Pathobiology and Disease

Code: SCI3049

2017/2018

Period 5

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General information

The course

In this course students will have an opportunity to learn how modern medicine has benefitted from our knowledge in the fields of (molecular) cell biology and immunology. Pathobiology is the field that deals with disturbance of normal physiological processes and the consequences of it for adequate functioning of our human body. Our challenge has been to arrange a program that offers insight in the nature, e.g. the causes and processes of disease.

The emphasis in this course is on diseases of the immune system and oncology. In this respect, this course builds on the knowledge obtained in the UCM course 'Immunology' and 'Cell Biology'. It is our hope that the acquired knowledge will furthermore enable you to better understand and appreciate the newest developments in treatment of these diseases.

The program comprises PBL tasks, workshops/practicals and assignments. PBL tasks will be presented to you in the form of tutorial group meetings and topic-related lectures. The tasks deal with 1) examples of diseases caused by unwanted reactions of the immune system, e.g. chronic inflammation and autoimmunity, and 2) with oncological diseases in which cells have gone astray, circumvent the body's defence mechanisms and give rise to cancer.

Workshops/practicals will address immunology- and oncology-related research highlights related to diagnostic, preventive and (immuno)therapeutic developments in immunological and oncological diseases.

Assignments consist of giving a presentation and writing an essay on a block-related topic.

Course objectives

- To gain insight in the field of pathobiology, particularly in immunological and oncological diseases
- To understand mechanisms underlying these diseases
- To get acquainted with possibilities for treatment or healing of these diseases
- To increase understanding of healthy living and genetic and environmental aspects that can jeopardize health
- To get more familiar with innovative research and with reporting scientific literature
- To provide extended basic and applied knowledge in the field of life sciences, supporting entrance of master courses in this field

Study instructions

A series of topics will be presented during the course. The study approach is that of problem-based learning and will be offered in various educational forms such as tutorial-group case discussions, workshops, practicals and lectures. Study books and other study materials from the basic courses 'Immunology' and 'Introduction to biology' can be used, but should be extended with more advanced recommended study material, i.e. books (see below) and research articles.

Readings

In addition to the main course books other books may be helpful in studying learning goals from the cases. Many of those are available in the UCM Reading Room and/or in the library at the FHML (UNS50). Students are encouraged to use these additional books or other sources of information.

Course reference books:

Oncology

- Alberts et al. (2015). *Molecular Biology of the Cell*. (6th ed).

Immunology

- Abbas, A.K., Lichtman, A.H., Pillai, S. (2014). *Cellular and Molecular Immunology*. (8th ed). Philadelphia: Elsevier

Other books:

- Weinberg R.A. (2014) The biology of Cancer (2nd ed.) Garland Science
- Abbas, A.K., Lichtman, A.H., Pillai, S. (2015 or later). *Basic Immunology*. *Functions and disorders of the immune system*. (5th ed). Philadelphia: Elsevier
- Male, D., Brostoff, J., Roth, D., Roitt, I. (2013). *Immunology.* (8th ed.). Philadelphia: Elsevier

Papers:

Case-related papers are indicated in the cases and/or available in the course on Eleum

Attendance requirements

In this course, tutorials, lectures, workshops/practicals and journal club alternate. The attendance requirement for the tutorials is 85%. If you miss more meetings, you will have to apply for an additional assignment (request forms are available at the Office of Student Affairs). In order to qualify for an additional assignment you have to have valid reasons for all missed sessions. If you don't meet the attendance requirement, you are not eligible for a resit. You automatically fail the course if you miss over 30% of the scheduled meetings.

The feedback session, the workshops/practicals and Journal club are mandatory meetings. The attendance requirement for these meetings is 100%. If 1 is missed an additional assignment should be made. Missing more than 1 results in a fail of the course.

Assessment

This course contains 3 elements of assessment:

1. a written exam consisting of open questions (60% of the overall course grade);
2. a presentation on an oncology topic (20% of the overall course grade) in pairs – for more information, see below;
3. a report on an immunological topic (20% of the overall course grade) in pairs – for more information, see below.

In addition, active participation is required in the course element: workshops, practical and journal club.

To pass the course a final grade of at least 5,5 is required,

Presentation Assignment

The presentation assignment in the first part of the course offers you an opportunity to discuss, develop and elaborate on an oncological topic together with a fellow student. You are free to pick any course-related topic within oncology that has your interest, but keep in mind that it has to be an academic presentation, including more than just a summation of facts as stated in the literature. In addition to that, you should move beyond the contents of the course. You are encouraged to come up with your own approaches and solutions to an oncological problem.

