potential measurements revealed an overall negative surface charge of A $\beta$  and  $\alpha$ -synuclein fibrils whereas SEVI amyloid has a positive charge. It is proposed that SEVI enhances virus infection by counteracting the electrostatic repulsion between the negatively charged viral and cellular membranes, thereby facilitating attachment of virus to the cells. Given that negatively charged A $\beta$ 1-42/  $\alpha$ -synuclein fibrils also enhance infection, we assume that the interaction of amyloid with negatively charged membranes of virions and cells is not the sole determinant for infectivity enhancement. Currently, we aim to clarify the mechanism underlying "brain amyloid" mediated infectivity enhancement, and to test the activity of patient derived amyloid on viral infection.

In summary our data demonstrates that fibrils associated with neurodegenerative disorders enhance HIV infection *in vitro*. However, their implication for ADC remains to be further elucidated.

International Graduate School In Molecular Medicine Ulm (grant to FA)

## PA 32

### The ubiquitin-proteasome system and proteolytic content in ATTR

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ATTR is a systemic conformational disease characterized by the extracellular deposition of transthyretin (TTR) amyloid fibers <sup>1,2</sup>. The most accepted model for this crippling and fatal disease relates TTR tetramer instability with TTR point mutations <sup>3</sup>. However there is increased evidence that non-genetic factors are involved in ATTR pathogenesis <sup>4</sup>. Due to its overall importance, the UPS is involved in many pathological developments and alterations of the intracellular ubiquitin–proteasome pathway are often found in neurodegenerative and inflammatory disorders. Although proteasomes are intracellular they are also detected in the blood serum (circulating proteasomes) and its concentration correlates with malignancies and severe inflammatory conditions <sup>5-7</sup>. Moreover, it was already observed that proteolytic activity is relevant in the context of amyloid diseases, such as Alzheimer and Parkinson's disease <sup>8,9</sup>, reason why we dedicated ourselves to understanding the involvement of proteasome in ATTR pathology.

Our intent is to explore the overall proteolytic content in V30M ATTR patients' plasma and to study the characteristics and functions of the ubiquitin-proteasome system as a crucial mechanism for removing altered proteins, using yeast as a model that expresses different TTR variants.

Plasma samples were collected from healthy subjects (controls) and ATTR patients heterozygous for the V30M mutation. Analysis was made by two-dimensional gel electrophoresis and western blot against transthyretin and fibrinogen. Different *S. cerevisiae* strains were transformed with several TTR variants. Overall proteolytic content was measured.

Using proteomics methodologies we observed higher number of protein fragments in plasma from ATTR individuals, indicative of increased proteolytic activity. In fact, we observed that there is an altered proteolytic content in the plasma samples from ATTR Portuguese V30M patients, regarding healthy subjects.

Working with transformed yeast allowed us to conclude that a specific proteolytic response is elicited only in the presence of amyloidogenic structures and that the ubiquitin-proteasome system appears to be involved in this response.

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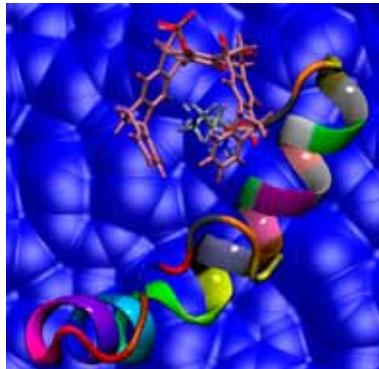
**PA 33****Mechanisms of aggregation and toxicity inhibition in amyloidogenic peptides by Molecular Tweezers: Molecular Dynamics and Quantum Mechanics/Molecular Mechanics studies**

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Despite the many scientific advances during the last decades, the development of effective therapeutic treatments of amyloid-related diseases like Alzheimer is still a big challenge. We have developed theoretical models for the interaction of amyloidogenic peptides with small molecules such as Molecular Tweezers (MTs). MTs have been introduced as promising agents that prevent aberrant aggregation and toxicity of amyloid peptides and proteins without inducing side effects.<sup>1,2</sup> They are small molecules with an electron-rich inner surface that allows them to host electron-poor residues such as the amino acid lysine, which is found in key positions in both the beta amyloid and the islet amyloid polypeptide. We use Molecular Dynamics simulations and Quantum Mechanics/Molecular Mechanics techniques to elucidate the action mechanisms of the Molecular Tweezers in the presence of amyloidogenic peptides.



Our models provide a molecular explanation for the selectivity of the MTs towards the amino acid lysine with respect to arginine. We also investigated the role of the N-terminus and the Cystein2 – Cystein7 disulfide bridge in the islet amyloid polypeptide. Furthermore, by using truncated model peptides we were able to understand the disaggregation activity of the tweezers even in the absence of Lysine. In addition, by investigating the interactions between the MT and the beta amyloid

monomers and oligomers we were able to observe the fast dissolution of the beta amyloid aggregates and the decreasing of the beta-sheet character in the tweezer – amyloid complex *in silico*. Our work at the interface between theory and experiment provides a better molecular understanding of amyloid aggregation and opens the way for the development of more efficient therapeutical agents.

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#### PA 34

#### Differences of histopathological features and amyloid components among various tissue sites of FAP patients after liver transplantation

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**Introduction:** It has been widely accepted that liver transplantation (LT) is a well-established therapy to halt the symptoms of familial amyloidotic polyneuropathy (FAP). However, even after LT, in some FAP patients, amyloid deposits are progressed gradually. Although, in those patients, wild type (WT) TTR has shown to continuously form amyloid deposition in heart, the pathological circumstances of long-term surviving FAP patients with LT are largely unknown. In this study, to evaluate the pathological circumstances of FAP patients with LT and compare with those without LT, we investigated histopathological features and amyloid components among various tissue sites of autopsied FAP patients.

**Methods:** We investigated histopathological characteristics and WT TTR proportions of amyloid deposition in various tissues of 11 autopsied FAP ATTR V30M patients (four patients died over 10 years after LT and seven patients without LT).

**Results:** All four patients with LT showed severe amyloid deposition in several tissue sites, such as heart, lung and tongue, while, less amyloid deposition was observed in more tissue site, such as thyroid gland, glomerulus of kidney, peripheral nerve and gastrointestinal tract (GIT). Electron microscopic analysis revealed that, although long, straight and parallel fibrils were observed in the patients without LT, short, rigid and random fibrils were observed in those with LT. WT TTR ratios of amyloid deposition were over 90% in most of tissue sites in the patient with LT, but below 25% in spinal cord. In the patients without LT, WT TTR ratios of amyloid deposition in heart, lung and tongue were higher than those in kidney, thyroid gland, peripheral nerve and GIT.

**Conclusions:** Pathological circumstances of FAP after LT are different from those without LT. The degree of TTR amyloid deposition is different in each tissue site. These differences may be caused by WT TTR and/or tissue factors.

#### PA 35

#### A study of the mechanism of fibril formation in spinal bulbar muscular atrophy

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Spinal bulbar muscular atrophy (SBMA) is a member of the polyglutamine (poly-Q) expansion family of diseases. These disorders are associated to the deposition in neuronal cells of aggregates of proteins carrying a poly-Q tract of a size longer than a threshold number of Q residues.

Androgen receptor (AR) is a nuclear hormone receptor that has an intrinsically disordered N-terminal transactivation domain bearing a polyQ tract. Aggregates of this protein have been observed in motor neurons of SBMA patients. Recent studies have indicated that AR aggregation takes place only in the presence of testosterone,<sup>1</sup> the hormone that activates AR, and that cleavage of the receptor by caspase-3 is a crucial event for cytotoxicity<sup>2</sup>.

To reproduce AR aggregation *in vitro* we have developed a strategy for the expression and purification of pathogenic as well as non-pathogenic fragments of the receptor. This has allowed us to characterize the kinetics of aggregation as well as the properties of the resulting aggregates using thioflavin-T binding and Transmission Electron Microscopy.

The results that we have obtained indicate that AR form amyloid-like fibrils with a rate that depend on the length of the poly-Q tract. Studies are now underway to understand the role of the regions flanking the poly-Q tract<sup>3</sup> with the long-term aim of understanding the inter-molecular interactions that cause AR aggregation in SBMA.

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#### PA 36

#### Heparan sulfate/heparin-HDL interaction dissociates serum amyloid A (SAA) from HDL-SAA complex leading to SAA aggregation

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**Background:** The acute-phase protein serum amyloid A (SAA) is an apolipoprotein associated with the high-density lipoprotein (HDL) in plasma. With still unclear causes, SAA can become deposited in organs, causing AA-amyloidosis, a serious complication of several chronic inflammatory disorders. Studies have shown an intimate anatomical and structural relationship between AA-amyloid and heparan sulfate (HS), a sulfated glycosaminoglycan, suggesting a role of HS in the AA-amyloid pathogenic process<sup>1</sup>. We have previously found that mice overexpressing human heparanase, a HS degrading enzyme, were resistant to experimental induction of AA-amyloidosis <sup>2</sup>, demonstrating a decisive role of HS in the disease.

**Objective:** In this study, we aimed to explore the molecular mechanisms of HS interaction with HDL-SAA.

**Methods and Result:** We found that incubation of heparin together with HDL-SAA (isolated from inflamed mouse plasma using ultra centrifugation) resulted in fibrilization of SAA at mild acidic condition (pH 5.0). Characterization of the interaction with surface plasmon resonance (SPR) technique revealed that heparin and HS induced a conformational change of HDL-SAA at pH 5.0, which resulted in the subsequent dissociation of SAA from the HDL lipoparticles. This activity of HS or heparin required a minimum chain length of 12-14 sugar units.

**Discussion/Conclusion:** These results offer an explanation for our early *in vivo* finding that the molecular size of HS is critical for SAA deposition, as shorter sugar chains are insufficient to cause dissociation of SAA from HDL-SAA complex. This finding may have potential value for preventing AA-amyloidosis by specifically targeting HS-SAA interaction.

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**PA 37****Heat shock factor 1 (Hsf1) plays a key role in AApoAII cardiac amyloidosis in mice**

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In mouse senile amyloidosis, misfolded serum apolipoprotein A-II (apoA-II) forms extracellular amyloid fibril deposits (AApoAII) throughout the entire body, excluding the brain. Heavy amyloid deposition in the heart and blood vessels is a characteristic feature of this disease and is associated with aging. Heat shock factor 1 (HSF1) is known to regulate the expression of heat-inducible proteins including a set of heat shock proteins (Hsps) that suppress the formation of protein aggregates and prevent proteotoxicity in many neurodegenerative disorders associated with misfolded intracellular proteins.

In this study, we evaluated the impact of HSF1 on extracellular deposition of AApoAII amyloid fibrils using HSF1-deficient and over-expressing mice. We crossed mice lacking the endogenous Hsf1 gene (*Hsf1*<sup>-/-</sup>) or transgenic mice expressing an activated form of human HSF1 (hHSF1 $\Delta$ RD Tg) with congenic R1.P1-Apoa2c (SAMR1C) mice carrying the amyloidogenic apoA-II allele (*Apoa2c/c*), resulting in R1xICR- *Hsf1*<sup>-/-</sup>, *Apoa2c/c* and R1xB6-hHSF1 $\Delta$ RD Tg, *Apoa2c/c* mice which were used in this study.

We induced amyloidosis through injection of AApoAII amyloid fibrils. After 2 and 4 months, we found that extracellular deposition of amyloid fibrils induces expression of Hsps (Hsp70, Hsp40 and Hsp27), and HSF1 deficiency results in the insufficient induction of Hsps expression, especially in the heart. Compared with wild-type mice, the degree of AApoAII amyloid deposition was significantly increased in the heart of HSF1-deficient mice. During the accelerated progression of cardiac amyloidosis, Hsf1 deficiency caused the deteriorating loss of the cytoskeletal protein  $\alpha$ -actin in the heart, as well as cardiac contractile dysfunction associated with hypertrophic changes. Conversely, over-expression of the active form of human HSF1 reduced amyloid deposition in the heart.

These results suggest that HSF1 expression may be an effective therapeutic strategy for systemic amyloidosis and for cardiac amyloidosis in particular.

**PA 38****Serum-free medium supports amyloid formation from human serum amyloid A in peripheral blood mononuclear cell cultures**

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**Background:** Mouse SAA1.1 was previously shown to undergo amyloid formation in cultures of human peripheral blood mononuclear cells (PBMC) in medium containing fetal calf serum (FCS); human SAA in this system did not form amyloid. However, human SAA-derived amyloid formation recently has been achieved using serum-free medium (SFM).

**Objective:** To understand why SFM is a more permissive milieu for amyloid formation than medium containing FCS.

**Methods:** PBMC were cultured in SFM or 15% FCS medium containing human SAA. Amyloid-enhancing factor was not used. The fate of SAA was compared in the two culture systems using SDS-PAGE, N-terminal sequencing, western blotting, molecular sieve chromatography, and Congo red staining.

**Results and Discussion:** SFM supported rapid and extensive amyloid formation from human SAA. Cell viability was equal to that in FCS medium. N-terminally truncated SAA fragments incapable of fibril formation were generated in FCS medium in absence of PBMC. The same fragments, however, were generated in SFM by PBMC, making it unlikely N-terminal truncation was a significant factor in

differential amyloid production. The biggest difference between SFM and FCS medium was the amount of full-length SAA remaining in medium after 2-day incubation with PBMC. Intact SAA was depleted from SFM during this time, while SAA fragments remained. FCS medium retained intact SAA, in addition to SAA fragments. Selective depletion of intact SAA from SFM coincided with accumulation of SAA in/on cells; cells maintained in FCS medium contained little or no SAA. Molecular sieve chromatography showed SAA in FCS medium and SFM to be a low molecular weight species. Therefore, its retention in FCS medium cannot be explained by HDL binding like that which occurs in medium supplemented with HDL.

**Conclusion:** Rapid transitioning of SAA from medium to cells in SFM but not FCS suggests FCS might contain molecules that block SAA association with cells, a critical step in amyloid formation.

#### PA 39

#### Cardiotoxicity of pre-fibrillar transthyretin oligomers and attenuation by doxycycline

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**Background:** Aggregation of transthyretin (TTR) can lead to amyloid fibril formation and deposition frequently occurring in the myocardium. Pre-fibrillar, oligomeric forms of TTR are cardiotoxic and may be important in TTR cardiac amyloid pathology.

**Objective:** To investigate the response of cardiac cells to amyloidogenic forms of TTR and test the effect of doxycycline on the observed changes.

**Methods:** Rat cardiomyocyte (H9C2) cells were treated with native and oligomeric forms of wild-type (wt), L55P, V30A, or V122I TTR, proteins associated with amyloidotic cardiomyopathy at varying ages of onset. To generate oligomeric TTR, proteins were incubated at 80 °C for 4 h and circular dichroism spectra showed progressive unfolding of TTR at this temperature and time interval. Cardiomyocytes were treated with TTR (0.2 mg/mL) heated for 0, 0.5, 1, 2, and 4 h, and incubated for 4 h in the presence of doxycycline (50:1, dox:TTR). Cell viability was assessed after 48 h treatment (ApotoxGlo Promega kit). Interaction between doxycycline and TTR was investigated using surface plasmon resonance (SPR). Data was analyzed using ANOVA and the unpaired two-tailed t-test; statistical significance was  $p < 0.05$ .

**Results:** Viability was significantly decreased in cells treated with aggregated (4 h) forms of V30A ( $p < 0.05$ ) and L55P TTR ( $p < 0.01$ ). Furthermore, the presence of doxycycline in V30A, L55P, and V122I samples heated for 4 h significantly increased cell viability ( $p < 0.01$ ). No significant decrease in viability was noted in cells treated with the heat denatured wt TTR. Interaction of TTR with doxycycline was confirmed by SPR measurements.

**Conclusions:** These data suggest that aggregated forms of L55P and V30A have a potent effect on cardiac cell viability. In addition, the presence of doxycycline may inhibit the formation of amyloidogenic L55P, V30A or V122I TTR oligomers. Interestingly, the aggregated forms showing the strongest effect on viability were the mutants with earlier disease onsets in the patient population (L55P and V30A).

Supported by NIH grant RO1AG031804 (LHC) and the Young Family Amyloid Research Fund

#### PA 40

#### Potential induction of vaccine-associated amyloid A amyloidosis in white young hens

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Avian amyloidosis is known as systemic AA amyloidosis that comparatively older birds develop secondarily to inflammatory disorders such as tuberculosis (Landman et al., 1998). Avian amyloidosis occurs most frequently in waterfowl such as Pekin ducks. Chicken amyloidosis is mainly known as

amyloid arthropathy in brown layer associated with infection of *Enterococcus faecalis*. There are reports that outbreak of systemic amyloidosis in young layer flocks has induced by the long-repeated inflammatory stimulations such as casein and vaccinations with oil-emulsified bacterins (Rampin et al., 1989). We encountered the irregular outbreak of white growing hen death in the large scale poultry farm from 2009 to 2011. We found AA amyloidosis in growing hen's carcass by postmortem examination. The outbreak in this farm was observed within about three weeks after inoculation of combined vaccine. Therefore, we started the surveillance to investigate the relationship between appearance of avian AA amyloidosis and vaccination program, and characterized etiological evidence of avian AA amyloidosis. Furthermore, to decrease the incidence of avian death with AA amyloidosis, hens were treated with antibiotics after the vaccination or inoculated with different vaccine composed of different ingredients in the combined vaccine. Finally we succeeded in reducing the number of hen death, and characterized avian AA amyloidosis induced by vaccination. From result, we found the potential amyloid deposits in healthy chickens. There is a possibility that the repeated vaccination induce systemic AA amyloidosis in young chickens.

This work was supported by a grant from the Intractable Disease Division, the Ministry of Health and Welfare, a Research Committee for Epochal Diagnosis and Treatment of Amyloidosis in Japan, and supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (Project Code: 22380165), Japan.

#### **PA 41**

#### **Interaction of ataxin 3 oligomers with rat cerebellar granule cells results in dysregulation of calcium homeostasis through different responses**

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Several human neurodegenerative diseases, including spinocerebellar ataxia type 3 Machado-Joseph's disease, Huntington's disease are associated to the expansion over a defined threshold of a polyglutamine (poly Q) encoding triplet in the relative genes. Typically, normal individuals have between 6 to 39 glutamine repeats in the relative genes, while affected individuals have repeats in the 36-180 range. Aggregates of the proteins with expanded poly(Q) display amyloid-like morphological and biophysical properties. Actually, early oligomers have been identified as the primary species responsible for the events associated to deregulation of biochemical homeostasis and in particular to the damage arising from abnormal neuronal Ca<sup>2+</sup> signaling.

In this paper we sought to further elucidate the correlation between the abnormal Ca<sup>2+</sup>-signaling, due to the interaction between neurons and aggregates containing expansions of poly(Q) tracts and the physical-chemical properties of the aggregates themselves. To this purpose we investigated the changes in the intracellular Ca<sup>2+</sup>-levels produced by the interaction of oligomers formed by variants of Ataxin 3 (AT3, provided from the group of Prof Tortora, University of Milan) with cerebellar granule cells.

In the present work we used two expanded forms of AT3 (AT3Q55, AT3Q26) and a truncated variant (AT3/291Δ). AT3Q26 is a non pathological protein variant while AT3/291Δ is truncated at residue 291, thus lacking the poly(Q) expansion. AT3Q55 is a pathological protein variant with an expanded CAG repeat showing a propensity to aggregate at 37°C into mature amyloid fibrils after 48 h incubation under aggregating conditions.

We exploited the different aggregation pathways of the pathological full length protein (AT3Q55), the non pathological protein (AT3Q26) and the truncated form AT3/291Δ to compare the effects produced by aggregates with different morphological and physical-chemical features (characterized by AFM analysis).

#### **PA 42**

#### **Transcriptional profiling of human cardiomyocytes in response to amyloidogenic transthyretin**

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The transthyretin amyloidoses are a subset of protein misfolding disorders characterized by the age-dependent deposition of amorphous aggregates and amyloid fibrils, derived from either wild type or mutant forms of the plasma protein transthyretin. The main sites of deposition are peripheral nerves and heart.

We have established tissue culture model systems to study the molecular basis of the transthyretin amyloidoses using cell lines derived from targets for deposition. Amyloidogenic transthyretin variants decrease cell viability, induce reactive oxygen species and caspase activation whereas a stable and non amyloidogenic transthyretin variant does not. The cytotoxic phenotype could be rescued using small molecules known to kinetically stabilize the native protein.

We have performed microarray studies to define the genes that are differentially expressed in human cardiac cell lines upon exposure to recombinant amyloidogenic transthyretin. Most transcriptional changes were found after 6hr of treatment and were slightly reduced after 24hr. At all time points analyzed (1h, 6h and 24h) there was an up-regulation of genes characteristic of an inflammatory response. This is also the most prevalent finding in the hearts of young transthyretin transgenic animals compared to age-matched non-transgenic animals (Buxbaum et al submitted). The transcriptional analysis in the cardiac cells indicates that at least a portion of the inflammatory transcripts seen in the heart (*in vivo*) comes from the cardiomyocytes themselves and not necessarily from other cells recruited to the heart. In addition, there was an increase in genes related to apoptosis and cell death, extracellular matrix, oxidoreductases, lipid metabolism and proteasomal degradation. Most of these groups of genes were prominent in the hearts of the young transgenic animals compared to age-matched controls. These results suggest that cellular model systems of the transthyretin amyloidoses can be a useful tool to understand the process of tissue damage and identify molecular markers that we can ultimately utilize to analyze the disease process *in vivo*.

#### PA 43

#### Investigation on the functional consequences of amyloidogenic light chains on *Caenorhabditis elegans*

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**Background:** In AL amyloidosis, cardiac involvement is common and is the leading cause of death (1). A direct toxic effect of soluble, pre-fibrillar light chains has been extensively reported. Using the nematode *Caenorhabditis elegans*, whose pharynx is considered an orthologous to the vertebrate heart (2), we designed a behavioral test to assess the cardiotoxic potential of amyloidogenic light-chains (LC), on the basis of their ability to reduce the muscular pumping motions of the nematode's pharynx.

**Methods:** Monoclonal LC were isolated from patients' 24 hours urine collections. In parallel to cardiotoxic amyloidogenic LC, non-cardiotoxic amyloidogenic LC and non-amyloidogenic LC were purified, as controls. The cardiotoxicity of LC were assessed in each patient from clinical, biochemical and instrumental parameters (3). Proteins were purified by ion-exchange chromatography, the homogeneity of the isolated species was assessed by SDS-PAGE and immunoblotting. N2 ancestral nematodes were fed with soluble LC (100 µg/ml) for 2 hours, transferred onto fresh NGM agar seeded with E. coli and the pharyngeal motion was measured (in terms of beats per minute) 20 hours after exposition.

**Results:** Hitherto, eight different LC were tested (4 cardiotoxic amyloidogenic LC, 2 non-cardiotoxic amyloidogenic LC and 2 non-amyloidogenic LC). The pumping rate was significantly impaired of 15% (p<0.05, Student's t-test) only in nematodes fed with the cardiotoxic amyloidogenic LC, whereas solutions containing non-cardiotoxic amyloidogenic LC and non-amyloidogenic LC had no effect.

**Conclusion:** The *C. elegans* was here used, for the first time, to investigate the tissue-specific toxicity of LC. This nematode-based assay is a promising surrogate model for investigating the heart-specific toxicity of amyloidogenic light chains, and could be used for assessing the mechanisms of disease and for a rapid screening of the biological effects of LC.

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**PA 44**

**An alternative fluorescence based systems for studying modulation of HTT amyloid-like aggregation in cells**

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Huntington's disease is an autosomal dominant inherited disease that leads to neurodegeneration. Its causative gene, huntingtin, encodes a protein containing between 6-35 glutamine residues in its N-terminal domain. This protein becomes pathogenic upon expansion of the glutamine rich tract ( $\geq 36$  residues), aggregating in cells as amyloid-like deposits, leading to synaptic and neuronal dysfunction and ultimately disease. In this project, 28 proteins were characterized as potential modifiers of huntingtin (HTT) aggregation. This prediction was based on earlier yeast 2 hybrid screening, siRNA experiments and computational analysis. The ability of the 28 candidate proteins to enhance or suppress HTT aggregation was tested in FRET and BiFC assays, in which fluorescent aggregates are formed following over-expression of the mutant HTT (Exon1). The study identified 8 consistent modifiers of mutant HTT aggregation; 7 suppressors and 1 enhancer. The proteins were involved in transcription regulation, RNA splicing and the ubiquitin-proteasome system, processes well known to regulate protein aggregation.

**PA 45**

**Characterization of ICCs in the GI tract of mouse model for Familial Amyloid Polyneuropathy**

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**Background and aims:** FAP is a fatal neurodegenerative disorder characterized by the extracellular deposition transthyretin (TTR) aggregates, particularly in the PNS. FAP initially presents with symptoms that are associated with sensory and autonomic nervous system (ANS) dysfunction. These include a loss of pain and temperature sensation in the distal limbs, impotence, gastrointestinal disturbances, bladder dysfunction and postural hypotension. As FAP progresses, sensory deficiencies extend to more proximal regions of limbs and cardiac insufficiency and mal-absorption from the gut become common.

Recently, a double-transgenic mouse line has been generated in our lab that expresses the Human mutant V30M TTR (HM30) in the absence of endogenous expression of the transcription factor, Heat shock factor 1 (Hsf1) hereafter referred to as HSFHM.

**Methods:** we used immunohistochemical and flow cytometry techniques in order to measure ckit levels in the GI tract of HM30 and HSFHM30 models, when compared to Wild Type controls at several ages.

**Results:** we show that ckit levels remain unchanged in the GI tract of wild type animals (except in the colon), whereas animal models for FAP present a significant loss of ICCs with age. This might be a reflection of TTR deposition in the GI tract.

**Conclusions:** ICCs are often referred to as pacemaker cells of the intestinal musculature and our results show that ICCs decrease at a higher rate in FAP animal models. We believe further investigation will allow us to understand how ICC's dysfunction is affected in FAP patients.

**PA 46**

**Transthyretin depositon in cultured cells**

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**Background:** Mutant (MT) forms of transthyretin (TTR) cause autosomal dominant hereditary systemic amyloidosis. In addition, wild-type (WT) TTR causes senile systemic amyloidosis, a sporadic disease seen in the elderly. Although destabilization of TTR tetramers is widely believed to be a critical step in amyloid formation, it remains to be fully elucidated how TTR makes amyloid in tissues and causes organ damage.

**Objective:** To investigate TTR deposition in cell culture and examine effects of deposition on cell morphology and viability.

**Methods:** Recombinant WT and MT TTR were expressed in *E. coli* and purified by ammonium sulfate precipitation and DEAE and Sephadex S-100 chromatography. TTR aggregates were formed in vitro by pretreating soluble, tetrameric TTR in 100 mM acetate buffer, pH 4.0, for varying lengths of time. Aggregate preparations were centrifuged to generate pellet and supernatant fractions or used without fractionation. Cultures of smooth muscle cells or cardiomyocytes were incubated with the various TTR preparations for 7 or 20 days. Cell lysates were examined by western analysis. Cultures were stained with Congo red. Cell viability was measured by MTS assay.

**Results:** TTR aggregates in non-fractionated pretreated preparations, as well as resuspended pelleted material, deposited on the surface of cells. The deposits stained weakly with Congo red but were not birefringent. Cultures incubated with non-treated TTR showed no deposits after 7 days and very small deposits after 20 days. Western analyses of cell lysates revealed that both non-treated TTR and TTR pretreated at pH 4.0 bound to and/or were internalized by cells. No decrease in viability was noted for cells incubated with nontreated TTR. Cells incubated with supernatant fractions of pH 4.0-pretreated TTR, however, showed significant cytotoxicity.

**Conclusions:** Pretreated TTR makes abnormal deposits in cultured cells and shows cytotoxic effects. These findings may help us understand pathogenesis of TTR amyloidosis.

#### PA 47

#### The association of macrophages and amyloid deposits in the pathogenesis of familial amyloidotic polyneuropathy

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**Background:** Histopathological observations demonstrated the close association of macrophage and amyloid fibrils in several types of amyloidoses including Alzheimer's disease, AA amyloidosis, and  $\beta$ 2m amyloidosis, macrophage has been hypothesized to play a key role in the pathogenesis of these disorders. However, in transthyretin-related amyloidosis, the association between macrophages and amyloid deposition is unclear because of the lack of systematic studies.

**Objective:** Our aim was to elucidate the association between macrophages and amyloid deposition in the pathogenesis of transthyretin-related FAP.

**Methods:** Autopsy tissue specimens of the heart, kidney, intestine, and liver from six patients with FAP ATTR Val30Met and six control patients were analysed by means of Congo red staining, immunohistochemistry, and immunoelectron microscopy. We also performed ex vivo assay using a macrophage cell line and sections from FAP patients to determine whether macrophages can phagocytose and degrade TTR amyloid fibrils.

**Results:** Double staining with anti-CD68 antibody immunostaining and Congo red staining demonstrated significant macrophage infiltration in tissues from FAP patients compared with control patients. Macrophages were closely associated with amyloid deposition; however, ultrastructural analyses produced no evidence of macrophage phagocytosis of amyloid fibrils. Also, the ex vivo assay using the macrophage cell line showed no degradation of transthyretin amyloid fibrils.

**Discussion:** Our data indicate that macrophages indeed infiltrated amyloid-laden tissue in familial amyloidotic polyneuropathy but that these cells did not phagocytose the amyloid fibrils. However, the increase in number of macrophages in tissues of FAP patients may affect the pathogenesis of FAP, because macrophages correlate well with production of oxidative stress and pro-inflammatory cytokines and with remodeling of extracellular matrix, which are intimately related to amyloidogenesis.

**Conclusion:** The association of macrophages and transthyretin amyloid deposits was demonstrated histopathologically. Further study is therefore needed to clarify the roles of these cells in the pathogenesis of FAP.

**PA 48****TTR V30M oligomeric aggregates inhibit proliferation of renal progenitor cells but maintain their capacity to differentiate into podocytes *in vitro***

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Familial Amyloidotic Polyneuropathy Type I is a neurodegenerative, autosomal dominant disease characterized by systemic extracellular amyloid deposition of a mutant transthyretin, TTR V30M [1]. Renal complications can occur, including nephrotic syndrome and end-stage kidney failure [2]. An unexplored mechanism is the toxicity of early oligomeric TTR aggregates.

A subset of renal progenitor cells (RPC) with self-renewal and multidifferentiation potential exist at the urinary pole of Bowman's capsule in the adult human kidney [3]. These resident progenitor cells can induce regeneration of podocytes and tubular structures of different portions of the nephron [4], which can be critical for preventing irreversible renal failure, and could also be useful in cell therapies.

To assess whether RPC are vulnerable to oligomer toxicity we assessed their growth, survival and differentiation *in vitro*, exposing them to TTRV30M oligomers. The proliferation of RPC, evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, was reduced by 16.3±9.7% and 32.6±6.3% after 48 and 72 hours, respectively, in the presence of the oligomers when compared to controls. However, the fluorescence-activated cell sorting (FACS) analysis of annexin V and propidium iodide did not show induction of apoptosis nor necrosis to any significant extent. Also, cell cycle progression was not significantly influenced by the oligomers. The expression level of the nephrin gene, a marker for podocytes, was evaluated by real time pcr and showed no alterations relatively to controls, so the inherent capacity of these progenitor cells to differentiate into podocytes was apparently not affected by the oligomers.

Further studies are needed to elucidate the mechanisms of toxicity in the kidney, particularly the role of oxidative stress. From this first attempt, we can say that TTRV30M oligomers inhibit the proliferation of renal progenitor cells but do not influence their differentiation into functionally mature podocytes, and thus should not compromise tissue regeneration.

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**PA 49****Predicting Amyloidogenic Propensity of Novel Transthyretin Variants**

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Transthyretin (TTR) is a 55 kDa homotetrameric protein found in human plasma and in the cerebrospinal fluid. More than 100 mutations in the TTR gene have been identified, and most of them considerably increase the amyloidogenic propensity of the protein<sup>1</sup>.

The aim of the present study was to determine the biophysical properties of four mutations identified by resequencing *TTR* in large Danish population samples ( $n > 15.000$ ). These four mutations were: three novel mutations: D38G, T59I and I107T, located at positions previously associated with amyloidosis, and H90N, a mutation suggested to associate with familial amyloid cardiomyopathy<sup>1</sup>.

Amyloidogenic propensity of TTR mutants is often linked to lower kinetic and thermodynamic stability than wild-type (wt)<sup>2</sup>. We determined optimal pH for aggregation, urea stability, and dissociation kinetics. The data was inserted in a combined stability score (CSS) to predict disease phenotype<sup>3</sup>.

The D38G, T59I and H90N mutations had the same range of optimal pH for aggregate formation, and a slightly lower midpoint of urea denaturation ( $C_m$ ) and faster half life ( $t_{1/2}$ ) of dissociation compared to wt. The D38G and T59I mutants had a CSS of 0.961 and 0.976, respectively. The I107T mutant behaved similarly to the most unstable variant reported to date (D18G)<sup>4</sup>, and was purified from inclusion bodies with even lower yields than D18G. When secreted, D18G is severely amyloidogenic *in vivo*, however the mutant protein is to the vast majority degraded in the ER<sup>3</sup>.

In conclusion, D38G and T59I have the potential to cause late-onset TTR cardiac amyloid disease as deemed from their respective CSS. More data remains to determine the relative stability of the H90N variant. We speculate that the I107T mutant is likely poorly amyloidogenic *in vivo* due to essentially complete degradation within the ER.

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**PA 50****Molecular basis of amyloid fibril recognition by the conformation-sensitive B10 antibody fragment**

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The unifying structural feature of amyloid fibrils is the cross-β conformation, differentiating this structure from natively folded polypeptide chains. While diagnosis of amyloid diseases and

development of therapeutic strategies critically rely on the recognition of this conformation with specific molecules such as Congo red, the molecular basis by which amyloid fibrils are recognized is only partially understood. This is specifically the case for the recently emerging group of conformation-specific antibodies.

Here, we address this question by biophysical analysis of the B10 antibody fragment, which was recently shown to recognize a large panel of different amyloid and amyloid-like fibrils in a conformation-specific manner. Hence, it differentiates A $\beta$  fibrils from soluble A $\beta$  oligomers or disaggregated A $\beta$  peptide. We find that this conformational specificity depends on positively charged residue within the B10 antigen binding site. Mutation of these basic residues and masking of negatively charged groups in amyloid fibrils abrogate B10-fibril interaction. Furthermore B10 also recognizes the highly regular and polyanionic biopolymers heparin and DNA. This specificity is also conserved in natural amyloid receptors from the innate immune system, the pattern recognition receptors. Analysis of such a receptor reveals close structural and functional similarities to B10. These data imply that the antigen specificity of B10 is based on a pattern recognition mechanism. This mechanism involves the binding of a highly regular and anionic surface pattern which is presented by many but not all amyloid fibrils and reflects the highly ordered and regular structure of these biopolymers. These results enable a broad range of further studies on the molecular mechanism of amyloid fibril formation, their structure and the targeting of amyloid fibrils by conformation sensitive antibodies.

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#### **PA 51**

#### **Novel fluorescent probes for the spectral assignment of a plethora of protein aggregates**

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The deposition of protein aggregates in various parts of the body is the cause of a wide range of diseases, such as Alzheimer's and the prion diseases. The development of detection agents that selectively label amyloid proteins is important to be able to understand the pathological mechanisms underlying protein aggregation diseases. For a long time, Congo red and Thioflavin T have been the primary choices for the study of protein misfolding pathways. However, these dyes cannot be used to discriminate morphologically distinct protein deposits and have shown limited ability to detect the spectrum of species found during the fibrillation pathway of amyloid proteins. We now present a new generation of amyloid ligands denoted luminescent conjugated oligo- and polythiophenes (LCOs and LCPs). Both LCOs and LCPs have a flexible thiophene backbone and the binding to protein aggregates restricts the conformation of the backbone, which is seen as a change in emission spectrum. Hence, a spectroscopic signature can be achieved for distinct protein aggregates. In addition, the length of the backbone as well as the type of substituents has been shown to influence their properties as amyloid ligands. We have now designed a library of probes with backbone lengths varying between 4-7 thiophene rings and hydrogen or carboxylic acid attached to the end thiophene units. In addition, we have also replaced one or more thiophene units with other molecular moieties. Herein, we present the comparison of probes in respect of their ability to detect early species in the fibrillation pathway of A $\beta$  as well as to spectrally discriminate A $\beta$  and tau in Alzheimer's disease brain tissue. Moreover, we also show how this novel set of probes can be used to study a plethora of protein aggregates found in various kind of human amyloid diseases.

**PA 52**

**Comparison of immunohistochemistry and mass spectrometry in amyloid typing (Ringstudy I)**

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Every amyloidosis identified on tissues needs to be typed in order to provide the clinician with a solid basis for therapeutic considerations. Amyloid typing can be performed in different ways. For routine amyloid typing, two methods are carried out on formalin-fixed paraffin tissue sections. These are immunohistochemistry (IHC), applying appropriate antibodies, and the more recent method of mass spectrometry (MS). Since both methods are based on different principles, their performance with regard to recognizing the chemical amyloid type(s) present is of fundamental interest with respect to an amyloid type specific therapy. Therefore, three groups (see authors' names) have agreed to conduct this comparison during 2003-2009 with MS being performed by S and IHC performed by L in a blinded fashion. Laboratory W submitted 38 samples with amyloids which were typed in agreement with the known chemical type in 14 cases (37%) by MS and in 34 cases (90%) by IHC. Laboratory S provided 10 samples which were typed in line with the known amyloid type in 9 cases (90%) by IHC. Laboratory L provided 48 samples containing 53 amyloids typed with IHC in all cases which were typed in agreement with the IHC data in 29 cases (54%) by MS. In general, most failing MS examinations did not produce any data. The advantage of IHC is not only the ease of performance, but mainly its higher sensitivity by utilization of the morphologic dimension. The advantage of MS is the presentation of amyloidogenic proteins as a guide for their identification. So, during this Ring study I, MS was crucial in the discovery of Semenogelin I amyloid. Therefore, both methods seem to be indispensable for amyloid typing, with IHC for the known amyloids and those which cannot be typed by MS, and MS for novel amyloids and those which cannot be typed by IHC.

R.P.L is owner of amYmed ([www.amymed.net](http://www.amymed.net))

**PA 53**

**Subtyping of amyloidosis by direct proteomic analysis of fixed biopsy samples**

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**Background:** Amyloidosis is a disease where insoluble deposits of specific proteins occur in tissues. Different classes of amyloidosis have been reported and their diagnostic relies on the identification of the associated proteins: up to 10 proteins can be targeted using histochemical approaches. However they can be inconclusive on certain cases, leading to a lack of information about the underlying etiology. Subtyping has been recently demonstrated combining laser capture microdissection (LCM) and mass spectrometry (1).

**Objective:** Here we show that ultrasonic treatment could help for the completion of enzymatic proteolysis (2) of raw biopsy samples even without LCM and to get closer to the clinical routine application for amyloidosis subtyping.

**Methods:** Paraformaldehyde-immobilized tissues (Bouin/AFA) from patients and controls were directly proteolyzed with an ultrasonic probe. Proteolytic peptide mixtures were analyzed by nanoLC-MS/MS (LTQ-FT-Ultra/ThermoFisher).

**Results:** Ultrasonic tryptic treatment on fixed raw tissues allowed performing subtyping of amyloidosis. The results were compared with data obtained with immunohistopathology using the whole series of antibodies for amyloidosis diagnosis. Some significant examples from different tissues and pathologies illustrate our results.

**Discussion:** Diagnosis and subtyping is unambiguous when amyloid deposits represent more than 80% of the tissue. On the opposite, when the percentage goes below 50%, amyloid diagnostic is possible but classification lacks some robustness. In such cases, LCM is still needed to confirm the subtyping.

**Conclusion:** Ultrasonic treatment combined with LC-MS/MS opens the way for an accurate amyloidosis diagnosis and subtyping directly from clinical samples and allows most of the time

avoiding the LCM step which could be time-consuming. This is of particular interest for classes that could not be distinguished by the classical histochemical analysis.

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#### **PA 54**

#### **The amyloidophilic peptide p5 binds rapidly and stably to visceral amyloid *in vivo* - A potential radiotracer for PET/CT imaging**

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**Background:** Visceral amyloid deposits contribute significantly to morbidity and mortality in a number of diverse disorders. Monitoring the extent of amyloid has prognostic value, but these capabilities are limited and generally unavailable outside Europe. Therefore, there is a need for specific, quantitative imaging radiotracers to assist in diagnosis, disease staging and monitoring response to therapy in patients.

**Objective:** The aim of the study was to examine the amyloid binding dynamics of peptide p5 that may be useful for PET/CT imaging using I-124.

**Methods:** We used dynamic PET/CT imaging to study peptide <sup>124</sup>I-p5 in AA and WT mice. Additionally, we performed serial SPECT imaging studies with <sup>125</sup>I-p5 peptide up to 72 h pi in mice with severe systemic AA amyloidosis. Peptide binding was quantified by image analysis and biodistribution methods, and the specific localization of the peptide with amyloid was confirmed at 72 h in micro-autoradiographs.

**Results:** In healthy mice, <sup>124</sup>I-p5 peptide was rapidly cleared by the kidneys with a peak uptake time of ~7 mins where it underwent dehalogenation. Liberated <sup>124</sup>I-iodide appeared in the stomach at a rate of 64 kBq/mL/min with additional uptake in the thyroid. In AA mice however, <sup>124</sup>I-p5 peptide bound rapidly in the liver ( $K_{fast} = 1.96 \pm 0.09 \text{ min}^{-1}$ ). Longer-term studies demonstrated that <sup>125</sup>I-p5 persisted in amyloid-laden organs (liver, spleen, and pancreas) for >72 h with tissue-to-muscle ratios of 168:1, 47:1, and 234:1 for the liver, pancreas and spleen, respectively.

**Discussion:** Radioiodinated p5 peptide rapidly accumulated in visceral AA amyloid deposits and persisted in these organs for >72 h pi. In WT mice, rapid dehalogenation of p5 peptide occurred in the kidneys, resulting in rapid clearance of unbound I-125. Sustained binding to amyloid deposits seen in diseased mice render this peptide suitable for visceral amyloid detection in patients by using PET imaging with <sup>124</sup>I-p5.

This work was supported a PHS grant R01DK079984 from the NIDDK  
EM, AS, TR, SK, and JW are co-founders of Solex LLC that has intellectual property associated with the p5 peptide

#### **PA 55**

#### **Diagnosing and typing early amyloidosis using Congo red fluorescence and immunohistochemistry**

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<sup>2</sup>*Reference Center of Amyloid Diseases, Martinsried, Germany*

Early diagnosis and classification of amyloidosis is the most important factor in many amyloid diseases for initiating and achieving a most successful therapy and, in addition, in preventing the otherwise necessary, further diagnostic measures. Unfortunately, it takes years from the onset of the amyloidosis when the clinician has the first suspicion until the bioptic proof of the presence of the amyloid disease. It has been published that this time gap is different in different amyloid diseases, for instance with AA amyloidosis in juvenile rheumatoid arthritis amounting to an average of 2.5 years, in AL amyloidosis

averaging 1.5 year and in ATTR (SSA) amyloidosis on the average of 3.5 years. Since most of the amyloidoses are progressive and fatal diseases, the therapeutic options continue to fade in relation to the duration of the disease without treatment. It is, therefore, absolutely necessary to follow the first suspicion and take appropriate biopsies very early during the onset of the disease. The second step includes the microscopic examination and competent evaluation in an expert laboratory with Congo red stained tissue sections which must be evaluated in bright light, in polarized light and in fluorescent light. In case of negativity, the third step will include the examination of 10 – 20 more sections in a similar manner in order to minimize the sampling error. The fourth step includes an immunohistochemical overlay of the amyloid containing sections, which is the precondition for typing minute amyloid deposits by recognizing the congruence of the two. The poster will show how the amyloid deposits in biopsies of patients with juvenile rheumatoid arthritis, which had been missed at the time of onset of the disease, have retrospectively been identified in biopsies taken at that time.

R.P.L. is owner of amYmed ([www.amymed.net](http://www.amymed.net))

#### **PA 56**

#### **Monitoring amyloid formation and maturation in vitro and in vivo using LCO fluorescence**

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During the latest decade it has become obvious that amyloidogenic proteins and peptides can adopt a number of different non-native conformations. The roles of these different conformations in the amyloid cascade and there respective contribution to disease, have been frequently discussed. Classic amyloid targeting probes like Thioflavine T and Congo Red have proven useful for detection of mature amyloid *in vivo* as well as amyloid-like structures *in vitro*. To meet a growing need for fluorescent probes targeting pre-amyloid states on the misfolding pathway, a new type of probe with the commune name luminescent conjugated oligothiophenes (LCOs) are being developed. This group of probes comprises a number of different molecules with different properties regarding size, color, side chain substitution and binding properties.

The aim of our study is to discriminate between different stages of amyloid formation *in vitro* and *in vivo*, using a selection of these probes. We have monitored *in vitro* amyloid formation of Aβ peptides and recombinant human and mouse prion protein. In addition we have used spectral imaging fluorescence microscopy (Spectraview®) in combination with atomic force microscopy (AFM) to evaluate the forming of different misfolded conformations. In addition we have used different mouse models of Alzheimer's disease at various ages to evaluate the plaque formation *in vivo* and change of amyloid constitution in mice over time. Size exclusion chromatography, Fluorescence lifetime imaging (FLIM) and 2-photon excitation microscopy has also been used as readouts. Mice infected with different prion strains have also been evaluated.

Using a combination of these techniques we are able to discriminate between early and late stages of *in vitro* fibrillation in our different assays. The knowledge, on molecular level, earned from *in vitro* experiments are correlated to findings in brain tissue from mouse models of these cerebral amyloid diseases ranging over different ages and stains.

#### **PA 57**

#### **Digitally reinforced Hematoxylin-eosin slides: First clue in detection of amyloid depositions**

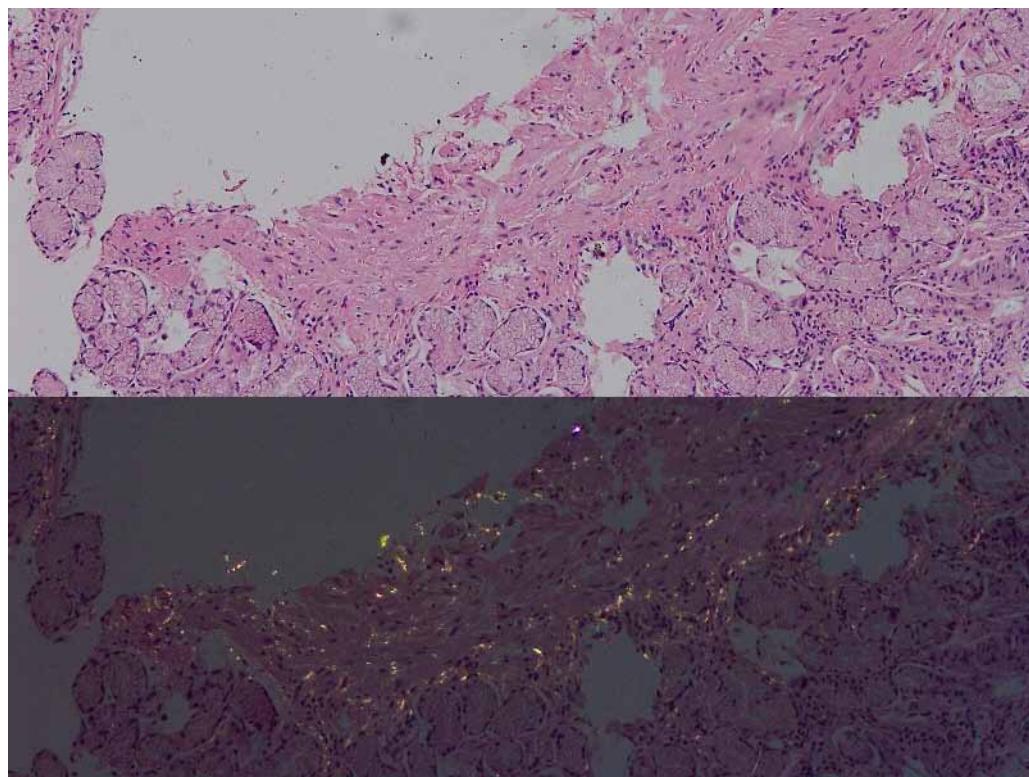
**Burcin Pehlivanoglu, Basak Doganavsargil, Banu Sarsik, Murat Sezak, Sait Sen**  
**Ege University Faculty of Medicine, Department of Pathology, Bornova, Izmir; Turkey**

**Background:** Amyloid has an amorphous, eosinophilic appearance on Hematoxylin-eosin (HE) and Congo-red is the gold standard for diagnosis. However, as in gastrointestinal (GI) endoscopic biopsies, tissue samples could be very small in size for additional staining processes. We recently realized that the Toluidin-blue stained amyloid deposits show birefringence in digitally photographed (digitally reinforced) sections. In this study, we searched the potential power of this technique to detect amyloid depositions in HE stained slides, the most widely used stain in pathology practice.

**Methods:** Ninety-one upper GI endoscopic biopsy specimens of 75 patients with amyloidosis and 20 consecutive control cases without gastric amyloidosis were reevaluated blindly using Olympus BX51 polarising microscope equipped with DP21 camera with stand alone system. Depositions which show green birefringence on HE with digitalized microscopy were considered as positive and results were confirmed using Congo-red.

**Results:** Amyloid deposition was seen on HE in 67 specimens and 10 biopsies were unqualified. The number of false negative cases was 12 while only one false positive case was observed. No amyloid deposition was found in the control group. The sensitivity, specificity, positive and negative predictive values were estimated as 85%, 96%, 99% and 65%, respectively.

**Conclusion:** We concluded digitalized HE sections can be used as a fast search method for diagnosis of amyloidosis. Further investigation is needed on this matter. However, as the technique improves, it may predict the positivity of Congo-red.



#### PA 58

#### Proteomics analysis of amyloid deposits with sequence/structure analysis of light chain proteins from AL patients

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Light chain amyloidosis (AL) is characterized by the secretion of monoclonal immunoglobulin light chains that misfold as amyloid fibrils, causing organ failure and death. Current treatments include chemotherapy and autologous stem cell transplant, resulting in median overall survival rates of 12 to 40 months [1]. The diagnosis of amyloidosis is traditionally made by histopathologic examination and histochemical stains such as Congo red [2]. We recently reported the development of a specific and sensitive novel test using laser microdissection of amyloid deposits analyzed by tandem mass

spectrometry (MS)-based proteomic analysis [3]. We applied this technology to compare amyloid deposits from AL amyloidosis patients enrolled in a Phase III clinical trial comparing the use of conventional dose chemotherapy with autologous stem cell transplant. We selected 14 patients that have differences in hematologic response after 1 year (complete response, partial response, stable disease (no response) and performed a proteomic analysis of the proteins associated with the light chain amyloid from these patients. We conducted a comparative analysis of the immunoglobulin light chain protein sequences found in the amyloid deposits with the sequences derived from the cDNA light chain sequencing of bone marrow plasma cells. We have analyzed the location of somatic mutations and compared it to an expanded sequence database from our previously published work. Our results show that we are able to match the sequence derived from plasma cell cDNA with peptides derived from the mass spectrometry analysis of the amyloid deposits in 10 cases. Some accessory proteins are common to all of the patients while there are some proteins that are uniquely found in patients with particular response. This proof of principle study provides important information regarding the role of both the light chain sequence and the proteins associated with light chain amyloid in AL amyloidosis.

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2. Howie, A.J., et al., *Physical basis of colors seen in Congo red-stained amyloid in polarized light*. Lab Invest, 2008. 88(3): p. 232-42.
3. Vrana, J.A., et al., *Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens*. Blood, 2009. 114(24): p. 4957-9.

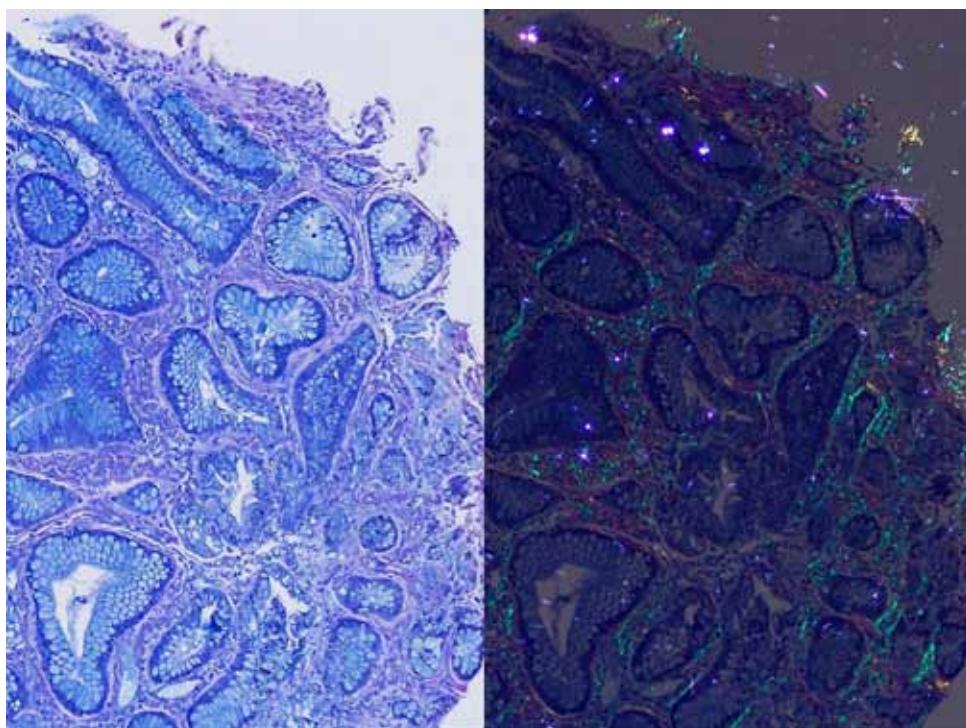
#### PA 59

#### **Digitally reinforced Toluidine blue can safely be used for detection of amyloid depositions**

**Sait Sen**, Banu Sarsik, Burcin Pehlivanoglu, Murat Sezak, Basak Doganavargil

*Department of Pathology, Ege University, School of Medicine, Izmir, Turkey*

Gastrointestinal (GI) amyloidosis is not infrequent and Congo-red is required for definitive diagnosis. However, GI endoscopic biopsies can be too small in size for additional stains. In our center, Toluidine-blue, which is also a metachromatic dye is used as a routine reflex stain for *H.pylori* detection.



We realized that Toluidin-blue stained amyloid deposits show birefringence in digitally photographed (digitally reinforced) sections in addition to expected metachromasia and we searched its potential value in detecting amyloid depositions.

Ninety-one upper GI endoscopic biopsy specimens of 75 patients with amyloidosis were blind-reviewed by using Olympus BX51 polarising microscope equipped with DP21 camera with stand alone system. Twenty consecutive patients without gastric amyloidosis were selected as control group. Depositions which show typical metachromasia and green birefringence with Toluidine-blue were considered as positive. Results were confirmed using Congo-red.

Of the 91 Congo-red confirmed amyloid positive biopsies, 10 were unqualified and 70 showed metachromasia and green birefringence with Toluidine-blue. One case was considered as false positive and the number of false negative cases was 8. No positivity was found in the control group. The sensitivity, specificity, positive and negative predictive values were estimated as 90%, 96%, 99% and 74%, respectively.

We concluded that digitally reinforced Toluidine-blue can be used safely in diagnosis of amyloidosis, particularly in laboratories which use Toluidine-blue routinely, by eliminating the need for extra Congo-red stain, thus providing opportunity to conserve tissue samples.

#### **PA 60**

#### **The novel polybasic peptide p5R can be used to identify murine and human amyloid deposits *in vitro* and *in vivo***

**Jonathan S. Wall**<sup>1,2</sup>, Tina Richey<sup>1</sup>, Alan Stuckey<sup>2</sup>, Angela Williams<sup>1</sup>, Ying Huang<sup>1</sup>, Emily Martin<sup>1</sup>, Sallie Macy<sup>1</sup>, and Stephen J. Kennel<sup>1,2</sup>

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**Background:** Although recent advances have been made in the translation of novel tracers for imaging A $\beta$  amyloid cerebral disease, routine methods for imaging the whole body distribution of visceral amyloidosis are unavailable in the USA.

**Objective:** To examine the efficacy of an arginine-substituted peptide, p5R, for detecting amyloid *in vivo* and *in vitro*.

**Methods:** Peptide p5R binding to *ex vivo* AA amyloid was assessed using a tissue extract binding assay. The dynamic distribution of  $^{124}\text{I}$ -p5R was measured in mice with AA by using dynamic PET imaging. Persistent reactivity of p5R in amyloid *in vivo* was demonstrated using serial SPECT imaging up to 72 hrs pi. Preferential binding of p5R with amyloid was confirmed by autoradiography. Binding characteristics were compared with those of the p5 peptide.

**Results:** In healthy mice, the p5R peptide was rapidly cleared from the circulation by the kidneys where it was catabolized and dehalogenated. In contrast, in mice with AA amyloidosis, p5R accumulated in amyloid-laden tissues within 1 h post-injection and remained visible in SPECT images as late as 72 h pi providing tissue-to-muscle ratios of 80:1 and 143:1 in the liver and spleen, respectively. *In vitro*, the  $^{125}\text{I}$ -p5R was found to bind via electrostatic interactions to amyloid extracts with high affinity -approximately 10-fold more avidly than p5. Both biotinylated and  $^{125}\text{I}$ -labeled p5R peptide could be used to accurately detect many amyloid forms in human, murine, canine, and feline formalin-fixed, paraffin-embedded tissue sections.

**Discussion:** Based on these findings, we posit that p5R will be an effective agent for detecting amyloid deposits *in vivo* and *in ex vivo*. Due to its faster clearance from the kidney and enhanced binding to amyloid, relative to p5, p5R is optimized for use as a radiotracer for the non-invasive molecular imaging of visceral amyloid deposits in patients.

This work was supported a PHS grant R01DK079984 from the NIDDK

EM, AS, TR, SK, and JW are co-founders of Solex LLC that has intellectual property associated with the p5 peptide

#### **PA 61**

#### **Tandem Mass Spectrometry Analysis of Protein Deposits in Human Subcutaneous Fat Tissues of a Patient with Immunoglobulin Light Chain Amyloidosis: *De novo* Sequencing and Post-translational Modifications**

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For the systemic amyloidosis of immunoglobulin (Ig) light chains (LCs), we have found that deposited LCs are extensively processed, especially at the C-terminus, leading to fragment patterns that differ from patient to patient. We are presently using both primary mass spectrometry (MS) and tandem (MS/MS) methods to compare LC fragments in amyloid deposits from fat biopsies.

The fat biopsy described herein showed deposition of Ig λLC proteins (3+ score, Congo red stain). No cDNA information of the LC gene is available. Proteins extracted from the biopsy sample were subjected to 2D gel analysis. Tryptic peptides derived from gel spots were analyzed with 1) a Reflex IV™ MALDI-TOF MS (Bruker), 2) an ultraflexeXtreme™ MALDI-TOF/TOF MS (Bruker) and 3) an LTQ-Orbitrap™ MS (ThermoFisher) with an Acuity nanoUPLC (Waters) and TriVersa NanoMate™ robot (Advion). Data was analyzed with MASCOT™; peak assignments were verified manually.

The amino acid sequence and post-translational modifications of the Ig LC were determined by de novo sequencing with TOF/TOF MS and with HCD fragmentation followed by detection in the Orbitrap. Mass fingerprinting searches on the MALDI-TOF MS data returned a hit for λLC constant region. Other proteins found in the spots included clusterin, serum amyloid P-component, APOA4 and APOE.

As the MW decreased, the abundances of the peaks for constant region peptides, e.g., C 4-22, diminished, while the serially truncated products from this peptide appeared. LC/MS/MS data confirmed the peak assignments. Sequences for some variable region peptides were assigned and aligned with germline gene IGLV1-51. MALDI-TOF/TOF MS revealed the oxidation of Trp (W) to kynurenine and N-formylkynurenine.

This research is supported by NIH grants P41 RR10888, S10 RR15942 and S10 RR20946 and the BUMC Amyloid Treatment and Research Program. We thank Bruker Daltonics for access to the Bruker ultraflexeXtreme™.

## PA 62

### Amyloid-reactive peptides bind MelA<sup>+</sup> melanocytes and extracellular melanin in human, canine and murine melanoma tumors

**Jonathan S. Wall**<sup>1,2</sup>, Amy K. LeBlanc<sup>2</sup>, Tina Richey<sup>1</sup>, Alan Stuckey<sup>2</sup>, Emily Martin<sup>1</sup>, Sallie Macy<sup>1</sup>, Robert Donnell<sup>3</sup>, Laurentia Nodit<sup>4</sup> and Stephen J. Kennel<sup>1,2</sup>

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**Background:** Melanoma is the most deadly form of skin cancer with >70,000 individuals diagnosed in 2011 in the USA. Melanocytes are the site of one of the naturally occurring amyloid fibrils formed by the Pmel17 protein in the low pH environment of the melanosome. During a routine screen of HSPG-reactive peptides binding to human tumors in formalin-fixed paraffin-embedded tissues, we identified certain reagents that bound melanocytic melanoma tumors.

**Objective:** To demonstrate the efficacy of heparin-binding peptides for targeting melanoma tumors in tissue sections and in vivo. We hypothesized that reactive peptides bind hypersulfated HSPG on the surface of melanoma tumor cells and may interact with intracellular amyloid fibrils composed of Pmel17 in the melanosome.

**Method:** Peptides were synthesized, purified by HPLC, labeled with biotin and used for histochemical staining of melanoma-laden FFPE tissue sections. Furthermore, radioiodinated peptides were used to detect pulmonary colonies of B16F10 cells in C57BL/6 mice by using small animal SPECT/CT imaging and micro-autoradiography.

**Results:** Biotinyl-peptides were shown to bind to human and canine melanin-producing (MelA<sup>+</sup>) melanoma cells in tissue sections. The <sup>125</sup>I-labeled peptides, but not control peptide, co-localized with pulmonary B16F10 murine melanoma tumor lesions. This was confirmed by using microautoradiography. Binding of the <sup>125</sup>I-labeled peptide was specific for MelA<sup>+</sup> melanocytes within pulmonary and metastatic tumors. Additionally, radiotracer was observed in extracellular melanin colocalized with perlecan.

**Discussion:** Our data indicate that amyloid-reactive peptides, such as p5R, also react with MelA<sup>+</sup> melanoma cells. Binding was dependent on the presence of melanin and therefore Pmel17 fibrils in

the melanosome. Although preliminary, these data demonstrate a phenotypic homology between amyloid and melanoma due to either the presence of common HSPG molecules or the amyloid-like fibrils. Regardless, these peptides, or similar reagents, may be novel probes for the radiodetection and targeted radiotherapy of melanoma *in situ*.

This work was supported by the University of Tennessee Molecular Imaging and Translational Research Program.

EM, AS, TR, SK, and JW are co-founders of Solex LLC that has intellectual property associated with the p5 peptide

#### PA 63

#### ***In vivo biodistribution of the amyloid-reactive peptide, p5, correlates with ex vivo amyloid quantitation based on Congo red tissue staining***

**Jonathan S. Wall**<sup>1,2</sup>, Tina Richey<sup>1</sup>, Emily B. Martin<sup>1</sup>, Alan Stuckey<sup>2</sup>, Angela Williams<sup>1</sup>, Sallie Macy<sup>1</sup>, and Stephen J. Kennel<sup>1,2</sup>

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**Background:** We have recently identified a peptide, designated p5 that binds preferentially and with high avidity *in vivo* to amyloid deposits and that can be used to image amyloid deposits *in situ* using a mouse model of inflammation-associated (AA) amyloidosis.

**Objectives:** To demonstrate that *in vivo* reactivity of radioiodinated peptide p5 correlates with amyloid load. This correlation would validate imaging with radioiodinated p5 as a non-invasive, quantitative assessment of amyloid load in patients with visceral amyloidosis.

**Methods:** We have compared the binding of <sup>125</sup>I-p5 in amyloid-laden tissues with amyloid burden, assessed using Congo red tissue staining. Mice with AA were administered ~ 20 µCi of <sup>125</sup>I-p5 and sacrificed 2 h pi. Tissues were analyzed for radioactivity and were also formalin-fixed, paraffin-embedded and sectioned for autoradiography and Congo red staining. The amyloid burden in each tissue was determined by Congo red scoring (0 – 4+) and by measuring the area occupied by Congo red-birefringent material ( $\mu\text{m}^3$ ) in microscope images.

**Results:** Peptide <sup>125</sup>I-p5 was shown by autoradiography to bind preferentially to amyloid, as evidenced by the coincidence of silver granules with Congo red-birefringent amyloid seen in consecutive tissue sections. Comparison of %ID/g <sup>125</sup>I-p5 values for the liver and spleen with Congo red measurements revealed a significant positive correlation (>0.84) with p values from  $10^{-5}$  -  $10^{-13}$ . Further, we demonstrated a significant positive correlation (0.7) between the amyloid load in the liver and that in the spleen.

**Discussion:** Our data demonstrate a significant correlation between the amount of radioiodinated peptide p5 bound *in vivo* and amyloid load based on Congo red staining. Our findings support the hypothesis that non-invasive imaging with radiolabeled p5 provides an accurate measure of tissue amyloid load as compared to the clinically used histological standard, Congo red, with the obvious advantage of non-dependence on tissue biopsy.

This work was supported a PHS grant R01DK079984 from the NIDDK

EM, AS, TR, SK, and JW are co-founders of Solex LLC that has intellectual property associated with the p5 peptide

#### PA 64

#### **Antibodies specific to AA76, the common species of AAs**

**Toshiyuki Yamada**, Jyunji Sato

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**Background:** In AA amyloidosis, carboxyl terminus-truncated serum amyloid A (SAA), AAs, deposit in tissues. Since the most common AA is 76 residue-length, specific detection of that AA species (AA76) would be useful for diagnosing AA amyloidosis. However, the currently utilized antibodies cannot distinguish AA76 from non-amyloidotic SAA and other AA specieses.

**Objective:** To develop antibodies specific to the AA76.

**Methods:** Rat was immunized with the peptide corresponding to carboxyl terminus of AA76 and monoclonal antibodies were developed by the conventional methods.

**Results:** Two clones (CF1 and CF6) were obtained by the initial screening as negative reactions with shorter and longer peptides than AA76. In immunohistochemistry, both antibodies reacted with AA amyloid deposits well, not with SAA leaked from vessels. Reactivity of both to AA fibrils were reduced largely by degenerative treatments such as SDS or guanidine. CF6 lost reactivity by trypsin treatment of specimens.

**Discussion and Conclusion:** AA76 may be a species appeared specifically during amyloidogenesis. The present antibodies can specifically detect, though in the limited, AA76. The antibodies should seek usefulness for diagnosis and investigative studies.

#### PA 65

#### Topo-optical histochemical analysis of amyloid deposits in Alzheimer, Morbus Down and Creutzfeldt–Jakob brain

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**Background:** Former results on tissue-isolated human amyloid fibrils (Appel et al., 2003 and 2005) showed a protein fibril co-localised with chondroitine and heparan sulfate.

**Objectives:** Can the same fibril constituents be shown in brain amyloid deposits? Do additional topo-optical reactions provide more information?

**Methods:** Formalin-fixed paraffin-embedded brain sections (2-5 µm) of Alzheimer, Morbus Down and Creutzfeldt-Jakob were investigated. Synthetic amyloid beta (1-40 and 1-42) fibrils served as controls. Our topo-optical reactions are indicative of linearly ordered functional groups. Only when the specific group is in repetitive linear order (like in amyloid fibrils) staining will occur. The aldehyde bisulfite toluidine blue reaction, also in its glycosaminoglycan (GAG)-specific variant 'chemically intensified basophilic reaction' (CIBR), is specific for linearly ordered vicinal OH groups in carbohydrates. The sialic acid specific reaction indicates sialic acid and the critical electrolyte concentration method (CEC) acidic groups like carboxyl and sulphate in GAGs.

**Results:** Neurofibrillary tangles, amyloid plaques and vessels in all three diseases (and controls) were stained with Congo red, N,N'-diethyl pseudoisocyanine chloride (PSI), pinacyanol, pinacyanol chloride, toluidine blue, 1,9-dimethyl methylene blue and rivanol for protein. Depending on whether the dye aggregated parallel or perpendicular to the longitudinal axis, the angle of the polarised light was affected positively (Congo red, PSI, pinacyanol, pinacyanol chloride) or negatively (others). All neurofibrillary tangles (NFTs) and plaques were resistant to potassium permanganate/trypsin, permanganate/pronase, performic acid/trypsin and performic acid/pronase digestion (24 hours). The ABT and sialic acid specific reactions were positive for plaques but negative for NFTs and synthetic Abeta fibrils. The linearly ordered OH-groups were perpendicular to the longitudinal axis. CIBR and the acidophilic CEC reaction was positive in NFTs and plaques at all ionic strengths pointing to strong acidic groups like sulphate in GAGs. After GAG-specific enzymatic treatment (heparinase, hyaluronidase and chondroitinase) the oriented binding of Congo red to plaques and NFTs was intensified.

**Conclusions:** Asn-linked carbohydrates (ABT and sialic acid specific reaction) were demonstrated in brain amyloid plaques, but not in NFTs and synthetic Abeta. GAG-specific reaction CIBR was positive in NFT and plaques, GAG-specific enzymatic treatment resulted in stronger NFT and plaque staining. Therefore, the NFTs and plaques have a protein core covered with GAGs in highly oriented fashion.

Josef Makovitzky was sponsored by NAR (Network Aging Research).



## Posters Tuesday PB 01 – 66

**13.00-14.00: Individual viewing and discussing of the posters ('Blauwe Patio')**

**18.00-19.00: Poster viewing and presenting in 5 groups ('Blauwe Patio')**

**19.00-19.30: Selected poster presentations PB 12, PB 41, PB 45, PB 47, and PB 66 ('Blauwe Zaal')**

Chairpersons Croockewit and Van Rijswijk

### Group 1 (Moderator Wall)

- PB 01 Electroneurophysiological pattern according to clinical profile in familial amyloid polyneuropathies – Adams
- PB 02 Heterogeneousness of phenotypes in TTR-Hereditary Amyloid Polyneuropathy: an explanation for a very late diagnosis – Adams
- PB 03 External Quality Assurance (EQA) of Immunofixation and serum free light chain measure in 9 laboratories participating in the Piedmont and Aosta Valley consortium for systemic amyloidosis – Baldovino
- PB 04 AA Protein in AL amyloidosis – Barros
- PB 05 Transthyretin-Related Familial Amyloid Polyneuropathy in the Majorcan Spot: Son Llàtzer Hospital Descriptive Study – Buades
- PB 06 New transthyretin variant Glu 54 Gln associated with familial amyloidosis – Coriu
- PB 07 Leukocyte Cell-Derived Chemotaxin 2 Amyloidosis Involving the Liver in 3 Patients – Darnell
- PB 08 Amyloidosis of lymph nodes: a proteomic analysis using tandem mass spectrometry – D'Souza
- PB 09 Renal amyloidosis associated with a novel fibrinogen A alpha chain mutation – Efebera
- PB 10 Subcutaneous amyloid deposition at insulin injection sites in two patients with type II diabetes mellitus – De Graeff
- PB 11 Amyloid arthropathy in a patient with ATTR-Val122Ile amyloidosis – Hazenberg
- PB 12 Neurological manifestations of senile systemic amyloidosis – Ikeda
- PB 13 Revised prevalence of TTR V122I in African-Americans with Cardiac Amyloidosis – Jacobson

### Group 2 (Moderator Gertz)

- PB 14 A simple amyloid typing procedure based on a proteomic approach – Kaplan
- PB 15 AA amyloidosis in patients with no known inflammatory condition – Kluge-Beckerman
- PB 16 Monoclonal gammopathy does not predict cardiac amyloid type – Maleszewski
- PB 17 Co-existence of TNFRSF1A and MEFV mutations causing AA amyloidosis as the sole manifestation: a case report – Mereuta
- PB 18 Diagnostic Value of Minor Salivary Gland Biopsy in Systemic Amyloidosis: Results of a Retrospective Study in 20 Patients – Moscetti
- PB 19 ApoA4: a novel form of amyloidosis? – Leung
- PB 20 Diagnosis of amyloid in urine cytology specimens – Picken
- PB 21 Diagnosis of amyloid in frozen sections – Picken
- PB 22 Apolipoprotein A-IV-associated amyloidosis: 3 new cases – Prokaeva
- PB 23 Cerebral amyloidotic angiopathy in young adults – Purrucker
- PB 24 Hereditary gelsolin amyloidosis: a novel variant N211K, and a review of the clinical, histological and SAP scintigraphic characteristics – Rowczenio
- PB 25 Familial amyloid polyneuropathy in Galician population – San Millán
- PB 26 Retinal microangiopathy as an initial manifestation of familial amyloid cardiomyopathy associated with transthyretin E89K mutation – Sandhu

### Group 3 (Moderator Merlini)

- PB 27 The distribution of age at onset of ATTRVal30Met FAP in Japan – Sekijima
- PB 28 Cardiac amyloidosis with reduced left ventricular ejection fraction and normal interventricular septal thickness – Suresh

- PB 29 Utility of the Heavy Light Chain (HLC) assay in patients with AL amyloidosis with a detectable serum monoclonal protein – Wechalekar
- PB 30 Normal heavy/light chain (HLC) and free light chain (FLC) ratios are associated with prolonged survival in patients with systemic AL amyloidosis – Wechalekar
- PB 31 Left atrial function assessment in patients with systemic light chains amyloidosis: a 3D speckle tracking imaging study – Petitalot
- PB 32 Identification and characterization of TTR amyloid associated molecules in FAP – Suenaga
- PB 33 Amyloid, a major player in pathogenesis of atherosclerosis? – Bodin
- PB 34 Piedmont and Aosta Valley Consortium of Systemic Amyloidosis: results of a four year experience – Baldovino
- PB 35 Ageing is associated with AA-type Amyloidosis in Patients with Familial Mediterranean Fever living in Germany – Blank
- PB 36 Baseline profile of patients undergoing tafamidis treatment in THAOS: the Transthyretin Amyloidosis Outcomes Survey – Coelho
- PB 37 Relationship between age at symptom onset and left ventricular wall thickness in ATTR Amyloid: THAOS survey – Rapezzi
- PB 38 Baseline Demographics and Clinical Characteristics in THAOS: the Transthyretin Amyloidosis Outcomes Survey – Suhr
- PB 39 The Pavia project for the integration of clinical data with a centralized biobank for systemic amyloidosis – Valentini

#### **Group 4 (Moderator Comenzo)**

- PB 40 Iatrogenic amyloid polyneuropathy after Domino FAP LT: characteristics and proposed criteria – Adams
- PB 41 Cardiac involvement in Cardiac AL Amyloidosis as measured by Equilibrium Contrast Cardiovascular Magnetic Resonance – Banypersad
- PB 42 Lessons from familial Mediterranean fever-amyloidosis kidney-transplanted patients – Ben-Zvi
- PB 43 Depression and Anxiety among patients with AL amyloidosis: the role of cardiac symptoms – Cappelli
- PB 44 Amyloidosis Of The Gastrointestinal Tract: A Case Series of 74 Patients – Cowan
- PB 45 Amyloid Deposits in the Bone Marrow of Patients with AL Amyloidosis Do Not Impact Stem Cell Mobilization or Engraftment – Cowan
- PB 46 Differentiating hypertensive heart disease and cardiac transthyretin isoleucine 122 (V122I) amyloidosis in Afro-Caribbean patients – Dungu
- PB 47 Determinants of Cardiac Severity in Patients with Systemic Light Chains Amyloidosis: An echocardiography and cardiac magnetic resonance imaging study – Ettaif
- PB 48 Production of plasminogen activator and it's receptor in organs from AL amyloidosis – Hata
- PB 49 Heavy / light chain analysis can replace IFE in a algorithm utilizing free light chain and urinary protein electrophoresis for identification of AL Amyloidosis Patients – Hazenberg
- PB 50 An exploratory case-control study of progression of AA amyloidosis in rheumatic disease – Hunter
- PB 51 High incidence of autoimmune disorders in patients with localized light-chain amyloidosis – Kimmich
- PB 52 Two Distinct Syndromes of Lymphoma Associated Amyloidosis – Kukreti

#### **Group 5 (Moderator Hawkins)**

- PB 53 Renal biopsy in familial Mediterranean fever with proteinuria: Is it justified? – Kukuy
- PB 54 Natural history of wild type transthyretin amyloidosis and possibility of developing an algorithm to differentiate it from isolated cardiac AL amyloidosis – Lachmann
- PB 55 Detection of serum IgA monoclonal components in patients evaluated for AL amyloidosis, using heavy chain/light chain immunoassay (HevyLite) – Lavatelli
- PB 56 Heart failure secondary to severe cardiomyopathy: clinical presentation of familial amyloid polyneuropathy with Val30Met mutation – Monteiro
- PB 57 Cardiac involvement in apolipoprotein A-1 amyloidosis (Leu75Pro) – Nardi
- PB 58 Acquired cutis laxa should be considered one of the cutaneous manifestations of plasma cell dyscrasia: A case report and review of the literature – Ravera

- PB 59 Urinary biomarkers for kidney disease in ATTR amyloidosis – Rocha  
 PB 60 Immunoglobulin D amyloidosis: a rare entity with a common phenotype – Roussel  
 PB 61 Diagnosis of cardiac AL amyloidosis: the “grey” area of patients with increased NT-proBNP and normal wall thickness – Salinaro  
 PB 62 Circulating Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Cardiac Amyloidosis: Novel Biomarkers of Cardiac Amyloidosis – Sam  
 PB 63 Cardiac pulmonary exercise testing (CPET) in patients with senile systemic amyloidosis (SSA) – Sam  
 PB 64 Quantification of the monoclonal protein using the heavy-lite test in patients with light chain amyloidosis – Schönland  
 PB 65 Clinical and laboratory features of leptomeningeal type of TTR amyloidosis – Ueda  
PB 66 Functional proteomics investigation of the mechanisms of cardiac damage in AL amyloidosis – Lavatelli

**PB 01****Electroneurophysiological pattern according to clinical profile in familial amyloid polyneuropathies**

Dodet Pauline, Mincheva Zoa, Cauquil Cécile, Kreib Anne-Marie, Theaudin Marie, Adams David, Lozeron Pierre

*French national reference centre for FAP (NNERF) and Department of Neurology, Assistance Publique-Hôpitaux de Paris, Université Paris Sud, France*

Familial amyloid polyneuropathies (FAP) are autosomal dominant small fibres sensory and/or vegetative neuropathies that evolve towards severe sensory motor neuropathies. Before disclosing a classical sensory motor axonal pattern, nerve conduction studies (EDX) are often normal in the early course of the disease, but atypical demyelinating EDX profiles have also been reported.

We report the results of the initial EDX performed in 56 consecutive FAP patients referred to our national reference centre between 2008 and 2010; and correlated them to their clinical profile: small fibre n=20, all fibres n=9, large fibres n=12 and multifocal n=9, other n=6. Criteria for EDX demyelination are those of EFNS/PNS 2010.

There were 35 men. Mean age at onset was 54 and 58 at initial EDX. Thirty one patients had a Met30 transthyretin mutation. At referral, the suspected diagnosis was FAP (n=23) including 10 with familial history, polyneuropathy (n=18), CIDP (n=8), other (n=7). Mean delay between initial EDX and symptoms' onset was 2.7 years. The five patients who had normal EDX (mean EDX delay 2.6y) had no specific clinical profile. EDX disclosed axonal loss in 50 patients, which was asymmetrical in 30. Twenty eight patients (50%) met criteria of definite demyelination, without correlation with disease duration. Demyelinating EDX was more common in large fibres and multifocal presentations (66% in each group) than in small fibres (35%) or all fibres presentations (33%). It was also slightly more common in non Met30 group (56 vs 45%). Half of the patients without familial history (n=9/18) fulfilled criteria for demyelination.

Whatever clinical profile and delay between symptoms' onset, demyelinating EDX is a common pattern in FAP patients.

**PB 02****Heterogeneousness of phenotypes in TTR-Hereditary Amyloid Polyneuropathy: an explanation for a very late diagnosis**

Adams D, Lozeron P, Mincheva Z, Theaudin M, Adam C, Kreib AM, Cauquil C, Adam C Maisonobe T, Vial C, Sole G, Signate A, Vallat JM, Lacour A, Delmont E, Misrahi M, Lacroix C  
*Neurology, Anatomopathology, Molecular Biology, Hôpitaux Universitaires Paris Sud, APHP, Univ Paris Sud, French Referral Center for FAP, French Network for FAP CORNAMYL, France*

**Background:** TTR-FAP are progressive and life-threatening neuropathies due to a point mutation of TTR gene with autosomal dominant transmission. They are classically described as a small fiber polyneuropathy (length dependent (LD) or predominantly autonomic) associated with Val30Met variant TTR with early onset (< 50y.) in Portugal. A LD-all fiber neuropathy has been reported in late onset. In France, the long diagnostic delay of TTR-FAP (3 years) could result from diagnostic traps.

**Objective:** To study and report the varieties of phenotypes in the FAP, according to the clinical presentation of neuropathies.

**Methods:** Monocentric Retrospective study of Referral Centre for FAP. We studied 60 consecutive cases of TTR-FAP referred between 2008 and 2010. Phenotypes of the neuropathy were defined with inaugural manifestations, topography and modalities of sensory loss, topography of motor deficit and autonomic dysfunction.

**Results:** The mean age was 59 y. (range 31-89). 24% were of Portuguese origin (Group 1), 76% of non Portuguese origin (Group 2).

In group 1, SF neuropathy was usual, with early onset and positive family story and Val30Met variant (100%).

In group 2, there were 4 phenotypes: SFN (26%), all fiber LD-PNP (26%), multifocal neuropathy upper limbs neuropathy (22 %), ataxic neuropathy (26%) with a late onset (89%) and positive family story (50%). Amyloid PNP was initially suspected in only 24% patients. Main misdiagnosis were idiopathic axonal polyneuropathy (n=11), CIDP (n=8), lumbar spinal stenosis (n=7). There were 11 TTR gene mutations: Met30 (46%), Tyr 77 (28%). Ataxic phenotype was mainly seen with Tyr77TTR variant (58%). Nerve biopsy was contributive for diagnosis in 24/29 pts (83%); labial salivary gland biopsy in 22/41 (54%).

**Discussion:** Varied presentation of TTR-FAP could result from varied pathogenic mechanisms.

**Conclusions:** A better knowledge of the phenotypes of FAP and the use of TTR gene analysis and biopsy will help to accelerate diagnosis.

### PB 03

**External Quality Assurance (EQA) of Immunofixation and serum free light chain measure in 9 laboratories participating in the Piedmont and Aosta Valley consortium for systemic amyloidosis**

**Simone Baldovino**, Domenico Cosseddu, Cristiana Marchese, Marco Manganaro, Madalina Mereuta, Savino Sciascia, Foco Miranda, Perrone Flavia, Napoli Patrizia, Bertone Carlo, Patrucco Giovanna, Crespi Ilaria, Ferrero Paola, Giarin Emanula, and Dario Roccatello  
*Piedmont and Aosta Valley consortium of systemic amyloidosis, Italy*

**Background:** The diagnosis of AL amyloidosis is based on histologic finding as well as on some laboratory tests. According to the most recent guidelines serum and urine immunofixation, and serum free light chain measure are of paramount importance.

**Objectives:** With the present work we evaluated the performance of the above mentioned tests in 9 laboratories belonging to the Piedmont and Aosta Valley consortium for systemic amyloidosis through an External Quality Assurance (EQA).

**Methods:** In 2010 all laboratories of Piedmont and Valle d'Aosta were invited to participate to an EQA in order to evaluate their performance in the identification of monoclonal components and the measure of free light chains in frozen serum samples. Nine laboratories joined the evaluation. Protein electrophoresis and immunofixation were performed in 3 laboratories by using capillary electrophoresis and in the remaining 6 by an automated gel electrophoresis. Sebia reagents (Evry, France) were used. Measure of free light chains was performed in 5 laboratories: 4 with the Binding Site Freelite method (Birmingham, UK) and 1 with New Scientific Company method (Fonegrò, Italy).

**Results:** The assessment of monoclonal component by serum proteins electrophoresis and immunofixation showed an overall sensitivity of 100% and an overall specificity of 61%. The quantification of the monoclonal component showed a good agreement in the results even though a laboratory did not performed quantification and 3 more laboratories showed only a percentage and not quantification in g/l. Assessment of free light chains showed variations coefficient greater than 40% between laboratories.

**Discussion:** While improved as compared to a previous EQA performed in 2009, the present EQA emphasized critical issues, especially in the determination of free light chains.

**Conclusion:** The adoption of the same analytical method by all laboratories is probably needed in order to compare data from different laboratories and improve diagnostic sensitivity.

*The abstract has been written on behalf of all participants in the consortium.*

**PB 04****AA Protein in AL amyloidosis**

**Francisca Barros**<sup>1</sup>, João Frazão<sup>1</sup>, Isabel Tavares<sup>1</sup>, Paulo Salamanca<sup>2</sup>, Cristiana Paulo<sup>3</sup>, Margarida Alvelos<sup>3</sup>, Ricardo Neto<sup>1</sup>, Raquel Vaz<sup>1</sup>, Manuel Pestana<sup>1</sup>

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**Introduction:** Renal amyloidosis is a detrimental disease caused by the deposition of amyloid fibrils, in which renal impairment may follow. Clinically evident renal involvement occurs mainly in AL, AA and some hereditary amyloidosis. We report a case of a patient whose kidney biopsy disclosed the coexistence of protein AA and immunoglobulin light-chain derived protein in amyloid deposits.

**Case report:** A 73-year-old male, sculptor, complaining of asthenia, peripheral oedema, decreased urine output and foamy urine was admitted to our hospital. Twenty years ago he was treated for boutonneuse fever (*Rickettsia conorii*). No history of other chronic diseases or infections. On admission he had nephrotic syndrome and severe renal failure, beginning of haemodialysis on admission. Analytical evaluation disclosed hypoalbuminemia 1.6 g/dL, proteinuria 20.19 g/24h, increased free light chain lambda 157 mg/dL (0.57-2.63), serum ratio free kappa / free lambda <0.01 (N 0.26-1.65) and immunoelectrophoresis with incomplete lambda monoclonal gammopathy. Bone marrow biopsy revealed interstitial marrow infiltration by plasmacytoma / multiple myeloma, without amyloid infiltration. Kidney biopsy was performed. Green birefringence was observed with Congo red staining under polarized light. The immunohistochemistry staining was extensively positive for lambda immunoglobulin light chains in glomerular and interstitial regions, and in some points, also for AA amyloid.

The patient was treated with 4 cycles of bortezomib and dexamethasone, with normalization of serum free light chains. There was no recovery of renal function. Eight months after diagnosis there's no evidence of progression of monoclonal gammopathy.

**Discussion:** We report the finding of AA amyloid as a minor constituent of amyloid deposits in a patient with AL amyloidosis. One possible explanation is that AA amyloid deposition was already present before the development of plasma cell disease.

Far as we know, the significance of protein AA in AL amyloidosis is unknown. In 1983 Falck and Westermark reported protein AA in kidney sections from five out of 14 cases of primary and myeloma associated amyloidosis, all having an immunoglobulin light chain derived protein as a major subunit<sup>1</sup>. They discussed that there might be some common pathogenetic mechanism working in both AL and AA type of systemic amyloidosis<sup>1</sup>.

**References:**

1. Falck HM, Westermark P. Protein AA in primary and myeloma associated amyloidosis. *Clin Exp Immunol* 1983; 53: 259-264.

**PB 05****Transthyretin-Related Familial Amyloid Polyneuropathy in the Majorcan Spot: Son Llàtzer Hospital Descriptive Study**

**Buades J**, Usón M, Ripoll T, Andreu H, Company M, Dieguez JM

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**Objectives:** Transthyretin-Related Familial Amyloid Polyneuropathy (TTR-FAP) is a public health problem in the Balearic Islands. The Balearic focus ranks among the five areas with higher FAP incidence worldwide. TTR Val30Met is the most frequent substitution. Son Llàtzer Hospital is a 350-bed community centre located in Palma. The first case diagnosed by our group was in 1991. The aim of this study was to describe the clinical and epidemiological aspects of our TTR-FAP population.

**Methods:** We retrospectively studied patient clinical records managed in our hospital between 2001 and 2011. Diagnostic criteria included: plasma TTR mutation, biopsy amyloid deposits and mixed polyneuropathy confirmed by Electromyography. Echocardiogram was performed when suspected cardiac involvement.

**Results:** Between 2001 and 2011 we diagnosed 107 subjects with TTR-FAP pathogenic mutations: 35 asymptomatic carriers and 72 symptomatic cases (male 54% vs. females 46%). Mean age-at-onset

was 47.3 years and mean duration 7.4 years. TTR-FAP family history was found in 100%. Val30Met was present in 100% patients; all were heterozygotes. Detection of amyloid in biopsies (58% intestinal, 18% abdominal fat, 18% sural nerve, 3% liver, 3% autopsy) was done in 40/72 patients. Polyneuropathy was the presenting manifestation in 80% patients, digestive dysfunction 14%, cardiac involvement 4%, ocular affection 1% and renal impairment 1%. Liver transplantation (LT) was undertaken in 47 patients (mean age 46 years), 3 of them were simultaneous double transplant (1 heart-liver and 2 kidney-liver). Nine patients (mean age 70.3 years) died after LT.

**Conclusions:** TTR-FAP is a rare and fatal neurodegenerative disease that constitutes a public health problem in Majorca. Clinical characteristics of our series are similar to that reported in the literature but with older age-at-onset (47 years) and with a relatively higher neurological and digestive involvement at presentation. The number of symptomatic TTR-FAP patients that underwent LT is high (65%).

## PB 06

### New transthyretin variant Glu 54 Gln associated with familial amyloidosis

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Transthyretin (TTR) amyloidosis is the most prevalent type of hereditary systemic amyloidosis and results from a mutation in the TTR gene. Over 100 such alterations have been described and now we wish to report yet another variation detected in two unrelated Romanian individuals with TTR amyloidosis. The first patient is a 54-year-old man with a progressive peripheral sensory motor polyneuropathy, autonomic dysfunction, and a restrictive cardiomyopathy. At 45, he had the onset of orthostatic hypotension, paresthesias of the lower extremities, dysphagia, and chronic diarrhea. Subsequently, there was rapidly progressive cardiac dysfunction, heart failure and pulmonary hypertension. Notably, his father was diagnosed with idiopathic cardiomyopathy at age 50 and died few years later.

The second patient is a 46- year- old woman who presented with restrictive cardiomyopathy and a bilateral carpal tunnel syndrome. At age 40 she developed symptoms of heart disease and also had a carpal tunnel syndrome treated surgically. Her father died at age 50 after a long standing history of cardiomyopathy and severe sensori-motor neuropathy.

In both cases, biopsies of abdominal subcutaneous fat and rectum showed after Congo red staining birefringent deposits. Direct genomic sequencing of the full TTR gene coding region indicated G- to - C transversion at the first base position in codon 54 (GAG -> CAG) which leads to the heterozygous mutation Glu54Gln in exon 3 of the gene. Mass spectrometric analysis of TTR immunoprecipitated from serum showed no molecular mass difference between wild type and TTR variant.

To the best of our knowledge these are the first reported cases of E54Q-associated TTR amyloidosis.

## PB 07

### Leukocyte Cell-Derived Chemotaxin 2 Amyloidosis Involving the Liver in 3 Patients

**Adam J. Darnell<sup>1</sup>**, Monika Fischer<sup>2</sup>, Raj Vuppalanchi<sup>2</sup>, Merrill D. Benson<sup>1</sup>, Oscar W. Cummings<sup>1</sup>

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**Background:** LECT2 (leukocyte cell-derived chemotaxin 2) is the newest member of the group of proteins that cause amyloidosis. Synthesized by the liver and a downstream target of  $\beta$ -catenin, LECT2 has multiple functions. Previously, LECT2 amyloidosis has been exclusively described in the kidney and notably for increased occurrence in Hispanics. Here we present 3 cases where LECT2 deposition was found in the liver.

**Objective:** The patients consisted of a 56 year old Hispanic woman with presumed autoimmune hepatitis, a 66 year old Hispanic man with presumed cirrhosis and a third patient with unknown demographics.

**Results:** The histology showed globular deposits of amyloid in the portal tracts in all patients. Negative staining for primary and secondary amyloidosis with recent recognition of LECT2 prompted immunohistochemical antibody stains for LECT2, which were positive.

DNA sequencing of the patients' peripheral blood revealed homozygosity for the G allele at nucleotide 172, forming the codon GTC (Valine) at position 40/58. A finding previously described in individuals with renal LECT2 amyloidosis.

**Conclusion:** The pathogenesis of LECT2 amyloidosis in the liver is unclear. Recent evidence suggests that  $\beta$ -catenin, an important regulator of LECT2 production plays an important role in various aspects of liver biology including the pathogenesis of liver cancer. According to a recent publication LECT2 amyloidosis was the 3<sup>rd</sup> most common type of amyloid identified in kidney biopsies, after AL and AA. We recommend LECT2 amyloidosis inclusion in the differential of any amyloidosis irrespective of the type of organ, after AL and AA are ruled out.

#### References:

1. Murphy, C.L., et al., *Leukocyte chemotactic factor 2 (LECT2)-associated renal amyloidosis: a case series*. Am J Kidney Dis, 2010. 56(6): p. 1100-7.
2. Benson, M.D., *LECT2 amyloidosis*. Kidney Int, 2010. 77(9): p. 757-9.
3. Ebert, E.C. and M. Nagar, Gastrointestinal manifestations of amyloidosis. Am J Gastroenterol, 2008. 103(3): p. 776-87.

#### PB 08

#### Amyloidosis of lymph nodes: a proteomic analysis using tandem mass spectrometry

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**Background:** Amyloidosis affecting the lymph nodes is uncommon. Lymph node involvement may occur in the setting of systemic amyloidosis or as a condition localized to the lymph nodes. Why some amyloid remains localized is unknown. We speculated that composition of the amyloid with these two presentations may differ.

**Methods:** We identified 44 patients with lymph node biopsy proven amyloidosis seen at our institution between May 1971 and October 2011. Using mass spectrometry, we analyzed the composition of amyloid in lymph nodes in order to identify differences in the amyloidogenic make-up between the systemic and localized subtypes.

**Results:** Table 1 shows the clinical and laboratory features of these patients. Using laser microdissection, Congo red positive areas of lymph nodes sections were removed, and analyzed using tandem mass spectrometry in 30 cases. Preliminary analyses demonstrate that all localized cases of immunoglobulin derived amyloid contained heavy chain amyloid (AH) whereas only 9 cases of systemic contained AH (p 0.006). Conversely, 18 systemic cases had light chain amyloid (AL) in their lymph nodes while only 4 localized cases had AL (p 0.009). Proteomic analysis also helped identified calcitonin in cervical node amyloid deposits in a patient with medullary thyroid carcinoma.

**Table 1.**

|                                 | Localized, n = 19<br>Median | Systemic, n = 25<br>Median |
|---------------------------------|-----------------------------|----------------------------|
| Gender, % male                  | 63                          | 56                         |
| Age at diagnosis, years         | 63.5                        | 61.5                       |
| Follow up, months               | 61.3                        | 30.3                       |
| Hemoglobin, g/dl                | 14.2                        | 12.5                       |
| White count, /cu ml             | 6.8                         | 7.7                        |
| Platelet, /cu ml                | 248                         | 271                        |
| Creatinine, mg/dl               | 1                           | 1                          |
| Alkaline phosphatase, IU/ml     | 124*                        | 154                        |
| Albumin, g/dl                   | 3.6                         | 2.9                        |
| $\beta_2$ microglobulin, mcg/ml | 1.8<br>N=8                  | 2.3<br>N=20                |
| Median overall survival, years  | 10.6**                      | 4                          |

\* p=0.001; \*\* p =0.05

**PB 09**

**Renal amyloidosis associated with a novel fibrinogen A alpha chain mutation**

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**Introduction:** We present an elderly patient with renal amyloidosis associated with a novel fibrinogen A alpha chain (AFib) mutation, who presented with slowly progressive renal insufficiency and proteinuria.

**Patient:** An 80 year old man who was known to have mild renal insufficiency since 1998, and low-level proteinuria since 2005, presented in October 2011 to the nephrology service with worsening creatinine over a six month period. His creatinine was 5.95 mg/dl (normal <1.3) and his urine protein was 6,379 mg/day (normal 40-225). He had no shortness of breath, dizziness, bruising, diarrhea or constipation. His past medical history was well controlled hypertension, hyperlipidemia and abdominal aortic aneurysm repair in 2004. He remained very active, and had no family history of renal disease. His parents were Russian and had died at age 72 and 75 years. His physical exam was unremarkable except for trace edema of the lower extremities.

**Investigations and Results:** Renal biopsy showed glomerular amyloid deposits that stained strongly with antibodies to fibrinogen. He did not have a monoclonal protein, and there was no cardiac, liver, gastrointestinal, neurologic or other organ involvement. Mass spectrometry confirmed the amyloid was of AFib-type. Direct DNA sequencing of the FGA gene revealed a novel fibrinogen mutation resulting from a single base substitution (c.1633G>A) altering the codon at position 526 from glutamic acid to lysine (E526K)

**Conclusion:** This is the first report of this novel fibrinogen mutation. The clinical presentation was indistinguishable from that of the most common AFib mutation (E526V- glutamic acid to valine), i.e. with a late onset of hypertension and proteinuric renal insufficiency without other amyloidotic organ involvement. The histological picture of major glomerular amyloid deposition was also similar. A major difference is that this patient had Russian ancestry while the majority of E526V mutations appear to be of British decent. Since his diagnosis, he continues to work full time and has not yet required dialysis.

**PB 10**

**Subcutaneous amyloid deposition at insulin injection sites in two patients with type II diabetes mellitus**

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Two patients with insulin dependent diabetes mellitus, one male aged 61 years old and one female aged 53 years old, developed a localized subcutaneous abdominal mass at their respective insulin injection sites. Both patients had been treated with recombinant human insulin or insulin analogues for more than 10 years. Biopsies were taken under the suspicion of malignant disease. However, histopathological examination of tumor biopsies demonstrated adipose tissue with deposits of amorphous eosinophilic material. After Congo red staining, samples showed apple-green birefringence in polarized light typical of amyloid. Antibodies against serum amyloid P (SAP) and serum amyloid A (SAA) stained negative. Additional evaluation including serum protein electrophoresis, serum immunoglobulin free light chain levels, bone marrow aspiration and <sup>123</sup>I-SAP scintigraphy revealed no signs of systemic AL amyloidosis. Finally, additional immunohistochemical staining revealed strong insulin positivity leading to the diagnosis of localized amyloidosis composed of iatrogenic amyloid insuline (Alns) type amyloid. In both patients, blood glucose levels markedly improved after they changed their injection site suggesting that in these cases, insulin resistance may be partly explained by accumulation of insulin in the amyloid mass, resulting in decreased bioavailability.

So far, twelve cases of localized insulin-derived amyloidosis have been described in literature. Given the high prevalence of insulin-dependent diabetes mellitus, its occurrence is likely to be

underestimated. This diagnosis should be considered in every patient using either porcine or recombinant human insulin presenting with a subcutaneous tumor at their injection site, especially in case of insulin-refractory disease. Alns amyloid is the exception of the rule that amyloid in subcutaneous abdominal fat tissue proves the presence of systemic amyloidosis.

## PB 11

### Amyloid arthropathy in a patient with ATTR-Val122Ile amyloidosis

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**Case:** A 66-year-old Afro-Caribbean woman was referred to our tertiary centre because of possible amyloid cardiomyopathy detected with cardiac ultrasound because of right-sided cardiac failure. Physical examination also disclosed prominent arthropathy of both shoulders ("shoulder pads") and both knees. Aspiration of subcutaneous abdominal fat tissue and shoulder joint fluid showed amyloid to be present at both sites. Because of the clinical picture of amyloid cardiomyopathy and amyloid arthropathy our thoughts initially went to AL amyloidosis. However, we did not find any sign of increased free light chain production in serum and urine, nor did we find a clonal plasma cell dyscrasia in the bone marrow. Because of cardiomyopathy we looked for a TTR gene mutation and found that our patient was homozygous for the TTR-Val122Ile mutation. The semi-quantitative amount of amyloid in the fat aspirate was 4+, whereas precursor quantification in this fat aspirate showed a TTR concentration of 936 ng/mg fat tissue ( $N < 4.4$  ng/mg fat tissue) with normal concentrations of amyloid A and kappa and lambda light chains. We therefore concluded that this patient had ATTR-Val122Ile amyloidosis.

**Discussion:** Typical amyloid arthropathy of shoulders as disease manifestation of ATTR amyloidosis has not been described before to our knowledge and can thus be added to the clinical manifestations of this disease. This case shows the benefits of performing a subcutaneous abdominal fat tissue aspiration as well as joint fluid aspiration for both detecting and typing of amyloid.

## PB 12

### Neurological manifestations of senile systemic amyloidosis

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**Background:** Senile systemic amyloidosis (SSA) is known to be caused by the deposition of wild-type transthyretin (TTR)-derived amyloid fibrils. Although main clinical picture of SSA is intractable heart failure with arrhythmia, our previous reports have suggested that carpal tunnel syndrome (CTS) might precede the cardiac manifestations in this disease. In this study we tried to clarify the natural course of SSA with a special attention to the neurological manifestations.

**Patients and Methods:** During past 5 years 20 patients who were supposed to be suffering from SSA were referred to us: they had a history of congestive heart failure. Echocardiography showed marked symmetrical thickening of ventricular walls and ventricular septum with hyperrefractile myocardial echoes. Myocardial technetium-99m pyrophosphate scintigraphy revealed a positive shadow. TTR gene analysis showed no mutation and TTR-related amyloid deposition was demonstrated by either endomyocardial or abdominal wall fat pad biopsy. We retrospectively analyzed the clinical records of these patients.

**Result:** The patients consisted of 14 men and 6 women and their ages at onset ranged from 60 to 97 years (average: 71.15 years). Eleven patients showed CTS as an initial manifestation in the disease and additional two patients were detected to have CTS when they were hospitalized for the treatment of congestive heart failure. Four patients were attacked by cerebral infarction (possibly due to atrial fibrillation) before the appearance of congestive heart failure.

**Conclusion:** Neurological complications, which have not been noted before, do not seem to be rare in the patients with SSA and among them CTS might be a common initial manifestation. Diflunisal or tafamidis is expected to slow the progression of SSA and thus, early diagnosis of the disease is

required for the use of both drugs. In this situation paying much attention to neurological manifestations in SSA is very important.

This study was supported by Grant-in-aid for Scientific Research in Japan (2059082 to SI, 20590695 to YS); a grant from the Intractable Disease Division, the Ministry of Health and Welfare, Amyloidosis Research Committee in Japan.

## PB 13

### Revised prevalence of TTR V122I in African-Americans with Cardiac Amyloidosis

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**Background:** In one large autopsy series,<sup>1</sup> cardiac amyloidosis was more prevalent in African-Americans than Caucasians. We reported that immunohistochemical and transthyretin (TTR) genetic analysis on tissues from that series revealed that 6/26 immunohistochemically TTR+ samples carried TTR V122I (23% familial amyloidosis); 77% contained another variant, or were normal-sequence TTR (true senile systemic amyloidosis, SSA).<sup>2</sup> The latter would imply a greater SSA prevalence in African-Americans than Caucasians. We subsequently found TTR L55P in several of the V122I-negative specimens, an implausible result. Our studies had begun before contamination risk in PCR was widely appreciated, and before aerosol-resistant pipette tips were in use. During our studies, our laboratory contained TTR L55P and wild-type genomic clones, which we found had contaminated some reagents.