Format of the presentation

Every duo has 20 minutes for the presentation, including questions and group discussion. A laptop and beamer will be available.

The presentation should contain at least the following items:

- Introduction (+/- minutes)
  - Introduction of research question/hypothesis,
  - Explanation of context and relevance,
- State of the art/results (+/- 6 minutes)
  - Overview of current research and findings as presented in the literature,
- Discussion (+/- 3 minutes)
  - Possible solutions (as provided in the literature or thought of by the students),
- Conclusion(s), (+/- 2 minutes)
- Group discussion (presenters lead the discussion). (+/- 5 minutes)

Assessment of the presentation

The assessment of the presentation will be based on:

- The quality of the introduction (are you able to explain the context and relevance of your research question in such a way that the audience has enough insight to understand the context and reasons for your topic?)
- The capacity to respect allocated time (15 minutes presentation and 5 minutes discussion)
- The quality of the presentation of the problem (are you able to explain the problem and capable of explaining why the solutions you provide are the best solutions?)
- The handling of questions/discussion (are you able to answer questions and discuss the problem?)
- The delivery of the presentation (quality of slides, speaking posture, etc.)

The presentation should be theoretically informed. You do not have to explain basic concepts discussed in the course, since you are presenting to students from your own discipline. However, don't take all background knowledge for granted!

The tutor will assess the presentations, supported by a second assessor. The presentation is the result of a cooperation of two students. There are no requirements as to who gives the presentation. You may present together, or decide to have one student present and have the other student lead the discussion and answer the questions. Either way, keep in mind that you will receive a group grade for this assignment. Your tutor will assess the final product; s/he cannot evaluate whether you divided the work equally. It is your own responsibility to assure each team member invests approximately the same amount of work.

Report Assignment

The report assignment in the second part of the course offers you an opportunity to discuss, develop and elaborate on an immunological topic together with a fellow student. You are free to pick any course-related topic within immunology that has your interest, but keep in mind that it has to be an academic report including more than just a summation of facts as stated in the literature. In addition to that, you should discuss the topic beyond the contents of the course. You are encouraged to come up with your own approaches and solutions to the immunological problem.

Format of the report

Every duo can write a report of about 4000 words (font Times Roman, letter size 12, line space 1,0), including figures and tables for the report. The report has to be handed in as a hardcopy and as an electronic file on ELEUM.

The report should contain at least the following items:

- Content page
- Introduction (+/-750 words)
  - Introduction of research question/hypothesis,
  - Explanation of context and relevance,
- State of the art/results (+/-2000 words)
  - Overview of current research and findings as presented in the literature,
- Discussion (+/-1000 words)
  - Possible solutions (as provided in the literature or thought of by the students),
- Conclusion (+/-250 words)
  - For now and elaboration on future aspects
- Reference list

Assessment of the report

The assessment of the presentation will be based on:

- The quality of the introduction (are you able to explain the context and relevance of your research question/problem)
- The quality of the results (are you able to bring in the results of your research in an organized and complete way)
- The handling of discussion (are you able to evaluate and discuss the results in the context of the research question/problem)
- The quality of the conclusion (are you able to draw conclusions from the presented results in the context of now and the future)
- The quality of the reference list (is the list sufficiently covering the research fields of the topic, are the references update, from scientifically relevant sources)
- The organization, text lay out and sufficient use of informative figures and tables)

The tutor will assess the reports, assisted by a second assessor. The report is the result of a cooperation of two students. There are no requirements for how the student team collaborates in making the report,

but there have to be a statement of which student(s) contributed to the different parts of the report. Either way, keep in mind that you will receive a group grade for this assignment. It is your own responsibility to assure each team member invests approximately the same amount of work in the report. In case of problems, you can discuss that with your tutor.

Feedback session and outline of the report

A feedback session for the report has been scheduled in meeting 10. When preparing your report, you can make use of the framework as presented for the presentation, as presented above. This framework is meant to help you think about your report in a structured way. You are expected to bring the report outline with you to the feedback session.

Resit

Students who initially fail the course (final grade < 5,5) but who have complied with the compulsory attendance requirement and took part in all of the assessments during the course, are eligible for a resit.

In order to receive a grade for the exam, the presentation and the report you will have to do a serious attempt for all 3. If it is not deemed a serious attempt you will not receive a grade and you will not qualify for a resit.

The overall grade for this course consists of a grade for the written exam (60%), a grade for the presentation (20%) and a grade for the report (20%).

The resit serves the purpose of lifting your overall grade to sufficient/above 5.5. For this aim, a resit will be arranged in consult with the course coordinator. Contact the course coordinator for arrangement of the resit.