**Objective:** To remove contamination and repeat our published work.

**Methods:** DNA was reisolated from available autopsy specimens. Previously-described work was repeated using modern methods to prevent contamination.

**Results:** Of the 26 original study samples, TTR allelotyping results could be obtained from only 12. Of these 12, the same six previously found to contain TTR V122I, and no others, were confirmed to carry this variant.

**Discussion:** Lab contamination with genomic TTR clones of normal sequence at codon 122 could lead to our original results, in which some samples actually containing no genomic DNA would appear to contain a normal codon 122. In contrast, such contamination could not lead to V122I false positives, as no V122I genomic clones were present in the lab, and no V122I contamination was found. Elimination of contamination could thus reduce the TTR V122I-negatives, but not positives, in our samples.

**Conclusion:** Our data suggest that V122I accounts for the excess of cardiac amyloidosis among African-Americans, compared with Caucasians. True SSA prevalence was similar in the two groups.

**References:** 1. ModernPathol 2:372-377, 1989. 2. New Engl J Med 336:466-473, 1997.

## PB 14

### A simple amyloid typing procedure based on a proteomic approach

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**Background and Objective:** Correct determination of amyloid type is crucial for treatment and prognostic purposes. Current antibody-based amyloid typing methods have restricted capability to check a long list of different amyloid proteins. In this respect, proteomic identification of amyloid proteins by mass spectrometry seems to be promising (Loo et al., 2011). However, this approach is by a few amyloid research centers, partially due to complex and time-consuming sample preparation procedures. In this study we present a new, simpler procedure for proteomic-based amyloid typing.

**Methods:** Amyloid proteins extracted from tissue specimens were separated by SDS-electrophoresis and electroblotted onto PVDF membranes (Kaplan et al., 2001&2009). Protein blots stained with Coomassie blue revealed prominent protein bands of MW < 25 kda. These bands are known to contain major amyloid protein subunits characterizing the amyloid type. Proteins eluted from the

excised bands were trypsinized and analyzed by capillary liquid chromatography tandem mass spectrometry (LC-MS/MS) (Orbitrap mass spectrometer, Thermo). The data were analyzed using the Sequest 3.31 software vs human section of the uniprot database. The identified proteins detected in the excised band were checked for the presence of sequences matching the particular amyloid protein type and its characteristic molecular weight.

**Results:** Mass spectral analysis of the selected protein bands revealed sequences belonging not only to amyloid but also to other tissue components and the tissue-contaminating serum proteins. Amyloid type was determined on a basis of the detected amyloidogenic sequences and MW of the excised protein band. The obtained results from the mass spectrometry data were in concordance with the previous typing of the same cases (Kaplan et al., 2004) using Western blotting and Edman degradation techniques. These cases included AA amyloidosis (n=2), AL-kappa amyloidosis (n=1), AL-lambda amyloidosis (n=1) and ATTR (n=1).

**Discussion and Conclusions:** In this study, we have demonstrated the utility of a new procedure for typing of amyloid proteins by proteomic approach. Comparing to other proteomic amyloid typing methods, the employed sample preparation is simple, inexpensive and requires only commonly used equipment available in most facilities.

## PB 15

### AA amyloidosis in patients with no known inflammatory condition

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**Background:** AA amyloidosis develops secondary to longstanding inflammation or sepsis. Common predisposing conditions include rheumatoid arthritis, periodic fever syndromes, Crohn's disease, bronchiectasis, and osteomyelitis.

**Objective:** To catalogue the underlying disease in AA amyloidosis patients seen at our center and raise awareness that amyloidosis of the AA type can afflict patients who have no known history of chronic inflammatory disease or sepsis.

**Methods:** Medical records of 22 patients with AA amyloidosis were reviewed. All patients had a Congo red-positive biopsy. Typing as AA amyloid was by immunohistochemistry and in some cases confirmed by protein sequence analysis of extracted fibrils.

**Results and Discussion:** Six of 22 patients with AA amyloidosis had no history of chronic inflammatory disease or sepsis. The initial presentation in 5 of the 6 patients was nephrosis and/or elevated creatinine. One of the 5 patients also had splenomegaly caused by a reactive process and an amyloid-infiltrated goiter. A sixth patient presented at age 13 with an amyloid-containing mesenteric mass. Episodic laboratory findings on 4 of these patients for whom detailed records were available showed elevated inflammatory markers, i.e., erythrocyte sedimentation rate and C-reactive protein. The 5 adult patients had complex medical histories which included anemia, obesity, osteoarthritis, coronary artery disease, and gastrointestinal disorders. None of the co-morbidities was considered to have an inflammatory component classically associated with development of AA amyloidosis. The underlying diseases identified in the other 16 patients were: rheumatoid arthritis (n=3), juvenile rheumatoid arthritis (n=4), ankylosing spondylitis (n=3), Crohn's disease (n=3), osteomyelitis (n=2), and familial Mediterranean fever (n=1).

**Conclusion:** When amyloidosis is diagnosed, the possibility that it is of the AA type should not be discounted solely on the basis of the patient having no history of chronic inflammation or sepsis.

## PB 16

### Monoclonal gammopathy does not predict cardiac amyloid type

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**Background:** The proper identification of cardiac amyloid type is essential for patient management, and has historically relied upon immunohistochemical or immunofluorescent methods. Due to the inherent difficulties of these assays, their interpretations have been assisted by serum and urine protein electrophoresis (SPEP and UPEP) with immunofixation electrophoresis (IFE). A newer ancillary test, free light chain immunoassay (FLC), has not been evaluated in this setting. The recent implementation of mass spectrometry based proteomic analysis for clinical amyloid typing allows us to determine the validity of these additional tests to help predict amyloid type.

**Objective:** To determine the validity of SPEP/UPEP/IFE and FLC assays in cardiac amyloid prediction.

**Design, Setting, and Participants:** Retrospective study of 2 tertiary care populations (n=109, 2001-2010) of biopsy proven cardiac amyloidosis with mass spectrometry based proteomic analysis.

**Main Outcome Measure** Test performance of SPEP/UPEP/IFE and FLC as predictors of cardiac amyloid type.

**Results:** Amyloid of transthyretin (ATTR) type was found in 75 of 109 patients (69%) and immunoglobulin light chain amyloid was found in the remaining 34 (31%, 21% lambda and 10% kappa). SPEP/UPEP/IFE detected a monoclonal gammopathy in 45 individuals, 26 with AL and 19 with ATTR amyloid, and was overall a poor predictor of AL amyloid in this patient population: specificity (70%; 95% CI, 57-81%) and positive predictive value (PPV 58%; 95% CI, 42-72%). The FLC assay detected an abnormal kappa/lambda ratio in 31 patients, 26 with AL and 5 with ATTR amyloid and was a better predictor of AL amyloid type in this patient population: specificity (89%, 95% CI, 76%-96%) and PPV (84%, 95% CI, 66-94%).

**Conclusions:** ATTR was the predominant amyloid type in this large cohort of endomyocardial biopsies characterized by mass spectrometry. Although serum FLC performs better than SPEP/UPEP/IFE, the performance characteristics of peripheral blood and urine studies for monoclonal proteins are not adequate to classify amyloid type.

## PB 17

### Co-existence of TNFRSF1A and MEFV mutations causing AA amyloidosis as the sole manifestation: a case report

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TNFRSF1A and MEFV mutations have been associated with two major autoinflammatory disorders (TNF receptor-associated periodic syndromes and Familial Mediterranean Fever, respectively) which may be complicated by AA amyloidosis.

A 22-year-old woman was presenting with a new-onset nephrotic syndrome with normal renal function during the 35th week of pregnancy. She recalled of no previous illness except for HCV infection and when she was 3 year-old, an esophageal burn by caustic soda treated surgically. Neither she nor any family member had a history of recurrent abdominal pain and/or fever. The patient presented with a normal physical examination except for significant leg edema. Delivery had been induced prematurely in the 36th week. Blood serum levels included: creatinine 0.5 mg/dl, albumin 2.1 g/dl, cholesterol 328 mg/dl, CRP 17 mg/dl. Daily urinary total protein excretion ranged between 3.6 g to 5.1 g. Kidney biopsy performed three weeks after delivery showed abundant Congo-red-positive material in the blood vessel walls of arteries and arterioles in conjunction with glomerular and tubulointerstitial deposition. Subcutaneous abdominal fat and rectal submucosa biopsies were also performed. The first one was negative for amyloid deposition whereas rectal biopsy was diagnostic for AA amyloidosis. Increased level of Serum amyloid A protein (SAA) was found (591 ng/ml). No laboratory or echo signs of cardiac involvement were seen. Mild peripheral neuropathy was found. DNA analysis evidenced the co-existence of heterozygous TNFRSF1A p.R92Q and MEFV p.M694I mutations associated with several MEFV polymorphisms which could increase the proinflammatory signals. This is a unique case of TNFRSF1A/MEFV signalling alteration leading to an autoinflammatory syndrome where AA systemic amyloidosis is the sole manifestation.

**PB 18****Diagnostic Value of Minor Salivary Gland Biopsy in Systemic Amyloidosis: Results of a Retrospective Study in 20 Patients**

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**Background:** Amyloidosis is a disorder characterized by deposition of an abnormal fibrillary extracellular protein with a  $\beta$ -sheet structure. Tissue biopsy stained with Congo red demonstrating amyloid deposits with apple-green birefringence is required for diagnosis. A less invasive but also less sensitive approach, such as fine-needle abdominal fat aspiration, is feasible, providing the diagnosis in about 85% of cases.

In the remaining patients, the minor salivary gland biopsy represents a simple and safe alternative to surgical ones.

**Objective:** To demonstrate the diagnostic value of salivary gland biopsy in patients with systemic amyloidosis and negative abdominal fat aspirate sample.

**Methods:** We retrospectively evaluated by minor salivary gland biopsy 20 patients (median age 67 yrs) with systemic amyloidosis and negative abdominal fat aspirate, referred to our Institution between March 2009 and June 2011. The diagnosis was previously assessed by involved organ biopsy (tongue, stomach, conjunctiva, and lung).

Salivary glands were removed with a biopsy forceps and the samples collected were fixed in formalin, stained with Congo red and analyzed by polarized light microscopy.

**Results:** In 17 cases (85%) minor salivary gland biopsy was diagnostic, whilst negative in 2 cases (10%, myocardial biopsy required). In one case the sample collected did not provide adequate material for histological analysis.

**Discussion:** Minor salivary gland biopsy is a safe and reliable tool for the diagnosis of systemic amyloidosis. In our experience, it was performed in all cases without adverse events, demonstrating an overall diagnostic sensitivity of 85 % in patients with negative fat aspirate.

**Conclusion:** Despite abdominal fat aspirate biopsy still remains the most common procedure used in the evaluation of patients with suspected amyloidosis, minor salivary gland biopsy has been proven to be effective and suitable as first-step diagnostic tool, especially in those patients who cannot undergo perumbilical fat aspiration due to previous abdominal surgery.

**PB 19****ApoA4: a novel form of amyloidosis?**

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**Background:** Amyloidosis is a disease characterized by protein misfolding that leads to deposition of amyloid fibrils in tissues. To date, at least 26 different proteins are known to form amyloid in humans. Recent introduction of liquid chromatography mass spectrometry (LCMS) has significantly improved the ability to subtype amyloidogenic proteins and has contributed to the identification previously indeterminate amyloidosis.

**Objective:** Recently, we identified a patient with cardiac amyloidosis in which ApoA4 is the dominant mass spectrometry signature. This study was undertaken to determine if ApoA4 is found in other tissues with amyloid deposits.

**Methods:** Between 2008 and 2010, all renal biopsies that were positive for amyloid deposits and underwent LMD-MS were included in the study.

**Results:** During the study period, 127 renal biopsies underwent LMD-MS with amyloid deposits. In 6 cases that were previously indeterminate by immunohistochemistry techniques, ApoA4 was the dominant spectra in 4 cases. In all of these cases, the amyloid deposits were restricted to the interstitial compartment with no glomerular or vascular compartment deposits. AApoA4 comprised of 3.1% of the cases. In comparison, AL/AH were noted in 52.5%, AA in 12.5% and AFib comprising of 5.5% of cases.

**Discussion:** In our limited case series, amyloid deposits with predominate ApoA4 was found in cardiac and renal tissue. It remains unknown whether the ApoA4 involved in amyloidogenesis is a mutant or a naturally occurring protein. Further studies are needed to better characterize this new subtype of amyloidosis.

**Conclusion:** ApoA4 appears to be a novel subtype of amyloid.

**PB 20**

**Diagnosis of amyloid in urine cytology specimens**

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Amyloidosis is perceived to be rarely seen in surgical pathology and there is a paucity of data regarding diagnosis in cytology preparations. This report presents the detection of unsuspected amyloid in a cytology specimen.

A 51 year old male presented with hematuria and underwent cystoscopy with bladder barbotage for cytology and a bladder biopsy. Thinprep cytology slides demonstrated benign urothelial cells and small clumps of amorphous material, which was subsequently shown to be amyloid by Congo red stain. Subsequently-available biopsy slides confirmed the presence of vascular and interstitial amyloid. Amyloid typing demonstrated the presence of amyloid derived from the lambda light chain – AL-lambda. There was no evidence of systemic amyloidosis or underlying plasma cell dyscrasia. The Figure below demonstrates a pauci-cellular specimen with Congo red positive and birefringent material diagnostic of amyloid.



This report illustrates the feasibility of amyloid detection in cytology thinprep preparations.

**PB 21**

**Diagnosis of amyloid in frozen sections**

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Amyloidosis is perceived to be rarely seen in surgical pathology and there is a paucity of data regarding diagnosis on frozen sections. This report presents 7 patients with unsuspected amyloidosis who were diagnosed at the time of frozen section. There were 4 females and 3 males, age ranging from 52 to 72. Two patients presented with laryngeal mass, one with ocular mass, two other patients with hematuria and two patients with intractable gastrointestinal bleeding. Four patients underwent surgical exploration with frozen section evaluation and planned immediate staging procedure based on frozen section diagnosis; three patients underwent a biopsy evaluated with frozen section for sample adequacy. Frozen sections demonstrated acellular deposits of amorphous material. Congo red stain performed on frozen section confirmed the presence of amyloid. Subsequent studies demonstrated localized amyloid in 5 patients and systemic amyloidosis (AL x1 and senile ATTR x1) in 2 patients.

Diagnosis of amyloid at the time of frozen section evaluation allowed the avoidance of unnecessary extended surgery and, hence, had a significant impact on patient management.

## PB 22

### Apolipoprotein A-IV-associated amyloidosis: 3 new cases

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**Background/Objectives:** Hereditary forms of amyloidosis are known to be associated with the deposition of apolipoproteins. Cases of Apolipoprotein (Apo) AI and AI<sup>I</sup> amyloid deposits have been described around the world. Recently, two reports of ApoA-IV-associated cardiac and renal amyloidosis were published in the literature. Here, we present clinical and genetic data on three new cases of amyloidosis associated with the deposition of ApoA-IV.

**Methods and Results:** Patient 1. An 81-year-old male with a 5-year history of renal insufficiency and no family history of amyloidosis presented with shortness of breath and minimal leg swelling. Clinical evaluation revealed an infiltrative cardiomyopathy with congestive heart failure. Endomyocardial biopsy showed nodular amyloid deposits within the myocardium. Mass spectrometry (MS) of Congo Red (CR)-positive laser micro-dissected areas of heart demonstrated ApoA-IV to be the main constituent of the amyloid deposits with a small amount of co-deposited SAP, ApoE, and ApoA-I proteins.

Patient 2. A 62-year-old male with no family history of amyloidosis presented with mild renal insufficiency. Kidney biopsy demonstrated isolated interstitial amyloid deposits in the deep medullary areas along with sparing of the cortex, vasculature, and glomeruli. Immunohistochemistry showed negative staining for TTR, Fibrinogen, AA, LECT2, immunoglobulin heavy chains, and kappa & lambda light chains. MS of peptides extracted from CR-positive laser micro-dissected areas of kidney revealed ApoA-IV, ApoE, and SAP to be the dominant amyloid-associated proteins.

Patient 3. A 57-year-old male with negative family history of amyloidosis presented with elevated creatinine. Kidney biopsy showed amyloid deposits predominantly involving medulla. MS of laser micro-dissected CR-positive material demonstrated deposition of ApoA-IV.

DNA sequencing of the ApoA-IV gene detected: patient 1 – two polymorphic homozygous sequence variants: c.87G>A (p.Thr29Thr) and c.440G>A (p.Ser147Asn); patients 2 & 3 – similar polymorphic heterozygous sequence variants. No sequence variants were found in the ApoA-I gene in patients 1 & 2.

**Conclusion:** ApoA-IV-associated amyloidosis may not be an uncommon entity and should be considered in patients with renal and/or cardiac amyloidosis of unknown origin.

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## PB 23

### Cerebral amyloidotic angiopathy in young adults

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**Abstract:** Cerebral amyloidotic angiopathy (CAA) is a progressive microvascular amyloidosis affecting the small and medium-sized arterioles and the capillaries of brain parenchyma and leptomeninges. CAA occurs mostly as a sporadic condition in the elderly, with incidence associated with increasing age, and is recognized as a cause of lobar intracerebral hemorrhage (ICH). The precursor protein in all sporadic cases is amyloid-β protein (Aβ). Pathologically, CAA is characterized by deposits of amyloid in cerebral vessel walls. We report two patients who experienced ICH due to Aβ-positive CAA at age 37 and 42 years, respectively.

Patient A. The 42-year-old patient experienced sudden left hemiparesis and conjugated ocular paresis to the left. A CCT scan showed large right frontal hemorrhage. A second CCT scan done four days later showed a new small right parietal bleeding. In the past, he had suffered from bilateral frontal and

right occipital bleedings. A meningeal biopsy was done for suspected vasculitis. Histologic examination showed congophilic angiopathy positive for A $\beta$  immunostaining. Eight months later, an MRT scan showed right frontal, parietal and occipital defects in addition to numerous cerebral microbleeds.

Patient B. The second patient came at age 43 years to our attention when he experienced a sudden left hemiparesis. A CCT scan showed right parietal ICH. A first left frontal ICH occurred at age 37 years, and in addition to this, a CCT scan at that time showed a defect from an earlier right occipital bleeding. A MRI scan disclosed defects in several locations, including left and right frontal, right temporal and parieto-occipital, and a large number of microbleeds in both hemispheres. A meningeal biopsy showed congophilic angiopathy with positive staining with antibodies against A $\beta$ .

**Discussion:** Sporadic CAA is a common pathologic finding in the elderly. It is typically considered in the differential diagnosis of lobar ICH in elderly patients. There are only a few reports in patients under 50 years old (1). To the best of our knowledge, patient B is the youngest patient with CAA reported in so far he was only 37 years old when he presented the first time with ICH. The CCT scan even showed an earlier ICH at that time.

Though we cannot exclude that our patients had hereditary CAA (patient A refused genetic testing, the results of genetic testing in patient B are pending), careful history examination revealed no evidence of an autosomal-dominant trait in the two families. In addition, unlike sporadic CAA, hereditary forms are exceedingly rare (2). We suggest that sporadic CAA is an underdiagnosed entity in younger adults with lobar ICH.

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#### PB 24

#### Hereditary gelsolin amyloidosis: a novel variant N211K, and a review of the clinical, histological and SAP scintigraphic characteristics

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**Background:** Familial amyloidosis, Finnish type (FAF) (MIM# 105120), is an extremely rare type of hereditary amyloidosis associated with variant gelsolin. Two amyloidogenic mutations have previously been reported, both resulting in substitution of Asp at position 214 (previously described as 187) by either Asn or Tyr.

**Objective:** We report here a third amyloidogenic mutation and review the clinical findings of all 10 patients with hereditary gelsolin amyloidosis followed at the UK National Amyloidosis Centre since 1991.

**Methods:** Evaluation of patients included clinical review, <sup>123</sup>I-SAP scintigraphy, echocardiography, DNA sequencing, histology and, where necessary, microdissection of amyloid deposits for mass spectrometry based proteomics (LDMS).

**Results:** A 61 yr old hypertensive, diabetic man of German ancestry presented with nephrotic syndrome and chronic kidney disease. There was no family history of renal disease, neuropathy or amyloid. Renal biopsy showed extensive glomerular amyloid and LDMS confirmed that the amyloid deposits were composed of variant gelsolin. DNA sequencing showed a novel gelsolin mutation (c.633C>A) encoding the variant N211K (nomenclature includes the 27 residues signal peptide omitted in the FAF literature).

Nine further patients were heterozygous for the gelsolin mutation encoding the amyloidogenic D214N (D187N) variant. Proteinuria was the presenting feature in two cases and the remainder were found to have corneal lattice dystrophy and/or cranial neuropathy. Cardiac amyloidosis did not occur. Median (range) age at presentation was 45 (32-60) years. SAP scintigraphy, performed in nine patients, showed abnormal renal uptake in every case and was corroborated by extensive glomerular amyloid in the two patients who underwent kidney biopsies.

**Conclusions:** We report a novel amyloidogenic gelsolin variant, N211K, and highlight the universal presence of renal amyloid deposits in patients with hereditary gelsolin amyloidosis.

## PB 25

### Familial amyloid polyneuropathy in Galician population

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**Background:** Transthyretin-related familial amyloidotic polyneuropathy (FAP) is a fatal inherited autosomal dominant disease resulting from mutations in the transthyretin (TTR) gene.

Liver transplantation (LT), which removes the main source of mTTR, must be performed early in the course of the disease, along with symptomatic treatment.

**Objective:** To evaluate the clinical, pathological and genetic features in patients of the galician community with FAP.

**Methods:** Probands suffered painful disesthesia and sensorial defects in distal limbs. Electrophysiology, cardiac examinations and sural nerve biopsies were performed with histochemical and immunohistochemical stainings including TTR. Post-mortem studies were realized in one patient. Exon 2 of TTR gene was sequenced in probands and relatives who asked for presymptomatic molecular diagnosis.

**Results:** Sural biopsies showed variable loss of nerve fibers. Congo red and thioflavin T affinity confirmed endoneurial and vascular amyloid deposits, positive for TTR. Ultrastructural examination demonstrated amyloid fibrils Postmortem studies revealed multi-organ involvement in one patient. Genetic analysis demonstrated amino acid substitution of Val 30 for Met in exon 2 of TTR gene in probands and several relatives.

**Discussion:** 14 families were diagnosed, with variable age at onset and illness duration. Ninety per cent of patients referred familial history. Cardiac and autonomic involvement was detected in some patients.

Our results are consistent with the Portuguese form of FAP first reported by C. Andrade in 1952.

### Conclusions

- FAP diagnosis should be considered in adult patients with neuropathic pain or sensory distal limb involvement.
- Familial history and geographic origin facilitate the diagnosis and sural nerve biopsy shows TTR positive amyloid deposits.
- Molecular analysis of *TTR* gene confirms diagnosis
- Genetic tests can be offered to at-risk family members, providing presymptomatic diagnosis
- FAP diagnosis is important for clinical follow-up, symptomatic management, genetic counseling and eventual indication of liver transplant or future effective pharmacologic treatment.

## PB 26

### Retinal microangiopathy as an initial manifestation of familial amyloid cardiomyopathy associated with transthyretin E89K mutation

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**Background:** Hereditary transthyretin amyloidosis (ATTR) is the most frequent form of familial amyloidosis. Although vitreous amyloid is frequently associated with familial amyloid polyneuropathy, it has only rarely ever been reported in familial cardiomyopathy.

**Objective:** To report a rare case of transthyretin familial amyloid cardiomyopathy due to a rare TTR variant (E89K), with retinal microangiopathy and vitreous amyloid as the initial manifestation.

**Methods:** A 54 year old female presented with bilateral retinal microangiopathy, presumed idiopathic retinal vasculitis. She subsequently developed retinal ischaemia, associated vitreous haemorrhage and was treated with panretinal laser photocoagulation. Clinical eye signs remained stable for 6 years with absence of overt inflammation. However, the patient developed chest pain and atrial flutter and

underwent echocardiography, cardiac magnetic resonance imaging and  $^{99m}$ Tc-3,3-Diphosphono-1,2-Propanodicarboxylic Acid (DPD) scintigraphy to investigate possible cardiac amyloidosis. Sequencing of the TTR gene was conducted, and a rectal biopsy performed for tissue diagnosis. A full neurological screen was also conducted.

**Results:** Cardiac investigations were highly suggestive of an amyloid cardiomyopathy. The rectal biopsy stained positive for Congo red with demonstration of apple green birefringence, confirming amyloid, and immunostaining confirmed the TTR subtype. Gene sequencing revealed heterozygous TTR mutation encoding E89K variant. No significant neuropathy could be detected.

**Discussion:** Vitreous amyloid has only rarely been reported in isolated familial amyloidogenic cardiomyopathy. Retinal microangiopathy and neovascularisation with associated vitreous haemorrhage is an uncommon complication of hereditary transthyretin amyloidosis and has previously been reported in patients with FAP. Treatment of ATTR is usually symptomatic. The patient maintains good vision at 6/5 corrected despite extensive vitreous amyloid.

**Conclusion:** Cardiac investigations were highly suggestive of an amyloid cardiomyopathy. The rectal biopsy stained positive for Congo red with demonstration of apple green birefringence, confirming amyloid, and immunostaining confirmed the TTR subtype. Gene sequencing revealed heterozygous TTR mutation encoding E89K variant. No significant neuropathy could be detected.

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#### PB 27

#### The distribution of age at onset of ATTRVal30Met FAP in Japan

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**Background:** ATTRVal30Met FAP is the most common type of FAP in Japan. Typically, Japanese ATTRVal30Met FAP patients are related to endemic foci and develop disease in the second or third decade of life. On the other hand, the existence of late-onset ATTRVal30Met FAP unrelated to endemic foci has been recognized in Japan.

**Objective:** To determine the distribution of age at onset of ATTRVal30Met FAP in Japan.

**Methods:** From 1980 to 2011, 281 patients were diagnosed as having FAP in the Shinshu University School of Medicine. Among them, 243 patients had Val30Met mutation in the *TTR* gene. We retrospectively analyzed the clinical records of these FAP patients.

**Results:** The age at onset ranged from 18 to 80 years and showed bimodal distribution with two distinct peaks; the first peak was 25 to 29 years and the second peak was 60 to 64 years. Based on the distribution of age at onset, ATTRVal30Met FAP patients were separated into two groups, i.e., early-onset (<50 years) and late-onset ( $\geq$ 50 years) group. The early-onset group consisted of 151 patients (82 men and 69 women), and 81.5 % of the patients were originated from endemic foci. On the other hand, the late-onset group consisted of 92 patients (67 men and 25 women), and only 12% of the patients were originated from endemic foci. Eighteen patients developed disease after 70 years old. From 1980 to 1999, only 23% of patients diagnosed as having ATTRVal30Met FAP were late-onset, while 62% of the patients were late-onset after 2000.

**Conclusions:** The age at onset of Japanese ATTRVal30Met FAP patients shows bimodal distribution with 2 distinct peaks, indicating that there is at least one genetic modifier or non-genetic factor which contributes to the pathogenesis and progression of FAP. The number of aged patients with ATTRVal30Met FAP is increasing in Japan.

This study was supported by Grant-in-aid for Scientific Research (20590695 to YS); a grant from the Intractable Disease Division, the Ministry of Health and Welfare, Amyloidosis Research Committee in Japan.

**PB 28****Cardiac amyloidosis with reduced left ventricular ejection fraction and normal interventricular septal thickness**

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Increased interventricular septal thickness (IVST) by echocardiography with low-voltage on electrocardiogram (ECG) is commonly used as an indicator of amyloid heart disease. We have observed a subset of patients with amyloid who have heart failure and normal IVST. All patients with amyloidosis and left ventricular ejection fraction (LVEF)  $\leq 40\%$  between 1983 and 2010 were analyzed to identify those with normal BMI-weighted IVST. Patients with a history of coronary artery disease were excluded. Nineteen (M=15,F=4) of 255 patients identified had normal IVST. The mean age of this group was  $65 \pm 10$  years. Eighteen had primary systemic amyloidosis and one had hereditary amyloidosis. All 19 patients had amyloidosis proven by biopsy. Edema, dyspnea, and weight loss were the most common presenting symptoms with mean duration from onset to presentation being  $5 \pm 3$ ,  $6 \pm 5$ , and  $7 \pm 7$  months, respectively. Common ECG findings despite normal IVST included low-voltage (26%) and a pseudoinfarct pattern (37%). Mean IVST, LV end diastolic dimension and LV mass index amongst this cohort were  $11.1 \pm 1.6$ mm,  $51 \pm 7$ mm, and  $114 \pm 23.8$ g, respectively. Grade 3/4 diastolic dysfunction was observed in 5 of 19 patients. Nine patients had a thickened mitral valve and of these 2 had a thickened tricuspid valve. Median survival from initial tissue diagnosis amongst the normal IVST cohort (group 1) was 4.5 months as compared to 3.5 months in age and gender matched controls with increased IVST and LVEF  $\leq 40\%$  (group 2). Mortality in both groups 1 and 2 is high with 81% and 79% dying within one year of onset of symptoms, respectively. A subset of patients with amyloidosis and cardiomyopathy present with normal IVS wall thickness and account for 7% of patients with amyloidosis and LVEF  $\leq 40\%$ . Amyloidosis must be considered in the differential diagnosis of patients with cardiomyopathy with reduced EF and normal IVST. The prognosis of these patients is as poor as those with increased IVST.

**PB 29****Utility of the Heavy Light Chain (HLC) assay in patients with AL amyloidosis with a detectable serum monoclonal protein**

**Ashutosh D Wechalekar<sup>1</sup>**, Philip Young<sup>2</sup>, Nancy Wassef<sup>1</sup>, Julian D Gillmore<sup>1</sup>, Simon DJ Gibbs<sup>1</sup>, Jennifer H Pinney<sup>1</sup>, Christopher P Venner<sup>1</sup>, Darren Foard<sup>1</sup>, Lisa Rannigan<sup>1</sup>, Thirusha Lane<sup>1</sup>, Carol J Whelan<sup>1</sup>, Helen J Lachmann<sup>1</sup>, Arthur Bradwell<sup>2</sup>, Stephen Harding<sup>2</sup> and Philip N Hawkins<sup>1</sup>

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**Background:** Patients with AL amyloidosis usually have subtle underlying clonal plasma cell dysrasias, and those who have a serum monoclonal immunoglobulin band detectable by standard serum protein electrophoresis (SPEP) typically have an M-protein concentration of just a few grams per litre. Quantitation of low level M-bands is inaccurate by SPEP, and this technique is therefore unsuitable for serial monitoring. Specific immunoassays have been produced that quantify Ig'κ/Ig'λ (heavy/light, HLC) in serum. We report here the use of HLC assays as an aid in identifying and quantifying monoclonal proteins in AL amyloidosis patients.

**Methods:** HLC (for IgG/IgA/IgM) ratios were measured in 152 patients with AL amyloidosis assessed at the UK National Amyloidosis Centre, in whom a monoclonal band was detectable by SPEP and/or immunofixation (IFE). We compared the results with 20 healthy controls samples.

**Results:** The median age of patients was 70 years (range, 35-85 years) with 96 (63%) males. The serum monoclonal immunoglobulin band was characterized by IFE as monoclonal IgG in 101 cases, monoclonal IgA in 33 cases, and monoclonal IgM in 18 cases. Abnormal HLC ratios were identified in 136/152 (89%) of patients, compared to 106/152 (70%) in whom monoclonal bands were quantifiable by SPEP. 103/106 patients with SPEP detectable M-protein bands had abnormal HLC ratios. Receiver operator curve (ROC) analysis of the patients and 20 healthy controls confirmed HLC ratio had a greater sensitivity than SPEP for the detection and quantification of intact immunoglobulin in AL amyloidosis (ROC AUC: HLC ratio=0.95 v 0.85, respectively). Data on quantitation will be presented.

**Conclusion:** HLC assays have greater sensitivity and quantitative potential than SPEP in AL amyloidosis. Management of patients with AL amyloidosis relies on accurate assessment of patients' clonal disease responses to treatment - further longitudinal studies are needed to determine the role of HLC analysis for this purpose.

PY and SH are employees of The Binding Site Group Ltd.

#### PB 30

#### **Normal heavy/light chain (HLC) and free light chain (FLC) ratios are associated with prolonged survival in patients with systemic AL amyloidosis**

**Ashutosh D Wechalekar<sup>1</sup>**, Philip Young<sup>2</sup>, Nancy Wassef<sup>1</sup>, Julian D Gillmore<sup>1</sup>, Simon DJ Gibbs<sup>1</sup>, Jennifer H Pinney<sup>1</sup>, Christopher P Venner<sup>1</sup>, Darren Foard<sup>1</sup>, Lisa Rannigan<sup>1</sup>, Thirusha Lane<sup>1</sup>, Carol J Whelan<sup>1</sup>, Helen J Lachmann<sup>1</sup>, Arthur Bradwell<sup>2</sup>, Stephen Harding<sup>2</sup> and Philip N Hawkins<sup>1</sup>

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**Background:** Serum free light chain (FLC) concentration at diagnosis is predictive of outcome in AL amyloidosis. We report the utility of heavy/light chain (Ig'k/Ig'λ, HLC) measurements in AL patients with and without abnormal FLC ratios.

**Methods:** HLC (IgG, IgA and IgM) were measured in two series of patients with AL amyloidosis: Group A – 147 unselected patients, and Group B - 146 patients selected for having normal or near-normal FLC ratios (normal FLC / total number patients; A 46/147, B 105/146).

**Results:** In both populations, abnormal FLC ratios were associated with poorer overall survival (OS) at 36 months (A: HR=2.2, p=0.037; B HR=2.1, p=0.006). Individually, IgG, IgA or IgM HLC ratios were not associated with OS in either population. However, a model based on combining presence (or not) of abnormal HLC and FLC ratios as a risk factor (0=both normal, 1 = either one abnormal and 2 = both abnormal) showed significant discriminatory power. Population A: patients with no risk factors (normal FLC & HLC ratios, n=23) had significantly better OS than patients with 1 or 2 risk factors (abnormal FLC and/or HLC ratios, n=123, p=0.04). At 36 months, mortality was only 9% in patients with no risk factors compared to 47% in patients with 1 or 2 risk factors. Population B: patients with 0 risk or 1 risk factor (n=127) had significantly better OS than those with both abnormal FLC and HLC ratios (n=21, p=0.007). At 36 months, the mortality was 34% in patients with 0 or 1 risk factors and 67% mortality in patients with 2 risk factors.

**Conclusion:** A combination of HLC and FLC ratios provides useful prognostic information in patients with AL amyloidosis – those with both normal HCL and FLC ratios have excellent outcomes. The clinical utility of this model needs to be confirmed in larger unselected AL populations.

PY and SH are employees of The Binding Site Group Ltd.

#### PB 31

#### **Left atrial function assessment in patients with systemic light chains amyloidosis : a 3D speckle tracking imaging study**

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**Background:** Echocardiography (TTE) is the most common test performed in patients with systemic light chains amyloidosis (SAL) and suspected cardiac involvement, characterized by advanced diastolic dysfunction and left atrial (LA) enlargement. Whether LA function is also decreased in such case has never been assessed. The aim of this study is to assess LA volumes, total LA emptying fraction (LAEF) and LA longitudinal function using 3D speckle tracking imaging (3D-STI-TTE), a recent technique coupling a 3D volumetric method with a strain imaging technique.

**Methods and Results:** Twenty-five consecutive patients (67±11 years, 64% male) in sinus rhythm with SAL and different degrees of cardiac severity (Mayo Clinic Staging) were included. Classical

echographic parameters were obtained along with 3D: LA volumes, LAEF and LA longitudinal function using a 3D probe for the Artida, Toshiba Medical Systems.

Comparing to patients with no- or minimal cardiac involvement (stage I), those with moderate and high risk MC (stage II and III) had significantly larger left atrial volumes and poorer 3D LAEF and STI longitudinal LA function (table).

**Conclusion:** LA volumes and function were significantly and progressively altered among SAL patients according to Mayo stage. Larger studies are warranted to evaluate the prognostic impact of these new echo parameters in SAL patients.

|   | Stage I<br>(7) | Stage II<br>(11) | Stage III<br>(7) |
|---|----------------|------------------|------------------|
| Age                                     | 67±12          | 65±3             | 70±4             |
| Male (%)                                | 71             | 55               | 71               |
| 2D LV EF (%)                            | 64±4\$         | 57±12            | 54 ±6            |
| <b>Inter ventricular septum (mm)</b>    | <b>12±3</b>    | <b>14±2</b>      | <b>15 ±2*</b>    |
| <b>Septal A' (cm/sec)</b>               | <b>11±3</b>    | <b>8±3</b>       | <b>4 ±3*,†,</b>  |
| <b>E/E' septal ratio</b>                | <b>9±4</b>     | <b>15±7</b>      | <b>17 ±8*</b>    |
| <b>2D max LA vol (mL)</b>               | <b>44±7</b>    | <b>62±11</b>     | <b>82 ±12*</b>   |
| <b>3D max LA vol (mL)</b>               | <b>46±8</b>    | <b>54±7</b>      | <b>86 ±13*,†</b> |
| <b>3D-total LA-EF (%)</b>               | <b>43±14</b>   | <b>36 ±13</b>    | <b>21 ±6*,†</b>  |
| <b>3D-ST LA longitudinal strain (%)</b> | <b>32±20</b>   | <b>20 ±12</b>    | <b>9 ±4*</b>     |

\*P≤ 0.01 compared with grade I

†P< 0.05 compared with grade II

## PB 32

### Identification and characterization of TTR amyloid associated molecules in FAP

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**Introduction:** Familial amyloidotic polyneuropathy (FAP) induced by amyloidotic TTR (ATTR), is characterized by systemic accumulation of amyloid fibrils. Recently, it has been shown that some of amyloid associated molecules, such as serum amyloid P component, apolipoprotein E, and proteoglycans, are co-localized with TTR amyloid fibrils and may play important roles in TTR amyloid fibril formation. However, the role of those co-localized proteins with TTR amyloid deposition is still largely unknown. In this study, we focused on the amyloid associated molecules and attempted to identify key molecules in TTR amyloid-laden tissues from FAP patients by using liquid chromatography tandem mass spectrometry (LC-MS/ MS).

**Materials and Methods:** Autopsy tissue specimens from FAP patients were analyzed. Congo red - positive areas were digested with Liquid Tissue (Expression Pathology, Inc., Gaithersburg, MD, USA) and trypsin, and analyzed by LC-MS/ MS. The existence of amyloid associated molecules in systemic organs was evaluated by immunohistochemical analysis. Serum samples of 25 FAP patients (18 of early onset cases and 7 of late onset cases; 16 males and 9 females) were also analyzed to determine the existence of identified molecules.

**Results and Discussion:** LC-MS/ MS analysis showed that various amyloid associated molecules were co-localized with TTR amyloid deposition. Of those various molecules, clusterin, a constitutively secreted extracellular molecular chaperone, was identified. Serum clusterin level of FAP patients was significantly higher than that of healthy volunteers. The mean level of early onset cases was significantly higher than that of age-matched healthy volunteers. Moreover, negative correlation was observed between the levels and severity of autonomic dysfunction.

**Conclusion:** LC-MS/ MS analysis identified clusterin as an amyloid associated molecule in FAP. Clusterin may play important roles in the pathogenesis of FAP.

**PB 33****Amyloid, a major player in pathogenesis of atherosclerosis?**

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Localized amyloid deposits are of increasing interest since, although generally small, they are understood to be of pathogenic importance in a number of distinct disorders. Among the most prevalent amyloid deposits are those in the aorta where medin-derived (AMed) amyloid occurs in almost everyone after 60 years of age. Less is known regarding the second common localized aortic amyloid which is clearly associated with atherosclerosis. Our hypothesis is that this form of amyloid takes part of the development of atherosclerosis.

**Material and Methods:** Aortic specimens were taken from 92 individuals above the age of 51 from 3 different places: aortic arch, aortic bifurcation and at the macroscopically most severe atherosclerotic lesion. Sections were studied for presence of amyloid after Congo red staining and the amount was estimated according to a four-graded scale (0 - +++). Since the composition of atherosclerotic amyloid may be more complex than we previously believed, we have also extracted amyloid, analyzed it by mass spectrometry. Furthermore, antisera against an identified peptide have been raised and used for immunohistochemistry (IH).

**Results:** Amyloid in atherosclerotic plaques occurred in two morphological forms, one compact with strong dye affinity and one diffuse, weakly stainable. Atherosclerotic amyloid was found in 41% of the individuals, most often in advanced lesions with necrosis and cholesterol crystals. Mass spectrometry analysis of purified amyloid repeatedly indicated presence of peptides of a larger well known protein precursor. IH analyses revealed immunolabelling not only of amyloid but also more diffusely in the atherosclerotic lesion.

**Discussion:** Amyloid deposits are very common in atherosclerotic lesions and a participation of aggregated protein in the pathogenesis can be highly suspected. The nature of the amyloid fibril protein will be discussed. It can be underlined that apolipoprotein CII, which has been proposed as a major component, was not found

**PB 34****Piedmont and Aosta Valley Consortium of Systemic Amyloidosis: results of a four year experience**

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The Piedmont and Aosta Valley Consortium of Systemic Amyloidosis was created in 2008 as a part of the Interregional Network of Rare Diseases.

The Consortium purposes included (1) census of patients with systemic amyloidosis, (2) survey of clinical facilities for patients, (3) dissemination of knowledge about amyloidosis and (4) specific training of physicians in order to improve diagnostic sensitivity, (5) promotion of sensitive diagnostic methods, (7) encouragement of a greater cooperation among health care providers, (8) development of specific diagnostic and therapeutic protocols, and (9) initiation of study protocols. Participation in the consortium was spontaneous. To date, the consortium is composed of 85 physicians representing 14 specialties and coming from 12 institutions.

From 2007 to 2011, 73 cases of amyloidosis have been reported. Considering a population of 4578000 people a prevalence of 16/1000000 and an incidence of 3.98/1000000/year can be estimated.

The Consortium has also made a survey on diagnostic and assistance facilities. The results were made available to patients and to general practitioners by editing both specific brochures and web sites.

The diagnostic sensitivity has been checked by an external quality evaluation of laboratory methods for primary amyloidosis (immunofixation and free light chain assay). The Consortium has also

promoted the development of genetic tests for major forms of familial amyloidosis (MEFV, MVK, TNFRA1, TTR, APOA1).

Currently, the consortium is developing diagnostic and therapeutic local Consensus Statements based on international guidelines and is promoting Continue Medical Education courses for physicians to increase the overall diagnostic sensitivity.

The abstract has been written on behalf of all participants in the consortium.

### PB 35

#### **Ageing is associated with AA-type Amyloidosis in Patients with Familial Mediterranean Fever living in Germany**

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**Background:** A delay in diagnosis of Familial Mediterranean Fever (FMF) might increase the risk for amyloidosis.

**Objective:** To analyze risk factors for the development of AA-type amyloidosis (AA) in FMF patients living in Germany.

**Methods:** Sixty-four patients were diagnosed with FMF based on the Tel Hashomer Criteria and MEFV mutations. FMF patients were screened for amyloidosis and patients with AA were screened for FMF. AA was confirmed by immunohistochemistry.

**Results:** Sixteen of 64 patients with FMF (25%) developed AA. In eleven of 16 patients (68%) AA and FMF were identified simultaneously. Patients who developed AA had a longer history of FMF attacks, were older at onset of FMF symptoms and had a higher FMF severity score but differences were not significant (median (95%CI), p). Importantly the median age at FMF diagnosis was 36.5 years in patients with AA compared to 22.0 years in patients without AA ( $p=0.002$ ). Twelve patients had their first FMF symptoms after age 30 and 11 of these patients also had AA (92%,  $p=0.0001$ ). Consecutively AA patients were significantly older at their last follow up (43.5 years) compared to patients without AA (31.0 years). Homozygous M694V mutations ( $n=21$ ) were associated with a younger age at FMF onset (5.5 years (3.9-16.6)) compared to a single M694V mutation ( $n=25$ ) 13.0 years (8.0-21.6,  $p=0.08$ ) or no M694V mutations ( $n=9$ ) 32.0 years (14.2-47.8,  $p=0.0005$ ).

| patients with FMF (n=64) | AA (n=16)        | no AA (n=48)     | p     |
|--------------------------|------------------|------------------|-------|
| History of FMF attacks   | 17.0 (3.8-28.0)  | 7.0 (2.1-11.0)   | 0.13  |
| Age at FMF onset         | 20.0 (6.8-35.4)  | 15.0 (8.5-16.5)  | 0.06  |
| FMF severity score       | 13.0 (8.0-16.4)  | 8.0 (7.0-12.4)   | 0.09  |
| Age at FMF diagnosis     | 36.5 (26.0-62.1) | 22.0 (19.0-29.5) | 0.002 |

**Conclusion:** Recognition of FMF and initiation of colchicine therapy is delayed for many years in a country with low FMF prevalence. Especially older patients are at high risk for AA.

### PB 36

#### **Baseline profile of patients undergoing tafamidis treatment in THAOS – the Transthyretin Amyloidosis Outcomes Survey**

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**Background:** The Transthyretin Amyloidosis Outcomes Survey (THAOS) is a worldwide registry of patients with transthyretin (TTR) amyloidosis (ATTR). Many of these patients are receiving tafamidis (Vyndaqel, Fx-1006A) treatment.

**Objective:** To describe the patient population in THAOS who have undergone treatment with tafamidis, including those with polyneuropathy (TTR-FAP) or cardiomyopathy (TTR-CM).

**Methods:** Demographics and baseline characteristics, including neurologic and cardiac exam measures were analyzed for both cohorts.

**Results:** Of 975 subjects enrolled in THAOS, 84 patients who were receiving tafamidis for treatment of ATTR were analyzed. At the time of enrollment, symptom duration was  $6.0 \pm 5.4$  years (mean  $\pm$  standard deviation) and length of treatment was  $2.4 \pm 0.8$  years. Of those with variant TTR, 93.0% (66/71) reported a family history of ATTR. 67 patients had TTR-FAP-associated mutations (including V30M, I107V, F64L, S77F, S77Y) with a mean age of  $43.4 \pm 14.1$  years and reported symptom onset at  $37.4 \pm 14.6$  years. Common types of symptoms reported included sensory neuropathy (98.5%), gastrointestinal disturbances (80.0%) and autonomic neuropathy (56.9%). Seventeen patients had either wild-type TTR or TTR-CM-associated mutations (V122I, L58H and T60A); they were older ( $74.6 \pm 6.8$  years) and reported later symptom onset ( $68.7 \pm 9.5$  years). Reported symptoms included cardiac problems (88.2%) and sensory neuropathy (64.7%). In patients with TTR-FAP-associated mutations, neurological assessments revealed deterioration consistent with length-dependent axonal degeneration. All patients with TTR-CM-associated genotypes had abnormal electrocardiograms.

**Discussion:** Follow-up data for all patients are currently being recorded, with the goal of demonstrating disease progression and disease-modifying effects of any treatment.

**Conclusions:** The THAOS registry can serve as a useful tool in characterizing disease profiles and monitoring disease progression in patients receiving treatment for ATTR.

Data for this abstract are part of the THAOS registry, which is sponsored by Pfizer Inc.

#### PB 37

#### Relationship between age at symptom onset and left ventricular wall thickness in ATTR amyloid – THAOS survey

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**Background:** Transthyretin amyloidosis (ATTR) is a rare hereditary disorder presenting primarily as polyneuropathy and cardiomyopathy. Multiple factors including TTR genotype, age, and geographic origin may influence phenotype. In particular, a relationship between age at symptom onset and development of cardiomyopathy has been suggested.

**Objective:** To utilize a large, heterogeneous cohort of individuals with ATTR amyloid to evaluate the association between age at symptom onset and cardiac manifestations, controlling for known confounders.

**Methods:** The association of echocardiographic measures of left ventricular (LV) wall thickness in symptomatic patients with ATTR from THAOS (Transthyretin Amyloid Outcomes Survey) with age of symptom onset, gender, mutation, blood pressure and liver transplant history was evaluated via multivariate regression analysis.

**Results:** Multivariate regression analysis (see Table) showed that age and male gender were independent positive predictors of increasing mean LV wall thickness in ATTR amyloid, whereas V30M mutation was a negative predictor.

| Variable                        | Parameter Estimate | P value |
|---------------------------------|--------------------|---------|
| Age (per 10 years)              | 0.54               | 0.0483  |
| Gender (Male vs Female)         | 1.96               | 0.0182  |
| Wild type vs Mutant             | -0.28              | 0.8071  |
| V30M vs non-V30M                | -2.8               | 0.0125  |
| Cardiac vs non-cardiac mutation | 1.27               | 0.2894  |
| Mean arterial pressure          | 0.04               | 0.1558  |
| Prior liver transplant          | -1.15              | 0.2626  |

Dividing the patients by age into late ( $\geq 50$  years) vs early symptom onset ( $< 50$  years), mean LV wall thickness was significantly higher in patients with late onset (15.7 vs 10.6 mm;  $P < 0.0001$ ). Late symptom onset was also associated with greater LV wall thickness than early onset ( $P < 0.01$ ) in both V30M and non-V30M patients.

**Discussion:** In ATTR, cardiac manifestations are more pronounced in patients with late symptom onset. The association between older age at symptom onset and more severe disease was seen regardless of the specific mutation(s) studied.

**Conclusion:** There is a strong association between age at symptom onset and cardiac involvement as determined by LV wall thickness in patients with ATTR.

Data for this abstract are derived from the THAOS registry, which is sponsored by Pfizer Inc.

### PB 38

#### Baseline Demographics and Clinical Characteristics in THAOS—the Transthyretin Amyloidosis Outcomes Survey

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**Background:** Transthyretin (TTR) amyloidosis (ATTR) is a rare systemic condition with two main forms: hereditary (associated with over 100 identified genetic mutations) and wildtype (WT) ATTR (associated with advanced age). Patients with ATTR present with polyneuropathic, cardiac, or mixed phenotypes, which can vary by genotype and geographic region. Established in 2007, the Transthyretin Amyloidosis Outcomes Survey (THAOS) is a global, multicenter, longitudinal, observational survey that collects data on the natural history of ATTR and aims to document the efficacy and safety of treatment modalities.

**Objective:** The present analysis describes baseline demographic and disease characteristics of subjects in THAOS as of September 1, 2011.

**Methods:** Symptomatic individuals with confirmed WT or variant ATTR and asymptomatic carriers of variant TTR are eligible for enrollment. Patient information collected includes cardiac and neurologic findings, renal function assessments, quality of life (QOL) assessments, hospitalizations, medications, and transplant history.

**Results and Discussion:** Data are available for 975 subjects, including 688 symptomatic patients with either WT (77 patients) or variant (611 patients) ATTR and 274 asymptomatic carriers of variant ATTR. Nineteen countries of origin are represented; the largest patient groups are from Portugal, the United States, and Italy. Forty-three TTR mutations reported were associated with multisystem involvement; ATTR phenotypes differed according to mutation. Val30Met was the most common mutation, and the majority of Val30Met patients (67.6%) were from Portugal.

**Table:** Comparison of health-related QOL as assessed by the EQ-5D Index in individuals with TTR mutations (symptomatic, asymptomatic and the Portuguese subset of the Val30Met population) and in the general population of the USA (Ref).

| Mean (SD) EQ-5D index |  |   |   |                          |
|-----------------------|--|---|---|--------------------------|
| Age range (years)     | All Symptomatic patients with a TTR mutation | Asymptomatic patients with a TTR mutation | Symptomatic patients with a Val30Met mutation in Portugal | General population (USA) |
| 18–34                 | 0.82 (0.15)*<br>n = 131                      | 0.92 (0.12)<br>n = 95                     | 0.86 (0.15)*<br>n = 186                                   | 0.92 (0.22)<br>n = 11752 |
| 35–49                 | 0.73 (0.20)*<br>n = 126                      | 0.88 (0.15)<br>n = 55                     | 0.77 (0.20)*<br>n = 141                                   | 0.88 (0.22)<br>n = 12157 |
| 50–64                 | 0.61 (0.28)*<br>n = 77                       | 0.80 (0.19)<br>n = 20                     | 0.55 (0.29)*<br>n = 39                                    | 0.84 (0.28)<br>n = 8375  |
| ≥ 65                  | 0.65 (0.28)*<br>n = 49                       | 0.76 (0.24)<br>n = 11                     | 0.47 (0.35)*<br>n = 14                                    | 0.79 (0.24)<br>n = 6394  |

\*p ≤ 0.0002 versus general population. Values given are means (SD).

Nearly all symptomatic Portuguese Val30Met patients (91.0%) reported sensory neuropathy while 26.6% reported motor neuropathy. Similar to the entire population of symptomatic patients with a TTR mutation, Portuguese Val30Met patients reported significantly worse QOL (EQ-5D assessments) compared to the general US population (Table).

**Conclusion:** THAOS demonstrates the value of an international registry to better understand presentations of ATTR.

**Reference:** Sullivan PW et al. A national catalog of preference-based scores for chronic conditions in the United States. *Med Care*. 2005;43:736-49.

Data for this abstract are derived from the THAOS registry, which is sponsored by Pfizer Inc.

#### PB 39

#### The Pavia project for the integration of clinical data with a centralized biobank for systemic amyloidosis

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**Background:** Biobanks are cryogenic facilities where biological samples are stored, archived and retrieved. In low-prevalence diseases as amyloidoses, the availability of large biobanks of optimally preserved and annotated samples is crucial for research advancement (1). The Pavia Amyloid Center coordinates a network of 70 peripheral centers nationwide. Our aim was to create a state-of-the-art centralized amyloid biobank, conjugating optimal specimen storage with development of bioinformatic tools for managing sample information.

**Methods:** Samples are stored at -80 °C, upon acquisition of patients' consent. Freezers are monitored through a web interface. Standard Operating Procedures (SOP) for sample handling were designed. Each individual is assigned a code and samples are de-identified. Specimens are immediately translated to the storage facility upon acquisition; samples shipped from peripheral Centers in dry ice are also collected. Plasma/serum, urine, fat tissue and bone marrow are acquired at diagnosis and follow-up visits, along with other tissues, when required by diagnostic workflow. A bioinformatic system is being implemented to support storage and information retrieval, based on the dedicated web-based electronic patient record "AMICA" (2).

**Results:** Samples from 1750 patients referred for suspected amyloidosis since 2008 are stored. About 850 of them were diagnosed with various systemic or localized amyloidoses. Serum, urine and plasma are available in all patients, fat tissue in 900 and bone marrow cells in 250. SOP guarantee an extremely controlled sample quality. The data model for the biobank is designed, together with a set of queries to allow retrieving, for a given sample(s), the data collected in the clinical sections of AMICA.

**Conclusions:** A large biobank of optimally preserved samples from amyloid patients is available, along with instruments for its interrogation. This biobank is a precious resource for studies on systemic amyloidoses, including proteomics and other high-throughput analyses.

#### References:

1. Public Health Genomics. 2011;14:96-103;
2. Amyloid 2011;S1:236-238.

#### PB 40

#### Iatrogenic amyloid polyneuropathy after Domino FAP LT: characteristics and proposed criteria

**Adams D**, Antonini T, Lacroix C, Mincheva Z, Lozeron P, Kreib AM, Theaudin M, Cauquil C, Blandin F, Karam V, Azoulay D, Adam R, Samuel D

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**Background:** Domino transplantation using the liver (DLT) from patient with familial amyloid polyneuropathy (FAP) has been developed to alleviate the graft shortage.

**Objective:** We studied 91 patients who consecutively received a FAP-DLT to assess the risk of developing de novo amyloid neuropathy.

**Methods:** 91 consecutive patients received a FAP domino graft between 1997 and 2007. All donors and recipients gave their written assent. Indication for DLT was cancer (n=59), cirrhosis (n=32). Systematic follow-up included sequential neurological evaluations with nerve conduction studies, a labial salivary gland biopsy (LSGB) to look for amyloid deposits 5 years after DLT and nerve biopsy in case of evolutive peripheral neuropathy. Domino liver donors had Met30TTR gene mutations in 70%.

**Results:** At first visit, 27/86 (31%) patients had already a peripheral neuropathy (due to diabetes (n=10), diabetes and/or alcohol (n=4), or iatrogenic (n=8)). During follow-up, 10 patients developed (n=7) or worsened neuropathy (n=3). Nerve biopsy disclosed endoneurial amyloid deposits in 4 patients (after 6–9 years from DLT); neuropathy of other origin (n=3) (severe diabetes, CIDP, AIDP). De novo amyloid polyneuropathy (n=4) mimicked inherited FAP: it started after a mean delay of 5.6 y. after DLT (3.5–8). Initial manifestations included intense pain in the feet (n=4), in association with major weight loss in 2, alternating diarrhea constipation in 2, fatigue in 1. 3 patients developed postural hypotension and 3 sexual impotence. They had a mean age of 63.5 y. (54–76). Three patients underwent a second LT from cadaveric donor. 23/44 patients (52%) had positive amyloid deposits on systematic LSGB. Electrophysiological studies showed a progressive axonal sensory polyneuropathy.

**Discussion:** peripheral neuropathy occurs in many DLT recipients with many possible mechanisms.

**Conclusion:** Long term neurological monitoring of FAP-DLT recipients and eventually nerve biopsy are required to prove de novo amyloid polyneuropathy and to take decision on the graft.

#### PB 41

#### Cardiac involvement in Cardiac AL Amyloidosis as measured by Equilibrium Contrast Cardiovascular Magnetic Resonance

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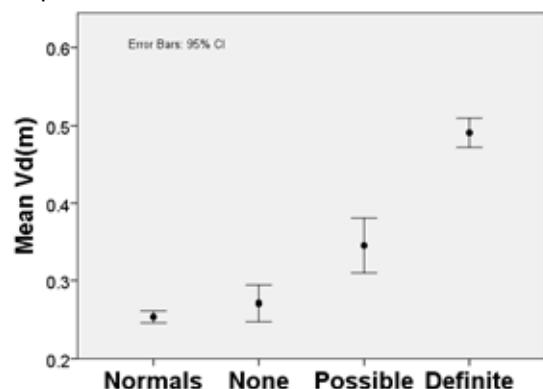
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**Background:** Amyloid is the exemplar of interstitial pathology, and cardiac involvement is a major determinant of outcome and the treatment options in systemic AL amyloidosis. Although echocardiography and cardiac magnetic resonance imaging are valuable investigations, neither can accurately quantify myocardial amyloid burden.

**Objective:** We report here the application to amyloidosis of Equilibrium Contrast Cardiovascular Magnetic Resonance (EQ-CMR), a method we have developed that enables the interstitial compartment of the heart to be quantified as the volume of distribution  $Vd_{(m)}$  of gadolinium contrast, hypothesising that expansion of the interstitium would closely reflect amyloid burden.

**Methods:** Patients with systemic AL amyloidosis (n=60, 65% male, median age 65 years) underwent conventional clinical CMR including late gadolinium enhancement, EQ-CMR, and a standard cardiac assessment including ECG, echocardiography, NT-proBNP and Troponin T measurements and functional assessment comprising the 6 minute walk test (6MWT). Results were compared to healthy controls. The conventional assessments ranked cardiac involvement as definite, probable or not suspected.



**Results:**  $Vd_m$  was significantly greater in amyloidosis patients than normal controls (0.40 vs 0.25,  $P < 0.001$ ). Conventional cardiac assessment, i.e. none, probable or definite, corresponded with a  $Vd_m$  of 0.276 vs 0.342 vs 0.488 respectively ( $P < 0.005$ ).  $Vd_m$  correlated with cardiac parameters by echocardiography (e.g. TDI S-wave  $R^2 0.27$ ,  $P < 0.001$ ) and conventional CMR (e.g. indexed LV mass  $R^2 0.31$ ,  $P < 0.001$ ). Significant correlations were also seen with NT-proBNP ( $R^2 0.47$ ,  $P < 0.001$ ) and Troponin T ( $R^2 0.28$ ,  $P = 0.006$ ).  $Vd_m$  also correlated with reduction in QRS voltages on ECG ( $R^2 0.33$ ,  $P < 0.001$ ).  $Vd_m$  correlated inversely with the functional 6MWT distance ( $R^2 0.13$ ,  $P = 0.03$ ).

**Conclusion:** Estimation of the size of the myocardial interstitial compartment ( $Vd_m$ ) using EQ-CMR in systemic AL amyloidosis demonstrates great potential to be the first non-invasive method for quantifying cardiac amyloid burden.

We currently receive funding from GlaxoSmithKline for some of our research studies. Dr Moon is supported by the Higher Education Funding Council for England.

## PB 42

### Lessons from familial Mediterranean fever-amyloidosis kidney-transplanted patients

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**Background:** Amyloidosis of familial Mediterranean fever (FMF) may lead to end-stage renal failure, culminating in some patients in kidney transplantation. In this study we aimed to assess demographic, clinical and genetic risk factors for the development of FMF-amyloidosis in a subset of kidney transplanted patients, and to evaluate the impact of transplantation on the FMF course.

**Methods:** Demographic, clinical and genetic data were collected from the files, interviews and examination of 16 kidney-transplanted FMF-amyloidosis patients, and compared with 18 FMF patients without amyloidosis.

| Clinical Data  | FMF with amyloidosis | FMF without amyloidosis | P value |
|--|----------------------|-------------------------|---------|
| <b>Number of patients</b>                            | 16                   | 18                      | ---     |
| <b>Male</b>  | 9 (57%)              | 6 (33%)                 | 0.05    |
| <b>Mean age (years)</b>                              | 49                   | 38                      | 0.025   |
| <b>North African origin</b>                          | 14 (87%)             | 8 (44%)                 | 0.01    |
| <b>Age at 1<sup>st</sup> attack</b>                  | 13±15                | 19±15                   | 0.34    |
| <b>Peritonitis</b>                                   | 14 (87%)             | 17 (94%)                | 0.6     |
| <b>Arthritis</b>                                     | 12 (75%)             | 16 (88%)                | 0.4     |
| <b>Pleuritis</b>                                     | 6 (37%)              | 10 (55%)                | 0.3     |
| <b>Attack Duration</b>                               | 24-72h               | 24-72h                  | 0.9     |
| <b>Pain score VAS (1-10)</b>                         | 7-10                 | 6-10                    | 0.9     |
| <b>Hospitalization during attacks (at least one)</b> | 9 (56.25%)           | 11 (61.1%)              | 0.8     |
| <b>Abdominal surgery</b>                             | 3 (18.75%)           | 8 (44.4%)               | 0.23    |
| <b>Time from disease to diagnosis</b>                | 10.5±8.73            | 7.67±8.42               | 0.34    |
| <b>Disease severity Score (9)</b>                    | 8±1<br>(moderate)    | 7±2<br>(moderate)       | 0.24    |

**Table:** Demographic and clinical parameters of FMF patients with amyloidosis

**Results:** Compliance with colchicine treatment in FMF-amyloidosis patients was much lower than in FMF without amyloidosis (50% vs. 98 %). Post kidney-transplantation, the frequency of typical FMF serosal attacks was significantly lower than before transplantation (a mean of 2214 days since last attack vs. 143 days, respectively). FMF-amyloidosis patients carried only M694V mutations in the FMF gene, while FMF without amyloidosis featured other mutations as well. Age of disease onset and clinical severity of the FMF-amyloidosis patients prior to transplantation were similar to FMF patients without amyloidosis.

**Conclusions:** Kidney transplantation in patients with AA amyloidosis of FMF seems to prevent FMF attacks. A protective role of immunosuppressive therapy in this regard cannot be excluded. Compliance with treatment and genetic makeup but not severity of FMF constitute the major risk factors for the development of amyloidosis in FMF.

#### PB 43

#### Depression and Anxiety among patients with AL amyloidosis: the role of cardiac symptoms

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**Background:** Amyloidosis is a rare disease group that affects 10 patients per million per year. Light chain (AL) amyloidosis represents the most common type of systemic amyloidosis and cardiac involvement determines the prognosis. Although among the different forms of cardiac amyloidosis, AL has the worse prognosis, no data are available about the incidence of disease related psychological impact in this population. In particular no data are available about the impact of diagnosis communication, cardiac symptoms onset and cardiac symptoms severity on anxiety and depression among AL patients.

**Objective:** The aims of this study was to evaluate the role that time gone by diagnosis communication, time gone by cardiac symptoms and actual cardiac symptoms severity have on level of anxiety, depression and psychological stress among cardiologic patients with AL.

**Methods:** Thirty-two AL patients with cardiac related symptoms were administered General Health Questionnaire, State- Trait Anxiety Inventory and Centre for Epidemiological Study-Depression Scale. Clinical variables such as months gone by diagnosis, months gone cardiac symptoms onset, cardiac symptoms severity measured with NYHA Scale were also collected.

**Results:** according to questionnaire normative values, AL patients presented severe psychological distress, severe anxiety and clinical depression. Moreover, levels of anxiety are determined by psychological distress ( $p<.001$ ) and months gone by cardiac symptoms onset ( $p<.01$ ) while depression levels are influenced by cardiac symptoms severity ( $p<.001$ ).

**Conclusion:** given the cardiac symptoms impact on anxiety and depression of AL patients, our results suggest to program psychological support for these patients keeping into consideration cardiac symptoms onset and symptoms severity. In our opinion, although psychological support should be proposed in all AL amyloidosis patients during the course of disease, it seems to be mandatory at cardiac symptoms onset more than at diagnosis communication, in order to help patients to accept AL disease when it becomes visible and present.

#### PB 44

#### Amyloidosis of the Gastrointestinal Tract: A Case Series of 74 Patients

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**Introduction:** Amyloidosis of the gastrointestinal tract (GI) is uncommon, reported in 7-8% of patients with AL amyloidosis. There are few reports on the incidence, presenting symptoms and outcome of patients presenting with biopsy-proven GI amyloidosis; we report on a series of patients.

**Methods:** This was a retrospective review of data prospectively collected from January 1998 to December 2011; 2,334 patients with all types of amyloidosis were evaluated during this time. Patients

with biopsy-proven GI amyloidosis, both systemic and localized, were included. Patients with isolated liver involvement were excluded.

**Results:** 74 patients were found to have amyloid involvement of the GI tract proven by biopsy. The median age was 68 years (range 37-83). Systemic amyloidosis with other organ involvement accounted for 59 (80%) patients. In contrast, amyloidosis localized to the GI tract without evidence of an associated plasma cell dyscrasia or other organ involvement comprised of 15 (20%) patients. Of 59 systemic cases, 51 (86%) had AL, 5 (8%) had mutant ATTR, and 3 (5%) had wild type ATTR. The most frequent symptoms for all patients were weight loss (45%, n=33), gastrointestinal bleeding (35%, n=26), heartburn (33%, n=24), and early satiety (32%, n=23); biopsies were most frequently gastric (49%, n=36) or small bowel (42%, n=31).

In systemic cases, multi-organ (46%, n=27) and neurologic involvement (37%, n=22) were common. Of localized cases, 12 (80%) had amyloid protein subtyping, and all had  $\lambda$  light chain disease. With a median follow-up time of 36 months (range, 1-143), none of the localized cases progressed to systemic amyloidosis. Of the 51 patients with systemic AL amyloidosis, treatment included high-dose melphalan and autologous stem cell transplantation (41%, n=21), oral melphalan (22%, n=11), and bortezomib (8%, n=4). Of the 15 patients with localized GI amyloidosis, supportive care was the mainstay of treatment and all are alive at present.

**Conclusions:** Patients with biopsy-proven GI amyloidosis present with weight loss and bleeding. In localized cases, all were due to  $\lambda$  light chain amyloidosis and none progressed to systemic disease during the period of follow-up.

Supported by the Amyloid Research Fund at Boston University

#### **PB 45**

#### **Amyloid Deposits in the Bone Marrow of Patients with AL Amyloidosis Do Not Impact Stem Cell Mobilization or Engraftment**

**Andrew J. Cowan**, David C. Seldin, Martha Skinner, Karen Quillen, Carl O'Hara, Kathleen T. Finn, Vaishali Sanchorawala

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**Introduction:** Amyloid deposits are often found in the blood vessels or interstitium of the bone marrow in patients with AL amyloidosis. The goal of this study was to determine whether this affects stem cell collection or engraftment following high dose melphalan and autologous stem cell transplantation (HDM/SCT).

**Methods:** A retrospective review of data collected from July 1994 to December 2011 at Boston Medical Center on patients with AL amyloidosis with Congo red staining of the pre-treatment bone marrow biopsy specimen who underwent HDM/SCT. Amyloid deposits in bone marrow biopsies stained with Congo red were graded as 0 (absent), 1+ (in vessels only), 2+ (interstitial deposits), and 3+ (extensive interstitial deposits). Data were collected and analyzed for stem cell yield, number of days of collection, neutrophil and platelet engraftment post SCT.

**Results:** 357 patients were eligible for analysis. 233 (65%) had amyloid in the bone marrow, of which 150 (64%) were 1+, 28 (12%) were 2+, and 55 (24%) were 3+. The median number of stem cells collected using GCSF mobilization for patients with amyloid deposits was  $6.8 \times 10^6$  CD34+ cells /kg (range 0.2 – 31.4), compared to  $6.9 \times 10^6$  (range 0.2 – 20.4) for those without amyloid deposits ( $p=0.98$ ). The median number of sessions of stem cell collection was 2 for both groups. The median time to neutrophil engraftment was D +10 for both groups. The median time to platelet engraftment was D +13 for patients with amyloid deposits, similar to D +12 for those without ( $p=0.18$ ). An analysis of the subgroups of patients with 2+ or 3+ amyloid deposits did not show any differences.

**Conclusions:** While amyloid involvement of the bone marrow is common, it does not negatively impact stem cell collection or neutrophil and platelet engraftment in patients with AL amyloidosis undergoing HDM/SCT. Even patients with extensive amyloid infiltration of the marrow interstitium can successfully undergo this procedure.

Supported by the Amyloid Research Fund at Boston University

**PB 46****Differentiating hypertensive heart disease and cardiac transthyretin isoleucine 122 (V122I) amyloidosis in Afro-Caribbean patients**

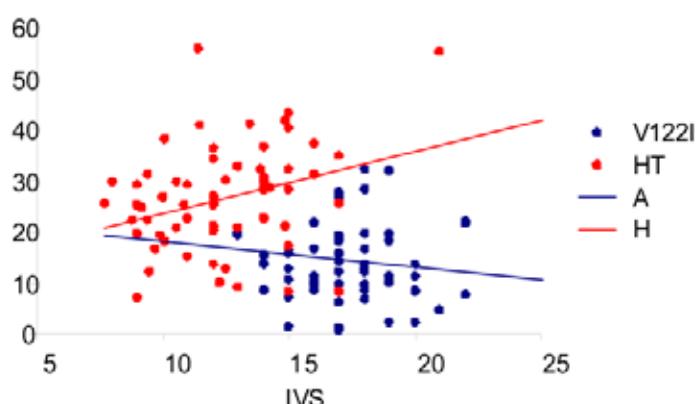
**J Dungu**<sup>1,2</sup>, O Valencia<sup>2</sup>, Prayman T Sattianayagam<sup>1</sup>, Simon DJ Gibbs<sup>1</sup>, Jennifer H Pinney<sup>1</sup>, Dorota Rowczenio<sup>1</sup>, Janet A Gilbertson<sup>1</sup>, Carol J Whelan<sup>1</sup>, Helen J Lachmann<sup>1</sup>, Ashutosh Wechalekar<sup>1</sup>, Julian D Gillmore<sup>1</sup>, A Baltabaeva<sup>2</sup>, TFT Antonios<sup>2</sup>, PN Hawkins<sup>1</sup>, LJ Anderson<sup>2</sup>

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**Background:** About 4% of African-Americans possess the isoleucine 122 (V122I) variant of transthyretin, which is associated with cardiac amyloidosis (ATTR). Ten percent of our local British Afro-Caribbean heart failure population have ATTR V122I, which had often been misdiagnosed as hypertensive heart disease (HHD). We sought to investigate the potential for ECG and echocardiography to differentiate these disorders.

## Precordial Voltage



**Figure:** Correlation between Interventricular septum wall thickness and ECG voltage in Afro-Caribbean patients with cardiac amyloidosis secondary to variant transthyretin V122I and hypertensive heart disease

**Methods:** Sixty-one Afro-Caribbean ATTR V122I patients were identified in collaboration with the UK National Amyloidosis Centre. Sixty-four Afro-Caribbean patients with hypertension were identified in the Blood Pressure Unit at St George's Hospital, London, UK. Precordial voltage (PV) was defined as the S wave in lead V<sub>1</sub> + R wave in lead V<sub>5</sub> or V<sub>6</sub>.

**Results:** Hypertension (46%) and normal ECG voltage (48.4%) were common findings in patients with ATTR V122I. Multivariate regression analysis demonstrated that increased age (74 vs 57 years, p<0.01), increased left ventricular (LV) wall thickness (17 vs 12mm, p<0.01) and decreased LV ejection fraction (35 vs 60%, p<0.01) supported a diagnosis of ATTR V122I. There was positive correlation between LV wall thickness and PV in HHD, but negative correlation in ATTR V122I. The septal/voltage ratio (Interventricular septal wall thickness (mm)/PV (mm)) was significantly increased in ATTR V122I with 83% sensitivity and 83% specificity at 0.7 cut-off.

**Conclusion:** The differential diagnosis of ATTR V122I over HHD is strongly supported when the interventricular septum thickness in mm exceeds the precordial voltage. Confirmation of ATTR with genetic testing and biopsy is ever more important given that various novel treatments are in development.

**PB 47****Determinants of Cardiac Severity in Patients with Systemic Light Chains Amyloidosis: An echocardiography and cardiac magnetic resonance imaging study**

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**Background:** Cardiac involvement in patients with systemic light chains amyloidosis (SAL) has major impact on prognosis; The Mayo clinic (MC) staging is usually used to assess patient prognosis, with patients on stage III, having the worse survival.

The aim of this study was to assess the main determinants of cardiac severity based on MC staging, using echocardiography (TTE) and cardiac magnetic resonance imaging (cMRI), in patients with AL.

**Methods and Results:** 35 consecutive patients (66±11 years; 69% male) with AL, underwent simultaneously, if clinically indicated a TTE and cMRI along with biological cardiac markers. Patients were divided into 3 groups according to MC staging.

Cardiac MRI imaging revealed (table) that, compared to patients with no or minimal cardiac involvement (stage I), those with moderate and high risk MC (stage II and III) had thicker interventricular and atrial septum, larger left atrial (LA) volumes, and lower LA total emptying fraction, (LAEF). Moreover, they had significantly lower Left ventricular (LV) 2D global longitudinal strain (GLS) despite similar LV ejection fraction; Late Gadolinium enhancement (LGE) was also more pronounced in MC stage II-III patients ( $p=0.03$ ).

**Conclusion:** MC staging is significantly associated with morphological cardiac systolic and diastolic alterations; further studies are needed to evaluate the impact on survival of these new imaging parameters in SAL patients.

|                                    | Stage I<br>(n=10)  | Stage II<br>(n=14)     | Stage III<br>(n=11)    |
|------------------------------------|--------------------|------------------------|------------------------|
| Age (Years)                        | 61±9 <sup>\$</sup> | 66±12                  | 71±10                  |
| Male (%)                           | 50                 | 64                     | 90                     |
| cMRI -interatrial septum (mm)      | 3±2 <sup>\$</sup>  | 4±2 <sup>\$</sup>      | 6±3                    |
| cMRI- Interventricular septum (mm) | 12±3               | 14±3 <sup>*</sup>      | 15±4 <sup>*</sup>      |
| cMRI -LVEF (%)                     | 61±8               | 54±13                  | 59±11                  |
| cMRI Max LA volume (mL)            | 59±15              | 83±21 <sup>*</sup>     | 97±41 <sup>*</sup>     |
| cMRI- LAEF (%)                     | 40±8               | 22±11 <sup>*</sup>     | 17±14 <sup>*</sup>     |
| cMRI- LGE (%)                      | 11                 | 50 <sup>*</sup>        | 71 <sup>*</sup>        |
| TTE- 2D-LV GLS (%)                 | -18.8±2.7          | -13.6±4.0 <sup>*</sup> | -10.5±3.4 <sup>*</sup> |

\*  $P<0.05$  compared to stage I; <sup>\$</sup>  $P<0.05$  compared to stage III

## PB 48

### Production of plasminogen activator and it's receptor in organs from AL amyloidosis

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**Background:** We previously reported that excessive fibrinolysis is a hallmark of AL amyloidosis (Amyloid 2009; 16, 89-93). However, mechanisms regulating fibrinolysis is not fully understood.

**Objective:** 1: Determination of key molecules regulating fibrinolysis, urokinase type plasminogen activator (uPA), and it's receptor, uPA-receptor (uPAR), in plasma cells and amyloid-deposited organs. 2: Analysis of roles of amyloid fibrils in regulation of uPA and uPAR-expression.

**Methods:** Plasma cells were purified by using anti-CD138 antibody-coated immunomagnetic beads from bone marrow samples. Gene expressions were analyzed by real time PCR. In some experiments, mRNA was extracted from fresh frozen organs from amyloidosis cases. Paraffin embedded samples were utilized for immunostaining of uPA. Amyloid fibrils were extracted from tongue of AL amyloidosis case. Induction of uPA or uPAR was analyzed by incubating HepG2 cells with amyloid fibrils.

**Results:** Expression of uPA was detected at low levels in plasma cells from both MGUS and Amyloid cases. Interestingly, uPA was more abundantly expressed in organs in AL amyloidosis than those in

familial amyloidosis at 30 to 100 times. Moreover, uPAR-expression was found more than 100 times in organs from AL amyloidosis comparing to familial amyloidosis. Expression of u-PA was induced by addition of amyloid fibrils in HepG2 cells.

**Conclusion:** Abundant expression of uPA and uPAR at organs in AL amyloidosis cases suggests conversion of plasminogen to plasmin occurs at amyloid lesion. Induction of u-PA gene by amyloid fibrils may lead to vicious cycle leading to production of plasmin which may disrupt extracellular matrix at amyloid lesion thus possibly enhancing organ damage. Further analysis of fibrinolysis should elucidate unknown mechanisms regulating progression of amyloidosis and lead to development of unique therapeutic approach.

#### PB 49

#### **Heavy / light chain analysis can replace IFE in a algorithm utilizing free light chain and urinary protein electrophoresis for identification of AL Amyloidosis Patients**

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Traditionally, serum free light chain (Freelite™, FLC), serum protein electrophoresis (SPEP) and urinary electrophoresis (UPEP) with confirmation by immunofixation (IFE) has been used to identify monoclonal immunoglobulin (M-Ig) production in patients with AL amyloidosis. Here we report on the utility of Ig'κ / Ig'λ (HLC) assays for the detection of M-Ig.

HLC (IgG, IgA and IgM) were retrospectively measured in sera taken at diagnosis from 94 patients having a detectable M-Ig in serum only and/or urine only, or no detectable M-Ig and normal free light chain ratio.

Monoclonal protein was identifiable by IFE in 63/94 patients (11 IgA, 29 IgG, 4 IgM, 19 FLC and 0/31 were negative (-ve)), by FLC in 69/94 patients (8/11 IgA, 24/29 IgG, 2/4 IgM, 19/19 FLC and 26/31 -ve IFE), by UPEP 60/94 patients (8/11 IgA, 21/29 IgG, 2/4 IgM, 16/19 FLC and 18/31 -ve IFE), and HLC in 56/94 patients (9/11 IgA, 26/29 IgG, 3/4 IgM, 10/19 FLC and 8/31 -ve IFE). 1/29 IgG patients had a monoclonal IgGκ with FLCλ identifiable by both FLC and UPEP, HLC ratio was negative. In addition there was 1 patient with a biclonal IgGλ and IgAk, an abnormal HLC ratio identified both clones. An algorithm of FLC + IFE and UPEP identified 28/31 patients with FLC + HLC and UPEP similarly identifying 28/31 patients.

HLC measurements and the inferred ratios can identify and quantify low level M production in patients with AL amyloidosis, including those with multiple clones. Additionally, abnormal HLC ratio identified 3 patients in whom no clonal abnormality was detectable by traditional test. An algorithm utilizing HLC + FLC and UPEP achieved the same sensitivity as the traditional tests and may be a quantitative alternative. Further work is required to assess the utility of this test compared to bone marrow assessments and in patient monitoring.

#### PB 50

#### **An exploratory case-control study of progression of AA amyloidosis in rheumatic disease**

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**Background:** Renal impairment is not uncommon in rheumatic diseases, but it is infrequently due to amyloidosis.

**Objective:** To demonstrate differences in progression of renal disease in patients with AA amyloid.

**Methods:** Patients with AA amyloidosis were identified from a West of Scotland registry. During the period patients were recruited to the registry, a control group of patients were identified because of referral for biopsy where amyloid was sought. Follow up data on the rate of deterioration of serum creatinine and time from recruitment to dialysis were obtained by case record review.

**Results:** 11 patients with AA amyloid (9 with rheumatoid arthritis, 1 ankylosing spondylitis & 1 undifferentiated polyarthritis) were identified, all diagnosed between 2000 & 2010. 16 control subjects had rheumatoid arthritis (12), undifferentiated polyarthritis (2), psoriatic arthritis (1), & ankylosing

spondylitis (1). Biopsies failed to show amyloid in rectal, renal, abdominal fat & carpal tunnel tissue in control subjects and 1 biopsy was declined because of anticoagulants. Median baseline serum creatinine was 134 micromol/l in control subjects & 125 in patients with AA amyloid. During follow up in controls (median 42 months), 5(31%) doubled serum creatinine but no patients were started on dialysis. During follow up of AA amyloid (median 27months) 5(45%) required dialysis & a total of 8(72%) doubled serum creatinine. 6 amyloid patients received biologic suppressive therapies (anti-TNF, rituximab & anakinra). Baseline serum creatinine failed to distinguish those with a better renal prognosis but no measurable urinary protein was present in 2 of 3 who neither doubled serum creatinine nor received dialysis therapy during between 54 & 108 months' follow up. Likewise 2 of these 3 received biologic therapies.

**Conclusion:** In this small study AA amyloid complicating inflammatory rheumatic diseases required renal replacement therapy more frequently than control patients with similar presentations.

## PB 51

### High incidence of autoimmune disorders in patients with localized light-chain amyloidosis

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**Background:** Case reports of localized pulmonary amyloidosis coinciding with Sjögren's syndrome (SS) have been published (1) and an association between SS and localized nodular amyloidosis of the skin has been hypothesized(2).

**Objective:** To assess whether an increased prevalence of autoimmune disorders and elevated autoantibody titers can be detected in localized light-chain amyloidosis (loc-AL).

**Methods:** Retrospective analysis of 43 patients with loc-AL presented to our amyloidosis referral centre between 2008 and 2011. Routine autoantibody screening was implemented in 2008. The control group consisted of 43 consecutive patients with systemic AL amyloidosis presenting in 2011.

**Results:** Patients with loc-AL were 21 males and 22 females, median age at first presentation was 60 (range, 36-82) years. Affected organs were respiratory tract 18, skin 8, urinary tract 5, larynx 5, GI tract 3 times and breast, lymphatic tissue and brain each once. Immunohistochemistry results were available for 39 patients: 29 loc-AL deposits were classified as lambda, 7 as kappa and 3 as unspecified. In patients with systemic AL 34 deposits were classified as lambda, 4 as kappa and 3 as unspecified. In several tissue samples loc-AL deposits were surrounded by monoclonal plasma cells as previously described (3). A history of autoimmune disorders (e.g. SS, SLE, CREST, AIH) was more common in patients with loc-AL than with systemic AL (15 vs. 6, p=0.024). Furthermore, elevated ANA titers were significantly more prevalent in loc-AL (12 vs. 0, p<0.001) compared to systemic AL. Interestingly, patients with loc-AL of the respiratory tract or skin had the highest prevalence of autoimmune disorders and elevated ANA titers.

**Conclusion:** Our findings suggest an association between loc-AL and autoimmune disorders which was not observed in systemic AL. We hypothesize that a polyclonal B-cell activation in autoimmune disorders might be the source for generation and local accumulation of monoclonal plasma cells leading to loc-AL deposition.

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1. Rajagopala et al., Respirology. 2010
2. Meijer et al., Arthritis Rheum. 2008
3. Setoguchi et al., Amyloid. 2000

## PB 52

### Two Distinct Syndromes of Lymphoma Associated Amyloidosis

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We present the clinical features and outcomes of patients with lymphoma-associated AL amyloidosis and describe two distinct syndromes of lymphoma-associated amyloidosis: 1) peritumoral amyloidosis in which amyloid deposition occurs in the immediate vicinity of the lymphoma and 2) systemic amyloidosis where deposition is in sites remote from the underlying lymphoma. Cases were identified retrospectively at Princess Margaret Hospital and International Consensus Criteria (ICC) was used to determine organ involvement.

We identified 13 patients with lymphoma-associated AL amyloidosis between 1997 and 2011. Median age was 69 (51-84); 54% were female. Seven had peritumoral amyloidosis with extranodal marginal zone lymphoma. A monoclonal immunoglobulin or abnormal free light chain was undetectable in three but present in trace amounts in the remaining (lambda restricted). All patients had lung involvement by amyloidosis. Four patients received systemic therapy including alkylator-based or rituxan combination chemotherapy with no complete responses. No patients with peritumoral amyloidosis were found to develop systemic amyloidosis.

Six patients had lymphoma with systemic amyloidosis. These patients all had lymphoma in the bone marrow with histology showing lymphoplasmacytic lymphoma or small B cell lymphoma with plasmacytic differentiation. An IgM M-protein was detectable in all. Amyloid was detected in a median of three organs including cardiac involvement in four patients. Four patients received multiple lines of therapy with first line being R-CVP. Organ response was not observed and none had a complete haematological response despite long follow-up.

In conclusion, patients with peritumoural amyloidosis have low level M-protein expression with predominant lambda restriction, symptoms confined to the site of their MALT lymphoma and poor radiological responses to systemic therapy. Patients with systemic amyloidosis have IgM isotype, lymphoma involving the bone marrow, multiorgan involvement by amyloid and worse outcomes. Future studies aimed at better defining prognosis and optimal treatment in these patients are warranted.

## PB 53

### Renal biopsy in familial Mediterranean fever with proteinuria – Is it justified?

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**Background:** Familial Mediterranean fever (FMF) is a genetically transmitted autoinflammatory disease, which might be complicated with reactive amyloidosis (AA). The kidney is one of the affected organs, and its involvement with amyloidosis is manifested initially with proteinuria. It is common practice to view proteinuria in FMF as resulting from amyloidosis and avoid kidney biopsy. However, nephropathy other than amyloidosis has been described in FMF, but its rate is unknown.

**Objective:** To appreciate the fraction of FMF patient with proteinuria unrelated to amyloidosis, and characterize its course and underlying pathology.

**Methods:** We have collected data retrospectively on FMF patients undergoing kidney biopsy for proteinuria above 0.5 gram/24 hours, over the last 10 years (since 2001).

Clinical, laboratory and genetic data were abstracted from patient's files, and patients were interviewed to complete the missing information. AA amyloidosis was confirmed with Congo Red and anti AA antibodies staining. Other kidney pathology was determined by experienced pathologist, using accepted methods as light, immunofluorescence and electron microscopy.

**Results:** From 27 patients referred to kidney biopsy, only 16 were diagnosed with AA amyloidosis (59.3%), 11 were diagnosed with another nephropathy. The amyloidosis and the other nephropathy group were comparable on most variables, but showed distinct characteristics with regard to the range of proteinuria (6.5 g vs. 2.4 g, p= 0.01), rate of development of end stage renal disease (75% vs. 27.2%, p=0.01) and severity of FMF (p= 0.01) respectively. Of note, in the amyloidosis group, the time interval from detection of FMF to ESRD was 44.7 years vs. 25.6 years in other nephropathy group (p=0.1).

**Discussion and conclusion:** Almost 50% of patients with FMF and proteinuria were diagnosed with nephropathy other than amyloidosis on the kidney biopsy, which includes entities with more favorable outcome. It is highly suggested to obtain biopsy from patients with FMF and proteinuria more than 0.5 gram/24 hours.

**PB 54****Natural history of wild type transthyretin amyloidosis and possibility of developing an algorithm to differentiate it from isolated cardiac AL amyloidosis**

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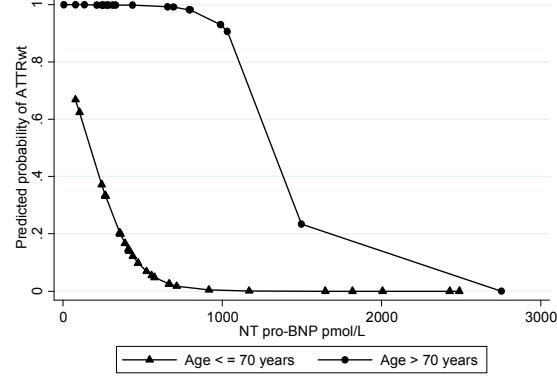
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**Background:** Wild-type transthyretin amyloidosis (ATTR) is thought to be common in older people and must be differentiated from cardiac isolated AL amyloidosis for clinical management.

**Methods:** Data were analyzed from 102 patients with biopsy proven ATTR and the 36 patients with isolated cardiac AL amyloidosis who were evaluated at our Centre between 2002 and 2011. Characteristics at baseline evaluation were used to attempt to develop an algorithm to determine the probability of ATTR versus AL.

**Results:** 88.8% of ATTR and 69.4% of AL patients were male ( $p = 0.015$ ). Mean age at presentation was 74 yrs for ATTR and 61 yrs for cardiac AL ( $p < 0.001$ ). All patients with isolated cardiac AL amyloidosis and 24.1% of patients with ATTR had evidence of a plasma cell dyscrasia. No ATTR patients had macroglossia. NYHA class III/VI symptoms were commoner in AL patients ( $p < 0.001$ ). Median survival from symptom onset was 6.07 years in the ATTR and 1.7 years in the AL group ( $P = 0.002$ ). Cox proportional hazards multivariable survival analysis showed that serum troponin  $> 0.03$  ng/ml and pacemaker insertion were both associated with a fivefold increased risk of death in ATTR patients. NYHA class IV symptoms conferred a hazard ratio of mortality of 15.44 (95% CI 3.59-63.80:  $P = < 0.001$ ). Using logistic regression the probability of ATTR in patients with isolated cardiac amyloidosis without macroglossia but with a plasma cell dyscrasia was calculated. The probability of ATTR was  $> 0.5$  in patients aged  $\leq 70$  years with an NT pro-BNP  $< 183$  pmol/L and in patients  $> 70$  years with an NT-proBNP of  $< 1420$  pmol/L (positive predictive value 90.0%, negative predictive value 85.2%).



**Conclusion:** Whilst factors including the presence of a plasma cell clone, macroglossia, age and NT-pro BNP concentration can help to distinguish ATTR from isolated cardiac AL amyloidosis, we have not yet validated an algorithm that can reliably omit the need for a cardiac biopsy.

**PB 55****Detection of serum IgA monoclonal components in patients evaluated for AL amyloidosis, using heavy chain/light chain immunoassay (HevyLite)**

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**Background:** AL amyloidosis patients often have small monoclonal components (MC), difficult to quantify by densitometry. IgA are most problematic, due to anodic migration and masking under  $\beta$ -zone proteins. HevyLite™ IgA assay (The Binding Site) separately quantifies IgAk and IgAl and infers clonality from IgAk/IgAl-ratio. This study aimed at assessing HevyLite performance in identifying serum IgA MC in a series of patients referred for suspected systemic AL amyloidosis, in whom agarose gel electrophoresis/immunofixation (IFE) had detected IgAk or IgAl MC, or IgA bands without identifiable associated light chains (LC), or increased IgA background without clear MC.

**Methods:** Serum MC were assessed by IFE and free light chain (FLC) quantification (Freelite™) in 35 consecutive patients. HevyLite reference ranges are: IgAk 0.48-2.82 g/L; IgAl 0.36-1.98 g/L; IgAk/IgAl-ratio 0.8-2.04.

**Results:** At IFE, 28/35 patients had complete IgA components (8 IgAk; 20 IgAl), 17 of which could be quantified by densitometry. 2 patients had IgA bands without identifiable LC; 5 had increased IgA background. Using HevyLite, IgAk/IgAl-ratio was elevated in all cases with IgAk MC at IFE; 6 of them also had high IgAk concentration (range: 2.94-30.1 g/L). IgAk/IgAl-ratio was low in 18/20 cases with IgAl MC (13 had increased IgAl; range: 2.01-22.4 g/L), normal in the remaining two. In both patients with IgA bands without detectable LC, IgAk/IgAl-ratio was normal; 2/5 patients with increased IgA background had high IgAk/IgAl-ratio, with normal  $\kappa/\lambda$  FLC ratio. AL amyloidosis was eventually diagnosed in 20 patients; in all, IFE showed complete IgA MC (quantifiable by densitometry in 12). In 18/20, IgAk/IgAl-ratio was abnormal; in the remaining two, who had IgAl at IFE, monoclonal  $\lambda$  FLC were detected by  $\kappa/\lambda$  ratio. No patients with IFE polyclonal background had AL.

**Conclusions:** HevyLite showed good diagnostic sensitivity (90%) in IgA AL amyloidosis, which can be improved with combination of FLC measurement.

## PB 56

### Heart failure secondary to severe cardiomyopathy: clinical presentation of familial amyloid polyneuropathy with Val30Met mutation

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**Background:** Conduction disturbances are the most common cardiac manifestations in familial amyloid polyneuropathy with Val30Met mutation (FAP ATTRV30M)<sup>1</sup>. Restrictive cardiomyopathy is uncommon in Portuguese patients.

**Objective:** To report a patient with TTR Val30Met mutation in whom the clinical presentation was a severe restrictive cardiomyopathy.

**Method:** Case report and literature review.

**Results:** A 47-years-old man, with diabetes and no known family history of cardiac or neurological disease, presented with one year of fatigue, dyspnea and lower limbs edema; on admission he had a heart failure class III on NYHA classification. He also reported erectile dysfunction, minor feet paresthesias, and more recently, reduced left visual acuity. Neurological signs including orthostatic hypotension were absent; there was no macroglossia. Echocardiography and cardiac magnetic resonance imaging revealed an infiltrative cardiomyopathy suggestive of amyloidosis. Endomyocardial biopsy was therefore performed, showing no staining with Congo red; salivary gland biopsy was also negative. Electromyography revealed bilateral carpal tunnel syndrome. Serum and urine immunofixation electrophoresis was negative; other causes of restrictive cardiomyopathy were excluded. DNA analysis of the TTR gene was performed and a Val30Met mutation was detected.

**Discussion:** The patient presented with a severe restrictive cardiomyopathy, associated with minor sensory and autonomic symptoms, raising the suspicion of AL amyloidosis, which was not confirmed either by biopsy or immunoelectrophoresis. Although it was not possible to detect amyloid on the examined tissues, TTR amyloidosis was suspected and unpredictably a Val30Met mutation was found<sup>1</sup>.

**Conclusion:** Heart failure due to restrictive cardiomyopathy is an atypical clinical presentation of FAP ATTRV30M. Our case report also highlights the need to consider FAP in the differential diagnosis of cardiac amyloidosis.

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**PB 57****Cardiac involvement in apolipoprotein A-1 amyloidosis (Leu75Pro)**

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Apolipoprotein A-1 (apo a1) amyloidosis (Leu75Pro) is a rare, autosomal dominant hereditary disorder. In order to evaluate cardiac involvement 109 patients (pts) (age 55±16 years, range 17-85 y, 57% male) with positive genetic test for apo a1 Leu75Pro mutation underwent clinical, electrocardiographic and echocardiographic examination. 66 pts (60%) were hypertensive. 20 pts (18%) had symptoms not explained by other conditions: 3 (2.7%) heart failure, 10 (9%) NYHA class II dyspnea, 9 (8%) arrhythmias (7 atrial fibrillation (AF), 2 supra ventricular tachycardia), and 2 (1.8%) dizziness. 7 pts had pace maker. 15 (14%) pts had valvular disease: 3 mitral and 1 tricuspid thickening , 10 mitral (9 ≤ moderate, 1 severe)and 5 aortic (3 mild, 2 moderate) regurgitation, 3 mild aortic stenosis. Mean left ventricular (LV) diastolic thickness was 10.2±1.9 mm, LV mass index 98±29 g/m<sup>2</sup>, LV diastolic diameter 48±5.4 mm, LV ejection fraction 60±6.9%, E/E' ratio 7.6±2.9, mitral systolic velocity 8.5±2.2 cm/sec, left atrial area 17.6±4.2 cm<sup>2</sup>, tricuspid systolic velocity 12.4±2.6 cm/sec, pulmonary systolic arterial pressure 29.5±6.9 mmHg , inferior caval vein diameter 15.7±4.4 mm. 24 pts (22%) had mean LV diastolic thickness ≥ 12mm, 21 were hypertensive and 7 trained; 16 (15%)had no signs of hypertrophy on ecg. 13 pts (11.9%) had a "restrictive" or "pseudonormal" LV filling pattern, while only 2 patients had LVEF < 50%. In 63 pts (57.8%) echo showed abnormalities suggesting amyloid localization: 33 (30%) interatrial septum thickening, 20 (18%) mild pericardial effusion, 48 (44%) increased right ventricular wall thickness and/or brightness, 23 (21%) increased LV thickness without arterial hypertension and/or with QRS low voltage at ECG, 5 (4%) valve thickening. Pts with cardiac abnormalities were significantly older than those without (59±15 vs 47±14 y). 84 pts (77%) had no symptoms nor clinical events possibly related to cardiac amyloidosis; pts whit cardiac symptoms or events were significantly older than pts without (66±13 vs 51±15y, p<0.001). In conclusion our data show a frequent (57.8%) involvement of heart in apo a1 amyloidosis, differing from other more common forms of cardiac amyloidosis, mainly consisting of minimal abnormalities, increasing with age, and rarely leading to overt cardiac disease.

**PB 58****Acquired cutis laxa should be considered one of the cutaneous manifestations of plasma cell dyscrasia: A case report and review of the literature**

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**Background:** Acquired cutis laxa (ACL) is a rare disease due to a loss of skin elastic fibers resulting in premature aging appearance. It has been associated to various conditions including hematologic disorder, these mostly represented by plasma cell dyscrasias.

**Objective:** We focus on the association between cutis laxa and plasma cell dyscrasia: description of a case recently observed in our Department and review of the literature.

**Case Report:** A 39 yrs old woman with a 2 yrs history of urticaria and angioedema (treated with different drug association) and 1 year history of cutis laxa (mainly involving face, neck, axillae, upper arms and groins and thighs) was admitted with nephrotic syndrome and rapidly progressive uremia. Monoclonal component was detected in serum and urine (sIF: IgA κ; uIF: κ; sFLC K 1576 mg/l; κ/λ 44.6). Renal biopsy revealed LCDD and cast nephropathy with segmental crescents and fibrinoid focal necrosis in > 50 % of the glomeruli. Bone marrow biopsy revealed 7% of plasma cell with κ restriction. Skeleton X-ray did not reveal osteolytic lesions. Histology of the involved skin showed findings consistent with the diagnosis of cutis laxa. Immunohistochemistry studies performed for kappa, lambda, IgG, IgM, IgD, and IgA only revealed deposition of IgA, with slight deposition of IgM, around

the elastic fibers in the reticular dermis. After haematology treatment (PEX, BDex, and two subsequent HDM/SCT) the patient achieved complete haematologic remission, partial recovery of renal function (last eGFR 25 cc/ m<sup>2</sup>), complete remission of urticaria and angioedema and stabilization of cutis laxa lesions.

**Review of the literature:** 26 cases of ACL associated with monoclonal gammopathy have been reported. Associated plasma cell disorders were symptomatic myeloma (5 pts), AL amyloidosis (6 pts), LCDD (2 pts), HCDD (3 pts), MGUS/asymptomatic myeloma (8 pts) and lymphocytic lymphoma (2 pts). In 16 pts the M-protein was IgG, in 3 pts IgA, in 2 pts (both with lymphocytic lymphoma) IgM, κ in 1 pt and λ in 3 pts. The M-protein light chain was κ in 11 pts and λ in 12 pts. Urticaria/angioedema preceded or accompanied cutis laxa in 6 pts. 24 pts underwent biopsies of the ACL involved areas: in 6 cases, all in AL amyloidosis group, biopsy revealed the presence of amyloid deposition. In the remaining pts the IF study of the skin, available in 12 pts, revealed in 8 the isolated presence of the Ig class of M-protein found in serum.

**Conclusion:** ACL should be considered one of the cutaneous manifestations of plasma cell dyscrasia and we suggest the search for an underlying plasma cell dyscrasia in any ACL new case. In these cases elastic fiber destruction could be induced directly by the M-protein or by AL amyloid deposition.

## PB 59

### Urinary biomarkers for kidney disease in ATTR amyloidosis

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**Background:** The detection and prognosis of nephropathy in amyloidosis associated with transthyretin (ATTR), V30M, depends on the presence of albuminuria and declined renal function. The increase of urinary levels of low molecular weight proteins as alpha 1-microglobulin (A1M) and beta-2 microglobulin (B2M) reflects changes in tubular function, as opposed to urinary loss of alpha-2 macroglobulin (A2M), which implies severe glomerular damage.

**Objective:** To investigate the variability of urinary A1M, B2M, and A2M compared to albumin aiming to detect proximal tubular dysfunction in ATTR V30M and distinguish the most likely to predict severity of nephropathy.

**Methods:** In 30 patients (12M/18F) and 11 asymptomatic gene carriers (2M/9F), urinary A1M, B2M, albumin and creatinine were simultaneously measured with serum creatinine, cystatine C and B2M. Albuminuria <30 (mg/g creatinine) was considered normal. In 13 patients serum A2M was assayed.

**Results/discussion:** Patients: age 49.4±12.6 years, evolution of neuropathy 5.1 years; urinary A1M was detected in 27 (>12mg/L in 17), A2M in 15; 5 had albuminuria between 30 and 300 and 20 >300. Asymptomatic: age 41.4±15 years; urinary A1M in 4, A2M in 1, albuminuria >30 in 1.

The excretion rates of A1M and B2M were positively correlated with albuminuria ( $P<0.001$ ), serum creatinine ( $P<0.05$ ) and cystatin C ( $P<0.001$ ). Excretion of A2M was almost exclusively found in the presence of albuminuria >30, although their levels do not correlate with the severity of albuminuria; the serum levels of A2M did not correlate neither with the degree of albuminuria or cystatin C; 5/14 patients who had simultaneous detection of A1M and A2M evolved to end-stage renal disease.

**Conclusions:** Unexpectedly, serum A2M was not a marker of severity of albuminuria. A potential approach to improve accuracy of detection and outcome of renal disease is the evaluation of urinary low molecular weight proteins.

**References:** Nephrol Dial Transplant (2008);23:1252-1256; Ren Fail (2011);33:176-83.

## PB 60

### Immunoglobulin D amyloidosis – a rare entity with a common phenotype

**Murielle Roussel**, Simon DJ Gibbs, Christopher P Venner, Jennifer H Pinney, Sanjay M Banypersad, Jason Dungu, Carol J Whelan, Helen J Lachmann, Julian D Gillmore, Philip N Hawkins and Ashutosh D Wechalekar

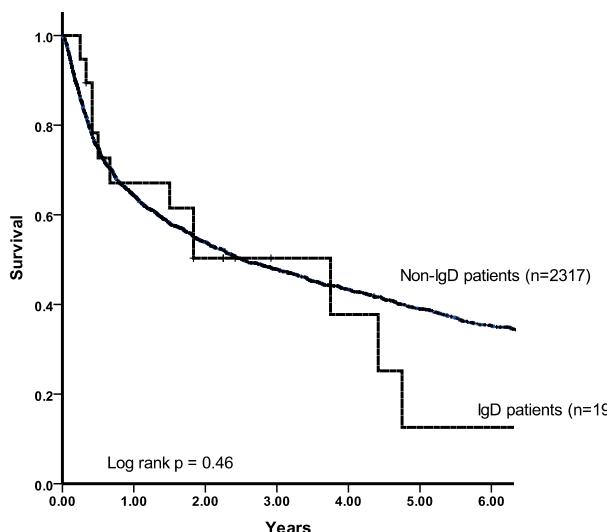
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AL amyloidosis is caused by deposition of particular light chains, mainly of lambda subtype. Intact immunoglobulins (Ig) are rarely IgM or IgD. Recently, 53 patients with IgD-related AL amyloidosis were reported by the Mayo Clinic. IgD patients may have a distinct phenotype, with lower incidence of cardiac and renal involvement but a similar overall survival (OS). We report here the characteristics of 19 patients with IgD-associated amyloidosis.