Staff information

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Overview of meetings, lectures, workshops, practical and deadlines

(Note that this is a preliminary schedule. Please check your schedule on MyUM and the announcements on EleUM on a regular basis)

Week 1	**Lecture**	**Introduction to the course and to Oncology**
	Lecture	**HPV and its role in cervical cancer**
	Meeting 1	**Prediscussion case 1**
	Meeting 2	**Postdiscussion case 1** **Prediscussion case 2**
Week 2	**Lecture**	**Introduction to Workshop Visualization**
	Workshop	**Visualization**
	Meeting 3	**Postdiscussion case 2** **Prediscussion case 3**
Week 3	**Lecture**	**Introduction Immunology, Intro Journal Club**
	Meeting 4	**Postdiscussion case 3** **Prediscussion case 4**
	KINGSDAY FRIDAY FREE	
Week 4	**Meeting 5**	**Postdiscussion case 4** **Prediscussion case 5**
	Meeting 6	**Presentations**
Week 5	**Lecture**	**Tolerance**
	Meeting 6	**Postdiscussion case 5** **Prediscussion case 6**
	ASCENCION DAY FREE	
Week 6	**Lecture 6**	**Multiple Sclerosis**
	Meeting 7	**Postdiscussion case 6** **Prediscussion case 7**
	Workshop	**Flow cytometry**
Week 7	**Lecture**	**Research**
	Lecture	**Immunotherapy**
	Meeting 8	**Postdiscussion case 7** **Prediscussion case 8**
	Workshop	**Journal Club**
Week 8	**Meeting 9**	**Postdiscussion case 8** **Course evaluation**

Feedback session
Report deadline

Exam

Introduction

Pathobiology of oncological diseases

In the UCM curriculum, students with interest in life sciences will have an opportunity to get acquainted with two exciting and nowadays quite popular disciplines: Molecular Cell Biology and Immunology. Molecular Cell Biology and Genetics have been profiting from the development and improvements of recombinant DNA technology and are nowadays driving forces in fundamental and biomedical research. This has resulted in recent years, for example, in some extraordinary developments, such as the sequencing of the whole genome of many organisms including humans, the cloning of different mammals and the development of DNA "chip" microarray and next generation sequencing technology to analyze the presence, expression and sequence of all human genes on a small glass slide or DNA chip. These new developments have found their way into the clinic and especially in the field of oncology these developments paved the way for the introduction of new diagnostic tools and treatment modalities in the clinic. Tailoring therapy to the individual patient has become a promising approach for maximizing efficacy and minimizing drug toxicity. Genomics and proteomics have provided a means for molecular profiling that allows tailoring of therapy.

In this respect, the first part of this course builds on the knowledge obtained in the UCM courses 'Introduction to Biology' and 'Cell Biology'. In addition, we will show you examples of how genetic alterations can lead to deregulation of these processes, leading cancer and how we can make use of our current understanding in the treatment of the disease.

The first workshop in this course will focus on the diagnostic tools to visualize cancer. The first part of the course ends with the production of a mind map by the tutorial group showing putative treatment proposal based on the various hallmarks of cancer. Writing an essay on cancer related subjects and a presentation the themes will be further deepened.

The last task of this course is intended as an integrative task, combining the fields of oncology and immunology. This task will focus on how our immune system can be used as a treatment modality of cancer.

Pathobiology of chronic inflammatory diseases

An increasing amount of diseases appears to be provoked by chronic inflammatory processes. These inflammatory processes are based on an immune response that, instead of leading to beneficial effects, leads to detrimental outcomes in tissues. The involved immune response can be considered an on-going unwanted or non-effective fight against tissue-related constituents. It can cause tissue damage, fibrosis, disruption of body homeostasis and specific organ failure.
The chronicity of these inflammatory processes is due to the fact that the immune system is continuously triggered by antigenic structures that that cannot be removed from the involved tissue. Antigenic structures belonging to this category are various, they are either produced by the body itself (autoantigens or newly formed metabolic compounds), or continuously present in or environment (allergens) or structures from persistent infectious micro-organisms.

In this course we address 2 types of chronic inflammatory diseases. 1) Autoimmune diseases caused by immune responses towards auto(self)antigens, and 2) Metabolic inflammatory diseases leading to impaired metabolic processes.

To understand the pathobiology of autoimmune diseases we first address the phenomenon of 'immunological tolerance', or in other words, how the immune system, in fact the adaptive immune system, guarantees that there is tolerance (no reactivity) of T- and B-lymphocytes to autoantigens.