Between 2000 and 2011, 2336 patients with AL amyloidosis were evaluated at the UK National Amyloidosis Centre; IgD paraprotein was isolated in 0.8 % of patients. Nine (47%) patients had underlying myeloma. Fifty-three percent were male and the median age was 65 years (range 51-84). Seventeen (89%) were IgD lambda. Median serum free lambda chains level was 308mg/L (range 53-6000); only 7 (37%) patients had measurable IgD, median 2g/L (range 1-3.5). Kidneys were the commonest organ involved (68%) with creatinine clearance <50ml/min in 32% and median 24-hour proteinuria of 2.2g (range 0.1-14.8g). Twelve (63%) patients had cardiac involvement with 10 Mayo stage II disease. Four (21%) patients had nerve involvement. No patient met criteria for liver involvement but 4 showed liver uptake on <sup>123</sup>I labelled SAP component scintigraphy.

Patients mainly received frontline cyclophosphamide-thalidomide-dexamethasone (CTD) chemotherapy (7/15 patients). Three patients died before receiving any treatment. Overall haematological response rate was 60% with complete and partial responses in 5 (33%) and 4 (26%), respectively. To date, 13 (68%) patients are dead, of whom 6 died within the first year. Although median OS was not significantly different, estimated 5-year OS was 13% for IgD patients compared to 37% for non-IgD patients (Fig. 1).

**Conclusion:** IgD-associated AL amyloidosis is a rare occurrence predominantly of the lambda subtype that causes the typical amyloidosis disease with the same overall survival.



**Fig. 1: Overall survival of patients with IgD-associated AL amyloidosis compared to non-IgD patients**

## PB 61

**Diagnosis of cardiac AL amyloidosis: the “grey” area of patients with increased NT-proBNP and normal wall thickness**

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**Background:** The diagnosis of cardiac amyloidosis is relatively simple in AL patients with increased wall thickness (WT) and raised NT-proBNP levels, but it is much more challenging in patients with still normal echo parameters and slightly increased cardiac biomarkers, who do represent a “grey” area.

**Objective:** To evaluate cardiac function in patients with normal WT and increased NT-proBNP.

**Methods:** We enrolled 292 consecutive never-treated subjects, in whom a first diagnosis of primary AL amyloidosis was concluded between 2008 and 2010. ECG and cardiac echo-colorDoppler data were evaluated at diagnosis. According to cardiac WT and NT-proBNP values, the cohort was divided into three groups: Group 0: WT <12 mm and NT-proBNP <332 pg/mL (n=50; i.e. patients with clearcut non-cardiac AL); Group 1: WT <12mm, NT-proBNP >332 pg/mL and normal renal function (n=27; i.e. patients in the “grey” area); Group 2: WT >12 mm and NT-proBNP >332 pg/mL (n=215; i.e. patients with clearcut cardiac AL).

**Results:** When compared with Group 0, despite comparable WT, chambervolumes and global function, Group 1 patients showed higher prevalence of regional systolic dysfunction and altered diastolic parameters, with lower mitral annulus longitudinal excursion (lateral:  $13.41 \pm 4.01$  vs  $15.18 \pm 2.67$  mm; septal:  $10.80 \pm 3.08$  vs  $13.21 \pm 2.52$  mm), and higher E/E' ratio ( $8.81 \pm 4.29$  vs  $6.04 \pm 2.83$ ) [ $p < 0.05$  for all]. Intermediate values of endocardial shortening fraction, transmitral E/A, and pulmonary vein S/D ratios were observed in Group 1 when compared with the other Groups, although these trends fell short of statistical significance. Notably, 1-year survival was 94% in Group 0, 78% in Group 1, and 60% in Group 2 patients ( $p = 0.0009$ ).

**Conclusion:** Beyond confirming NT-proBNP diagnostic value, this study underscores the limitations of the currently used echocardiographic diagnostic criteria (i.e. wall thickness >12 mm). Systo-diastolic dysfunction is already evident in the “grey” area of patients with amyloidosis and subclinical cardiac involvement.

## PB 62

### Circulating Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Cardiac Amyloidosis: Novel Biomarkers of Cardiac Amyloidosis

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**Background:** Cardiac amyloidosis is characterized by amyloid fibril deposition in the heart, resulting in a cardiomyopathy (CMP) with heart failure (HF) and/or conduction disturbances. Immunoglobulin light chain related CMP (AL-CMP) features rapidly progressive HF and an extremely poor prognosis compared to CMP due to deposition of mutant (ATTR amyloidosis) or wild-type (senile systemic amyloidosis, SSA) transthyretin (TTR) proteins. Amyloid fibril deposition in the heart disrupts myocardial extracellular matrix (ECM) homeostasis, which is regulated, partly, by matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). We therefore tested the hypothesis that circulating MMPs and TIMPs at initial presentation may discriminate between patients with AL-CMP and TTR-CMP and predict their prognosis.

**Methods and Results:** 50 AL-CMP and 50 TTR-CMP (SSA and ATTR) patients were enrolled from the Amyloid Treatment and Research Program at Boston Medical Center between August 1996 and July 2010. Clinical and laboratory evaluations, including echocardiography, were performed at the first visit. Serum MMP-2, MMP-9, TIMP-1, and TIMP-2 levels were determined by ELISA. Patients with AL-CMP had higher levels of BNP, TnI, MMP-2, TIMP-1, and the ratio of MMP-2 to TIMP-2 (MMP-2/TIMP-2), despite less left ventricular (LV) hypertrophy and relatively preserved LV ejection fraction compared with TTR-CMP. Prognosis was worse in AL-CMP vs. TTR-CMP (log-rank  $P < 0.01$ ). MMP-2/TIMP-2, combined with BNP and TnI, showed the highest discriminative ability for identifying patients with AL-CMP from TTR-CMP at initial presentation. NYHA functional class (HR 6.484;  $P < 0.01$ ), BNP (HR 1.001;  $P < 0.01$ ), and TIMP-1 (HR 1.006;  $P = 0.012$ ) were predictors for mortality in AL-CMP and TTR-CMP. In addition to NYHA and BNP, female gender was a predictor of death in AL-CMP (HR 2.343;  $P < 0.05$ ).

**Conclusions:** Circulating MMPs and TIMPs at initial presentation may differentiate between AL-CMP and TTR-CMP patients and add prognostic information. In addition to a worse NYHA class and higher BNP, female gender increased the risk of death in AL-CMP.

This work was supported by funding from the National Institutes of Health HL095891 (to F.Sam), RO1AG031804 (L.H.Connors) and the Gerry Foundation, the Young Family Amyloid Research Fund and the Amyloid Research Fund at Boston University

**PB 63****Cardiac pulmonary exercise testing (CPET) in patients with senile systemic amyloidosis (SSA)**

**Flora Sam**, Sujata Ramamurthy, Eric H. Awtry, John L. Berk, Martha Skinner, and Lawreen H. Connors

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**Background:** SSA induces a cardiomyopathy (SSA-CMP) with progressive heart failure (HF). In non-amyloid HF patients, CPET quantifies disease severity and prognosis. It is unknown whether measures of left ventricular (LV) remodeling are associated with CPET in SSA-CMP. We tested the hypothesis that echocardiographic measures of LV diastolic function correlated with CPET measures in SSA-CMP.

**Methods:** Data were collected from patients enrolled in a longitudinal study of SSA-CMP at Boston Medical Center, between 2008 and 2010. All cases had biopsy-proven amyloidosis and immunohistochemical evidence of wildtype transthyretin deposition. Clinical, laboratory evaluations and echocardiography were performed. Data are presented as mean $\pm$ S.E.M.

**Results:** Sixteen patients with SSA-CMP completed CPET on their initial visit to the Amyloid Clinic and were included in this study. SSA-CMP patients were white men age  $77\pm1.5$  yrs. NYHA functional class was  $2.5\pm0.5$ . Most patients (94%) were not on usual HF therapy such as ACE-inhibitors, digoxin or  $\beta$ -blockers; but 88% were on a diuretic. BNP ( $418\pm52$  pg/mL) and TnI levels ( $0.18\pm0.1$  ng/mL) were elevated. LVEF was  $47\pm3\%$  with LV mass of  $313\pm17$  gms. Doppler echocardiography demonstrated an elevated E/A ratio of  $2.2\pm0.3$  and tissue e' of  $4.4\pm0.3$  cm/s, consistent with a restrictive LV filling pattern.

|                                       | <b>Control</b><br>N=10 | <b>SSA-CMP</b><br>N=16 | <b>P value</b> |
|---------------------------------------|------------------------|------------------------|----------------|
| <b>Age</b>                            | $75\pm2$               | $77\pm6$               | 0.37           |
| <b>Gender (male: female)</b>          | 7:3                    | 10:0                   | <0.01*         |
| <b>VO<sub>2</sub>max (ml/kg/min)</b>  | $15\pm1.2$             | $14\pm1.3$             | 0.55           |
| <b>Exercise duration</b>              | $7.1\pm1.4$            | $6.9\pm0.6$            | 0.93           |
| <b>VE/VC<sub>CO</sub><sub>2</sub></b> | $35\pm4$               | $46\pm3$               | <0.05*         |

Increased E/e', reflecting increased left atrial pressure, was inversely correlated with a decline in VO<sub>2</sub>max ( $R=-0.59$ ,  $P=0.016$ ). Neither BNP nor TnI predicted VO<sub>2</sub>max in SSA-CMP.

**Conclusions:** CPET exercise duration and VO<sub>2</sub>max were comparable in SSA-CMP vs. controls. In SSA-CMP: (1.) VE/VC<sub>CO</sub><sub>2</sub>, a measure of systemic disease severity and inversely related to cardiac output during peak exercise is greater vs. control; (2.) Doppler echocardiography demonstrated severe diastolic dysfunction and restrictive LV filling; (3.) Increased E/e' correlated with decreased VO<sub>2</sub>max. Whether CPET or diastolic dysfunction findings also provide prognostic information in SSA-CMP remains to be determined.

This work was supported by funding from the National Institutes of Health HL095891 (to F.Sam), RO1AG031804 (L.H.Connors) and the Young Family Amyloid Research Fund and the Amyloid Research Fund at Boston University

**PB 64****Quantification of the monoclonal protein using the heavy-lite test in patients with light chain amyloidosis**

**Stefan Schönland**, Christoph Kimmich, Tilmann Bochtler, Markus Zorn and Ute Hegenbart  
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**Background and objective:** Monoclonal gammopathy can be best quantified using the free light chain (FLC) test in most patients with systemic light chain (AL) amyloidosis. About 5-10% of AL patients have only low levels of amyloidogenic FLC in serum (e.g. < 50 mg/l) but a heavy chain is present in immunofixation (IFE). However, quantification of the monoclonal immunoglobulin using

serum electrophoresis might be difficult due to the small size of the M protein, especially if a nephrotic syndrome is present.

**Methods:** We retrospectively assessed a consecutive cohort of 48 AL patients (with a positive IFE for IgA or IgG at diagnosis) and analyzed 55 serum samples using the IgA or IgG heavy/light chain (HLC) assay (normal HLC ratio for IgA 0,8-2,04 and for IgG 0,98-2,75). Seven patients were tested at diagnosis, all other patients during or after chemotherapy. Three IgA and 3 IgG samples had FLC levels below 50 mg/l at diagnosis and were therefore not suitable for remission evaluation using FLC.

**Results:** Main results are shown in **Table 1**. At time of HLC analysis IgA and IgG levels were elevated in 5 and 2 samples, respectively. For IgG median HLC was 1,3 (range, 0,1-24,8) and for IgA 0,81 (range, 0,01-5,6). An abnormal HLC ratio was detected in 17 and 15 pts, respectively, but in none of the patients with CR.

**Conclusion:** The HLC test is a valuable tool to quantify the paraprotein in patients with AL amyloidosis with a positive IFE at diagnosis. Especially in patients with IgA, a quantification of the monoclonal intact immunoglobulin is more likely with HLC assay as compared to M component measurement in the serum electrophoresis. Further prospective studies are needed to evaluate whether the HLC test should be incorporated into AL remission criteria. In a next step this might influence therapeutic decisions.

| <b>Table 1: Patient characteristics</b>        | <b>IgA</b> | <b>IgG</b> |
|--|------------|------------|
| Number of patients                             | 22         | 26         |
| Number of samples                              | 27         | 28         |
| Samples with detectable M-Gradient             | 2          | 13         |
| Patients with nephrotic syndrome               | 10         | 8          |
| Patients with CR at HLC evaluation             | 0          | 5          |
| Samples with abnormal HLC ratio                | 17         | 15         |
| Samples with CR and abnormal HLC ratio         | na         | 0          |
| Samples with normal FLC and abnormal HLC ratio | 7          | 6          |

This project was funded in part by a grant by Binding Site company who gave the HLC test kit for free.

## PB 65

### Clinical and laboratory features of leptomeningeal type of TTR amyloidosis

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To date, over 100 transthyretin (TTR) mutations have been identified as the cause of hereditary systemic amyloidosis. It has been reported that TTR amyloidosis can be classified into several clinical phenotypes, such as peripheral neuropathy type, cardiomyopathy type, and leptomeningeal (LM) type, which correlate with types of TTR mutations.

In the present study, we investigated clinical and laboratory features, such as blood tests, cerebrospinal fluid tests, MRI, nerve conduction velocity test, echocardiography, and biopsies, in LM type of TTR amyloidosis patients (Gly30, Arg53, Ser64, His69, and Cys114). We also presented an autopsy case with TTR Gly30.

In most of those patients, the initial symptom was transient stroke-like symptom or seizure. They also had dizziness, headache, cerebral hemorrhage, transient coma, amnesia, and blurred vision. In some patients, vitreous opacities were the first manifestation. Several patients had hydrocephalus and underwent a shunt operation, which could delay progression of symptoms such as seizure. Brain MRI showed diffuse abnormal leptomeningeal enhancement without focal parenchymal mass lesion. Protein concentrations in cerebrospinal fluid of selected patients were over 100 mg/dl. Serum TTR concentrations in LM amyloidosis patients were about a half of those in healthy volunteers. In most of the patients with TTR Gly30, symptoms and laboratory findings in other organs than central nervous system and eyes were not observed or very mild. In most of the patients with TTR Gly30, amyloid deposition was not observed in the skin and rectal biopsy specimens. In an autopsy case with TTR Gly30, severe leptomeningeal amyloid deposits were observed. However, no amyloid deposition was observed in other tissue sites.

To make an accurate and timely diagnosis of the disease, we should be aware that tissue biopsies (skin, G.I., fat, cardiac) may be of limited value for detecting amyloid deposits.

## PB 66

### Functional proteomics investigation of the mechanisms of cardiac damage in AL amyloidosis

**Francesca Lavatelli**<sup>1</sup>, Esther Imperlini<sup>2,3</sup>, Paola Rognoni<sup>1</sup>, Giuseppina Palladini<sup>1</sup>, Veronica Valentini<sup>1</sup>, Mario Nuvolone<sup>1</sup>, Francesco Salvatore<sup>3,4</sup>, Stefano Perlini<sup>1</sup>, Stefania Orrù<sup>2,5</sup>, Giampaolo Merlini<sup>1</sup>

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**Background and Objective:** In systemic amyloidoses, fibril deposition causes cell and organ dysfunction. A key pathogenetic role of soluble prefibrillar species is emerging (1, 2). In AL amyloidosis, the peculiar organ tropism of each light chain (LC) suggests specific interactions with cell and/or tissue components. Soluble LC causing human heart amyloidosis trigger damage in rodent cardiac cells (2). We investigated the cardiotoxicity mechanisms by evaluating the interactome of amyloidogenic cardiotoxic LC in rat cardiomyocytes through functional proteomics.

**Methods:** Monoclonal LC are purified by chromatography from urines of very well characterized patients with AL amyloidosis or multiple myeloma. Ventricular rat cardiomyocytes are isolated from perfused hearts, pelleted, pooled and homogenized. Upon precleaning, LC are incubated with the homogenate, followed by immunoprecipitation (IP) with monoclonal anti-LC antibodies. After SDS-PAGE separation, eluted proteins are subjected to in-gel digestion and liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based identification. Lists of identified interactors are analyzed by bioinformatics.

**Results:** LC ( $\lambda$  isotype) with different *in vivo* toxicity (amyloidogenic cardiotoxic and non-amyloidogenic) are tested in parallel. The two latter LC types represent controls. The IP system efficiently captures both LC, with no significant antibody leaking in the eluate. SDS-PAGE separation and LC-MS/MS analysis of eluted proteins shows multiple species in the IP of LC complexes, some of which specifically captured by anti-LC antibodies. Bioinformatic analysis showed that most of the amyloidogenic cardiotoxic LC interactors are localized in the mitochondria and specifically involved in definite metabolic processes that are essential for cardiomyocytes functioning.

**Discussion and conclusions:** We developed an in-vitro system for functional proteomics investigation of AL amyloidosis. Functional characterization of LC protein interactors could be key to elucidate mechanisms linking extracellular misfolded proteins to cell toxicity and to design novel targeted therapies for amyloidoses.

#### References:

1. Palladini et al., Blood, 2006;107(10):3854-8.
2. Shi et al., PNAS, 2010;107(9):4188-93.

## Posters Wednesday PC 01 – 66

**13.30-14.30: Individual viewing and discussing of the posters ('Blauwe Patio')**

**16.30-17.15: Poster viewing and presenting in 5 groups ('Blauwe Patio')**

**17.15-17.45: Selected poster presentations PC 08, PC 28, PC 45, PC 50 and PC 61 ('Blauwe Zaal')**

Chairpersons Minnema and Kuks

### Group 1 (Moderator Ando)

- PC 01 Cardiac pacing in familial amyloid polyneuropathy: the French experience – Algarrobo
- PC 02 Right ventricular function in AL amyloidosis: characteristics and prognostic implication – Cappelli
- PC 03 Early treatment has a significant impact on renal survival in light chain deposition disease (LCDD) – Economo
- PC 04 Identification of factors contributing to poor outcomes in patients with AL amyloidosis with high serum free light chain concentration at diagnosis – Gunasekera
- PC 05 Primary Systematic (AL) Amyloidosis is Associated with Increased Arterial Stiffness Independently of Organ Involvement or Other Cardiovascular Risk Factors: A Marker of Vascular Involvement by AL Amyloidosis? – Kastritis
- PC 06 Autologous peripheral blood hematopoietic cell transplantation in the treatment of AL amyloidosis – Kim
- PC 07 Malnutrition is common and is associated with poor outcome in AL amyloidosis – Lane
- PC 08 Quality of life as an outcome measure in AL amyloidosis following chemotherapy – Lane
- PC 09 Troponin and BNP are Predictors of Survival in both AL and ATTR Cardiac Amyloid – Maurer
- PC 10 Similar Central Hemodynamics in AL and ATTR Cardiac Amyloidosis and their association with Outcomes – Maurer
- PC 11 MCP-1 and VEGF Markers in the Evaluation of Systemic AL Amyloidosis Response to Upfront Melphalan-Dexamethasone Association – Moscetti
- PC 12 Prognostic value of fragmented QRS in cardiac AL amyloidosis: a further advantage of 12-lead ECG analysis – Musca
- PC 13 Longitudinal left ventricular function for prediction of survival in systemic light-chain amyloidosis – Schönland

### Group 2 (Moderator Sanchorawala)

- PC 14 Cardiac arrhythmias among patients attending the National Amyloidosis Centre, UK – Whelan
- PC 15 Detection of microbleeds in hereditary cerebral amyloid angiopathy associated with amyloidogenic transthyretin Tyr114Cys using susceptibility-weighted imaging – Yamashita
- PC 16 Clinical outcomes for biopsy-proven cardiac AL Amyloidosis: Experience of a single institution – Jimenez-Zepeda
- PC 17 Gender differences and variability of age-of-onset in familial amyloid polyneuropathy (ATTRV30M) in Portugal: a reappraisal – Coelho
- PC 18 Population-based Resequencing of APOA1 in 10,330 Individuals: Spectrum of Genetic Variation, Phenotype, and Comparison with Extreme Phenotype Approach – Haase
- PC 19 A Cohort Analysis of Patients from Australian Amyloidosis Clinics – Kwok
- PC 20 Anticipation is a true biological mechanism observed in a large group of Portuguese FAP ATTRV30M kindreds – Lemos
- PC 21 Cardiac AL Amyloidosis: Winning the Battle and Losing the War – Mackie
- PC 22 Cardiac AL Amyloidosis and synchronous meningeal Alzheimer's identified at autopsy: incidental versus related? – Mackie
- PC 23 "How can I Understand": A qualitative research study of the information needs of patients with AL amyloidosis and their carers – Neely
- PC 24 Familial amyloidosis of the Finnish type (FAF): first steps towards treating the underlying condition? – Van Overbeke
- PC 25 Systemic AA amyloidosis in a patient with newly diagnosed tuberous sclerosis – Hegenbart
- PC 26 HLA Typing in Amyloidosis of familial Mediterranean Fever – Shinar

**Group 3 (Moderator Van Gameren)**

- PC 27 A stabilizing variant in transthyretin, T119M, associates with reduced risk of ischemic vascular events, later age at onset, and increased age at death – Stig Hornstrup
- PC 28 25 Years of Amyloidosis in the UK– A Single Centre Experience of 5100 Patients – Wechalekar
- PC 29 Serum amyloid A in the assessment of patients with longstanding Rheumatoid Arthritis – El Mansoury
- PC 30 Amyloidosis with cardiomyopathy characterized by normal interventricular septal thickness: Description of a new entity – Rahul
- PC 31 Sudden Death in AL Amyloidosis: Insights from Multimodality Cardiac Imaging – Falk
- PC 32 Analysis of TTR-related amyloidosis in the field of orthopedics – Yanagisawa
- PC 33 Safety and efficacy of triplet regimens in newly diagnosed light chain (AL) amyloidosis – Barley
- PC 34 Resolution of AL hepatic amyloidosis – Benson
- PC 35 Treatment of AL Amyloidosis with Autologous Stem Cell Transplantation: Results in a Series of 47 Patients From a Single Institution – Cibeira
- PC 36 Cardiac Safety and Tolerability, and Effects on Cardiac Function, of Tafamidis in Patients With Non-V30M TTR-FAP – Damy
- PC 37 First domino liver transplant alone without kidney transplant for Fibrinogen A- $\alpha$  chain renal amyloidosis – Fix
- PC 38 Ten Year Survival Following Autologous Stem Cell Transplantation for Immunoglobulin Light Chain Amyloidosis – Gertz
- PC 39 Refinement in Patient Selection Can Reduce the Treatment-Related Mortality from Stem Cell Transplantation in Amyloidosis to <2% – Gertz

**Group 4 (Moderator Dispenzieri)**

- PC 40 Subcutaneous bortezomib is effective and well tolerated for the treatment of systemic AL amyloidosis – Gibbs
- PC 41 Light chain deposition disease: improved patient survival in the era of novel agents – Gibbs
- PC 42 Case report: A 63-year-old man with amyloidosis presenting a predominant diffuse lymphadenopathy and a paraproteinemia IgM  $\kappa$  – Di Girolamo
- PC 43 Green Tea Halts Progression of Cardiac Transthyretin Amyloidosis: A Pilot Study – Kristen
- PC 44 Lenalidomide, Cyclophosphamide And Dexamethasone (CRd) For Light Chain Amyloidosis: Long term Results From A Phase 2 Trial – Kumar
- PC 45 Improved Hematologic Responses Following Risk Adapted Stem Cell Transplant (SCT) and Bortezomib Consolidation in Systemic Light-Chain Amyloidosis (AL) is Associated with Long Term Organ Improvement – Landau
- PC 46 Bortezomib and Dexamethasone Consolidation Results in Rapid Free Light Chain Control in Patients with Less Than a CR Following Stem Cell Transplant (SCT) in Systemic Light-Chain Amyloidosis (AL) – Landau
- PC 47 Urinary Excretion of Epinephrine and Dopamine Correlates with Efficiency of G-CSF Mobilized Stem Cells in Patients with AL Amyloidosis – Landau
- PC 48 The Depth of Renal Response Correlates with Overall Survival in AL amyloidosis – Leung
- PC 49 Transthyretin Stabilization, Efficacy and Safety of Tafamidis for the Treatment of Transthyretin Amyloidosis – Merlini
- PC 50 Melphalan and dexamethasone (MDex) vs. bortezomib, melphalan and dexamethasone (BMDex) in AL amyloidosis: a matched case control study – Palladini
- PC 51 Treatment and outcome of 150 patients with IgM-related AL amyloidosis – Roussel
- PC 52 Autologous stem cell transplantation in POEMS syndrome: the Spanish experience – Rovira

**Group 5 (Moderator Obici)**

- PC 53 Treatment of AL Amyloidosis with 2 Cycles of Induction Therapy with Bortezomib and Dexamethasone Followed by Bortezomib-High Dose Melphalan Conditioning and Autologous Stem Cell Transplantation – Sanchorawala

- PC 54 Phase I study of MLN9708, a novel, investigational oral proteasome inhibitor, in patients with relapsed or refractory light-chain amyloidosis (AL) – Sanchorawala
- PC 55 Revlimide-Dexamethasone in patients with relapsed light chain amyloidosis previous high-dose chemotherapy does not impair response and revival – Schönland
- PC 56 Using nutritional status measured by BMI and mBMI for monitoring clinical progress in patients with transthyretin familial polyneuropathy: data from two tafamidis studies – Suhr
- PC 57 Updated Experience with Upfront Cyclophosphamide, Bortezomib and Dexamethasone (CVD) in the Treatment of AL Amyloidosis – Venner
- PC 58 Stringent Patient Selection Improves Outcomes in Patients with AL Amyloidosis Undergoing Autologous Stem Cell Transplantation – Venner
- PC 59 Basic and clinical significance of Interleukin 6 (IL-6) in AA amyloidosis – Song
- PC 60 Light Chain Deposition disease: Novel strategies for a rare disorder – Jimenez-Zepeda
- PC 61 Autologous Stem Cell Transplant is an effective therapy for carefully selected patients with AL Amyloidosis: Experience of a single Institution – Jimenez-Zepeda
- PC 62 Bortezomib-containing regimens for the treatment of AL amyloidosis: Impact on hematological response – Jimenez-Zepeda
- PC 63 Successful treatment of small plasma cell clonal proliferation with improvement of target organ function: beyond amyloid – Picken
- PC 64 Bortezomib/dexamethasone followed by autologous stem cell transplantation as front line treatment for light chain deposition disease – Tovar
- PC 65 Tc-99m Pyrophosphate for Identifying ATTR Cardiac Amyloid and Associations of Myocardial Update with Disease Severity – Maurer
- PC 66 SOM0226: A reprofiled drug intended for the prevention and treatment of familial transthyretin amyloidosis (ATTR) – Centellas

**PC 01****Cardiac pacing in familial amyloid polyneuropathy: the French experience**

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All authors belong to the french reference center for FAP (NNERF)

**Background:** Familial amyloid polyneuropathy (FAP) is a dominantly inherited disease caused by mutated transthyretin. The FAP cardiopathy is characterized by cardiac infiltration leading to conduction disorders, with increased risk of sudden death. Prophylactic cardiac pacing may be considered in asymptomatic patients exhibiting conduction disorders. However, the potential benefits remain to be documented.

**Objective:** This retrospective study documented conduction disorders in a large series of FAP and tested the incidence of permanent AV block in FAP patients with prophylactic pacemaker implantation.

**Methods:** From January 1999 to January 2010, 262 patients with genetically proven FAP were evaluated. Prophylactic PM was implanted in patients with HV interval  $\geq$ 70ms, or HV interval >55ms associated with either a fascicular block on ECG or a nodal conduction disorder. After implantation, the spontaneous AV conduction was analyzed in a subset of patients by using temporary pacemaker inhibition and the device memory collected at each follow-up visit.

**Results:** As compared to patients with prophylactic PM (n=100) and patients implanted for high grade heart block (n=18), the patients who did not require PM implantation (n=144) were younger in age and displayed less severe cardiac involvement. Follow up after prophylactic PM implantation was analyzed in 95/100 patients over  $45\pm35$  months. Temporary pacemaker inhibition indicated pacemaker dependency with high-degree AV block in 24/95 patients (25%). On the basis of pre operative cardiac evaluation, a risk of evolution towards permanent AV block was observed with nodal conduction disorders (hazard ratio 3.3, 95% CI, 1.2.-9.07) while microvoltage on surface ECG reduced the risk (hazard ratio 0.21, 95% CI, 0.06-0.71).

**Conclusion:** In FAP with conduction disorders, prophylactic pacemaker implantation prevented major cardiac events in 25% patients over a 45 months mean follow-up. It is suggested that prophylactic PM implantation prevented symptomatic bradycardia in these patients.

## PC 02

### Right ventricular function in AL amyloidosis: characteristics and prognostic implication

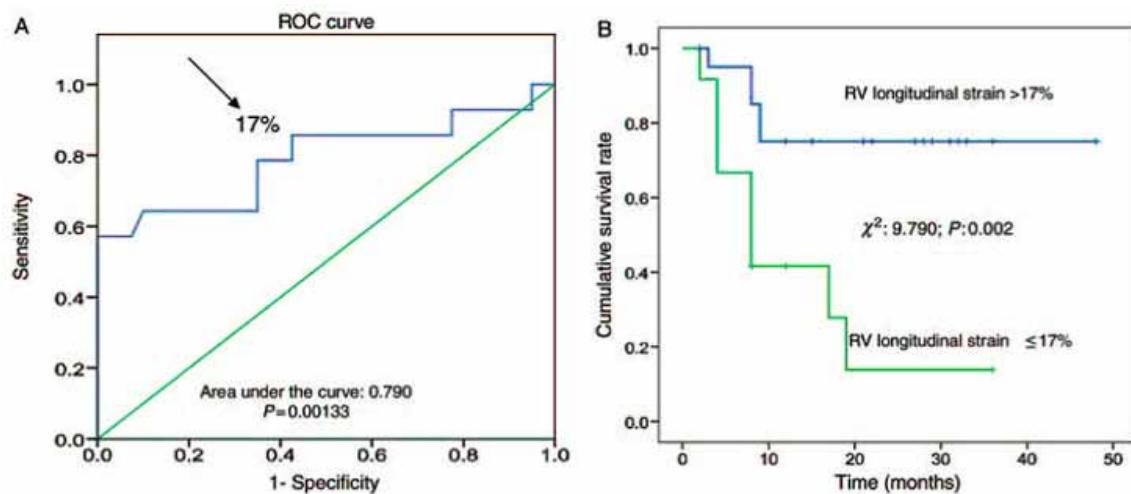
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**Background:** The importance of right ventricle (RV) dysfunction in AL amyloidosis has often been underestimated.

**Objective:** This study was designed to comprehensively evaluate RV function and its prognostic role in patients with AL amyloidosis with and without echocardiographic evidence of cardiac involvement.

**Method-results:** Fifty-two biopsy-proven AL amyloidosis patients underwent a thorough echocardiographic evaluation. Twenty-seven patients (CA) met the international echocardiographic criteria for cardiac involvement [left ventricular (LV) wall thickness >12 mm], whereas 25 patients had no cardiac amyloidosis features (NCA). Patients were compared with sex and age-matched controls. Patients and controls underwent traditional, tissue Doppler (TDI), and speckle-tracking echocardiographic evaluation of both ventricles. No difference was observed in RV diastolic diameter. CA patients showed increased RV free wall thickness ( $P<0.0001$ ). When compared with both controls and NCA patients, RV longitudinal systolic function was significantly ( $P<0.0001$ ) depressed in CA patients, according to traditional echocardiography, TDI, and speckle-tracking evaluation. Conventional Doppler flow evaluation did not show any difference in diastolic RV filling, whereas at tricuspid annulus TDI analysis, CA subject showed significantly lower E' and A' values, with increased E/E' ratio ( $P<0.0001$ ). Over a 19-month median follow-up period, 18 patients died. According to Cox multivariate analysis, N-terminal pro-Brain Natriuretic Peptide and RV longitudinal strain (RVLS) were the strongest death predictors. RVLS had the most reliable diagnostic power for survival compared with other echocardiographic parameters, a 17% cut-off value yielding a 65.5% sensitivity, and a 90% specificity (figure 1A). Moreover, this cut-off discriminated two groups with a highly significant survival difference: 9 months vs. not reached (Figure 1B;  $P<0.0001$ ).



**Conclusion:** In patients with AL amyloidosis, RV longitudinal strain was the only echocardiographic predictor of prognosis. We suggest that RV function analysis should be performed routinely as a part of echocardiographic evaluation in these patients.

**PC 03****Early treatment has a significant impact on renal survival in light chain deposition disease (LCDD)**

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**Introduction:** LCDD is a rare systemic disease in which renal involvement is dominant. When not early recognized and treated, renal disease rapidly evolves to ESRD.

**Aim, Patients and methods:** Retrospective analysis of 15 cases of LCDD diagnosed and treated in our Center between August 2004 and December 2011: evaluation of renal survival in relation to the severity of renal involvement at the diagnosis and at the beginning of the treatment.

**Results:** 15 patients (7♂; 8♀); median age 61.8 yrs (range 39.4-76.7). Four patients with severe extrarenal involvement: heart (3 pts, one of which with AL amyloidosis), liver (1 pt), skin (1 pt with cutis laxa).

Three pts with MM; only one with symptomatic MM. All the patients had monoclonal gammopathy (11 k, 4 λ). Serum electrophoresis alone failed in revealing M-protein in 5/15 pts; serum free light chain essay, always performed since it was available, revealed a pathologic k/λ ratio in all the cases. A bone marrow biopsy was obtained in 13/15 pts: in two cases there was no evidence of monoclonal plasma cells.

At presentation all the patients had renal failure (median s-Cr 3.68; range 1.04-9.8 mg/dl) and proteinuria (median 4.15; range 0.37-10 g/24h), 14 pts had significant microhematuria and 10 pts severe hypertension. A kidney biopsy was obtained in 14/15 cases (1 pt diagnosed by liver biopsy).

13 pts received haematology treatment (CyBorDex, MDex, Dex, VDex, VMdex, BDex, VAD, plasmapheresis); 4/13 pts HDM/SCT. No patient experienced severe treatment related toxicities.

s-Creatinine at the diagnosis was <5 mg/dl (median 2.01; range 1.04-4.2) in 10/15 pts; 9 had haematology treatment. Two pts died early as a consequence of severe extrarenal involvement, 1 pt achieved CR but renal disease progressed to ESRD, 6 pts improved or stabilized renal function (median s-Cr 1.98; range 1-4.7 mg/dl) with normalization of urinalysis.

s-Cr at the diagnosis was > 5 mg/dl (median 7.03; range 5.4-9.8). In 5/15 pts. Only one of the 4 treated pts recovered renal function and is off-dialysis. The other pts rapidly progressed to ESRD.

**Conclusions:** In LCDD patients' serum Creatinine value at the diagnosis and at the beginning of haematology treatment, is the main determinant of renal survival. To prevent ESRD, diagnosis should be done in the early phases of renal involvement. Therefore, extensive use of all the diagnostic tests available to detect monoclonal component is recommended in evaluation of pts with nephropathy. Haematology treatment is effective and no patient experienced severe treatment related toxicities.

**PC 04****Identification of factors contributing to poor outcomes in patients with AL amyloidosis with high serum free light chain concentration at diagnosis**

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**Introduction:** The absolute level of the involved free light chain (iFLC) is an independent marker of poor prognosis in systemic AL amyloidosis, but the reasons for its negative impact on outcomes remain poorly understood. We have previously reported presenting iFLC >500mg/L as a poor prognostic indicator.

**Methods and Results:** Ninety five serial patients with systemic AL amyloidosis with presenting iFLC ≥500mg/L, assessed at the UK National Amyloidosis Centre from July 2007-Dec 2011 were studied. Median age at diagnosis was 65 yrs (range 59–90). The organ involvement was: cardiac in 61 (64.2%), renal in 68 (71.6%), and liver in 21 (22.1%). 20/42 (47%) patients with baseline bone marrow data had >10% plasma cell infiltration. Baseline Mayo staging was: stage 1 – 7%, stage 2 – 35% and stage 3 - 58%, compared with stage 1/2/3 of 18%/42%/40% respectively in our ALchemy prospective

cohort of 'all comers' with AL. 81 patients were included in an intention to treat analysis, of whom 5 died or became too ill to proceed. On an intention to treat basis, 28/81 (34%) patients achieved haematological response – 16/81 (20%) with very good partial response (VGPR) or complete response (CR), compared to overall and VGPR/CR of 57% and 33% respectively in the ALchemy cohort. 49 (52%) patients died. The median overall survival was 18 months, compared to median not reached in the ALchemy series at 24 months; estimated OS of stage 3 patients was 31% at 18 months.

**Conclusion:** A high proportion of patients presenting with iFLC >500 mg/L have stage 3 disease, higher bone marrow plasma cell burden, and poor haematological response rates. Advanced disease, possibly due to greater abundance of the amyloid fibril precursor protein, and inferior treatment responses, due to higher clonal/disease burden, contributes to poor outcomes in this group of patients.

#### PC 05

#### **Primary Systematic (AL) Amyloidosis is Associated with Increased Arterial Stiffness Independently of Organ Involvement or Other Cardiovascular Risk Factors: A Marker of Vascular Involvement by AL Amyloidosis?**

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**Background:** Systemic AL amyloidosis is characterized by the deposition of light chain-derived amyloid fibrils in the microvasculature. Although, vascular involvement in AL is relatively common, is often unrecognized due to the lack of validated markers in routine clinical practice. Arterial stiffness, a process associated with early vascular wall damage, is associated with increased risk of cardiovascular events but the effect of AL amyloidosis on arterial stiffness has never been assessed.

**Methods:** Seventy-three patients with systemic AL were consecutively and prospectively studied. Forty-four individuals without AL and with comparable traditional cardiovascular risk profile (age, gender, hypertension, hyperlipidemia and diabetes prevalence) were used as controls. Carotid-to-femoral pulse wave velocity (PWV) was used as an index of arterial stiffness and pulse wave analysis was used to assess aortic blood pressures (BP). Consensus criteria were used for the definition of organ involvement.

**Results:** Fifty-one percent of the AL patients had cardiac and 77% renal involvement, 33% were Mayo stage-III, 37% stage-II and 30% stage-I. Compared to controls AL patients had significantly higher PWV ( $10.6 \pm 3.3$  vs.  $8.2 \pm 3.1$  m/sec,  $p < 0.001$ ) while peripheral diastolic (pDBP,  $73.0 \pm 11.3$  vs.  $77.2 \pm 12.5$  mmHg, respectively,  $p = 0.078$ ) and aortic systolic BP (aSBP,  $110.2 \pm 19.2$  vs.  $119.5 \pm 23.2$ , respectively,  $p = 0.065$ ) were marginally lower. By multivariate linear regression analysis, AL was significantly associated with increased PWV (model R<sup>2</sup>=0.317, beta=0.349,  $p < 0.001$ ) independently of traditional cardiovascular risk factors including age and BP. PWV was not correlated to heart, renal or liver involvement. Furthermore, within the AL group, the only independent determinant of PWV was pSBP( $p = 0.014$ ).

**Conclusions:** In patients with AL, arterial stiffness is increased compared to controls with a comparable cardiovascular risk profile perhaps due to amyloid deposition in the arterial wall. Further research is needed to assess whether PWV may serve as an index of arterial involvement and/or a risk marker in AL patients.

#### PC 06

#### **Autologous peripheral blood hematopoietic cell transplantation in the treatment of AL amyloidosis**

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**Background:** The immunoglobulin light-chain amyloidosis (AL) is the most common type of systemic amyloidosis resulting in organ dysfunction and death. AL amyloidosis still remains an "orphan disease"

with unmet pharmacologic needs. Since high-dose chemotherapy followed by autologous peripheral blood hematopoietic stem cell transplantation (ASCT) was reported to show high rate of hematologic and organ response, ASCT has been regarded as one of standard frontline treatment approaches for AL amyloidosis.

**Patients and method:** We retrospectively analyzed 17 patients with AL amyloidosis undergoing high-dose melphalan followed by ASCT at the Samsung Medical Center.

**Results:** The median age of patients was 55 years old (range: 38-58 years), and the male to female was 13:4. The most commonly involved organ was kidney (n = 11). Cardiac involvement was found in 3 patients and gastrointestinal tract involvement was also found in two patients. Some patients had combined involvement. The immunophenotype was as follows: IgA lambda (n = 4), IgA kappa (n = 1), IgG kappa (n = 1), IgG lambda (n = 3), lambda light chain (n = 4), kappa light chain (n = 3), unknown (n = 1). Ten patients underwent frontline ASCT whereas 7 patients underwent ASCT as a salvage treatment setting. The hematologic responses (n = 12) including 8 complete response were achieved after ASCT. Among them, four patients showed the improvement of organ function. However, the other four patients progressed to end stage renal disease requiring hemodialysis. The outcome of frontline ASCT was better than ASCT in the salvage setting. There was no transplantation-related mortality. All patients showed successful engraftment. At the time of data analysis, 13 patients were alive and four patients died due to amyloidosis.

**Conclusions:** High-dose chemotherapy followed by ASCT is a feasible treatment with acceptable toxicity, especially as a frontline treatment for AL amyloidosis.

## PC 07

### Malnutrition is common and is associated with poor outcome in AL amyloidosis

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**Background:** Weight loss is common in patients presenting with systemic AL amyloidosis but the impact of malnutrition on survival and quality of life (QOL) has been little studied.

**Methods:** One hundred and ten consecutive, newly-diagnosed, treatment-naïve patients with AL amyloidosis attending the UK National Amyloidosis Centre in 2009 were enrolled into a prospective observational nutrition study. Using the Patient-Generated Subjective Global Assessment (PG-SGA),<sup>1</sup> which comprises a patient questionnaire and a clinician assessment, we examined nutritional status and its relationship with biochemical and clinical parameters, quality of life (QOL) as measured by the EORTC QLQ-C30 questionnaire<sup>2</sup> and patient survival.

**Results:** At study entry, 72/110 (66%) patients had a PG-SGA score >4, indicating malnutrition requiring specialist nutritional intervention. Number of amyloidotic organs, elevated alkaline phosphatase, presence of an autonomic neuropathy and higher Mayo Stage<sup>3</sup> were significantly and independently associated with poor nutritional status ( $p<0.05$ ). QOL was significantly poorer among those with higher PG-SGA scores ( $p<0.01$ ). Furthermore, PG-SGA score was the only independent predictor of patient survival ( $p=0.02$ ) in a multivariate model including, amongst other parameters, the Mayo staging system and number of organs involved by amyloid.

**Conclusions:** Malnutrition is prevalent among patients with systemic AL amyloidosis prior to treatment, especially when there is multisystem organ involvement. Malnutrition is associated with a poor quality of life, and there is a strong correlation between its severity and patient survival. The PG-SGA would be an appropriate tool to gauge whether early and aggressive nutritional intervention in malnourished patients presenting with systemic AL amyloidosis could improve QOL and/or survival.

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**PC 08**

**Quality of life as an outcome measure in AL amyloidosis following chemotherapy**

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**Background:** Quality of life (QoL) is of paramount importance in chronic diseases, but almost no data exist regarding QoL in AL amyloidosis. We sought to prospectively evaluate QoL in patients with AL amyloidosis, and compare it with other outcome measures following chemotherapy.

**Methods:** As part of our prospective UK-wide observational study of chemotherapy in AL amyloidosis (ALchemy), we ascertained QoL at diagnosis (baseline), during treatment, and at 12 and 24 months using the EORTC QLQ-C30, which is designed to capture data on global health, functionality and symptomatology.

**Results:** More than 350 patients have been recruited into the study and data for the first 150 is presented herein. At baseline, mean scores for global health status and all functional and symptomatic scales were worse than age-matched scores from the general population, indicating poorer overall QoL amongst these patients. Among the 92 patients re-evaluated after their third cycle of chemotherapy, there was deterioration in 3 out of 5 functional scales (role, social and physical function); all symptom scale scores were unchanged apart from fatigue, which worsened. In the 75 patients re-evaluated at 12 months, role functioning had improved to better than baseline levels, and social function returned to the baseline value; physical function and global health had not improved, nor had any symptom scores apart from pain. There appeared to be an indication that early clonal response was associated with improved QoL, however the influence of this and other parameters will be examined in a multivariable analysis, and results will be presented in Groningen.

**Conclusion:** The findings indicate that AL amyloidosis and its treatments impact substantially on quality of life, and support the potential for using the EORTC QLQ-C30 questionnaire as an outcome measure in clinical trials.

**PC 09**

**Troponin and BNP are Predictors of Survival in both AL and ATTR Cardiac Amyloid**

Christopher Russo, Phillip Green and **Mathew S. Maurer**

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**Background:** Troponin and b-type natriuretic peptide (BNP) have previously been described as predictors of survival in AL cardiac amyloidosis. However, the prognosis associated with these cardiac markers has not been well described in transthyretin (ATTR) cardiac amyloidosis.

**Objective:** To evaluate the prognostic impact of troponin and BNP on survival in a cohort of patients with AL and ATTR cardiac amyloidosis.

**Methods:** Subjects included patients with AL or ATTR cardiac amyloidosis followed at Columbia University Medical Center from 1997-2011. Biomarkers (BNP and troponin-I) were collected at time of diagnosis. Cox proportional hazards modeling was used to evaluate the impact of biomarkers and amyloid type on time to death or cardiac transplantation, with cross-products of the biomarker and amyloid type (AL or ATTR) used to test for effect modification.

**Results:** 158 patients (mean age  $64 \pm 12$  years, 43% ATTR, median survival 1083 days (95% CI 558-1427) had an average baseline troponin and BNP of  $0.28 \pm 0.53$  ng/ml and  $1142 \pm 1625$  pg/ml respectively. In univariable modeling, the amyloid type, troponin, and BNP were significantly associated with time to death or transplantation. There was no significant interaction between amyloid type and either BNP or troponin. In a model that included BNP, troponin, and amyloid type, amyloid type was not, but BNP (HR 1.2 95% CI 1.1-1.4, p=0.008, per 500 pg/ml increase in BNP) and troponin (HR 2.2 95% CI 1.4-3.5, p=0.0004, per 1 ng/ml unit increase in troponin-I) were both significant predictors of time to survival or transplantation.

**Conclusion:** These findings suggest that troponin and BNP are prognostic indicators in both AL and ATTR amyloidosis.

**PC 10****Similar Central Hemodynamics in AL and ATTR Cardiac Amyloidosis and their association with Outcomes**

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**Background:** A right heart catheterization (RHC) is performed at the time of endomyocardial biopsy to quantify central hemodynamics and cardiac output in patients with cardiac amyloidosis. Such data may have prognostic significance.

**Objective:** To evaluate if parameters from RHC differ between subjects with ATTR and AL cardiac amyloidosis and to determine if such parameters are predictive of adverse outcomes.

**Methods:** Study subjects included patients (n=98) seen at Columbia University Medical Center who had ATTR (n=53) or AL (n=45) and underwent RHC were analyzed. Differences in RHC between AL and ATTR were evaluated. The association between each parameter and time to death or OHT was evaluated using cox modeling.

**Results:** The table below demonstrates that overall hemodynamics do not differ between AL and ATTR patients. Significant ( $p<0.05$ ) univariate predictors of time to death or OHT in overall cohort included RAP, pulmonary artery diastolic pressure (PADP), pulmonary capillary wedge pressure (PCWP), Pulmonary Artery Saturation (Pasat). The likelihood ratio test showed that adding PADP, PCWP, or PASat (individually) to RA did not significantly improve predictive value.

The Hazard ratio for death in those with a right atrial pressure (RAP)  $> 10$  mm Hg as compared those  $< 10$  mm Hg was 2.3 (95% CI 1.13-4.57,  $p=0.02$ ). There was no interaction between amyloid type (AL or ATTR) and RAP.

|             | AL   |      | ATTR |      | Pt†  |    | P†   |
|-------------|------|------|------|------|------|----|------|
|             | Mean | Std  | N    | Mean | Std  | N  |      |
| RA          | 10.3 | 5.2  | 52   | 10.3 | 6.7  | 44 | 0.99 |
| PASP        | 40.3 | 12.3 | 53   | 45.8 | 12.7 | 45 | 0.03 |
| PADP        | 18.2 | 7.1  | 53   | 17.4 | 6.2  | 45 | 0.51 |
| PCWP        | 19.2 | 7.2  | 53   | 18.1 | 7.4  | 45 | 0.46 |
| PASat       | 58.8 | 9.6  | 51   | 58.9 | 7.9  | 43 | 0.97 |
| CO          | 3.8  | 1.36 | 50   | 3.41 | 0.99 | 45 | 0.11 |
| CI          | 1.98 | 0.6  | 50   | 1.75 | 0.46 | 41 | 0.49 |
| PVR (woods) | 2.58 | 1.63 | 39   | 3.74 | 2.13 | 33 | 0.01 |

† P value of t-test comparing AL to ATTR

\* Significant ( $p<0.05$ ) univariate predictors of time to death or OHT in overall cohort

**Discussion:** Central hemodynamics show elevated filling pressures and reduced cardiac outputs in patients with cardiac amyloid but do not differ between AL and ATTR patients. Among data available from right heart catheterization, right atrial pressure was associated with adverse outcomes.

**Conclusion:** Since right atrial pressure is predictive of outcomes and available via physical exam, the importance of a right heart catheterization in this population is not clear.

**PC 11****MCP-1 and VEGF Markers in the Evaluation of Systemic AL Amyloidosis Response to Upfront Melphalan-Dexamethasone Association**

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**Background:** Systemic AL amyloidosis is characterised by the widely deposition of fibrillary aggregates of immunoglobulin light chains with  $\beta$ -sheet conformation leading to organ failure and

death. Our previous studies demonstrated that MCP-1 and VEGF could be considered markers of local inflammation and new angiogenesis in systemic AL amyloidosis<sup>(1-3)</sup>.

**Objective:** To evaluate treatment related changes on endothelial inflammatory activity by VEGF and MCP-1 serum levels in 6 patients (median age 72.8 yrs) with systemic AL amyloidosis.

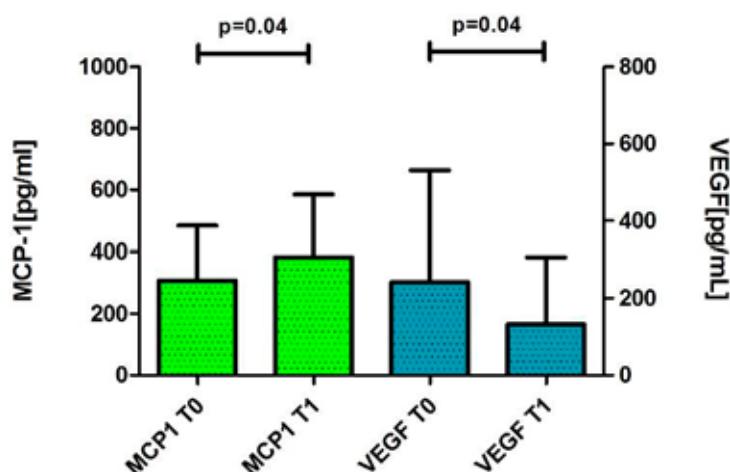
**Methods:** MCP-1 and VEGF were detected by Kit Randox Evidence Biochips Arrays. According to age and disease risk stratification all patients were treated with upfront oral Mel-Dex association (Melphalan 9 mg/sm, Dexamethasone 20mg day 1-4 q28).

Two samples of peripheral blood were performed (T0 at presentation of disease; T1 at conclusion of the first cycle of therapy). The sera were frozen to -80°C until their use. The results were analyzed by paired t test and Person correlation, *p* values ≤ 0.05 were considered statistically significant.

**Results:** MCP-1 serum levels (M±SD) were significantly (*p*=0.04) higher at the end of the first cycle of therapy (T1: 410.10 ± 159.70 pg/mL) compared to baseline (T0: 333.20 ± 148.80 pg/mL), while VEGF serum levels were significantly (*p*=0.04) decreased (M±SD: T1: 165.40 ± 207.40 pg/mL vs. T0: 242.50 ± 275.00 pg/mL, see figure). No correlation was found between the two parameters.

**Discussion:** VEGF serum levels decrease suggests the inhibition of new angiogenesis with consequent reduced interactions between neoplastic plasma cells and bone marrow microenvironment. The significant increase of MCP-1 serum levels could be related to increased migration of white blood cells after treatment.

**Conclusion:** Further studies are necessary to better evaluate the MCP-1 increase in relation to treatment, while VEGF decrease could represent an useful marker to select early the responder patients.



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#### PC 12

#### Prognostic value of fragmented QRS in cardiac AL amyloidosis: a further advantage of 12-lead ECG analysis

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**Background:** In cardiac AL amyloidosis, the 12-leads electrocardiogram (EKG) reflects the generalized infiltrative nature of the disease, with low peripheral voltages, pseudoinfarction patterns, and conduction abnormalities such as fascicular block or varying-degree atrioventricular block. Moreover, it is not unusual to see “aspecific” abnormalities of the QRS complexes, such as notches and RsR' pattern in the absence of QRS prolongation, a pattern that has been defined as fragmented QRS complexes (fQRS). In the setting of ischemic heart disease and dilated cardiomyopathy, fQRS have been associated with regional myocardial scars.

**Objective:** Since cardiomyocyte damage and interstitial fibrosis are associated with cardiac amyloid deposition, the study was aimed at analyzing the prevalence and the potential prognostic role of fQRS in cardiac AL amyloidosis.

**Methods:** We enrolled 456 consecutive untreated subjects in whom the diagnosis of AL amyloidosis was concluded between 2008 and 2010, who were divided according to the presence (n=307) or absence (n=149) of heart involvement, as defined by echo-derived wall thickness >12 mm, and increased NT-proBNP levels. To avoid any possible interference on fQRS presence, patients with a positive history of coronary disease were excluded.

**Results:** The prevalence of fQRS was significantly higher in patients with cardiac AL amyloidosis than in patients without cardiac involvement (28.5% vs. 11.7%; p=0.0008). After a median follow-up of 477 days, mortality was significantly higher in the fQRS group when compared with the “normal” QRS group, both in the general AL population (p=0.0065) and in the group with cardiac involvement (p= 0.0189). No association was found between the presence of fQRS and the duration of PQ, QRS, and QTc intervals, the presence of peripheral low voltages, pseudonecrosis, NT-proBNP levels or cardiac wall thickness.

**Conclusion:** The presence of fQRS at diagnosis has a prognostic value both in the general population of AL patients and in cardiac amyloidosis.

## PC 13

### Longitudinal left ventricular function for prediction of survival in systemic light-chain amyloidosis

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**Background:** Systemic light chain (AL) amyloidosis is associated with a high incidence of cardiovascular events. Reduced myocardial longitudinal function is one of the hallmarks of myocardial involvement in AL amyloidosis. The aim of the study was to determine whether longitudinal left ventricular (LV) function provides prognostic information.

**Methods and Results:** In this prospective observational study 206 consecutive patients with biopsy proven AL amyloidosis were investigated in our Interdisciplinary Heidelberg Amyloidosis Center. Standard echocardiographic imaging parameters, mean tissue Doppler derived longitudinal strain (LS) and two-dimensional global longitudinal strain (GLS) of the LV, the biomarker cardiac troponin-T (cTNT) and NT-proBNP as well as comprehensive clinical disease characteristics were assessed. Primary endpoint was all-cause mortality or heart transplantation. After a median follow-up period of 1207 days LS and 2D-GLS were significant predictors of survival in AL amyloidosis. In multivariable echocardiographic Cox models only the parameters for diastolic dysfunction and longitudinal function (2D-GLS) remained as independent predictors of survival. In comprehensive clinical models 2D-GLS (p<0.0001), diastolic dysfunction (p<0.01), the pathologic free light chains (p<0.05), cTNT (p<0.01) and the Karnofsky index (p<0.001) were independent predictors. 2D-GLS was also associated with worse outcome in patients evaluated prior to firstline chemotherapy (n=113, p<0.0001) or with preserved LV ejection fraction (EF≥50%; n=127, p<0.01). In multivariable Cox models, LS and 2D-GLS both offered significant incremental information for the assessment of outcome compared to clinical variables (age, Karnofsky index and NYHA class) and serological biomarkers.

**Conclusion:** In the largest serial investigation reported so far, reduced LV longitudinal function serves as an independent predictor of survival in AL amyloidosis and offers incremental information beyond standard clinical and serological parameters.

**PC 14****Cardiac arrhythmias among patients attending the National Amyloidosis Centre, UK**

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**Background:** Patients with cardiac amyloidosis are presumed to have a high incidence of arrhythmia-related deaths, but the value of 24 hour Holter monitoring for detecting significant arrhythmias in patients with suspected cardiac amyloidosis is not clear.

**Methods:** Holter monitors were routinely fitted to patients attending the NAC in whom there was a suggestion of cardiac amyloidosis. Patients were given a diary to record symptoms. The clinician filled out a proforma during the clinical assessment detailing previous arrhythmias and current medications. The monitors were read by two experienced technicians and abnormal results were assessed by the cardiology consultant. If there was an abnormality, a recommendation was made in the clinic letter and the Holter report was sent to the referring doctor or local cardiologist.

**Results:** 333 patients underwent Holter monitoring between May 2010 and November 2011. 199 patients had AL amyloidosis, 22 had senile ATTR amyloidosis, and 21 had hereditary ATTR amyloidosis. Non-sustained ventricular tachycardia (NSVT) was identified in 21 patients; atrial fibrillation (paroxysmal, persistent or permanent) occurred in 21 patients; supraventricular or atrial tachycardia occurred in 23 patients and non-significant sinus pauses (<3 seconds) occurred in 5 patients. Of the 21 patients with NSVT, 18 had cardiac amyloidosis (10 with AL and 8 with ATTR type), whereas one had hereditary fibrinogen amyloidosis with no cardiac involvement and 2 others were found subsequently not to have systemic amyloidosis.

**Conclusion:** 65 (19.5%) patients were found to have significant cardiac arrhythmias on 24 hour Holter monitoring, including non-sustained ventricular tachycardia in 21 cases. This cohort of patients will be reassessed following pharmacological and device interventions. These preliminary findings support all patients with systemic amyloidosis being offered 24 hour Holter monitoring.

**PC 15****Detection of microbleeds in hereditary cerebral amyloid angiopathy associated with amyloidogenic transthyretin Tyr114Cys using susceptibility-weighted imaging**

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**Background:** Patients with hereditary cerebral amyloid angiopathy (CAA) associated with amyloidogenic transthyretin (ATTR) Tyr114Cys present with rapidly progressive dementia and fatal lobar hemorrhage. Cerebral microbleeds (CMBs) are potential risk factor for intracranial hemorrhage with recent increased attention in amyloid β-related CAA. However, CMBs are not well elucidated in CAA with ATTR Tyr114Cys. Susceptibility-weighted imaging (SWI) is a newer technique that is more sensitive than conventional T2\* gradient echo imaging in detecting CMBs.

**Objective:** To examine CMBs in CAA with ATTR Tyr114Cys patients using SWI.

**Patients and Methods** A 49-year-old man (patients 1) and a 47-year-old woman (patient 2) with ATTR Tyr114Cys mutation were examined neuroradiologically using SWI at 3.0 tesla. Patients 1 developed vitreous opacity at the age of 30, underwent liver transplantation at the age of 39, and showed slowly progressive intellectual deterioration subsequently. Patient 2 developed vitreous opacities at the age of 31, underwent liver transplantation at the age of 34, and showed slowly progressive intellectual deterioration at the age of 39.

**Results:** SWI demonstrated hypointense signals in the cerebellum of patients 1, and bilateral subcortical white matter and the thalamus of patient 2 indicating CMBs.

**Discussion** In this study, we demonstrated that CAA ATTR Tyr114Cys patients developed CMBs. Liver transplantation for this disease prevented fatal lobar hemorrhage caused by CAA removing ATTR in the circulating blood. However, transplanted patients could show slowly progressive intellectual deterioration and CMBs because of continuing amyloid fibril formation from circulating wild-type TTR produced by the transplanted liver trapped by pre-existing deposits of amyloid or form ATTR in the

cerebrospinal fluid produced by the choroid plexus. In addition to liver transplantation, disease modifying therapy such as tetramer stabilizers is needed for CAA ATTR Tyr114Cys.

**Conclusion:** CAA ATTR Tyr114Cys patients developed CMBs locating in the cerebellum, the subcortical white matter, and the thalamus.

## PC 16

### Clinical outcomes for biopsy-proven cardiac AL Amyloidosis: Experience of a single institution

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**Background:** Recently, cardiac biomarkers, B-type natriuretic peptide (BNP) and troponins, have been used to predict survival for AL amyloidosis patients, including those undergoing treatment with HDM/ASCT.<sup>1</sup>

**Objective:** Here we report the clinical outcomes of patients with biopsy-proven cardiac AL amyloidosis.

**Table 1.** Clinical and Laboratory characteristics of patients with biopsy-proven cardiac AL amyloidosis

| Clinical Characteristics               | N  | Median | Range                | %              |
|--|----|--------|----------------------|----------------|
| Age (years)                            | 34 | 57     | 38-77                |                |
| Gender                                 | 34 |        |                      |                |
| -Male                                  | 19 |        |                      | 55.9%          |
| -Female                                | 15 |        |                      | 44.1%          |
| -IgG                                   | 6  |        |                      | 17.6%          |
| -IgA                                   | 6  |        |                      | 17.6%          |
| -Light-Chain only                      | 16 |        |                      | 47.1%          |
| -Not detected on SPEP                  | 6  |        |                      | 17.6%          |
| Kappa/lambda ratio                     | 24 | 6.5    | 0.01-90              |                |
| Renal Involvement                      | 23 |        |                      | 67.6%          |
| Cardiac Involvement                    | 34 |        |                      | 100%           |
| Hepatic involvement                    | 3  |        |                      | 8.8%           |
| GI involvement                         | 5  |        |                      | 14.7%          |
| Soft tissue involvement                | 8  |        |                      | 23.5%          |
| ≥3 Organs involved by AL               | 6  |        |                      | 17.6%          |
| Hemoglobin (g/L)                       | 34 | 126    | 63-161               |                |
| Creatinine (μmol/L)                    | 34 | 139    | 65-836               |                |
| Alkaline phosphatase, units/L          | 34 | 96     | 34-261               |                |
| B2-Microglobulin (μmol/L)              | 28 | 413    | 136-1980             |                |
| 24 Hr Proteinuria (g/d)                | 34 | 1.95   | 0.02-10.54           |                |
| ***BMPC (%)                            | 34 | 9      | 2-70                 |                |
| Intraventricular Septal Distance (mm)  | 34 | 15     | 10-21                |                |
| Heart Failure                          | 34 |        | No, 12<br>Yes, 22    | 35.3%<br>64.7% |
| Diastolic dysfunction (echocardiogram) | 28 |        | No, 8<br>Yes, 20     | 28.6%<br>71.4% |
| Ejection Fraction, %                   | 34 | 55     | 35-77                |                |
| MRI                                    | 8  |        | Positive<br>Negative | 62.5%<br>37.5% |
| Troponin I ng/mL (normal <0.07)        | 22 | 0.12   | 0.03-2.8             |                |
| **BNP (pg/mL) (normal <98)             | 17 | 498    | 7-2927               |                |

\*\*Brain Type Natriuretic Peptide

\*\*\* BMPC: Bone marrow plasma cells

**Methods:** We retrospectively reviewed the records of all patients with biopsy proven AL Amyloidosis seen at Princess Margaret Hospital between 01/97-12/11. Hematological Response and Organ Response were assessed according to the Consensus Opinion from the 10th International Symposium on Amyloid.<sup>2</sup> The effect of troponin-I and BNP on overall survival was examined using the Cox proportional hazards model.

**Results:** 34 patients were identified. Patients' characteristics are shown in Table 1. Sixty-six percent of patients presented with heart failure and with 71.4% showing echocardiographic signs of diastolic dysfunction. Only one patient was classified as stage III based on the Mayo Clinic Criteria.<sup>2</sup> However, Troponin-I and BNP were available both in only 17 cases. Treatment was given to all but 6 patients and 2 received a heart transplant. ASCT was performed in 14 patients (41%). Patients receiving ASCT achieved a  $\geq$ VGPR of 71%. Median survival for patients with  $\text{BNP} \geq 300 \text{ pg/mL}$  or  $\text{Troponin-I} \geq 0.07 \text{ ng/mL}$  was 32 months and 33 months, respectively and median survival for patients undergoing ASCT with either  $\text{BNP} \geq 300 \text{ pg/mL}$  or  $\text{Troponin-I} \geq$  decreased to 8.8 months and 12.20 months, respectively. Patients progressed at a median of 29.3 months (7.9-50). Median PFS was longer in patients who received ASCT (41 months,  $p=0.0025$ ).

**Conclusion:** Outcomes for patients with cardiac amyloidosis especially those with advanced disease remain poor. This study confirms the role of cardiac biomarkers in assessing cardiac involvement which correlates with the pathological findings.

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#### PC 17

#### Gender differences and variability of age-of-onset in familial amyloid polyneuropathy (ATTRV30M) in Portugal: a reappraisal

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**Background:** In 1952, Andrade described FAP in Portugal as a disease occurring between 25-35 yrs. This AD disorder was later shown to present remarkable differences in mean age-at-onset (AO) between clusters. More emphasis has been put in these differences than on the variability within each focus. In Portugal the disease have been characterized by early onset (<40yrs) and high penetrance. However late-onset ( $o. \geq 50$ yrs) cases have been increasingly ascertained. Moreover, when analyzing all families ascertained, 40% of probands had no affected parent at the time of the diagnosis. These probands are not due to *de novo* mutations but rather to incomplete penetrance in previous generation.

**Objective:** To assess gender differences in AO in families where the proband had no affected parent.

**Methods:** A longitudinal analysis of 2440 patients (566 kindreds) diagnosed between 1939 and 2010, who had their AO established by the same group of neurologists.

**Results:** Age-at-onset ranged from 17 to 82yrs, with a mean of 35,1yrs (SD 10,7). Women had a later onset than men - 37,4 (10,3) vs. 33,1 (10,7) ( $P < 0,001$ ). Probands who, at the time of their diagnosis, ignored the existence of the disease in previous generations (216 families), had a mean AO of 46,2 (12,5), vs. 33,5 (8,8) in probands of 347 families where the disease was already known ( $P < 0,001$ ). However, in those 216 families no differences were found between male and female probands - 45,9 (13,0) vs. 46,7 (11,6), whereas in the group of 347 probands with one affected parent, women had a later onset than men 36,5 (9,1) vs. 31,0 (7,8).

**Discussion/Conclusion:** No gender differences in AO were found in probands with no affected parent. However, they have a higher mean AO, meaning that a protective factor may exist in some families. Our approach to genetic modifiers should focus on differences between parents and their offspring.

**PC 18****Population-based Resequencing of *APOA1* in 10,330 Individuals: Spectrum of Genetic Variation, Phenotype, and Comparison with Extreme Phenotype Approach**

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**Background:** Rare genetic variants, identified by in-detail resequencing of loci, may contribute considerably to complex traits. We used the apolipoprotein A-I gene(*APOA1*), a major HDL gene, and population-based resequencing to determine the spectrum of genetic variants, the phenotypic characteristics of these variants, and how these results compared with results based on resequencing only the extremes of the apoA-I distribution.

**Methods:** First, we resequenced *APOA1* in 10,330 population-based participants in the Copenhagen City Heart Study (CCHS). The spectrum and distribution of genetic variants was determined as a function of the number of individuals resequenced. Second, the apoA-I and HDL cholesterol phenotype was determined for 24 nonsynonymous and synonymous variants identified, and validated in the Copenhagen General Population Study (n=45,239). Third, genetic variants identified and the corresponding observed phenotypes were compared with those predicted using an extreme phenotype approach based on the apoA-I distribution.

**Results:** First, population-based resequencing of *APOA1* identified 40 variants of which only 7(18%) had minor allele frequencies >1%, and most were exceedingly rare. Second, approximately 0.3% of individuals in the general population were heterozygous for nonsynonymous variants which were associated with substantial reductions in apoA-I (up to 39 mg/dL) and/or HDL cholesterol (up to 0.9 mmol/L), and surprisingly 0.4% were heterozygous for variants previously associated with amyloidosis. Third, using the extreme apoA-I phenotype approach, nonsynonymous variants correctly predicted the apoA-I phenotype observed in the population-based resequencing. However, using the extreme approach, between 79%(screening 0-1<sup>st</sup> percentile) and 21%(screening 0-20<sup>th</sup> percentile) of all variants were not identified, among these variants previously associated with amyloidosis.

**Conclusion:** Population-based resequencing of *APOA1* identified a majority of rare genetic variants which were cumulatively relatively frequent. Approximately 0.3% of the population were heterozygous for rare variants in *APOA1* associated with substantial reductions in apoA-I and HDL cholesterol, and 0.4% were heterozygous for variants previously associated with amyloidosis.

**PC 19****A Cohort Analysis of Patients from Australian Amyloidosis Clinics**

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<sup>2</sup>Princess Alexandra Hospital, Brisbane, Australia

**Background:** The Westmead Hospital and Princess Alexandra Hospital Amyloidosis Clinics (WAC, PAAC) were established in 2007 and 2009 as centres for diagnostic subtyping and management advice for patients with amyloidosis.

**Objective:** To describe the characteristics, management and outcomes of patients from Australia's amyloidosis clinics.

**Method:** A retrospective cohort analysis was performed of WAC and PAAC patients seen prior to November 2011.

**Results:** 123 patients were available for analysis. Median follow-up from diagnosis was 16 months. 68 patients had AL amyloidosis (AL), 16 localised (LA), 14 familial (FA), 11 senile systemic (SSA) and 11 AA amyloidosis (AA). 3 remain indeterminate. For AL patients, 60 had primary AL, 6 multiple myeloma and 3 a lymphoproliferative neoplasm. An average of 2 organs were involved (range 1-7); most commonly kidney (76%), heart (59%) and nerves (37%). Cyclophosphamide, dexamethasone and thalidomide was the preferred induction therapy. 9 underwent autologous stem cell transplant. 56% of

treated AL patients (n=37/66) attained a haematologic response after a median of 5 months. 30% achieved an organ response after a median of 6 months. The median overall survival for all AL patients by Kaplan Meier analysis was 66 months. LA most often affected the respiratory tract (n=9), gastrointestinal tract (n=4), bladder and skin (both n=2). The 14 FA cases belonged to 10 families. 3 were index cases. Mutations affected the TTR (n=7), lysozyme (n=2) and fibrinogen (n=1) gene regions. All SSA cases had cardiac involvement, 4 had multiorgan disease. Diflunisal was prescribed for 1 inherited TTR and 1 SSA case. All AA cases had renal amyloidosis and 45% required dialysis. 4 had multiorgan disease. The majority had autoimmune disease (n=8). No inflammatory disease could be identified in 2.

**Discussion and Conclusion:** This cohort of Australian amyloidosis clinic patients reflects the recognised epidemiology and heterogeneity of the amyloidosis disorders.

## PC 20

### Anticipation is a true biological mechanism observed in a large group of Portuguese FAP ATTRV30M kindreds

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**Background:** Familial amyloid polyneuropathy (FAP) ATTRV30M is an autosomal dominant systemic amyloidosis. A wide variability in age-at-onset (AO) has been uncovered, including among Portuguese patients [17-80 yrs]. Early (=40) and late-onset (=50) cases are not separate entities, often coexisting in the same family, with offspring showing anticipation -a much earlier AO than their affected parent. Historically, anticipation was mostly ascribed to ascertainment biases. Previous studies with Portuguese, Swedish and Japanese families have shown the presence of a true marked anticipation.

**Objective:** Our aim was to study anticipation in a larger number of kindreds than assessed before, removing possible biases and to gain more insight into parent-of-origin effects.

**Methods:** From the UCP-registry, we analysed 926 parent-offspring pairs, both clinically observed and with well-established AO.

**Results:** Women had a statistically significant higher AO than men, either for daughters (mean, SD - 33.7, 6.08) vs. sons (29.43, 6.84) or mothers (39.73, 11.85) vs. fathers (36.15, 11.48). Also, 291 parent-offspring pairs showed marked anticipation (=10 years) and the transmitting parent was the mother in 203 pairs. Conversely, among the 22 offspring showing a 10 years higher AO, 19 had a transmitting father. Mother-son pairs showed larger anticipation (10.43, 9.34) while the father-daughter pairs showed only residual anticipation (1.23, 9.77). Both offspring and parent's gender were highly significant factors (with no interaction). To remove possible biases, we repeated these analyses: 1) excluding the proband, 2) in a random sample (60% of cases) and 3) excluding offspring born after 1960. Anticipation was found in all subsamples and the same trend of parent-of-origin effects was observed. Noteworthy, no parent with AO = 40 had an offspring with AO =50.

**Discussion/Conclusion:** These findings confirm anticipation as true biological phenomenon. The study of genetic modifiers should focus on families, aiming to unravel mechanisms of anticipation that may have important clinical implications.

## PC 21

### Cardiac AL Amyloidosis – Winning the Battle and Losing the War

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A 58 year-old-woman, who was a lifetime nonsmoker with no prior cardiac history, presented with decompensated heart failure. Symptoms were present for several months.

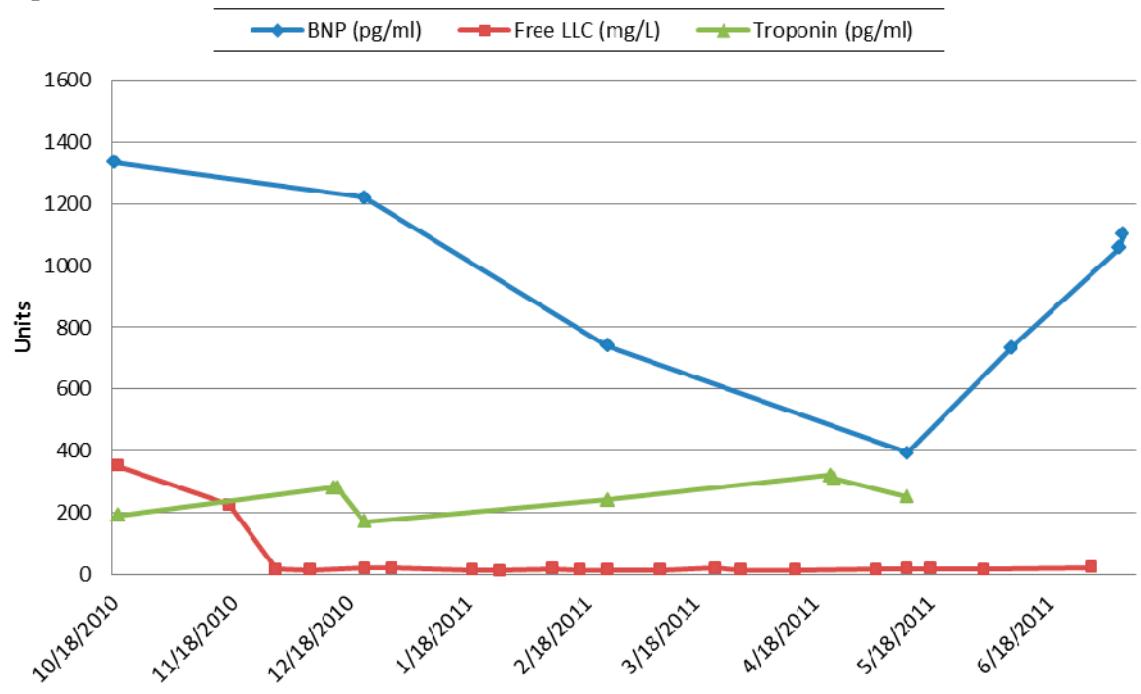
On initial presentation, BUN was 28 with creatinine of 1. Troponin-I was 160pg/ml and BNP was 1336pg/ml. Twenty-four-hour urine protein was 761mg/24h. Initial CXR had large right and smaller left pleural effusions. Initial echocardiogram demonstrated an 18mm interventricular septal wall thickness. Serum free lambda light chains were 350 mg/L and abdominal fat pad biopsy was positive

for AL amyloid. Bone marrow biopsy demonstrated a lambda monoclonal plasma cell population with 5% plasma cells.

The reported median survival of patients with cardiac AL amyloidosis with heart failure is 5 months<sup>1</sup>. Could this patient have been a heart transplant candidate? Although criteria for initial cardiac transplantation evaluation exists<sup>2</sup>, our patient was felt not to be a candidate for cardiac transplantation because of probable renal involvement and possible pulmonary involvement of amyloidosis.

The patient was treated with bortezomib and dexamethasone and quickly achieved a sustained reduction in her lambda light chains (Figure 1). Cardiac markers remained persistently elevated, suggesting that the degree of damage from the AL light chains was irreversible.

**Figure 1**



Ultimately, the patient continued to have recurrent pleural effusions despite therapeutic thoracenteses and heart failure management, and ultimately, she died from cardiopulmonary arrest. The poor prognosis of patients with advanced cardiac amyloid is well-established<sup>3</sup>. Nonetheless, it is somehow more devastating when a patient exhibits a complete hematological response of the abnormal light chains yet still displays progressive and irreversible organ dysfunction. Unfortunately, despite our best clinical efforts, we are all left broken-hearted.

**References:** 1. Kapoor et al. Am J Med. 2011. 2. Lacy et al. J Heart Lung Transplant. 2008. 3. Varr et al. J Heart Lung Transplant. 2011.

## PC 22

### Cardiac AL Amyloidosis and synchronous meningeal Alzheimer's identified at autopsy – incidental versus related?

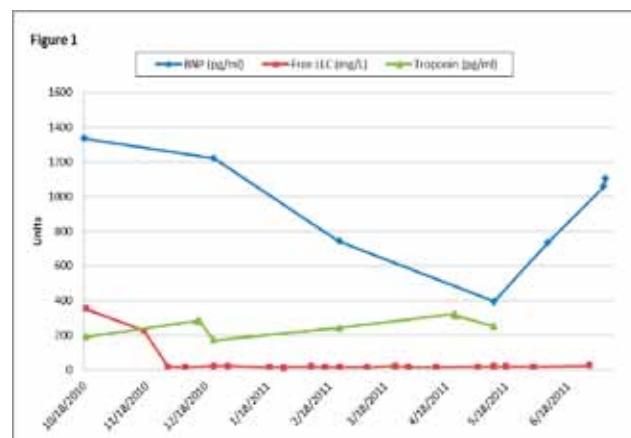
**Kelly K. Mackie<sup>1</sup>**, Kevin Barton<sup>2</sup>, Maria M. Picken<sup>3</sup>, Erin E. Coglianese<sup>4</sup>, John M. Lee<sup>3</sup>

<sup>1</sup>Internal Medicine, <sup>2</sup>Hematology/Oncology, <sup>3</sup>Pathology, <sup>4</sup>Center for Heart and Vascular Medicine, Cardiology, Loyola University Medical Center, USA

A 58 year-old-woman, a lifetime non-smoker with no prior cardiac history presented with decompensated heart failure. On initial presentation, BUN was 28 with creatinine of 1. Troponin-I was 160pg/ml and BNP was 1336pg/ml. Twenty-four hour urine protein was 761mg/24h. Initial CXR reported large right and smaller left pleural effusions. Initial echocardiogram demonstrated an 18 mm interventricular septal wall thickness. Serum free lambda light chains were 350 mg/L and abdominal

fat pad biopsy was positive for AL amyloid. Bone marrow biopsy demonstrated a lambda monoclonal plasma cell population with 5% plasma cells.

Following treatment with Bortezomib and dexamethasone, a sustained reduction in lambda light chains was quickly achieved (Figure 1). However, cardiac markers remained persistently elevated, suggesting that the damage from the AL light chains was irreversible.



Over the course of disease progression, the patient's mental status changed. She began having hallucinations, paranoia, and became withdrawn and depressed. The patient was seen by psychiatry and treated with antidepressants without improvement. She continued to have recurrent pleural effusions and died from cardiopulmonary arrest. Autopsy showed cortical neuritic amyloid plaques in numbers diagnostic of Alzheimer's disease. There was also amyloid in the vessels in the dura and choroid plexus. Abundant amyloid deposits were seen in the heart (the interventricular septum measured 2 cm), lungs and the kidneys. While neuritic plaques are associated with the deposition of Beta amyloid protein, the involvement of the dura and choroid plexus most likely represented light chain deposition from the systemic amyloidosis, given that these areas do not contain a blood-brain barrier. Little is known about whether there is an increased incidence of Alzheimer's disease in AL amyloid patients. Whether the finding of cerebral parenchymal and systemic amyloidosis, in our patient, is incidental or related, remains an open question. It is feasible that an amyloidogenic-specific environment can exist within both the brain parenchyma and systemically, in particular in the heart.

## PC 23

### "How can I Understand" - A qualitative research study of the information needs of patients with AL amyloidosis and their carers

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**Background:** AL amyloidosis is a rare and devastating disease. There appears to be no research that looks specifically at the needs of patients and carers dealing with a diagnosis AL Amyloidosis in Australia.

**Objective:** A qualitative study was designed to identify: a) How patients and carers gain their information about their disease; b) How useful the information is c) How information is used to assist in decision-making; d) The most effective method of information provision and delivery.

**Methods:** The patient cohort consisted of participants on the Australasian Leukaemia and Lymphoma Group (ALLG) MM8 study (a prospective clinical trial of risk-adapted melphalan in patients with AL amyloidosis). Patients and their carers were offered the choice of taking part in a 45-minute separate recorded interview by phone or in person at diagnosis and at 6 months. Longitudinal qualitative methodology using semi-structured interviews was used. Interview audio recordings were transcribed and analysed by SATURATE Qualitative Computer Aided Analysis software. Transcript coding was developed driven by the exact words of the participants.

**Results:** 11 patients, 6 male and 5 female, and 7 carers, consented to be interviewed. 3 patients had no carers. 1 carer declined. At 6 months 5 patients had died, 1 failed the trial, 5 patients and 5 carers were re-interviewed. In all 22 interviews were conducted. The quality of the recording of 5 of the 22 tapes was poor. 17 interviews were used.

All participants expressed confusion, loss of control and fear that they might die at diagnosis. Information was sought from a number of sources, much of which was not useful. Participants identified the need for the provision of simple factual information, presented verbally and in writing that allowed them to ask questions and gave them hope. By 6 months participants were seeking much more information to enable them to participate in decision-making.

**Discussion:** This study provides the first systematic assessment of the information needs of patients with newly diagnosed AL Amyloidosis. Information gained was subsequently used in the production of the Leukaemia Foundation of Australia's booklet "Understanding Amyloidosis" launched in December 2010.

#### PC 24

#### Familial amyloidosis of the Finnish type (FAF): first steps towards treating the underlying condition?

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Familial amyloidosis of the Finnish type (FAF) is an autosomal dominant inherited disorder characterized by deposits of gelsolin amyloid in multiple organs. A point mutation in the gelsolin gene (G654A/T) leads to two consecutive aberrant proteolytic cleavages that degrade the secreted form of mutated gelsolin. Furin proteolysis generates the C68 fragment that is secreted and subsequently cleaved by MT1-MMP to generate 8 and 5 kDa amyloidogenic peptides. We aim to reduce the amyloid buildup by using *Cameloid* single-domain antibodies directed against gelsolin (and gelsolin fragments). Gelsolin nanobody cDNAs were modified with an endoplasmic reticulum targeting sequence and subsequently cotransfected with mutant plasma gelsolin cDNA in HEK293T cells. Immunostaining showed a clear colocalisation of ER-modified nanobodies with mutant plasma gelsolin. Western blot analysis of the medium of transfected cells showed that gelsolin nanobodies, targeted to the ER, reduced formation of C68 from these cells. The epitope of the gelsolin nanobodies was determined and the data suggested that the nanobodies might interfere with furin proteolysis of mutant plasma gelsolin. To confirm this hypothesis, the recombinant gelsolin nanobodies were tested in their ability to reduce furin proteolysis of recombinant mutant plasma gelsolin. The *in vitro* furin assay showed that the nanobodies indeed interfere with furin proteolysis. Apart from the effect on furin, the nanobodies were also tested on a potential effect on MT1-MMP cleavage. To this end, gelsolin nanobodies were modified to bind albumin by means of an albumin binding peptide or a bispecific format. Linking albumin to the nanobody increases the size of the molecule and its potential to promote steric hindrance. We found that albumin linked formats of gelsolin nanobody are able to reduce MT1-MMP proteolysis of recombinant C68. Our findings show that gelsolin nanobodies may be of great value in reducing the amyloid burden in FAF patients.

#### PC 25

#### Systemic AA amyloidosis in a patient with newly diagnosed tuberous sclerosis

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**Case report:** A 45-year-old Greek woman with supposed infantile brain damage and a 10 year history of renal AA amyloidosis presented to our outpatient clinic. As cause for AA amyloidosis an inflammatory bowel disease (IBD) had been diagnosed and treated with systemic steroids and mesalazine. 9 years later she developed pelvic masses and underwent hysterectomy. Histology

showed myoma and again AA amyloid. The patient suffered from terminal renal insufficiency and started dialysis in October 2010.

On presentation, the patient had no gastrointestinal symptoms and recently repeated rectosigmoidoscopy showed no evidence of active IBD. Blood analysis showed elevated CRP (74 mg/L; normal <5 mg/L) and serum amyloid A protein levels (SAA) (151 mg/L; <10 mg/L). Echocardiography displayed septum thickness of 14 mm and decreased ejection fraction indicating cardiac amyloidosis. The patient did not report abdominal attacks suggesting Familial Mediterranean fever and *MEFV* gene analysis did not reveal any mutations. Body examination was normal except reddish spots on the right cheek and a large palpable tumor of the left flank. CT-scan showed multiple cystic lesions in both kidneys suspicious of carcinoma and a two-step bilateral nephrectomy was performed. Histological workup revealed clear cell carcinoma of both kidneys (pT2b, pNx, G2, R0 and pT1a, pNx, G2, R0) and glomerular AA amyloid. Tumor-free renal parenchyma showed numerous mesenchymal lesions consistent with lymphangioleiomyomatosis. Phakomatosis was suspected and *TSC1* gene analysis revealed a pathogenic point mutation (IVS-I1G>A). Retrospectively, the infantile brain damage and the facial skin lesions can be attributed to tuberous sclerosis (TS). Interestingly, CRP levels normalized after the second nephrectomy. Six months later CRP and SAA levels still remained normal and ejection fraction improved.

**Conclusion:** We report an uncommon association of phakomatosis and AA amyloidosis. We assume that the TS associated kidney pathology caused a chronic inflammation resulting in systemic AA amyloidosis.

## PC 26

### HLA Typing in Amyloidosis of familial Mediterranean Fever

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<sup>1</sup>Heller Institute of Medical Research, <sup>2</sup>Medicine F, <sup>3</sup>Tissue Typing Unit, Sheba Medical Center, Israel

**Background:** Amyloidosis is a lethal manifestation of FMF that remains a major threat even in the era of colchicine treatment and of genetic risk alerts, due to incomplete predictive criteria. The HLA system is a cardinal module of the inflammatory response whose role in FMF is not established.

**Objectives:** To determine if certain HLA antigens increase the risk to develop amyloidosis of FMF.

**Methods:** HLA-A, HLA-B, HLA-C and HLA-DR antigens frequencies were compared between a cohort of 33 FMF patients referred for kidney transplantation due to amyloidosis at the Sheba Medical Center, Tel-Hashomer, and populations of matching origins. HLA-based patient subgroups were compared for FMF manifestations, demographic data and *MEFV* genotypes, based on data abstracted from medical files and questionnaires. Patients lacking genotyping were screened for the three most common mutations.

**Results:** Nineteen of 33 FMF patients with amyloidosis were North African Jewish (NAJ) and 12 were Arabs. NAJ patients carried the B55 and the C1 5 fold and 3 fold more often than their unaffected counterparts, ( $p=0.043$  and  $p=0.032$ , respectively). Arab amyloidotic patients over presented the B38 antigen ( $p=0.032$ ), B39 ( $p=0.006$ ) and B57 ( $p=0.018$ ). The DR4 antigen rate was lower than expected in both NAJ and Arab FMF-amyloidosis patients ( $p=0.02$ ) and in a subgroup of 19 NAJ homozygous for the p.M69V mutation. Other *MEFV* genotypes were associated with higher frequency of A30 ( $p=0.03$ ), B39 ( $p=0.0005$ ), and DR11 ( $p=0.03$ ). Clinically, in patients stratified according to the frequent versus normal HLA antigens, FMF manifestations were at large, comparable.

**Conclusion:** Several HLA-B antigens were over represented in the amyloid patients but none were common across both the NAJ and Arab ethnic groups. The lower representation of HLA-DR4 argues against its contribution to the risk of developing amyloidosis in NAJ and Arab FMF patients.

## PC 27

### A stabilizing variant in transthyretin, T119M, associates with reduced risk of ischemic vascular events, later age at onset, and increased age at death

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**Background:** Senile systemic amyloidosis, which clinically may present as ischemic heart disease (IHD), is a common aging phenomenon caused by deposition of wildtype transthyretin (TTR) fibrils mainly in the heart. A common mutation in *TTR*, T119M, results in a more stable, less amyloidogenic protein; thus mimicking the effect of drugs currently under investigation for the treatment of amyloid disease. We tested the hypothesis that *TTR* T119M protects against ischemic vascular events in the general population.

**Methods:** We genotyped T119M in two large cohorts of the general population, the Copenhagen City Heart Study and the Copenhagen General Population Study, totalling app. 70.000 participants, of which 7,146 had ischemic heart disease (IHD) and 4,174 had ischemic cerebrovascular disease (ICVD).

**Results:** In heterozygotes versus noncarriers, the T119M mutation (present in 1:200), predicted odds ratios (OR) for risk of IHD, ICVD and any ischemic event of 0.88 (95% confidence interval (CI): 0.60-1.30), 0.50 (0.26-0.95), and 0.77 (0.54-1.10), respectively. When stratified by age (<70 or ≥70 years of age), the risk reduction was largest in individuals <70 years (IHD: OR 0.52 (0.27-0.98); ICVD: OR 0.29 (0.09-0.91); any ischemic event: OR 0.48 (0.27-0.83). Furthermore, mean age at event in heterozygotes versus noncarriers was increased by 8, 7, and 7 years, respectively, for IHD ( $P=0.04$ ), ICVD ( $P=0.21$ ) and any ischemic event ( $P=0.02$ ). Finally, age at death and age at death after any ischemic event was increased by 5 and 6 years, respectively ( $P$ -values: 0.04 and 0.002).

**Conclusion:** Compared with noncarriers, *TTR* T119M associated with reduced risk of ischemic vascular events, with later age at onset of events, with increased age at death, and with increased age at death after any ischemic event. This suggests a potential beneficial effect of stabilizing transthyretin in the general population.

## PC 28

### 25 Years of Amyloidosis in the UK– A Single Centre Experience of 5100 Patients

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Medicine has changed remarkably during the past quarter century, and we report here our 25 year experience in the field of amyloidosis. We reviewed all patients with a confirmed diagnosis of amyloidosis who were referred to the UK amyloidosis centre from 1987-2012. 5100 individuals with amyloidosis or amyloidogenic mutations were identified. AL amyloidosis was commonest accounting for 3468 (67%) of all cases - 2869 (56%) with systemic AL and 599 (11.6%) with localised AL. AA was next most frequent, representing 633 (12%) of all cases, followed by transthyretin amyloidosis (ATTR), accounting for 507 (10%) cases. Two-thirds (339) of the ATTR patients had a TTR gene mutation and 168 were wild-type. Other types included: AFib 87 (1.7%), AB<sub>2</sub>M 93 (1.8%), ApoAI 40 (0.8%), AGel 9 (0.2%), ALECT2 16 (0.3%), ALys 17 (0.3%), ACys C 4 (0.1%), and Alns 3 (0.1%). In addition, 55 patients with light chain deposition disease were assessed. The type of amyloid in 208 (4%) patients could not be typed definitively either due to lack of tissue or inconclusive immunohistochemistry. The proportion of patients with systemic AL amyloidosis has remained steady, ~55% of all patients evaluated each year, as has the 20% early mortality in AL. There has been a remarkable progressive decrease in patients referred with AA amyloidosis from 32% of all cases in 1987-1995 to 6.8% in 2009-2012. Frequency of senile ATTR amyloidosis has increased strikingly from 0.2% until 1999 to 6.4% of all cases in 2009—2012. The 10 yr survival over the whole period for systemic AL has been 20%, localised AL 73%, AA 40%, ATTR (hereditary) 54%, ATTR (wild type) – 31%, AFib 59% and Apo AI 64%. This 25 year experience reflects progress in identification and treatment but highlights the unmet need in advanced AL amyloidosis and substantial proportion of patients with other types of amyloidosis for whom no treatments are yet available.

## PC 29

### Serum amyloid A in the assessment of patients with longstanding Rheumatoid Arthritis

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**Objective:** To evaluate serum amyloid A protein (SAA) as a marker of disease activity in longstanding RA in comparison to other acute phase reactants.

**Methods:** 121 Patients (112 women and 9 men) with RA (> 5 years, fulfilling the ACR criteria) were consecutively collected from the outpatient clinics of two university hospitals and assessed clinically and radiologically. Tenderness and swelling were assessed in the joints and the joint areas (Ritchie method). The ESR was measured in mm/hr. The IgM rheumatoid factor, C-reactive protein (CRP), and serum amyloid A (SAA) were measured by ELISA ( $N < 10$ ) IU/l, 2.1 mg/l, and 4.2 mg/l respectively.

**Results:** The age of the patients ranged from 16 to 75 years (median 48 years). The disease duration ranged from 5 to 27 years (median 10 years). The age at onset ranged from 9 to 63 years (median 34 years). 9 JCA patients were included in the study. The number of swollen joints ranged from 0 to 12 (median 5). The number of tender joints ranged from 0 to 30 (median 12). The morning stiffness ranged from 0 to 214 (median 30) min.

No serum was available in 9 of the patients. Rheumatoid factor was negative in 9 patients (8%) and ranged from 12 to 1450 (median 118) IU/l. The SAA ranged from 1 to 455 (median 21) mg/l. The CRP ranged from 2 to 610 (median 21) mg/l. The ESR ranged from 10 to 145 (median 61) mm/hr.

|                   | SAA         |       | CRP  |      | ESR         |        |
|-------------------|-------------|-------|------|------|-------------|--------|
|                   | r           | p     | r    | p    | r           | p      |
| N Swollen Joints  | <b>0.28</b> | 0.002 | 0.20 | 0.04 | 0.18        | 0.06   |
| N Tender Joints   | 0.17        | 0.08  | 0.13 | 0.16 | <b>0.31</b> | 0.0005 |
| Morning Stiffness | 0.12        | 0.19  | 0.22 | 0.02 | <b>0.29</b> | 0.002  |

**Conclusion:** Similar to studies in patients with early RA, SAA is superior to ESR and CRP in the assessment of the number of swollen joints, the most objective clinical measurement of disease activity. The ESR remains valuable in the assessment of tender joints and morning stiffness. Therefore, SAA should be measured in addition to the ESR in the evaluation of patients with longstanding rheumatoid arthritis.

#### PC 30

#### Amyloidosis with cardiomyopathy characterized by normal interventricular septal thickness: Description of a new entity

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Increased interventricular septal thickness (IVST) by echocardiography with low-voltage on electrocardiogram (ECG) is commonly used as an indicator of amyloid heart disease. We have observed a subset of patients with amyloid who have heart failure and normal IVST. All patients with amyloidosis and left ventricular ejection fraction (LVEF)  $\leq 40\%$  between 1983 and 2010 were analyzed to identify those with normal BMI-weighted IVST. Patients with a history of coronary artery disease were excluded. Nineteen (M=15,F=4) of 255 patients identified had normal IVST. The mean age of this group was  $65 \pm 10$  years. Eighteen had primary systemic amyloidosis and one had hereditary amyloidosis. All 19 patients had amyloidosis proven by biopsy. Edema, dyspnea, and weight loss were the most common presenting symptoms with mean duration from onset to presentation being  $5 \pm 3$ ,  $6 \pm 5$ , and  $7 \pm 7$  months, respectively. Common ECG findings despite normal IVST included low-voltage (26%) and a pseudoinfarct pattern (37%). Mean IVST, LV end diastolic dimension and LV mass index amongst this cohort were  $11.1 \pm 1.6$ mm,  $51 \pm 7$ mm, and  $114 \pm 23.8$ g, respectively. Grade 3/4 diastolic dysfunction was observed in 5 of 19 patients. Nine patients had a thickened mitral valve and of these 2 had a thickened tricuspid valve. Median survival from initial tissue diagnosis amongst the normal IVST cohort (group 1) was 4.5 months as compared to 3.5 months in age and gender matched controls with increased IVST and LVEF  $\leq 40\%$  (group 2). Mortality in both groups 1 and 2 is high with 81% and 79% dying within one year of onset of symptoms, respectively. A subset of patients with amyloidosis and cardiomyopathy present with normal IVS wall thickness and account for 7% of patients with amyloidosis and LVEF  $\leq 40\%$ . Amyloidosis must be considered in the differential diagnosis of patients with cardiomyopathy with reduced EF and normal IVST. The prognosis of these patients is as poor as those with increased IVST.

## PC 31

### Sudden Death in AL Amyloidosis: Insights from Multimodality Cardiac Imaging

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**Background:** Microvascular angina is classically characterized by exertional ischemia on the electrocardiogram, often with minimal changes on routine nuclear stress imaging. It generally carries a good prognosis, rarely progressing to myocardial infarction. Patients with AL amyloidosis can have microvascular angina possibly due to perivascular amyloid deposition in the intramyocardial blood vessels. The clinical significance of microvascular angina in amyloidosis is not clear.

**Case Report:** A 61-year-old man with clinically stable moderate angina and stable AL amyloid cardiomyopathy 5 years after stem cell therapy was vigorously exerting himself when he became profoundly hypotensive and developed atrial fibrillation. Shortly after transportation to the emergency room he sustained a cardiac arrest due to electromechanical dissociation. He was successfully resuscitated, without evidence of new cardiac damage. ECG immediately prior to cardiac arrest demonstrated acute changes consistent with extensive myocardial ischemia, typical of the appearance of severe epicardial coronary disease. Angiography revealed no obstructive epicardial coronary disease and echocardiography demonstrated moderate to severe left ventricular thickening, typical of amyloid cardiomyopathy. Imaging, utilizing positron emission tomography (PET) with adenosine vasodilation, demonstrated profound global subendocardial ischemia with transient ischemic dilation of the left ventricle. He remains stable with amiodarone and avoidance of vigorous exertion. A similar imaging picture of global myocardial ischemia in the absence of structural cardiac disease was subsequently found in two other, untreated, patients with AL amyloidosis, one of whom had angina and the other congestive heart failure without angina.

**Conclusions:** These cases illustrate that, in patients with AL amyloidosis, global myocardial ischemia may occur in the absence of obstructive epicardial coronary artery disease and can be severe enough to cause cardiac arrest. The example presented illustrates the importance of multimodality imaging to determine mechanisms of aborted sudden death and sheds light on the well-recognized phenomenon of sudden death in AL amyloidosis that can occur even in the presence of a prophylactic implanted defibrillator.

## PC 32

### Analysis of TTR-related amyloidosis in the field of orthopedics

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**Background:** Transthyretin (TTR) amyloid deposition has been shown to be found in ligaments and tendons in the field of orthopedics, especially in elderly people. However, detailed incidence and clinical significance of TTR amyloid deposition in ligaments and tendons remains largely unknown. In this study, we analyzed TTR-related amyloidosis in the field of orthopedics.

**Objective and Methods:** We investigated 126 specimens of patients (46 - 83 years old) with carpal tunnel syndrome (54 specimens), rotator cuff tears (21 specimens), and lumbar spinal canal stenosis (LSCS) (51 specimens). TTR amyloid deposition was detected by Congo red and immunohistochemical staining. Serum TTR levels in 15 patients with LSCS were measured. Existence of mutated TTR in serum was analyzed by surface-enhanced laser desorption/ ionization time-of flight mass spectrometry (SELDI-TOF MS).

**Results and Discussion:** We identified 61 amyloid positive samples including 46 TTR cases (18 cases in tenosynoviums, 5 cases in rotator cuff tendons, and 23 cases in yellow ligaments). The mean age in TTR cases was higher compared to non-TTR cases in tenosynoviums and yellow

ligaments. A statistically positive correlation between amount of amyloid deposits in the ligamentum flavum and age was observed. Seven TTR cases with LSCS did not have any mutated TTR, and serum TTR levels were not significantly changed. Because the incidence of senile systemic amyloidosis (SSA) was lower than that of the ligaments and tendons, these findings suggest that the WT TTR amyloid deposition in the ligaments and tendons might be localized form of amyloidosis. Meanwhile, the mean age in TTR cases was younger than that of the patients with SSA, suggesting these manifestations may be initial symptoms of SSA.

**Conclusion:** We should carefully follow the clinical course of the patients with WT TTR-related amyloidosis in the field of orthopedics, because a part of those patients could develop to SSA.

### PC 33

#### Safety and efficacy of triplet regimens in newly diagnosed light chain (AL) amyloidosis

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The prognosis of patients with systemic AL amyloidosis, particularly cardiac, is poor. Treatments have been derived from multiple myeloma (MM), but there are few studies using triplet regimens in AL amyloidosis owing to concern of greater toxicity than seen in MM.

We retrospectively reviewed newly diagnosed AL amyloidosis patients who were initially treated with a triplet regimen to assess their safety and efficacy. Of the 9 patients included, the median age was 64 and 8 were ineligible for stem cell transplant. At least 2 organs were involved in 4 patients, including 7 with kidney and 4 with heart—2 of whom had NYHA class 3 heart failure.

All patients were initially treated with a 28 day cycle of bortezomib 1.3 mg/m<sup>2</sup> weekly \* 3 (either SQ or IV), dexamethasone equivalent of 4-20 mg IV or PO weekly, and either lenalidomide 10-15 mg daily for 21 days or cyclophosphamide 250-300 mg/m<sup>2</sup> IV on days 1 and 8.

With a median follow up of 11 months (range 3.2-30), 8 of 9 patients had a hematologic response, including 2 CRs, with a median time to response of 2.7 months. An organ response was seen in 6 of 9 patients, including all 4 patients with cardiac involvement. To date, there have been no deaths and only 1 patient with progressive disease.

The major grade 3 and 4 toxicities observed were fluid overload and syncope - and these were seen only in the patients with baseline heart failure (either ischemic or amyloid). Of note, even these patients went on to achieve hematologic and organ responses.

In conclusion, with dose attenuated triplet regimens, the toxicities related to baseline organ dysfunction are not only manageable, but due to impressively rapid hematologic responses, are reversible. Dose-attenuated bortezomib based triplet regimens should be tested prospectively in clinical trials.

### PC 34

#### Resolution of AL hepatic amyloidosis

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**Background:** Treatment of AL amyloidosis is targeted to control of the offending plasma cell dyscrasia so that progression of amyloid deposition is stopped. Whether tissue amyloid can then be reduced and organ function restored is a question posed for each individual patient.

**Objective:** We report five patients who presented with massive hepatomegally and AL amyloid proven by liver biopsy. Patients were monitored from four to 27 years to evaluate response to chemotherapy aimed at control of the plasma cell dyscrasia.

**Methods:** Ages of subjects at time of diagnosis ranged from 45 – 67 years. All were male. Three patients also had renal involvement with nephrotic syndrome, one with advanced renal insufficiency. Four were treated with melphalan and corticosteroid, two oral medications, two IV melphalan with stem cell rescue.

**Results:** Liver size in each patient returned to normal in 24 – 36 months and repeat liver histology for three patients demonstrated decrease in amyloid, one with complete resolution. Survival was four to 27 years without recurrence of amyloid.

**Discussion:** Massive hepatomegally from AL amyloid deposition is associated with a grave prognosis. Unfortunately progressive enlargement of the liver is ignored or unappreciated by many patients. This results in delayed diagnosis and, therefore, seriously impacts treatment options. Even so, favorable response may be obtained in the patients with a plasma cell clone that is responsive to chemotherapy. Resolution of hepatic amyloid may be related to the high level of metabolic activity of this vascular organ.

**Conclusion:** These cases demonstrate that hepatic amyloid, even with massive hepatomegally, can resolve and normal liver function restored if the plasma cell clone responds to therapy.

#### PC 35

#### Treatment of AL Amyloidosis with Autologous Stem Cell Transplantation: Results in a Series of 47 Patients From a Single Institution

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Treatment of immunoglobulin light chain (AL) amyloidosis is a real challenge. Long-term experience on high dose melphalan and autologous stem cell transplant (HDM/SCT) from single referral centers has shown its potential to obtain durable hematologic responses, a high organ response rate, and prolonged overall survival, with a decreased transplant related mortality (TRM) in recent years. We analyzed a series of 47 consecutive patients (median age: 57; range, 34 to 71) treated with HDM/SCT at a single center from November 1997 to April 2011. Median number of involved organs was 2 (range, 1 to 5), with cardiac involvement in 62% and >2 organs involved in 51% of patients. Median time from diagnosis to SCT was 8 months and 24 patients had received previous therapy. A reduced dose of melphalan (140 mg/m<sup>2</sup>) was administered as conditioning regimen in 16 (34%) patients according to age, heart involvement, renal dysfunction and/or performance status. In an intention-to-treat analysis, the overall hematologic response rate was 34% (15% complete remissions) and 47% achieved an organ response. Sixteen patients died during the first year following SCT and the overall TRM was 25.5%, decreasing from 43% during the first 5 years to 18% for the last 33 patients ( $p=0.14$ ). Median time to progression and overall survival were 3.5 and 6.5 years, respectively. Our results support the efficacy of SCT in AL and the fact that a careful selection of patients and experienced management is crucial to reduce the TRM.

#### PC 36

#### Cardiac Safety and Tolerability, and Effects on Cardiac Function, of Tafamidis in Patients With Non-V30M TTR-FAP

**Thibaud Damy,** <sup>1</sup> Daniel Judge, <sup>2</sup> Ahmet Dogan, <sup>3</sup> Karine Berthet, <sup>4</sup> Violaine Planté-Bordeneuve<sup>1</sup>  
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**Background:** Transthyretin familial amyloid polyneuropathy (TTR-FAP) is an autosomal dominant disease characterized by extracellular amyloid deposition in the nerves and heart. Orthotopic liver transplant (OLT) is recommended to remove the source of mutated TTR and stop amyloid deposition. However, progressive cardiomyopathy due to continuing amyloidosis has been described following OLT in patients with non-V30M mutations. Tafamidis prevents dissociation of TTR into monomers and formation of amyloid.

**Objectives:** To evaluate cardiac safety and tolerability of tafamidis in patients with non-V30M TTR-FAP.

**Methods:** Patients (N=21) with TTR-FAP due to non-V30M TTR mutations and no OLT history were studied in a phase 2 open-label trial. Cardiac assessments included ECG, 24-hour Holter monitoring, echocardiogram, and cardiac biomarkers (troponin I and NT-pro-BNP) at baseline and 6 and 12 months.

**Results:** Of the 21 patients enrolled, mean (SD) age, LVEF, troponin I, and NT-pro-BNP at baseline were 63.1(9.86) years, 60.3(9.96) %, 0.023(0.04) ng/mL, and 1248.9(1529.4) pg/mL, respectively. Nine patients had a history of cardiac events. Six of these 9 experienced peripheral edema or dyspnea related to heart failure while on treatment, and 3 patients were hospitalized for other cardiovascular events (AV block, coronary stenosis, TIA). Eighteen patients completed the study, with no significant changes in troponin I, LVEF, or cardiac remodeling. NT-pro-BNP, while elevated at baseline, remained stable with no clinically relevant changes. The pattern of Holter monitoring abnormalities was similar at baseline and while on treatment (eg, atrial tachycardia, 52.4% [11/21] vs 44.4% [4/9]). The percentage of patients with normal heart rate variability (HRV) increased from 21% (4/19) at baseline to 42% (8/19) at month 12.

**Discussion:** This study showed no deleterious effects of tafamidis on cardiac function among a cohort of treated TTR-FAP patients. The number of patients with normal HRV improved.

**Conclusion:** Tafamidis was safe and well tolerated in patients with non-V30M TTR-FAP.

This study was sponsored by FoldRx Pharmaceuticals, acquired by Pfizer Inc in October 2010.

#### PC 37

#### First domino liver transplant alone without kidney transplant for Fibrinogen A- $\alpha$ chain renal amyloidosis

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**Background:** Fibrinogen A- $\alpha$  chain amyloidosis (AFib) is caused by variant fibrinogen produced by the liver but deposited predominantly in the kidneys. Kidney transplantation for this disease is followed by amyloid deposition in the graft by 1 to 5 years. Liver and kidney transplantation can be curative but has been associated with high morbidity for patients on hemodialysis prior to transplantation. Liver transplantation alone for AFib has not been reported but may arrest the disease by removing the source of amyloid production.

**Results/Case:** A 58-year-old woman presented with nephrotic syndrome, hypertension and urine protein excretion 3.4 g/day. Renal function rapidly declined, with creatinine 1.39 to 1.97 mg/dL in 10 months. Kidney biopsy demonstrated glomerular amyloid deposits identified as AFib by mass spectrometry. Genomic DNA sequencing confirmed the Glu526Val mutation associated with AFib. She wished to avoid kidney transplantation and advocated for liver transplantation alone. She received priority for liver transplantation to minimize further renal damage, and domino liver transplantation was performed 4 months after listing. Creatinine was 2.85 mg/dL prior to transplantation, peaked at 3.77 mg/dL in the immediate postoperative period without requiring hemodialysis, and was 2.12 mg/dL at discharge 1 week later. Acute cellular rejection occurred 4 months after transplantation and resolved with treatment. She is doing well 18 months after transplantation with creatinine 2.4 mg/dL. The recipient of her liver was a 61-year-old woman with primary sclerosing cholangitis complicated by severe pruritis that resolved after transplantation; she now has normal liver and kidney function.

**Discussion:** In this first report of liver transplantation alone for the treatment of AFib, the patient's kidney function stabilized after liver transplantation compared to the rapid progression of renal amyloidosis before transplantation.

**Conclusion:** Liver transplantation alone for AFib may arrest the disease process and is a viable treatment option prior to the initiation of hemodialysis.

#### PC 38

#### Ten Year Survival Following Autologous Stem Cell Transplantation for Immunoglobulin Light Chain Amyloidosis

Stefan Cordes, Angela Dispenzieri, Martha Q. Lacy, Suzanne R. Hayman, Francis K. Buadi, David Dingli, Shaji K. Kumar, WJ Hogan and **Morie A. Gertz**

Mayo Clinic Rochester MN, USA

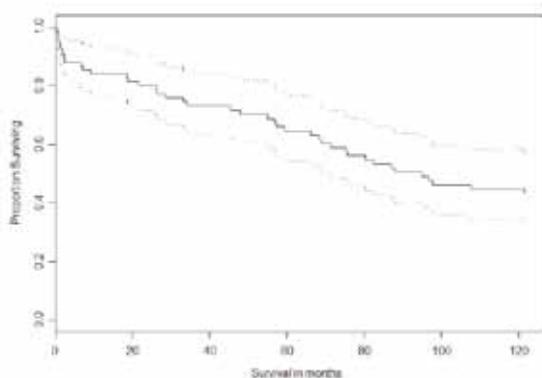
**Methods:** All patients with AL amyloidosis who underwent autologous stem cell transplantation at the Mayo Clinic over the five-year interval between July 27, 1996 and July 28, 2001 were included in this study (n=74).

**Results:** We report on 74 patients who underwent high dose melphalan treatment supported by autologous stem cell transplantation prior to August 2001. Of these, 32 (44%) survived for longer than 10 years. In this study we have identified 4 characteristics with statistically significant differences in the ten-year survivor group: (1) Number of Organs Involved, (2) Septal Thickness, (3) Total cholesterol, and (4) Urine total protein.

**Conclusion:** We find that the numbers of organs involved is the only predictor in multivariable analysis. Depth of the response to therapy, as measured by the lowest post-transplant free light chain is the most significant indicator of *durability* of response.

Unfortunately, since this cannot be assessed before therapy it has no predictive value in terms of selecting patients. It should be noted that NT pro BNP was available only in 34 of the 74 patients and therefore could not be systematically assessed for impact in this model.

*Survival of all patients transplanted prior to July 2001.*



#### PC 39

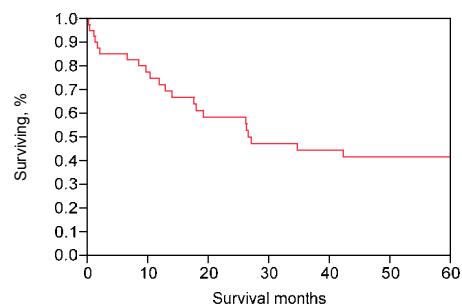
#### Refinement in Patient Selection Can Reduce the Treatment-Related Mortality from Stem Cell Transplantation in Amyloidosis to <2%

**Morie A. Gertz**<sup>1</sup>, Martha Q. Lacy<sup>1</sup>, Angela Dispenzieri<sup>1</sup>, Shaji K. Kumar<sup>1</sup>, David Dingli<sup>1</sup>, Nelson Leung<sup>2</sup>, William J. Hogan<sup>1</sup>, Francis K. Buadi<sup>1</sup>, Suzanne R. Hayman<sup>1</sup>  
Divisions of <sup>1</sup>Hematology as well as <sup>2</sup>Nephrology and Hypertension, Mayo Clinic, Rochester, MN, USA

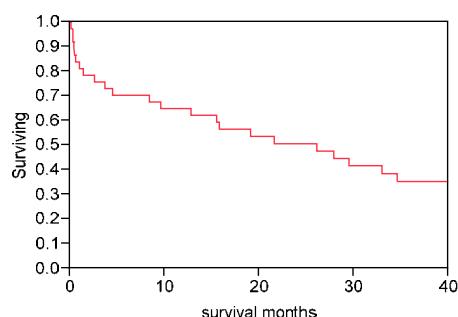
**Introduction:** This study was undertaken to develop selection guidelines for transplant centers to determine eligibility for high-dose therapy in patients with light chain amyloidosis.

**Materials and Methods:** 499 transplanted patients were reviewed comparing two cohorts—one with a treatment-related mortality of over 10% and one with a treatment-related mortality of 1% to determine patient differences. A second comparison was undertaken among the 44 patients that died prior to day +100 to determine features that would identify early deaths.

*Survival of those patients transplanted with an NT proBNP > 500 pg/mL*



*Overall survival of patients transplanted with the troponin T level >0.06 mg/mL*



**Results:** Cardiac involvement was the major determinant of treatment-related mortality. One-quarter of patients transplanted with an NT-proBNP >5000 pg/mL had died by 10.3 months. When the serum troponin T was >0.06 ng/mL, 25% died at 3.7 months.

**Conclusion:** Mayo staging, although highly predictive for overall survival, is not useful for selecting patients for stem cell transplantation. Patients who have a serum troponin T >0.06 ng/mL or an NT-proBNP >5000 pg/mL (not on dialysis) should not be considered acceptable candidates for stem cell transplantation due to an unacceptable early mortality rate. Application of these selection criteria is capable of reducing treatment-related mortality to <2%.

#### PC 40

#### Subcutaneous bortezomib is effective and well tolerated for the treatment of systemic AL amyloidosis

**Simon DJ Gibbs**, Christopher P Venner, Darren Foard, Lisa Rannigan, Jennifer P Pinney, Thirusha Lane, Murielle Roussel, Sanjay Banpersad, Carol J Whelan, Jason Dungu, Helen J Lachmann, Julian D Gillmore, Ashutosh D Wechalekar and Philip N Hawkins

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**Background:** Subcutaneous (SC) bortezomib has similar efficacy to intravenous administration (IV) for the treatment of multiple myeloma, but is associated with significantly lower rates of neuropathic complications (Moreau *et al*, 2011).

**Objectives:** We report here our preliminary experience of SC bortezomib for treatment of systemic AL amyloidosis (AL) and light chain deposition disease (LCDD) with emphasis on neuropathic manifestations.

**Results:** Eleven patients received SC bortezomib since June 2011, 10 for AL and one for LCDD. Treatment was as first-line in 7 patients, and in 4 for relapsed/refractory disease. 8 were male, 3 female. Median age was 72 years (range 54-82). Nine had Mayo Stage 3 disease. Two received SC bortezomib monotherapy, four with dexamethasone, two with cyclophosphamide/prednisolone, two with melphalan/prednisolone, and one with cyclophosphamide/dexamethasone. Two patients had pre-existing peripheral neuropathy, and 4 had autonomic neuropathy. Four patients were initially treated with IV bortezomib (range 0.5-5 cycles) before switching to SC administration. The doses of SC bortezomib varied from 1mg/m<sup>2</sup> weekly to 1.3 mg/m<sup>2</sup> twice weekly. The number of SC cycles administered ranged from 1-5 at time of censor.

Clonal responses to SC bortezomib were evaluable in 9 patients: overall haematological response was 78%, with dFLC VGPR of 56%, including 44% CR. These response rates are similar to those reported by our Centre in association with IV treatment. One patient who received only SC monotherapy achieved CR. Two patients developed peripheral neuropathy following SC bortezomib, after cycles 3 and 5 respectively, and several reported minor injection site reactions. One patient reported a reduction in neuropathic symptoms and another less diarrhoea after switching from IV to SC therapy.

**Conclusions:** These preliminary findings suggests that SC bortezomib is a convenient and well tolerated mode of administration with similar efficacy to IV therapy in AL amyloidosis, and support further studies including elderly patients and those with advanced cardiac involvement.

#### PC 41

#### Light chain deposition disease: improved patient survival in the era of novel agents

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**Background:** Light chain deposition disease (LCDD) is a systemic disorder caused by deposition of monoclonal immunoglobulin light chains in a non-fibrillary conformation. Reports of renal and patient

survival in the era of serum free light chain (SFLC) analysis, cardiac biomarkers and imaging, and novel therapeutic agents are scant.

**Objectives:** To analyse the use of SFLC analysis, cardiac magnetic resonance imaging (CMR) and biomarkers, and update renal and patient survival in LCDD.

**Results:** LCDD was confirmed in 33 patients by electron microscopy. Median age at diagnosis was 52 years (range 26-72). Deposits were derived from kappa light chains in 73%. Renal involvement was universal, 96% had haematuria and hypertension, and evidence of cardiac, liver, and gastrointestinal involvement was suspected in 8, 5, and 3 cases respectively. Seven patients had end-stage renal failure at diagnosis.

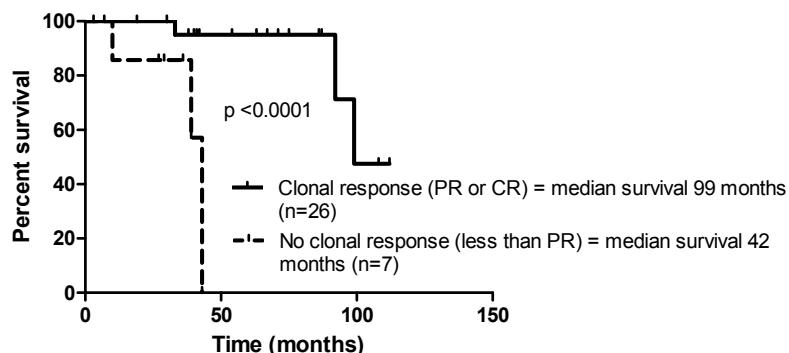
Median NT pro-BNP concentration at diagnosis was 5933ng/L, and 84% were above 332ng/L. Troponin T concentrations above the upper limit of normal (0.055ug/L) were observed in 39%. CMR was performed on 8 patients, 4 with suspected cardiac involvement, none demonstrated the late gadolinium enhancement characteristically observed with cardiac amyloidosis.

Median follow-up was 42 months. Estimated median renal survival was 5 years. Improvement in renal function occurred in 7 patients, and improvements in mean cardiac interventricular wall thickness >2mm were observed in 2 of 8 patients with cardiac involvement, all of whom had achieved at least partial clonal responses (PR) by international consensus criteria.

Median patient survival was 8.25 years. Adverse prognostic features were age >65 years, extra-renal disease and achieving less than PR. Two patients in complete clonal responses underwent renal transplantation, with excellent graft function after 20 and 34 months.

**Conclusions:** Survival is better than previously reported, possibly reflecting improved clonal monitoring with SFLC analysis and treatment with novel agents and autologous stem cell transplantation. Effective clonal suppression can improve renal and cardiac function. Renal transplantation is feasible in selected patients with good clonal responses.

#### *Overall Survival according to clonal response*



AB is an employee of the Binding Site

#### PC 42

**Case report:** A 63-year-old man with amyloidosis presenting a predominant diffuse lymphadenopathy and a paraproteinemia IgM κ

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**Background:** Diagnostic criteria, treatments, and prognosis in the Waldenström macroglobulinemia (WM) were recently reviewed<sup>1</sup>. It is still a matter of debate whether these patients should receive or not the same chemotherapy regimens as other patients with AL amyloidosis.

**Objective:** In a review of some case of IgM amyloidosis we evaluated a patient unusually unresponsive to a prolonged chemotherapy and radiotherapy, continuing to be well during follow-up.

**Methods:** A 63-year-old man presented in 1990 an asymptomatic MGUS IgM κ. Four years later (1994) a palpable mass appeared in the laterocervical region. The CT scan showed extensive lymphadenopathy (supraclavicular, axillary, thoracic, mesenteric, iliac, retroperitoneal, inguinal sites). No other visceral organs were affected. On a biopsy specimen a massive deposition of AL amyloid

fibrils subversing lymph node structure, with no other abnormal findings of lymphoproliferative disorders was observed. The feature was considered as AL amyloidosis localized at the lymph nodes on the basis of M-protein and abnormal plasma cells (6%) in the bone marrow.

The therapeutic approach was a long-lasting chemotherapy for one year followed by radiotherapy on the mediastinum and laterocervical, axillary, inguinal sites.

**Results:** During the following four years, CT control showed no obvious change in the size of lymph nodes, with clinical conditions persistently good and lab analyses in the normal range.

The case here reported, started more than 10 years ago and recently reviewed, stresses that the lack of therapeutic response was more likely related to the progressive and quick substitution of tissue nodes with amyloid materials, obviously unresponsive to pharmacological and radiologic therapy performed at the first diagnosis.

**Conclusion:** Systemic amyloidosis can occur rarely as multiple masses with replacement of lymph nodes, causing lymphadenopathy<sup>2-5</sup>. Only an appropriate work-up in the follow up procedure should be undertaken to minimize the risk of a potential pitfall about the best therapeutic choice.

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#### PC 43

#### Green Tea Halts Progression of Cardiac Transthyretin Amyloidosis – A Pilot Study

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**Background:** Treatment options in patients with cardiac TTR amyloidosis, especially of the senile form, are limited. Epigallocatechin-3-gallate, the most abundant catechin in green tea (GT), inhibits fibril formation from several amyloidogenic proteins in vitro. Thus, it might also prevent amyloid fibril formation from human TTR and thus, halt progression of the disease. This prospective, open-label, single center interventional trial was performed to evaluate the therapeutic potential of green tea in patients with transthyretin (TTR)-related cardiomyopathy.

**Methods:** 19 patients with cardiac TTR amyloidosis were evaluated by standard blood tests, echocardiography, and cardiac MRI (n=9) while consuming GT for 12 months.

**Results:** 5 patients were not followed-up for reasons of death (n=2), discontinuation of GT consumption (n=2), and heart transplantation (n=1). After consumption of GT for 12 months a no increase of LV wall thickness and LV myocardial mass was observed by echocardiography. In the subgroup of patients evaluated by cardiac MRI a decrease of LV myocardial mass was detected in all patients resulting in a mean decrease of -12.5%. This was accompanied by an increase of mitral annular systolic velocity of 9%. In all 14 patients total cholesterol ( $191.9 \pm 8.9$ mg/dL vs.  $172.7 \pm 9.4$ mg/dL; p<0.01) and LDL cholesterol ( $105.8 \pm 7.6$ mg/dL vs.  $89.5 \pm 8.0$ mg/dL; p<0.01) decreased significantly during the observational period. No serious adverse side-effects were reported by any of the participants.

**Conclusions:** Consumption of the polyphenol epigallocatechin-3-gallate within GT appears to represent a promising therapeutic tool to halt the progression of the cardiac amyloid load in patients with cardiac amyloidosis of TTR origin.

This work was supported financially by Peter Waldmann Amyloidose-Stiftung (Küssnacht, Switzerland). Capsules of GTE were a gift from Dr. Loges + Co. GmbH (Winsen/Luhe, Germany) and GT was provided by Projektwerkstatt GmbH (Berlin, Germany).

#### PC 44

#### **Lenalidomide, Cyclophosphamide And Dexamethasone (CRd) For Light Chain Amyloidosis: Long term Results From A Phase 2 Trial**

**Shaji K. Kumar**, Suzanne R. Hayman, Francis K. Buadi, Vivek Roy, Martha Q. Lacy, Morie A. Gertz, Jacob Allred, Kristina M. Laumann, Leif P. Bergsagel, David Dingli, Joseph R. Mikhael, Craig B. Reeder, A. Keith Stewart, Steven R. Zeldenrust, Philip R. Greipp, John A. Lust, Rafael Fonseca, Stephen J. Russell, S. Vincent Rajkumar, Angela Dispenzieri.

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Light chain (AL) amyloidosis remains incurable despite recent therapeutic advances. Given the activity of lenalidomide in combination with alkylating agents in myeloma, we designed a phase 2 trial combining lenalidomide, dexamethasone and cyclophosphamide. The treatment consisted of 4 week-cycles of lenalidomide (15 mg PO days 1-21), cyclophosphamide (300 mg/m<sup>2</sup> PO days 1, 8, 15) and dexamethasone 40 mg PO days 1, 8, 15, 22 (weekly continuously). Thirty-five patients with AL, including 24 previously untreated patients, were enrolled. Nearly half of the patients had cardiac stage III disease and 28% had 3 or more organs involved. The median duration on study was 7.1 months with a median of 7 cycles of therapy administered. The overall hematological response ( $\geq$ PR) rate was 60%, including 40% with a very good partial response or better. Using serum free light chain for assessing response, 77% patients had a hematological response. Organ responses were seen in 29% of patients and were limited to those with a hematological response. Hematological toxicity was the predominant adverse event, followed by fatigue, edema and gastrointestinal symptoms. The median hematologic PFS was 28.3 months and the median overall survival was 37.8 months. The median overall survival was not reached for patients in stage I compared with 37.8 months (95%CI: 17.5-NA) and 7 months (95% CI: 4.2-12.3) for patients in stage II and III respectively; log rank P < 0.001. The cumulative incidence rates of initiation of new therapy were 20%, 26%, and 36% at 6, 12, and 24 months post study entry respectively. Seven patients (20%) died on study; death was related to advanced cardiac amyloidosis in five patients and possibly treatment related in two patients. CRd is an effective combination for treatment of AL amyloidosis and is associated with durable hematologic responses as well as organ responses and manageable toxicity.

Clinical trial support provided by Celgene

#### PC 45

#### **Improved Hematologic Responses Following Risk Adapted Stem Cell Transplant (SCT) and Bortezomib Consolidation in Systemic Light-Chain Amyloidosis (AL) is Associated with Long Term Organ Improvement**

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**Background:** Risk-adapted melphalan dosing has improved the safety but may compromise efficacy of SCT for patients with AL. In a phase II trial we studied bortezomib and dexamethasone (BD) as consolidation following risk-adapted SCT.

**Methods:** Untreated patients with AL involving  $\leq$  2 organs were assigned MEL 100, 140 or 200mg/m<sup>2</sup> SCT, based on age, renal function and cardiac involvement. Hematologic response (HR) was assessed at 2-3, 12 and 24 months post-SCT. Pts with persistent clonal plasma cell disease received

consolidation with BD. Organ responses (OR) and overall survival (OS) were assessed at 12 and 24 months.

**Results:** We treated 40 patients with renal (71%), cardiac (51%), liver/GI (15%) or nervous system (12%) disease; 49% had 2 organs involved. Four patients with advanced cardiac AL died within 100 days of SCT, resulting in 10% TRM. With median follow-up 41 months, OS at 12 and 24 months post-SCT was 88% and 82%. In patients with cardiac AL, OS was 76% and 64% at 12 and 24 months, versus 100% in all others. At 2-3 months post-SCT, 50% had  $\geq$  partial response (PR) with 31% patients achieving CR; 66% then received BD consolidation. At 12 and 24 months, 97% (30/31) and 81% (17/21) had  $\geq$  PR with 77% and 55% CR. Four patients progressed or relapsed. Of patients who received BD, 90% had improved responses. OR evolved over time, and occurred in 69% and 95% at 12 and 24 months; organ progression occurred in two patients. None of the four patients with serologic progression had organ impairment.

**Conclusions:** In newly diagnosed AL, BD following risk-adapted SCT achieves unprecedented HR, CR and OR rates. Organ improvement occurs over time and is maintained, enabling early identification of hematologic relapse prior to recurrent organ compromise. We speculate that maintenance therapy may prolong the durability of response.

HL, HH and RC have received research funding and have served on the advisory board of Millenium Pharmaceutical.

#### PC 46

#### Bortezomib and Dexamethasone Consolidation Results in Rapid Free Light Chain Control in Patients with Less Than a CR Following Stem Cell Transplant (SCT) in Systemic Light-Chain Amyloidosis (AL)

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**Background:** Updated hematologic response (HR) criteria in AL are based on the serum free light chain (FLC) assay because control of the involved FLC is associated with improved survival. We evaluated the FLC responses in patients with newly diagnosed AL achieving <CR following risk-adapted melphalan (MEL) who received bortezomib and dexamethasone (BD) as consolidation on a phase II study.

**Methods:** Patients with newly diagnosed AL involving  $\leq 2$  organs were assigned to MEL 100, 140 or 200mg/m<sup>2</sup> SCT, based on age, renal function and cardiac involvement. HR was assessed at 2-3 months, and patients with persistent clonal plasma cell disease received consolidation with BD (up to 6 cycles). The primary endpoint was response at 12 months following SCT.

**Results:** Twenty-five (69%) of 36 patients who underwent risk-adapted SCT achieved <CR and were eligible to receive BD consolidation; one declined and one with SD was removed from study. BD was administered for a median of 6 cycles (range 1-6). Early discontinuation was due to neuropathy (N=3), infection (N=2), CHF (N=2), GI toxicity (N=2) and thrombocytopenia (N=1); one patient with stage III heart disease died. Of 23 patients who received BD, 20 are evaluable at 12 months post-SCT. Ninety percent (18/20) achieved deeper responses following BD, including 74% with CR. Maximal FLC reduction was achieved following 1 cycle of BD in all but one patient.

**Conclusions:** BD following risk-adapted SCT results in rapid FLC reduction and improves HR in 90% of AL patients with suboptimal HR following SCT. The sequence of BD following MEL may be particularly effective. The optimal duration of BD consolidation is unclear. However, 1-2 cycles may adequately control disease and limit toxicity. The durability of HR with BD consolidation continues to be assessed; maintenance therapy following FLC control may extend durability of HR.

HL, HH and RC have received research funding and have served on the advisory board of Millenium Pharmaceutical.

**PC 47****Urinary Excretion of Epinephrine and Dopamine Correlates with Efficiency of G-CSF Mobilized Stem Cells in Patients with AL Amyloidosis**

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**Background:** Hematopoietic stem cell (HSC) migration is essential for peripheral blood HSC collection. Sympathetic signaling regulates HSC egress from bone marrow. We prospectively studied catecholamines and the efficiency of HSC collection in patients with AL undergoing G-CSF mobilization prior to high dose melphalan on a phase II study.

**Methods:** 24h urine samples were analyzed for epinephrine (EPI), NE and dopamine (DA) excretion before G-CSF administration and after HSC collection was completed. Statistics included Spearman rank coefficient (r), Wilcoxon rank sum and Signed rank tests.

**Results:** In 39 patients, median CD34 cells collected was  $8.3 \times 10^6/\text{kg}$  (IQR 5,12.3) in a median of 2 (IQR 2,3) collections. The median CD34 cells infused on day 0 was  $4.7 \times 10^6/\text{kg}$  (IQR 3.8, 6); time to neutrophil engraftment ( $\text{ANC} > 500 \times 2 \text{ days}$ ) was 9 days (IQR 9, 11). Baseline urinary excretion of EPI and DA correlated with total CD34 cells collected ( $r=0.33$ ,  $P=0.005$ ;  $r=0.47$ ,  $P=0.05$ , respectively). An optimal collection defined as  $5 \times 10^6 \text{ CD34 cells/kg}$  in 2 collections was achieved by 25/39 patients and was associated with higher baseline EPI (7 vs 4mcg/24h,  $P=0.02$ ) and DA (220 vs 156mcg/24h,  $P=0.05$ ) but not NE. Only DA significantly changed from baseline to after HSC collection ( $P=<0.0001$ ).

**Conclusion:** Sympathetic signals regulate HSC egress from their niche, and we found baseline EPI and DA excretion are associated with greater and more efficiently collected HSCs following G-CSF in patients with AL. Reduced DA excretion following G-CSF in our study support other evidence that DA plays a role in progenitor migration (Nat Immunol. 2007) and indicates that DA is important in G-CSF mobilization in patients with AL. Modulation of the sympathetic system to enhance HSC mobilization and the use of catecholamine values to guide clinicians with respect to the need for plerixafor or chemo-mobilization should be explored.

**PC 48****The Depth of Renal Response Correlates with Overall Survival in AL amyloidosis**

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**Introduction:** Renal response is an important endpoint for patients with immunoglobulin light chain amyloidosis (AL). Current definition defines it as a >50% reduction in proteinuria with <25% decrease in renal function.

**Objective:** The intent of this study is to better understand the components of the renal response criteria and their interplay.

**Methods:** Patients who underwent autologous stem cell transplantation from 1995 to 2010 were screened. Inclusion criteria were > 1 year of follow-up and a baseline 24 hour urine protein > 1 g/d. Those who were dialysis dependent or died before 1 year were excluded.

**Results:** Of the 435 patients screened, 152 met criteria. In univariate analysis, >50% proteinuria reduction ( $p < 0.001$ ), serum free light chain (sFLC) reduction by >50% ( $p = 0.002$ ), <25% increase in serum creatinine (Scr) ( $p = 0.0003$ ), and a 50% proteinuria reduction within 15 months of treatment ( $p = 0.04$ ) were associated with better OS. Multivariate analysis showed sFLC reduction by >50% (0.006), <25% increase in serum creatinine (Scr) ( $p = 0.0005$ ) and >50% proteinuria reduction ( $p = 0.0006$ ) were independently associated with OS. OS was significantly better among patients achieving >85% reduction in proteinuria,  $p = 0.03$ . Patients with >75% reduction in sFLC were more likely to achieve >50% proteinuria reduction ( $p = 0.009$ ). Finally, only patients who had <85% proteinuria reduction had a worse OS when there was a > 25% increase in Scr.

**Conclusion:** The depth of renal response correlated with OS. Patients with 85% reduction in proteinuria had better OS than those with >50%. The negative impact of >25% decrease in renal function was only pertinent in patients with <85% reduction in proteinuria. These findings should be helpful in better refining the definition of renal response criteria in AL patients.

**PC 49**

**Transthyretin Stabilization, Efficacy and Safety of Tafamidis for the Treatment of Transthyretin Amyloidosis**

**Giampaolo Merlini**<sup>1</sup>, Teresa Coelho<sup>2</sup>, Rodney H. Falk<sup>3</sup>, Daniel P. Judge<sup>4</sup>, Ilise Lombardo<sup>5</sup>

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**Background:** Transthyretin (TTR) amyloidosis (ATTR), characterized by progressive neuropathy (TTR-FAP) and cardiomyopathy (TTR-CM), results from wild-type or variant TTR tetramer dissociation into monomers, ultimately forming amyloid deposits.

**Objective:** To examine kinetic TTR stabilization by tafamidis across clinical trials and evaluate its efficacy and safety in ATTR patients.

**Methods:** Data were analyzed from a Phase II/III double-blind, randomized, trial (Fx-005, n=125), an open-label extension (Fx-006, n=71) in V30M TTR-FAP patients and Phase II open-label studies in non-V30M TTR-FAP patients (Fx1A-201, n=21), and in wild-type or V122I patients with TTR-CM (Fx1B-201, n=35). Pharmacodynamics were assessed by proportion of patients with TTR tetramer stabilization. Clinical outcomes, including Norfolk Quality of Life-Diabetic Neuropathy scores (TQOL), modified Body Mass Index (mBMI), Neuropathy Impairment Score-Lower Limb (NIS-LL), and cardiac status were also assessed.

**Results:** Stabilization of plasma TTR was achieved in the majority of patients receiving tafamidis across all clinical trials. This included 97.9% of tafamidis patients vs 0% of placebo patients ( $p<0.0001$ ) after 18 months (Fx-005); 94.1% (tafamidis-tafamidis) vs 93.3% (placebo-tafamidis) at 12 months (Fx-006); and 100% and 87.5% at 12 months in patients receiving tafamidis (Fx1A-201 and Fx1B-201, respectively). In V30M patients, tafamidis resulted in less deterioration in NIS-LL (least square mean  $\pm$  standard error, tafamidis:  $2.8\pm1.0$ , placebo:  $5.8\pm1.0$ ,  $p=0.0271$ ), TQOL ( $2.0\pm2.3$  vs  $7.2\pm2.4$ ;  $p=0.12$ ) and mBMI ( $39.3\pm11.5$  vs  $-33.8\pm11.8$ ;  $p<0.0001$ ). These effects were sustained over an additional 12 months' treatment of tafamidis. Similar benefits were observed in non-V30M TTR-FAP patients. Incidence of cardiovascular hospitalization/death at 12 months was lower in TTR-CM patients vs non-randomized, historical controls (25.7% vs 44.8% respectively,  $p=NS$ ).

**Discussion:** Tafamidis stabilized TTR in most patients with wild-type or mutant TTR, and tafamidis-treated patients experienced less neurologic or cardiac deterioration, and maintained quality-of-life and nutritional status.

**Conclusions:** Treatment with tafamidis results in TTR stabilization and slower disease progression.

This study was sponsored by FoldRx Pharmaceuticals, acquired by Pfizer Inc in October 2010.

**PC 50**

**Melphalan and dexamethasone (MDex) vs. bortezomib, melphalan and dexamethasone (BMDex) in AL amyloidosis: a matched case control study**

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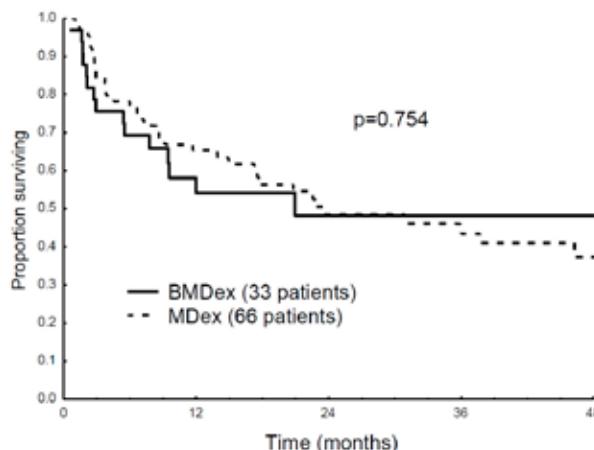
**Background:** Despite two phase III trials comparing MDex and BMDex that are ongoing, many patients with AL amyloidosis are currently treated with combinations of bortezomib and alkylators, based on promising results from uncontrolled studies.

**Methods:** We compared 33 unselected newly-diagnosed subjects treated with BMDex before the phase III trial started, with 66 controls treated with MDex. Treatment schedule was: melphalan 0.22

mg/Kg and dexamethasone 40 mg (20 mg in stage III patients and in 28 subjects with fluid retention >3% of body weight) on days 1-4, and bortezomib 1.3 mg/m<sup>2</sup> (1.0 mg/m<sup>2</sup> in stage III) on days 1, 4, 8 and 11, in 28-day cycles. Controls were matched for organ involvement (cardiac: 82%, renal: 47%), systolic blood pressure (<100 mmHg: 9%), NYHA class (≥III: 49%), NT-proBNP (>10000 ng/L: 22%), Mayo stage (I: 12%, II: 46%, III: 42%), eGFR (≥60 mL/min: 77%, 30-59 mL/min: 14%, 15-29 mL/min: 4%, <15 mL/min: 5%), and dFLC (>500 mg/L: 30%). There was no significant difference in median age (65 vs. 66 years), ejection fraction (56% vs. 52%) and plasma cell infiltrate (17% vs. 16%). Hematologic response rate was 48% (CR 18%, VGPR 21%) with BMDex and 50% (CR 8%, VGPR 17%) with MDex ( $p=0.899$ ). However, dFLC decrease in responders was greater with BMDex (median 95% vs. 83%,  $p=0.018$ ). There was no difference in NT-proBNP response (cases 14% vs. controls 24%,  $p=0.230$ ), SAE (21% vs. 29%,  $p=0.682$ ), and deaths occurring in the first 3 (25% vs. 16%,  $p=0.402$ ) and 6 months (29% vs. 22%,  $p=0.633$ ). Overall, 51% of patients died and survival was no different in the two groups (Figure 1).

**Conclusion:** These data support the need to perform the ongoing controlled trials in order to better define the role of novel agents in the upfront treatment of AL amyloidosis.

Figure 1. Overall survival according to treatment type



G.P.: honoraria from Janssen-Cilag and Celgene

## PC 51

### Treatment and outcome of 150 patients with IgM-related AL amyloidosis

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AL amyloidosis is associated with IgM-paraproteinemia in 5% of cases. Response to alkylating agents is poor and there is no standard of treatment. We describe here the treatment and outcome of 150 patients with IgM-related AL amyloidosis, with particular focus on the impact of lymphoma-like therapeutic approaches.

150 consecutive IgM patients with AL amyloidosis were evaluated between 1988 and 2011 at the UK National Amyloidosis Centre. 74% of patients had underlying lymphoma, mainly of lymphoplasmacytic subtype; lymph node amyloid was present in 27%. Serum FLC ratio was abnormal in 83/119 evaluable patients with baseline dFLC >50 in 66 cases.

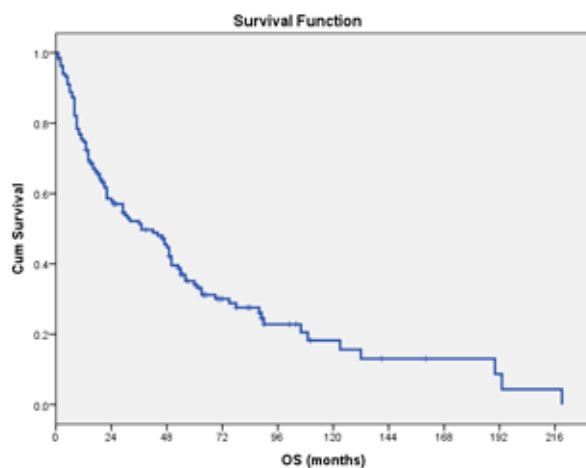
135 patients required therapy, of whom 124 were evaluable for frontline regimen: chlorambucil/melphalan in 57, rituximab-based in 28 (R-CVP 9, FCR 7, RCD 5, R-CHOP 3, others 4), purine analogs in 9 (FC 5), CTD in 8, VAD in 8, CHOP/CVP in 5, CVD in 1, and HD melphalan in 2. Median time to next treatment was 10 months with a better outcome for frontline HDMel, CHOP/CVP and FCR (median 49, 16 and 13mo, respectively) with 50% responders.

Considering all lines of therapy (>2 regimens in 60 patients), 47 patients received Rituximab, 36 purine analogs, 5 bortezomib and 5 HDMel. Median OS was 37 months (Fig 1) with a survival advantage for patients receiving HDMel, CVD (median OS not reached) and FCR (78mo) compared to

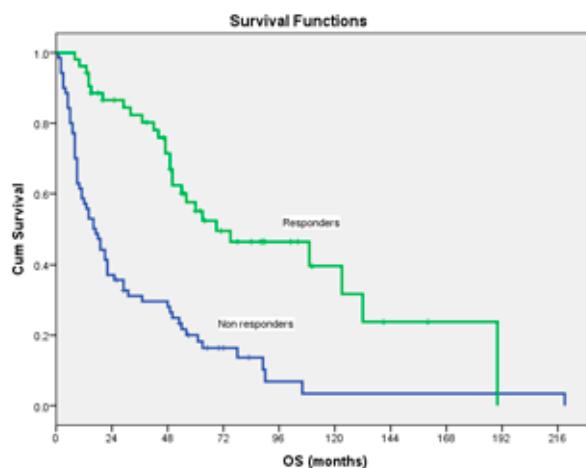
(R)CHOP/CVP or FC regimens (median OS 42 and 31mo). Approximately 45 % of patients achieved hematologic and dFLC >PR (6 dFLC-VGPR) with a median OS of 69 vs 16 months for non responders (Fig 2).

Patients with IgM related AL amyloidosis should be treated with appropriately tailored regimens for the underlying clonal disorder to achieve at least PR. Exposure at some stage to CVD, FCR or HDM appears to be associated with better survival.

**Fig 1: Overall Survival**



**Fig 2: OS by treatment response**



## PC 52

### Autologous stem cell transplantation in POEMS syndrome: the Spanish experience

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**Background:** POEMS syndrome results from a clonal plasma cell proliferation producing a small monoclonal protein usually of λ type. Its major clinical feature is a progressive peripheral neuropathy. Single osteosclerotic lesions are treated with radiation only. In disseminated disease, high-dose melphalan followed by autologous stem cell rescue (ASCT) has shown to be effective in short series, but with significant morbidity.

**Patients and methods:** Between December 1999 and September 2009, 19 patients with POEMS syndrome (median age: 54 years; 12 female/7 male) were treated with melphalan-200 (16 patients) or melphalan-140 (3 patients) followed by ASCT at 9 Spanish institutions. All patients presented an M protein (16 IgA-λ; 3 IgG-λ) and 18 peripheral polyneuropathy. Other clinical features included osteosclerotic lesions (12 patients), organomegaly (16), endocrinopathy (7), skin lesions (18), extravascular volume overload (14), papilledema (6), pulmonary hypertension (4), portal hypertension (2), Castleman's disease (3), thrombocytosis (12) and polycythemia (2). The median number of prior therapies was 2 (range, 0-4). Median time from diagnosis to ASCT was 8 months (range, 2-95).

**Results:** No transplant-related-mortality (TRM) was observed. After a median follow-up of 45 months (range, 3-89), one patient has died of progression 90 months post-ASCT. Five patients presented an engraftment syndrome and two a primary graft failure resolved, one needing a back-up infusion on day + 26 and the other achieving a delayed engraftment. Sixteen patients were evaluable for response: 8 obtained a complete hematologic response (CR), 7 a near-CR and one had disease progression. All patients experienced a significant organic improvement (including pulmonary and portal hypertension), observed at 4-6 months post-ASCT.

**Conclusions:** In this series, ASCT proved to be a highly effective therapy for patients with disseminated POEMS syndrome. Despite that no TRM was observed, these patients may have a delayed hematopoietic recovery and may develop an engraftment syndrome that must be promptly treated.

**PC 53**

**Treatment of AL Amyloidosis with 2 Cycles of Induction Therapy with Bortezomib and Dexamethasone Followed by Bortezomib-High Dose Melphalan Conditioning and Autologous Stem Cell Transplantation**

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Achievement of a hematologic complete response (CR) is a critical determinant of outcomes after high-dose melphalan and autologous stem cell transplantation (HDM/SCT) in AL amyloidosis. We are conducting a prospective trial to determine whether incorporating bortezomib (B) into induction therapy for 2 cycles followed by B-HDM/SCT would improve CR in newly diagnosed patients with AL. The objectives of the trial are hematologic responses, tolerability and survival. Patients receive 2 cycles of induction with B 1.3mg/m<sup>2</sup> and dexamethasone 20mg D1,4,8,11 every 21 days followed by conditioning regimen of B 1mg/m<sup>2</sup> on D -6,-3,+1,+4 and HDM at 140-200mg/m<sup>2</sup> in two divided doses on D -2 and -1. Eighteen patients were enrolled between Jan 2010 to Nov 2011. The median age is 55 (range, 35-65), 12 (67%) are women, 14 (78%) have lambda isotype, 10 (56%) have multi-organ involvement, 9 (50%) have cardiac involvement, of which 6 (33%) had cardiac biomarker stage II and 3 (17%) stage III disease. Sixteen patients are eligible for response and toxicity assessment. Of these, 4 developed grade 3-4 toxicities requiring dose reductions (n=3) and discontinuation (n=2) during induction. Hematologic responses with normalization of serum free light chain concentrations occurred in 9 (56%) patients and additional 4 (25%) had 50% reduction of involved free light chain levels after induction treatment. Of the 16 patients, 14 proceeded with SCT, while 2 did not due to worsening of autonomic neuropathy leading to syncope and development of ESRD requiring dialysis. Mortality within 100 days after SCT was 6% (1/16), while 100% of the 11 evaluable patients achieved a hematologic response (55% CR and 45% VGPR) at 6 months. By intention-to-treat analysis, hematologic response occurred in 79% (43% CR and 36% VGPR) of patients. The median survival for all 18 patients has not yet been reached. In conclusion, incorporating bortezomib into induction and conditioning yielded a high rate of hematologic response with tolerable toxicity. Two patients (11%), who were eligible for SCT at enrollment, did not proceed to SCT due to clinical deterioration during induction treatment.

Supported in part by Millennium Takeda Oncology Company

**PC 54**

**Phase I study of MLN9708, a novel, investigational oral proteasome inhibitor, in patients with relapsed or refractory light-chain amyloidosis (AL)**

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**Background:** The proteasome inhibitor bortezomib provides durable hematologic responses in relapsed AL patients. This study (NCT01318902) assessed MLN9708, an oral, potent, reversible, and specific 20S proteasome inhibitor.

**Objective:** Determine the safety, maximum tolerated dose (MTD), pharmacokinetics (PK) and recommended phase 2 dose of oral MLN9708 in patients with relapsed/refractory AL.

**Methods:** Patients with cardiac biomarker risk stage I/II relapsed/refractory AL and measurable major organ (heart/kidney) involvement received increasing doses of oral MLN9708 (standard 3+3 dose-escalation), up to the MTD determined in multiple myeloma,<sup>1</sup> on days 1, 8, and 15 of 28-day cycles for up to 12 cycles.

**Results:** At data cut-off (Dec 1, 2011), 9 patients (5 M, 4 F) had been enrolled; 6 to the 4.0 mg and 3 to the 5.5 mg fixed dose level. Median age 65 years (range, 54–74). Median number of prior therapies 3 (range, 1–7); 3 received prior bortezomib. Major organ involvement included 3 patients with renal, 2 with cardiac, and 4 with both organs. Patients received a median of 3 cycles (range, 1–6); 2 received ≥5 cycles. Eight patients had ≥1 AE, with drug-related AE in 6. Most common drug-related AE included nausea (n=3; 1 grade 1, 1 grade 2, 1 grade 3), anemia (n=2; 1 grade 2, 1 grade 3), and thrombocytopenia (n=2; both grade 3). There was 1 dose-limiting toxicity, prolonged thrombocytopenia, at 4.0 mg. Three patients had a drug-related grade ≥3 AE; 1 patient had a drug-related serious AE. No on-study deaths or discontinuation due to AE. Seven patients continue on treatment; 2 discontinued for disease progression. Preliminary hematologic responses included 3 VGPR, 2 PR, and 3 no change (1 too early to evaluate).

**Conclusion:** Recruitment is ongoing. Updated safety, efficacy and PK data will be presented.

**References:** 1. Kumar S, et al. ASH 2011, abstract 816.

VS – Research support: Celgene and Millennium Pharmaceuticals, Inc. JAZ – Research support: Celgene and Millennium Pharmaceuticals, Inc. RC – Research support: Genentech and Millennium Pharmaceuticals, Inc.; Consultant for Millennium Pharmaceuticals, Inc., and Neotope. DB, GL, NG, A-MH – Employment: Millennium Pharmaceuticals, Inc.

## PC 55

### Revlimide-Dexamethasone in patients with relapsed light chain amyloidosis previous high-dose chemotherapy does not impair response and revival

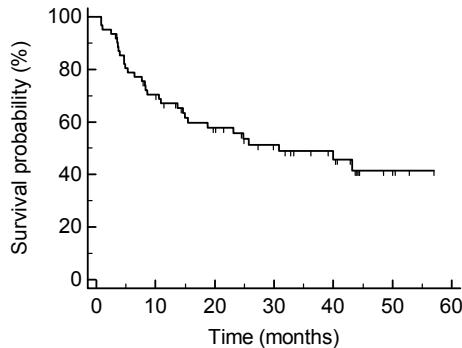
S. Dietrich, U. Hegenbart, T. Bochtler, AV. Kristen, H. Goldschmidt, AD. Ho, S. Schönland, University Hospital, Amyloidosis Centre, Heidelberg, Germany

**Background:** Despite of many improvements in the therapy of systemic light-chain (AL) amyloidosis patients continue to relapse. We have conducted a retrospective study of 62 relapsed AL patients who were uniformly treated with revlimide and dexamethasone (RD).

**Patients:** The median age was 61 (range 42–67) years. The starting dosage of R was 15 mg and of D 20 mg (day 1–4). 29 patients had received previous high dose melphalan (HDM). Number of previous therapy lines was 2 (range: 0–5). Pts. received a median of 5 cycles (range, 1–15).

**Results:** Median overall survival (OS) for the entire group was 31 months (Figure 1) and median follow up was 39 months. Overall, 13 of 47 (27%) pts. responded already after 3 cycles and 18 of 36 (50%) responded after 6 cycles of RD leading to a significantly prolonged OS ( $p=0.04$ , HR 0.3). A multivariable logistic regression analysis for HR including the covariates: age, deltaFLC, previous HDM, number of previous therapy lines and creatinine clearance revealed by backward selection only deltaFLC as a borderline significant predictor for HR ( $p=0.13$ , OR 1.2). Therefore, previous HDM or number of previous therapy lines did not impair HR. Multivariable cox regression analysis revealed elevated NT-ProBNP levels, Karnofsky Index (KI), advanced age and high deltaFLC levels as significant predictors for OS (Table 1). In line with the results obtained for HR previous HDM as well number of previous therapy lines did not impact on OS.

**Figure 1. Overall survival**



**Table 1.** Multivaribale analysis for OS (n=56)

| Covariates              | P             | HR            | 95% CI of HR             |
|-------------------------|---------------|---------------|--------------------------|
| NT-ProBNP (>4400 ng/dl) | <b>0.0048</b> | <b>4.2005</b> | <b>1.5583 to 11.3223</b> |
| CreaCl (ml/min)         | 0.3193        | 0.9937        | 0.9815 to 1.0061         |
| <b>Age (years)</b>      | <b>0.0008</b> | <b>1.107</b>  | <b>1.0491 to 1.2955</b>  |
| Previous HDM            | 0.9169        | 0.9498        | 0.3627 to 2.4872         |

We observed that NT-ProBNP levels rose during therapy with RD ( $p=0.01$ ). Among patients who received at least 3 cycles of RD an increase of NT-ProBNP was associated with a significantly reduced OS ( $p=0.03$ , HR 1.9).

**Summary:** We could confirm that RD induces response in a significant number of AL patients. Previous HDM seems not to impair response and survival after RD.

S. Schönland: Research grant funded by Celgene

## PC 56

### Using nutritional status measured by BMI and mBMI for monitoring clinical progress in patients with transthyretin familial polyneuropathy: data from two tafamidis studies

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**Background:** In addition to polyneuropathic and cardiomyopathic symptoms, transthyretin familial polyneuropathy (TTR-FAP) is characterized by degenerating gastrointestinal symptoms such as involuntary weight loss, malnutrition and cachexia. Deterioration in nutritional status reflected by a reduced body mass index (BMI: kg/m<sup>2</sup>) and/or a modified body mass index (mBMI; BMI x serum albumin: kg/m<sup>2</sup>\*g/L) is indicative of increasing severity of TTR-FAP and patient prognosis.

**Objective:** Evaluate nutritional status in patients with TTR-FAP in the tafamidis clinical trials.

**Methods:** Changes in BMI and mBMI were recorded over the course of a double blind 18-month clinical trial of tafamidis in patients with TTR-FAP (005) and an open label 12-month extension of that trial (006).

**Results:** In study 005, mean mBMI was decreased after 18 months in patients administered placebo (N=61) (LSMean [±SE] change from baseline: -33.8±11.8,  $P<0.05$ ), whereas in patients treated with tafamidis (N=64), mean mBMI was increased (39.3±11.5,  $P<0.01$  vs baseline;  $P<0.0001$  vs placebo). Changes in BMI demonstrated a similar trend as mBMI in both groups (placebo: -0.1±0.21, tafamidis: 0.3±0.2 vs baseline) although differences from baseline and between-group differences were not statistically significant. Worsening of nutritional status in patients receiving placebo during the double blind study 005 was reversed following the initiation of tafamidis in the open label extension study 006 (N=33). Mean mBMI and BMI were significantly improved from 006 baseline at 12 months of treatment (mBMI: 70.6±14.9,  $P<0.0001$ ; BMI: 0.7±0.2,  $P<0.01$ ) and demonstrated a trend toward improvement from levels at baseline for the double blind study (change from 005 baseline: mBMI 27.8±18.7; BMI 0.3±0.3).

**Discussion:** Nutritional status as measured by BMI and mBMI may be used as a clinical indicator to reflect disease progression and treatment benefits in patients with TTR-FAP.

**Conclusion:** Tafamidis therapy results in improvement in patients' nutritional status, suggesting its potential in improving clinical outcomes in patients with TTR-FAP.

This study was sponsored by FoldRx Pharmaceuticals, acquired by Pfizer Inc in October 2010.

**PC 57****Updated Experience with Upfront Cyclophosphamide, Bortezomib and Dexamethasone (CVD) in the Treatment of AL Amyloidosis**

**Christopher P Venner**, Thirusha Lane, Darren Foard, Lisa Rannigan, Simon DJ Gibbs, Jennifer H Pinney, Carol J Whelan, Helen J Lachmann, Julian D Gillmore, Philip N Hawkins, Ashutosh D Wechalekar

*Centre for Amyloidosis and Acute Phase Proteins, University College London Medical School, UK*

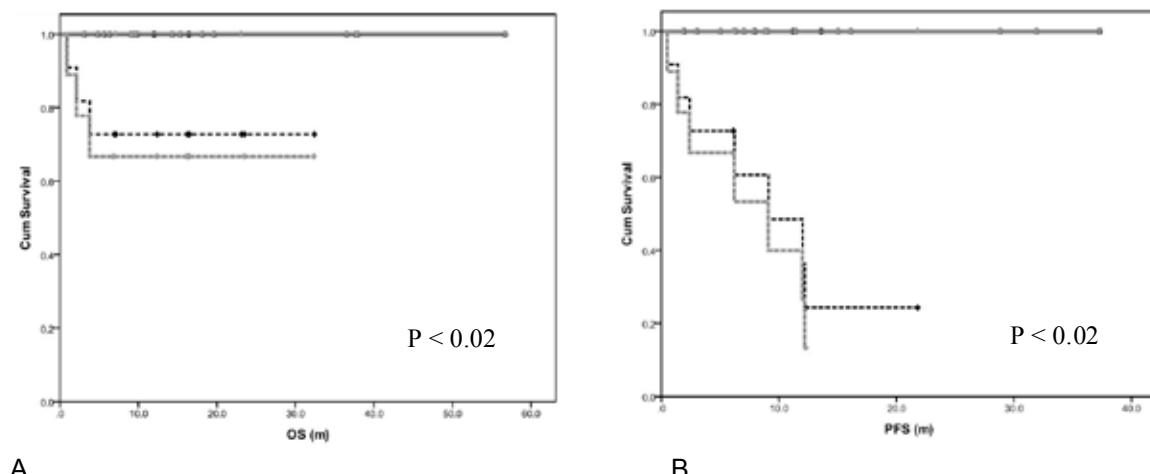
**Background:** Bortezomib combinations have shown great promise in the treatment of AL amyloidosis. Here we present an updated series of patients from the UK National Amyloidosis Centre treated with CVD in the upfront setting.

**Objective:** Characterise the response of CVD when used in the upfront treatment of AL amyloidosis.

**Patients and Methods:** The primary cohort comprises 30 patients referred to the National Amyloidosis Centre in London from 2006-2011. Complete information for staging by the Mayo clinic criteria was available in 28 patients, and 47% were stage III based on values obtained prior to the initiation of CVD. Haematologic, dFLC and organ responses were measured. Progression free survival (PFS) and overall survival (OS) were determined from the start of treatment.

**Results:** Median follow-up was 12.2m. Median number of cycles given was 4.5. All 30 patients were assessable by haematologic response criteria, 28 of whom were assessable for dFLC response. Overall hematologic response rate (RR) was 87% (CR = 63% and dFLC-VGPR = 68%). 23 patients were assessable for a BNP response based on a pre-treatment NT-proBNP  $> 660$  ng/L. BNP responses were seen in 16 patients (38%). 3 deaths have been reported and there were no treatment related mortalities. The time to maximal response was 3.5m. Median PFS has not been reached. The estimated 1-year PFS was 79%. Attaining both a CR and dFLC-VGPR correlated with a significant improvement in PFS and OS (figure 1).

**Conclusion:** This retrospective series lends further support to the use of bortezomib containing regimens in the treatment of AL amyloidosis, especially in the upfront setting. CVD is a safe and effective treatment option supporting previous findings presented by our own group as well as others. In addition, it emphasizes the importance of attaining deep clonal responses to maximize outcome. Larger phase III studies are warranted and are underway.



**Figure 1:** Overall and event free survival post-upfront CVD based on response. OS is shown in (A) and PFS in (B). Curves are separated based on CR (black solid line) vs non-CR (black dashed line), dFLC-VGPR (grey solid line) vs non-dFLC-VGPR (grey dashed line).

ADW has received honoraria from Jansen Cilag

**PC 58****Stringent Patient Selection Improves Outcomes in Patients with AL Amyloidosis Undergoing Autologous Stem Cell Transplantation**

**Christopher P Venner**, Thirusha Lane, Darren Foard, Lisa Rannigan, Simon DJ Gibbs, Jennifer H Pinney, Carol J Whelan, Helen J Lachmann, Julian D Gillmore, Muriel Roussel, Philip N Hawkins, Ashutosh D Wechalekar

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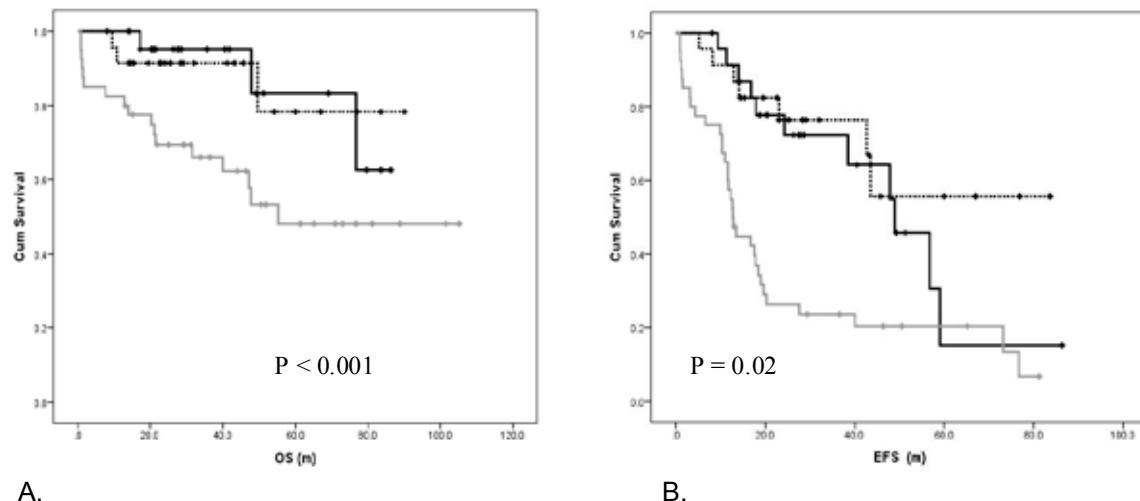
**Background:** Autologous stem cell transplantation (ASCT) in AL amyloidosis leads to both complete and durable responses. We report our experience with patients seen at the UK National Amyloidosis Centre who underwent transplantation after modern risks stratification.

**Objectives:** Characterize outcomes post-ASCT after implementation more stringent risk stratification.

**Patients and Methods:** 88 patients were transplanted since 2003. >2 organ involvement, ECOG>1, eGFR≤45mL/min, significant cardiac involvement, autonomic neuropathy, gastrointestinal involvement and TnT≥0.05ng/mL were considered relative contraindications to transplant. 24% had cardiac involvement (mean ejection fraction=61%). 70% had renal involvement (10% pre-ASCT GFR<45mL/min) with 39% having nephrotic range proteinuria. 38% underwent transplantation upfront. Hematologic, dFLC and organ responses were assessed. GFR by 12m post-transplant was also examined. Progression free survival (PFS) was determined from the date of ASCT. Overall survival (OS) was calculated from both diagnosis and time of ASCT. TRM was defined as death within 100 days of transplant.

**Results:** Median follow-up was 31.8 months (m). Median OS from time of ASCT was not reached and was 131.5m from diagnosis. Median PFS was 27.6m. By intention-to-treat hematologic response rate was 80% (CR=28% and dFLC-VGPR=54%). CR and dFLC-VGPR correlated with superior PFS and OS (figure 1). TRM was 6.8% (non-nephrotic patients=3.7%, nephrotic patients=12%). Renal responses were observed in 30%. 2 patients become dialysis dependent both of whom had nephrotic syndrome. In patients whose baseline GFR was >45mL/min 11% had a post-ASCT GFR<45mL/min, one of whom required dialysis. One patient (13%) with baseline GFR<45mL/min required dialysis.

**Conclusion:** ASCT remains an effective treatment option in AL amyloidosis. TRM has improved with more stringent patient selection. Depth of response positively impacts survival. Kidney injury remains a source of morbidity and should be considered when discussing high-dose therapy. Further trials are needed to assess the role of high-dose therapy in the modern era.



**Figure 1:** Overall and event free survival in patients post-ASCT based on response. OS is shown in (A) and PFS in (B). Curves are separated based on CR (solid line), dFLC-VGPR (dashed line) and ≤ PR (grey line).

**PC 59****Basic and clinical significance of Interleukin 6 (IL-6) in AA amyloidosis****S-N J. Song**, T. Matumura-Nishikawa*Member of Japanese AA Amyloidoses Clinical Research Group*

AA amyloidosis has been a progressive and fatal disease with deposition of AA amyloid fibril on systemic organs, especially on kidney, thyroid and intestine in chronic inflammatory disease. Therefore, we expect to have an effective therapy for AA amyloidosis.

Serum amyloid A (SAA), a precursor molecule of AA protein, is produced and augmented with cytokines mainly in hepatocyte. Recently we proved that interleukin 6 (IL-6) activated STAT3, a transcriptional factor, essentially induces SAA mRNA, and that NF- $\kappa$ B p65 complementally augments SAA mRNA induction by TNF- $\alpha$  or IL-1 combined with IL-6 stimulation. Since IL-6 is a pivotal cytokine on induction of SAA, IL-6 blockade may completely inhibit the production of SAA.

It has been known that serum level of SAA and CRP are decreased and normalized after the IL-6 blocking therapy, on the contrary, SAA levels are decreased, but hardly reach into the normal range by the TNF- $\alpha$  blocking therapy.

Gillmore *et al.* suggested when the deposited AA fibril is decreased, it may be necessary to block the elevation of SAA in serum for long period. Therefore, an anti IL-6R antibody, Tocilizumab or Actemra, seems to be an ideal therapeutic reagent for prevention and improvement of AA amyloidosis by the inhibition of SAA production. We carry out the clinical study for the treatment with Tocilizumab to the patients with AA amyloidosis.

In this symposium, I present the role of IL-6 on SAA expression and the inhibition mechanism with IL-6 blockade on SAA production. Then, I also show the clinical efficacy of Tocilizumab therapy in AA amyloidosis.

We hope we will overcome AA amyloidosis, an incurable disease, near future.

I have disclosed the support of interest from Chugai Pharmaceutical Co.

**PC 60****Light Chain Deposition disease: Novel strategies for a rare disorder**

**Victor H Jimenez-Zepeda**<sup>1</sup>, Suzanne Trudel<sup>1</sup>, Andrew Winter<sup>1</sup>, Donna E Reece<sup>1</sup>, Christine Chen<sup>1</sup>, and Vishal Kukreti<sup>1</sup>

<sup>1</sup>Princess Margaret Hospital, Department of Medical Oncology and Hematology, Toronto, ON, CA.

**Background:** Light chain deposition disease (LCDD) is a monoclonal gammopathy characterized by non-amyloid deposition of light chains in various organs. The use of novel agents has shown a more rapid response with a dramatically important reduction of light chains. Furthermore, autologous stem cell transplantation (ASCT) has been reported as a feasible strategy in LCDD.

**Objectives:** In this series, we reviewed a single center experience in the treatment of LCDD with emphasis on the role of ASCT and the use of novel agents such as bortezomib.

**Methods:** Between 06/05 and 02/11, 12 patients with LCDD were evaluated. LCDD was diagnosed by renal biopsy in all patients and a hematologic response (HR) was defined according to standard criteria<sup>1</sup>. Primary Endpoint was response rate.

**Results:** Non-transplanted patients (6) received treatment with bortezomib (2), lenalidomide (1) and conventional chemotherapy. Six patients underwent ASCT.(Table 1) Prior to transplantation, patients received dexamethasone alone (N=3) or dexamethasone plus bortezomib (N=3); Bortezomib was administered at 1.3 mg/m<sup>2</sup> once weekly for a median of 4 cycles. Transplanted patients had a median time to  $\geq$  ANC 0.5  $\times 10^9$ /L of 13 days (11-14), median time to platelets  $\geq 20 \times 10^9$ /L of 14 days (13-22) and median time to discharge of 17 days (13-30). Treatment-related mortality was 0%. At day-100 post ASCT overall response rate was 100%, 4 patients achieved complete HR (66.7%), 1 patient exhibited nCR (16.7%) and one more attained PR (16.7%). At 6 months post-ASCT all six patients showed organ response.Two other patients who received bortezomib without transplant exhibited PR and VGPR respectively. Additionally, 1 patient discontinued bortezomib due to autonomic neuropathy.

**Conclusion:** The use of drugs such as bortezomib and ASCT is a feasible and efficacious strategy in LCDD leading to a rapid hematologic and organ response.

**References:**

<sup>1</sup>Gertz, M.A. et al., Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. Am J Hematol, 2005; 79(4): p. 319-28.

**Table 1.** Clinical characteristics of patients with Light Chain Deposition Disease who underwent Autologous Stem Cell Transplant

| Clinical characteristic n=6  | Median | Range     | %     |
|------------------------------|--------|-----------|-------|
| Age (years)                  | 56     | 45-65     |       |
| Male                         |        |           | 33%   |
| Female                       |        |           | 67%   |
| Hemoglobin (g/L)             | 114    | 100-146   |       |
| Creatinine (μmol/L)          | 205    | 124-307   |       |
| B2-microglobulin ((μmol/L)   | 412    | 137-631   |       |
| Albumin (g/L)                | 37     | 33-41     |       |
| Calcium                      | 2.18   | 1.96-2.35 |       |
| LDH (IU/L)                   | 260    | 207-297   |       |
| IgG                          |        |           | 33%   |
| No detected                  |        |           | 67%   |
| Light Chain detected         |        |           | 100%  |
| Kappa                        |        |           | 83.3% |
| Lambda                       |        |           | 16.7% |
| Kappa                        |        |           | 59.9% |
| Lambda                       |        |           | 34.3% |
| Biclonal                     |        |           | 2%    |
| ND                           |        |           | 3.9%  |
| Organ involvement            |        |           |       |
| Kidney                       |        |           | 100%  |
| Heart                        |        |           | 16.7% |
| Induction Treatment:         |        |           |       |
| Bortezomib and dexamethasone |        |           | 50%   |
| Dexamethasone alone          |        |           | 50%   |

**PC 61**

**Autologous Stem Cell Transplant is an effective therapy for carefully selected patients with AL Amyloidosis: Experience of a single Institution**

**Victor H Jimenez-Zepeda**<sup>1</sup>, Norman Franke<sup>1</sup>, Andrew Winter<sup>1</sup>, Diego Delgado<sup>2</sup>, Donna E Reece<sup>1</sup>, Suzanne Trudel<sup>1</sup>, Christine Chen<sup>1</sup>, Rodger Tiedemann<sup>1</sup>, Joseph Mikhael<sup>3</sup> and Vishal Kukreti<sup>1</sup>

<sup>1</sup>Division of Hematology, Princess Margaret Hospital, Toronto, ON, Canada, <sup>2</sup>Division of Cardiology and Heart Transplantation, University Health Network, Toronto, Ontario, Canada, <sup>3</sup>Mayo Clinic in Arizona, Division of Hematology and Oncology, Scottsdale, AZ, USA

**Background:** Autologous stem-cell transplant (ASCT) has been widely used to treat patients with AL amyloidosis. However, transplant-related mortality (TRM) rates are high, and a recent randomized trial suggested that non-ASCT regimens produced comparable results with less toxicity.<sup>1</sup>

**Objective:** In order to define the role of patient selection in ASCT, we evaluated 78 consecutive AL amyloidosis patients transplanted at our center.

**Methods:** The AL amyloidosis diagnosis was confirmed in all patients. We included only patients who had received ≤ 2 previous courses of chemotherapy, who did not have myeloma, and who had an ECOG performance-status scores of 0 to 2. Seven categories of organ involvement were recorded. Troponin-I and brain natriuretic peptide (BNP) values at baseline were obtained. CHR and OR were assessed according to the Consensus Opinion from the 10th International Symposium on Amyloidosis.<sup>2</sup> The effect of troponin-I and BNP on overall survival (OS) was examined using the Cox proportional hazards model. Survival curves were constructed according to the Kaplan-Meier method.

**Results:** Clinical characteristics are shown in Table 1. Twenty-percent of the patients transplanted at our center had ≥ 3 organs affected. Most patients did not receive induction therapy (76.9%). Blood stem cells were mobilized using growth factors only in all patients. TRM occurred in 11.5%. Complete

hematological response (CHR) and organ response (OR) were achieved in 50%, and 60% respectively. Median overall survival (OS) was significantly lower for patients with brain-type natriuretic peptide (BNP)  $\geq 300$  pg/ml (17.5 months vs NR) ( $p=0.0004$ ), troponin-I  $\geq 0.07$  ng/ml (13.5 months vs NR) ( $p=0.00001$ ) and those not achieving a CHR (88 months vs NR) ( $p=0.0345$ ); High BNP and troponin-I were the most important predictive factors in a multivariate analysis.

**Conclusions:** Based on this study, patients with BNP $<300$  pg/ml and/or normal levels of troponin-I ( $<0.07$ ) should be considered transplant candidates.

#### References:

1. Jaccard, A., et al., *High-dose melphalan versus melphalan plus dexamethasone for AL amyloidosis*. N Engl J Med, 2007. 357(11): p. 1083-93.
2. Gertz, M.A., et al., *Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004*. Am J Hematol, 2005. 79(4): p. 319-28.

**Table 1.** Clinical and Laboratory characteristics of patients with AL Amyloidosis undergoing ASCT

| Clinical Characteristics                      | N  | Median | Range      | %     |
|---|----|--------|------------|-------|
| Age (years)                                   | 78 | 57     | 33-74      |       |
| Gender  |    |        |            |       |
| Male  | 53 |        |            | 68%   |
| Female  | 25 |        |            | 32%   |
| IgG Kappa                                     | 5  |        |            | 6.5%  |
| IgG Lambda                                    | 20 |        |            | 26%   |
| IgA Lambda                                    | 4  |        |            | 5.1%  |
| IgM Kappa                                     | 2  |        |            | 2.7%  |
| IgM Lambda                                    | 2  |        |            | 2.7%  |
| Free Kappa                                    | 10 |        |            | 13%   |
| Free Lambda                                   | 34 |        |            | 44%   |
| Renal Involvement                             | 56 |        |            | 71.8% |
| Cardiac Involvement                           | 37 |        |            | 47.4% |
| Hepatic involvement                           | 18 |        |            | 23.1% |
| GI involvement                                | 8  |        |            | 10.3% |
| $\geq 3$ organs involved by AL                | 16 |        |            | 20.5% |
| Hemoglobin (g/L)                              | 78 | 138    | 104-182    |       |
| Creatinine ( $\mu\text{mol}/\text{L}$ )       | 78 | 129    | 37-700     |       |
| Albumin g/L                                   | 78 | 35.1   | 25-58      |       |
| Alkaline phosphatase, units/L                 | 78 | 146    | 42-810     |       |
| B2-Microglobulin ( $\mu\text{mol}/\text{L}$ ) | 78 | 348    | 70-2900    |       |
| 24 Hr Proteinuria (g/d)                       | 78 | 5.6    | 0-24.9     |       |
| ***BMPC (%)                                   | 78 | 8      | 1-30       |       |
| Intraventricular Septal Distance (mm)         | 78 | 13     | 9-23       |       |
| Ejection Fraction, %                          | 78 | 62     | 45-79      |       |
| Troponin I ng/ML (normal <0.07)               | 62 | 0.12   | 0.03-2.8   |       |
| **BNP (pg/mL) (normal <98)                    | 45 | 178    | 10-1670    |       |
| Kappa/lambda ratio                            | 54 | 4.34   | 0.001-71.2 |       |

\*\*Brain Type Natriuretic Peptide

\*\*\* BMPC: Bone marrow plasma cells

**PC 62****Bortezomib-containing regimens for the treatment of AL amyloidosis: Impact on hematological response**

**Victor H Jimenez-Zepeda**, Faraz Zaman, Donna E Reece, Suzanne Trudel, Christine Chen, Rodger Tiedemann and Vishal Kukreti

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**Background:** New treatment options are required for systemic AL amyloidosis (AL). Recently, Bortezomib has been shown to be the most active single agent in the treatment of AL.

**Objective:** In this study, we aimed to assess the efficacy of bortezomib for AL amyloidosis patients. In addition, we explored the rapidness of hematological (HR) and organ response (OR) in this series.

**Table 1.** Clinical and Laboratory characteristics of patients with AL Amyloidosis treated with bortezomib-containing regimens

| Clinical Characteristics                                     | N  | Median | Range     | %   |
|--|----|--------|-----------|-----|
| Age (years)  | 25 | 57     | 42-76     |     |
| Gender   |    |        |           |     |
| Male   | 16 |        |           | 64% |
| Female   | 9  |        |           | 36% |
| Kappa  | 6  |        |           | 24% |
| Lambda   | 19 |        |           | 76% |
| Renal Involvement  | 13 |        |           | 52% |
| Cardiac Involvement  | 20 |        |           | 80% |
| Hepatic involvement  | 9  |        |           | 36% |
| GI involvement   | 9  |        |           | 36% |
| ≥3 organs involved by AL                                     | 8  |        |           | 32% |
| Hemoglobin (g/L)   | 25 | 134    | 111-177   |     |
| Creatinine (μmol/L)  | 25 | 140    | 58-668    |     |
| Albumin g/L  | 25 | 34     | 14-45     |     |
| Alkaline phosphatase, units/L                                | 25 | 182    | 36-685    |     |
| B2-Microglobulin (μmol/L)                                    | 25 | 274    | 112-1068  |     |
| 24 Hr Proteinuria (g/d)                                      | 25 | 4.3    | 0.1-24.5  |     |
| ***BMPC (%)  | 24 | 8      | 2-23      |     |
| Intraventricular Septal Distance (mm)                        | 25 | 14     | 8.9-25    |     |
| Ejection Fraction, %   | 25 | 59     | 36-79     |     |
| Troponin I ng/mL (normal<0.07)                               | 24 | 0.11   | 0.06-0.32 |     |
| **BNP (pg/mL) (normal <98)                                   | 23 | 414    | 13-1459   |     |
| Bortezomib regimens:   |    |        |           |     |
| -CyBorD+ (Bortezomib 1.3mg/m <sup>2</sup> weekly)            | 10 |        |           | 40% |
| -Bortezomib alone 0.7mg/m <sup>2</sup> weekly                | 2  |        |           | 8%  |
| -Bortezomib plus dexamethasone (1mg/m <sup>2</sup> weekly)   | 1  |        |           | 4%  |
| -Bortezomib alone 1mg/m <sup>2</sup> weekly                  | 1  |        |           | 4%  |
| -Bortezomib plus dexamethasone (1.3mg/m <sup>2</sup> weekly) | 4  |        |           | 16% |
| -Bortezomib alone 1.3 mg/m <sup>2</sup> weekly               | 5  |        |           | 20% |
| -Bortezomib alone 1.5 mg/m <sup>2</sup> weekly               | 2  |        |           | 8%  |

\*\*Brain Type Natriuretic Peptide

\*\*\* BMPC: Bone marrow plasma cells

+CyBORD (Cyclphosphamide, Bortezomib and Dexamethasone)

**Methods:** Patients with documented symptomatic AL who received treatment with bortezomib-containing regimens were identified.<sup>1</sup> TnI and BNP values at baseline, 3 and 6 months were recorded. HR and OR were assessed according to the more recent validation of the criteria response.

**Results:** A total of 25 patients with AL amyloidosis treated with bortezomib from 12/2005 to 10/2011 were identified. Cardiac involvement was documented in 20 cases. Clinical characteristics and treatment combinations are shown in Table 1. Prior therapies included: Stem cell transplant (ASCT)

(9), Melphalan and Dexamethasone (Mel/Dex) (4), ASCT and Cyclophosphamide, Thalidomide and Dexamethasone (1), ASCT and Cyclophosphamide/Prednisone (1), ASCT and Mel/Dex (1) and none (9). After a median of 7 cycles (1-56), a HR was seen in 23 cases (92%) including: Complete Response in 8 (32%), Very Good Partial Response in 14 (56%) and Partial Response in 1 (4%). At 6 weeks, 16 patients had already achieved  $\geq$ PR. OR at 6 months was documented in 20 cases (80%). With respect to cardiac response, a  $\geq$ 50% decrease of Troponin-I was seen in 9 of 13 evaluable patients while a decrease of BNP of  $\geq$ 50% was observed in 11 of 13 evaluable cases at a median of 6 and 5 months, respectively. Only one patient discontinued therapy due to toxicity (autonomic neuropathy) and 23 patients remained progression free.

**Conclusion:** Bortezomib is a safe and tolerated therapy for AL patients showing rapid HR and cardiac responses assessed by BNP and TnI.

#### References:

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Victor H Jimenez Zepeda received honoraria from Janssen Ortho

#### PC 63

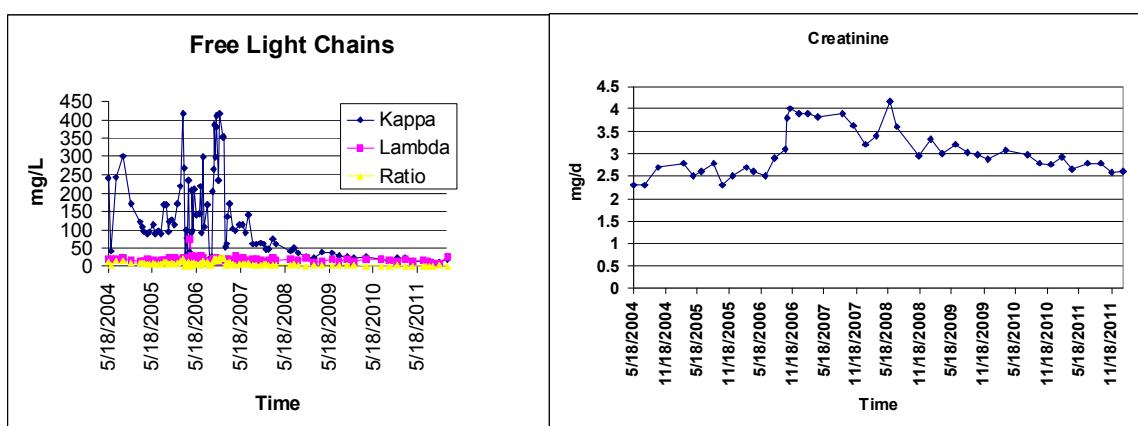
#### Successful treatment of small plasma cell clonal proliferation with improvement of target organ function - beyond amyloid

Kevin Barton and **Maria M. Picken**

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Plasma cell dyscrasia may be associated with amyloid and other related disorders, including light chain deposition disease and crystal storing histiocytosis. In a subset of patients, the tumor load may be very small such that the question may arise whether treatment of the underlying plasma cell proliferation should be considered. We report here on a patient who initially developed renal failure of unclear etiology. Light chain proximal tubulopathy (LCPT) was subsequently identified in the renal transplant after a slow decline in allograft function.

A 58-year-old woman underwent living, related, kidney transplantation in 1997. In January 2002, worsening kidney function led to kidney biopsy, which identified the tubulopathy. Bone marrow aspirate and biopsy did not identify any B cell clonal process and plasma cells were less than 1%. Serum immunofixation identified a small IgG kappa monoclonal protein, and urine immunofixation identified kappa free light chains. Therapy was initiated with pulses of dexamethasone, and with relatively stable renal function.



Serum free light chain assays were used for tracking her disease, beginning in 2004. In March 2005, she was switched to a bortezomib-based therapy because of increased free light chains. This was further adjusted to cyclophosphamide, bortezomib, and dexamethasone in April 2006. In late 2006, she developed fevers and sepsis of unclear etiology and therapy was halted temporarily with worsening of her renal function.

In December 2006, after resolution of her acute illness, she was begun on lenalidomide. Apart from some muscle cramping, she tolerated this therapy quite well. She showed a slow, steady improvement of her kappa free light chains and achieved complete hematologic remission with slow improvement of renal function.

**Conclusion:** Treatment of small clones is feasible and can lead to improvement of target organ function in at least some renal transplant patients using IMIDs such as lenalidomide.

#### PC 64

#### Bortezomib/dexamethasone followed by autologous stem cell transplantation as front line treatment for light chain deposition disease

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**Background:** Treatment of light chain deposition disease (LCDD) remains controversial since the results obtained with conventional chemotherapy used for multiple myeloma are unsatisfactory. However, and based on the promising results obtained by treating patients with light chain amyloidosis with bortezomib/dexamethasone, new treatment options appear in LCDD.

**Objective:** To report our experience with induction with bortezomib in combination with dexamethasone followed by high dose melphalan/autologous stem cell transplantation (HDM/ASCT) as front line treatment in three patients with LCDD.

**Methods and discussion:** We describe three patients who presented with a monoclonal gammopathy and rapid renal function impairment, being diagnosed with a LCDD by kidney biopsy. All of them were treated with bortezomib plus dexamethasone followed by HDM/ASCT with no major complications (see details in Table 1). In one case, given the sustained complete hematologic remission, but persistence of the renal impairment the patient received kidney transplantation with excellent response. There is no consensus regarding treatment in LCDD. Results of conventional chemotherapy commonly used for multiple myeloma are unsatisfactory. Treatment with high dose intravenous melphalan and autologous stem cell transplantation is nowadays the most frequent treatment used in younger patients with LCDD. Recently, two reports with successful results on the use of bortezomib in the treatment of LCDD have been published, with only one of them followed by ASCT. Our small series confirm and expand the results on the use of bortezomib in combination with dexamethasone followed by ASCT as a safe and well tolerated treatment for patients with LCDD.

**Conclusion:** Given the results obtained by this and other small series, induction treatment with bortezomib/dexamethasone followed by ASCT should be considered the treatment of choice in younger patients with LCDD. Additionally, renal transplant should be taken into consideration in those patients requiring hemodialysis after achieving hematologic CR with ASCT

**Table.** Patient's characteristics and outcome

| Case | Age | Gender | Involved organs | Response to bortezomib | Plasma exchange | Response to ASCT | Follow-up from ASCT |
|------|-----|--------|-----------------|------------------------|-----------------|------------------|---------------------|
| 1    | 63  | female | Kidney          | CR unsustained         | YES             | CR               | 32 months           |
| 2    | 51  | female | Kidney Heart    | NO                     | No              | CR               | 38 months           |
| 3    | 38  | female | Kidney          | PR                     | No              | Near CR          | 7 months            |

JB received honoraries for lectures and Advisory Boards from Janssen.

**PC 65****Tc-99m Pyrophosphate for Identifying ATTR Cardiac Amyloid and Associations of Myocardial Update with Disease Severity**

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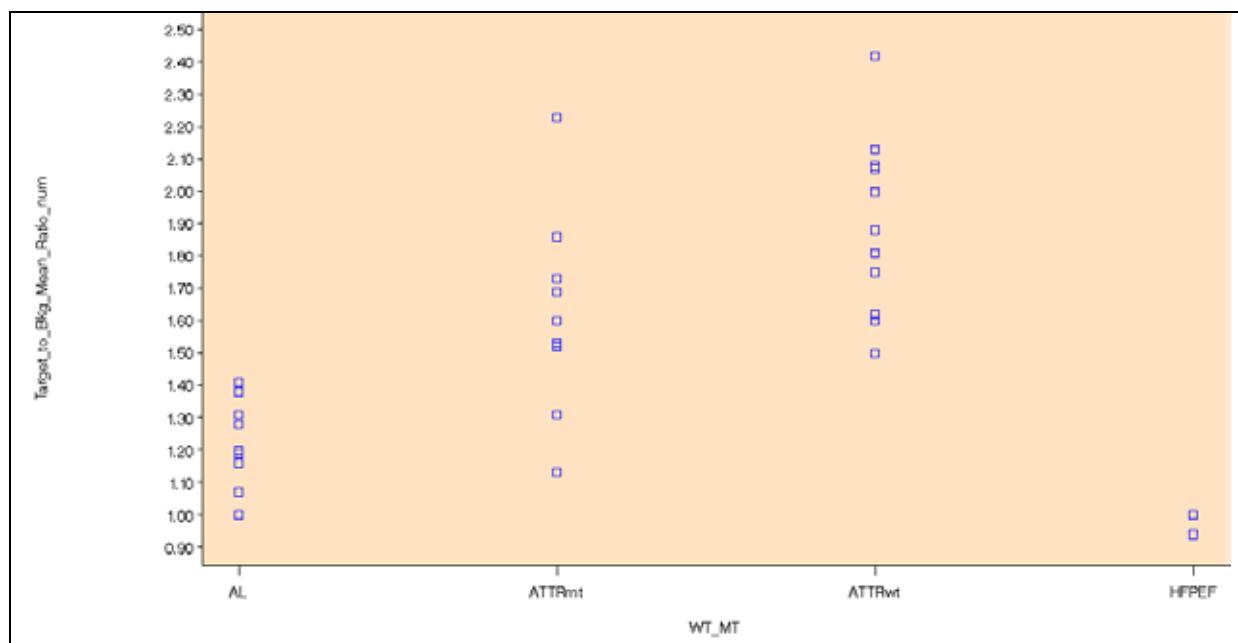
**Background:** ATTR cardiac amyloid is an under-recognized and under-diagnosed cause of heart failure with a preserved ejection fraction perhaps in part because definitive diagnosis requires an invasive endomyocardial biopsy. A non-invasive method for identifying patients with ATTR cardiac amyloid would be a significant advance.

**Objectives:** We evaluated the ability of technetium-99m pyrophosphate (Te 99m PYP) to identify ATTR cardiac amyloidosis and its association with disease severity.

**Methods:** 35 subjects were studied including 21 subjects with ATTR cardiac amyloid (10 with mutations and 11 with wild type disease), 10 subjects with AL cardiac amyloid and 4 controls. Subjects underwent planar imaging with technetium-99m pyrophosphate (Tc 99m PYP) performed on dual head Phillips Precedence camera equipped with low energy, high resolution collimators. Patients received 20-30 mCi of Tc 99m PYP intravenously and one hour later two supine views were obtained: anterior/lateral and left anterior oblique. About 750,000 counts were collected over 5 minutes from anterior/lateral and LAO views. Quantitative analysis of heart retention was calculated by drawing ROI over the heart in comparison to the contralateral thorax. All ROI's were corrected for background counts. Image analysis was independently performed by an experienced nuclear cardiologist blinded to all patient data.

**Results:** The cardiac uptake was diffuse and the ratio of target to background was significantly different among the cohorts (see below). The ratio of target to background of >1.5 had a sensitivity of 90% and specificity of 100% for identifying ATTR cardiac amyloid with an AUC of 0.973. Among the subjects with ATTR cardiac amyloid, total cardiac uptake in the region of interest was correlated with troponin ( $r=0.71$ ,  $p=0.02$ ) and with BNP ( $r=0.64$ ,  $p<0.05$ ) among subjects with mutations while the target to background ratio was significantly associated with wall thickness ( $r=0.82$ ,  $p<0.01$ ) in wild type subjects. The ratio of target to background was inversely associated with the modified BMI ( $r=-0.41$ ,  $p=0.07$ ) a marker of overall disease severity.

**Conclusion:** Cardiac imaging with Tc 99m PYP discriminates between ATTR from AL amyloid and HFPEF and is associated with measures of disease severity.



**PC 66****SOM0226: A reprofiled drug intended for the prevention and treatment of familial transthyretin amyloidosis (ATTR)**

**Marc Centellas<sup>1</sup>, Raúl Insa<sup>1</sup>, Núria Reig<sup>2</sup>, Núria Gavaldà<sup>1</sup>, Antoni Planas<sup>3</sup>**

<sup>1</sup>R&D and <sup>2</sup>Intellectual Property, SOM Biotech, University of Barcelona, <sup>3</sup>Laboratory of Biochemistry, IQS, Universitat Ramon Llull, Spain

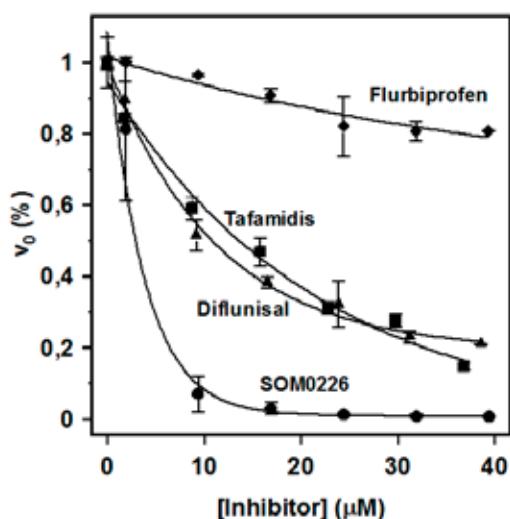
**Background:** SOM0226 is a reprofiled drug for the treatment and prevention of familial transthyretin amyloidosis (ATTR). The drug has been tested *in-vitro* and is planned to enter a phase II clinical trial after positive *ex-vivo* results. As a reprofiled drug, SOM0226 has already passed a significant number of toxicity tests and hence its safety is known and the risks of failure for toxicology reasons are reduced. SOM Biotech, a start-up established at the Barcelona Science Park, University of Barcelona, has the mission to discover and develop new indications of already known drugs through a proprietary virtual screening platform.

SOM0226 presents a better efficacy and safety profile than the most advanced drug for ATTR.

**Objective:** The purpose of the project was to identify an available treatment for ATTR.

**Methods:** Tafamidis was used as the reference compound in our ligand and receptor-based virtual screening approach. The physicochemical and binding-target site properties of tafamidis were compared with a database comprising molecules that have been in the market. Thirty compounds were selected as potential active drugs and tested *in-vitro* using a kinetic turbidity<sup>1</sup>. *Ex-vivo* tests<sup>2</sup> are ongoing to evaluate the ability of the compound to bind TTR and modulate different steps of the process of TTR amyloid fibril formation.

**Results:** Relative initial rates of fibril formation ( $V_0$ , %) are plotted against inhibitor concentration for SOM0226, tafamidis, diflunisal and flubiprofen.



**Conclusion:** SOM0226, a drug already in the market for a non-amyloidogenic indication, has promising activity as a TTR amyloid fibril inhibitor.

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Marc Centellas, Núria Reig, Núria Gavaldà and Raúl Insa are full-time employees at SOM Biotech. They also own a patent describing the new use for SOM0226.

This reprofiling project is partially supported by a grant from the Spanish Ministry of Health and the Ministry of Innovation.

**PC 67****An assessment of the prospective potential of ligand efficiency indices to guide drug discovery for TTR amyloidosis**

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We have previously reported the design and synthesis of ligands that effectively stabilize Transthyretin (TTR)<sup>1</sup> in order to obtain therapeutically active compounds for Familial Amyloid Polyneuropathy (FAP). Now we are currently engaged in a program to optimize these ligands to target TTR amyloidosis, through the following steps: a) SAR analyses of the ligands described previously for the TTR tetramer, classified in families and mapping them in Chemico-Biological Space (CBS) using Ligand Efficiency Indices (LEIs); b) drug design / optimization of TTR ligands through docking in the TTR tetramer three-dimensional structure, and through optimization of physicochemical / pharmacokinetic / selectivity properties; c) comparative structural analyses of selected amyloidogenic and non-amyloidogenic TTR mutants and native TTR structures; and d) virtual screening of commercially available ligands and therapeutically active compounds (repurposing) towards wild-type and mutant TTR tetramer structures. Progress results in steps a) and b) of this drug design program will be reported.

Our approach relies on Ligand Efficiency Indices (LEIs) to map the different chemical series in CBS. First, we used an efficiency index, BEI<sup>2</sup>, based on “predicted” binding affinity related to the Molecular Weight (MW) of the compound, combined with a surface-binding efficiency index (SEI)<sup>2</sup> based on Polar Surface Area (PSA). This prospective mapping highlighted a series of compounds that were synthesized for biological evaluation<sup>3</sup>. We will illustrate here the use of these indices, combining three crucial variables (potency, MW and PSA)<sup>4</sup> in a 2D graphical representation of chemical space, to perform a retrospective mapping of SAR data for our current TTR inhibitors database. Based on the results that assess the prospective potential of the methodology<sup>4</sup>, we suggest strategies and approaches for future drug design efforts for TTR ligands.

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## Presenting author index

- Ackermann, Elizabeth - OP73  
 Adams, David - OP58, PB01, PB02, PB40  
 Algalarrondo, Vincent - OP30, OP47, PC01  
 Arnold, Franziska - PA31  
 Baldovino, Simone - PB03, PB34  
 Banpersad, Sanjay - PB41  
 Barley, Kevin - PC33  
 Barros, Francisca - PB04  
 Benson, Merrill - PC34  
 Ben-Zvi, Ilan - PB42  
 Bergstrom, Joakim - PA02  
 Berk, John - OP69  
 Blank, Norbert - PB35  
 Bodin, Kael - PB33  
 Buades, J. - PB05  
 Buxbaum, Joel - OP05  
 Campos, Raul - PA49  
 Cappelli, Francesco - PB43, PC02  
 Centellas M. - PC66  
 Chiesa, Giulio - PA35  
 Cibeira, Teresa - PC35  
 Coelho, Teresa - OP74, PB36, PC17  
 Coker, Alun - PA03  
 Comenzo, Raymond - OP41  
 Connors, Lawreen - OP48  
 Coriu, Daniel - PB06  
 Cowan, Andrew - PB44, PB45  
 Csoka, Levente - PA04  
 Da Costa, Gonçalo - PA05, PA32  
 Damy, Thibaud - PC36  
 Darnell, Adam - PB07  
 Davies, Hannah - PA06  
 Davis, Peter - PA07  
 Del Pozo Yauner, Luis - PA08  
 Desport, Estelle - OP44  
 Dessain Scott K - OP24  
 DiCostanzo, Ara Celi - PA09  
 Digre, Andreas - PA10  
 Dispenzieri, Angela - OP64  
 Dogan, Ahmet - OP26  
 Dorbala, Sharmila - OP37  
 D'Souza, Anita - PB18  
 Dungu, Jason - OP28, OP49, PB46  
 Econimo, L. - PC03  
 Efebera, Yvonne - PB09  
 El Mansoury, Tarek M - PC29  
 Ettaif, Hind - PB47  
 Falk, Rodney - PC31  
 Fändrich, Marcus - OP03  
 Ferreira, Nelson - OP70  
 Fix, Oren - PC37, OP53  
 Fonseca, Daniel - PA11  
 Garceau, Denis - OP68  
 Geneste, Ambre - PA12  
 Gertz, Morie - PC38, PC39  
 Gibbs, Simon - PC40, PC41  
 Gilbertson, Janet - OP27  
 Girolamo di, Marco - PC42  
 Gillmore, Julian - OP39  
 Goncalves, Nadia - OP13  
 Graeff de, Pauline - PB10  
 Greene, Michael - PA13  
 Gu, Xiaohong - PA14  
 Gunasekera, Chrishan - PC04  
 Gursky, Olga - PA15  
 Haase, Christiane L. - PC18  
 Hata, Hiroyuki - PB48  
 Haupt, Christian - PA50  
 Hazenberg, Aldert - OP52  
 Hazenberg, Bouke - OP23, PB11, PB49  
 Higuchi, K. - PA37  
 Hunter, John - PB50  
 Hutt, David - OP29  
 Ihse, Elisabet - OP06  
 Ikeda, Shu-ichi - PB12  
 Ishii, Wataru - OP18, PA38  
 Jaccard, Arnaud - OP62  
 Jacobson, Daniel - PB13  
 Jonson, Maria - PA16  
 Kaplan, Batia - PB11  
 Kastritis, Efstatios - OP61, PC05  
 Katoh, Nagaaki - PA17  
 Kim, Kihyun - PC06  
 Kimmich, Christoph - PB51  
 Klimtchuk, Elena - PA18  
 Klingstedt, Therese - PA51  
 Kluge, Barbara - PB15  
 Koch, Clarissa - PA39  
 Kristen, Arnt - PC43  
 Kukreti, Vishal - PB52  
 Kukuy, Olga - PB53  
 Kumar, Shaji - OP36, PC44  
 Kwok, Fiona - PC19  
 Lachmann, Helen - OP51, PB54  
 Landau, Heather - PC45, PC46, PC47  
 Lane, Thirusha - PC07, PC08  
 Lavatelli, Francesca - PB55, PB66  
 Lemos, Carolina - PC20  
 Leung, Amy - OP14  
 Leung, Nelson - PB19, PC48  
 Liepnieks, Juris - PA19  
 Linke, Reinhold - OP22, PA52  
 Liuu, S. - PA53  
 Lopes, Alice - OP55

Lopez del Amo, Miguel Juan - OP01  
Lorenzen, Nikolai - OP04  
Loureiro, Joana - PA20  
Lu, Yanyan - PA61  
Mackie, Kelly - PC21, PC22  
Madine, Jillian - OP02  
Makovitzky, Josef - PA21, PA65  
Maleszewski, Joseph - PB16  
Martin, Emily - PA54  
Maurer, Mathew - OP46, OP50, PC09, PC10, PC65  
Mereuta, O. - PB17  
Merlini, Giampaolo - PC49  
Michels, Hartmut - PA55  
Milani, Paolo - OP63  
Misumi, Yohei - PA47  
Mollee, Peter - OP25  
Monteiro, Cecilia - PB56  
Moreira, Luciana - PA48  
Moscetti, Alessandro - PB18, PC11  
Murakami, Tomoaki - PA40  
Musca, Francesco - PC12  
Nardi, Matilde - PB57  
Neely, Patricia - PC23  
Noborn, Fredrik - PA36  
Noordzij, Walter - OP31  
Nystrom, Sofie - PA56  
Obayashi, Konen - PA23  
Obici, Laura - OP71  
Ohshima, Toshinori - PA34  
Oliva, Laura - OP10  
Oskarsson, Marie - PA24  
Otzen, Daniel - PA25  
Overbeke Van, Wouter - PC24  
Ozawa, Daisaku - PA22  
Palladini, Giovanni - OP35, OP42, PC50  
Pehlivanoglu, Burcin - PA57  
Pellistri, Francesca - PA41  
Perlini, Stefano - OP38  
Petitalot, Vincent - PB31  
Picken, Maria - PB20, PB21, PC63  
Picotti, Paola - OP09  
Prokaeva, Tatiana - PB22  
Purrucker, JC - PB23  
Radujkovic, Aleksander - PC25  
Ramella, Nahuel - PA26  
Ramirez-Alvarado, Marina - OP11, PA27, PA58  
Rapezzi, Claudio - PB37  
Ravera, S. - PB58  
Reixach, Natalia - PA42  
Rheenen van, RWJ - OP32  
Riccardo, Porcari - PA28  
Rocha, Ana - PB59  
Rodrigues, Catia - OP12  
Rognoni, Paola - PA43  
Rojas, Eugenia - PA44  
Roussel, Murielle - PB60, PC51  
Rovira, Montserrat - PC52  
Rowczenio, Dorota - OP45, PB24  
Salinaro, Francesco - PB61  
Sam, Flora - PB62, PB63  
San Millan, Beatriz - PB25  
Sanchez-Garcia, Elsa - PA33  
Sanchorawala, Vaishali - OP65, PC53, PC54  
Sandhu, Binnie - PB26  
Saraiva, Maria - PA45  
Sawashita, Jinko - PA29  
Schönland, Stefan - OP40, PB64, PC13, PC55  
Segal, Daniel - OP15  
Sekijima, Yoshiki - PB27  
Sen, Sait - PA59  
Shinar, Yael - PC26  
Sigurdson, Christina J. - OP07  
Sjolander, Daniel - OP21  
Snow, Alan - OP67  
Soria, Cristina - OP16  
Sponarova, Jana - OP19  
Stangou, Arie - OP54  
Stenvang, Marcel - PA30  
Stig Hornstrup, Louise - PC27  
Su, Yu - OP72  
Suenaga, Genki - PB32  
Suhr, Ole - OP57, PB38, PC57  
Suresh, Rahul - PB28, PC30  
Swiecicki, Paul - OP59  
Tasaki, Masayoshi - OP75  
Tovar, Natalia - PC64  
Ueda, Mitsuharu - PA46, PB65  
Usmany, Shariq - OP08  
Valentini, Veronica - PB39  
VandenAkker, Corianne - PA01  
Venner, Christopher - PC57, PC58  
Wall, Jonathan - OP20, OP33, PA60, PA62, PA63  
Ward, Jennifer Ellis - OP17  
Warsame, Rahma - OP43  
Wechalekar, Ashutosh - OP60, PB29, PB30, PC28  
Wells, KJ - OP34  
Whelan, Carol - OP56, PC14  
Yamadanij, Toshiyuki - PA64  
Yamashita, Taro - PC15  
Yanagisawa, Akihiro - PC32  
Yoshizaki, K. - PC59  
Zepeda, Victor - PC16, PC60, PC61, PC62  
Zhou, Ping - OP66