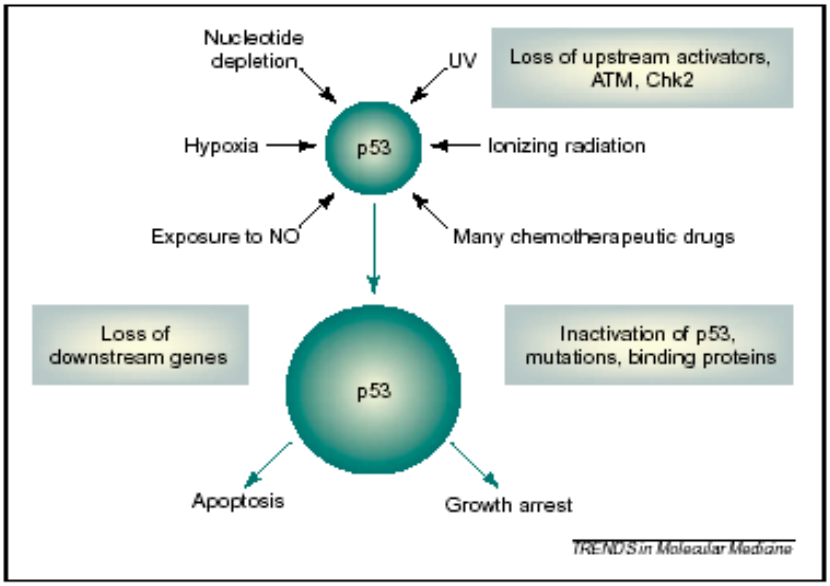
With obtained knowledge about this we will address the pathobiology of autoimmune diseases, and in particular Multiple Sclerosis (MS) and Diabetes Mellitus type 1 (juvenile diabetes), and related current and innovative treatment of these diseases.

The pathobiology of chronic inflammatory diseases will be addressed by examples of diseases such as obesitas, atherosclerosis and Diabetes Mellitus type 2 (adult-onset diabetes, a worldwide expanding disease in young people). These diseases can be considered as provoked by low grade inflammatory processes as the result of an imbalance of metabolic products. Novel research about potential new therapeutic approaches in these diseases , will also be addressed.

Case Studies

Case study 1. 'Guardian of the Genome'

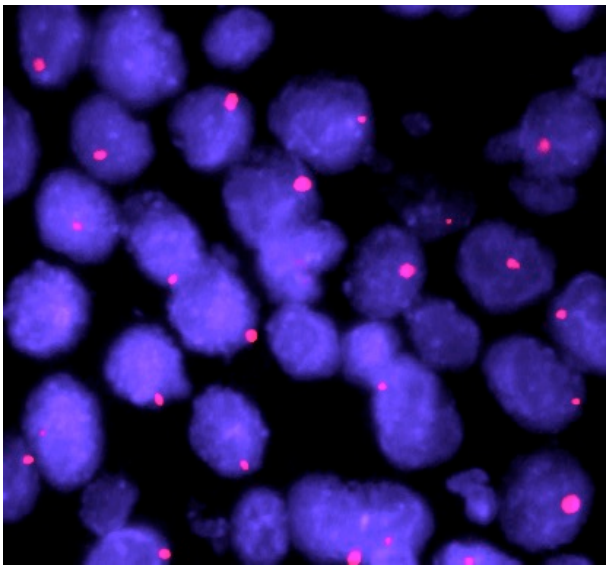
In a television documentary some years ago researchers from the University of Maastricht tested the effect of UV light (from the sun) on the skin of two groups of students lying on the beach. One group received UV-protection cream, while the other group got a non-protecting cream. Since UV-light can cause alterations in the DNA, skin tissue of the latter group showed an increased synthesis of the protein p53, also called "the guardian of the genome". The program clearly stated, that p53 functions as part of the cell-cycle control system that determines at certain DNA damage checkpoints if DNA may be repaired prior to cell division, or if a cell is so much damaged that it should be condemned to death (by a process called apoptosis). Such an important function implicates, that inactivation of p53 would lead to increased DNA damage in cells and a high chance to develop chromosome alterations, ultimately resulting in cancer. Indeed, approximately 50% of all human cancers show p53 inactivating alterations.



P53 activation and inactivation

(Lane D, 2002)

However, in the case of anogenital cancers, such as uterine cervical carcinoma, and ~50% of oropharyngeal cancers usually no mutations are detected in the p53 and pRb genes, and one assumes that in this case the human papillomavirus (HPV) is responsible for deregulation of the function of both genes.



Localization of HPV type 16 (red signals) in cancer cell nuclei (blue)

Recommended literature

Papers

Goh AM et al. The role of mutant p53 in human cancer. J Pathol 2011; 223: 116–126.

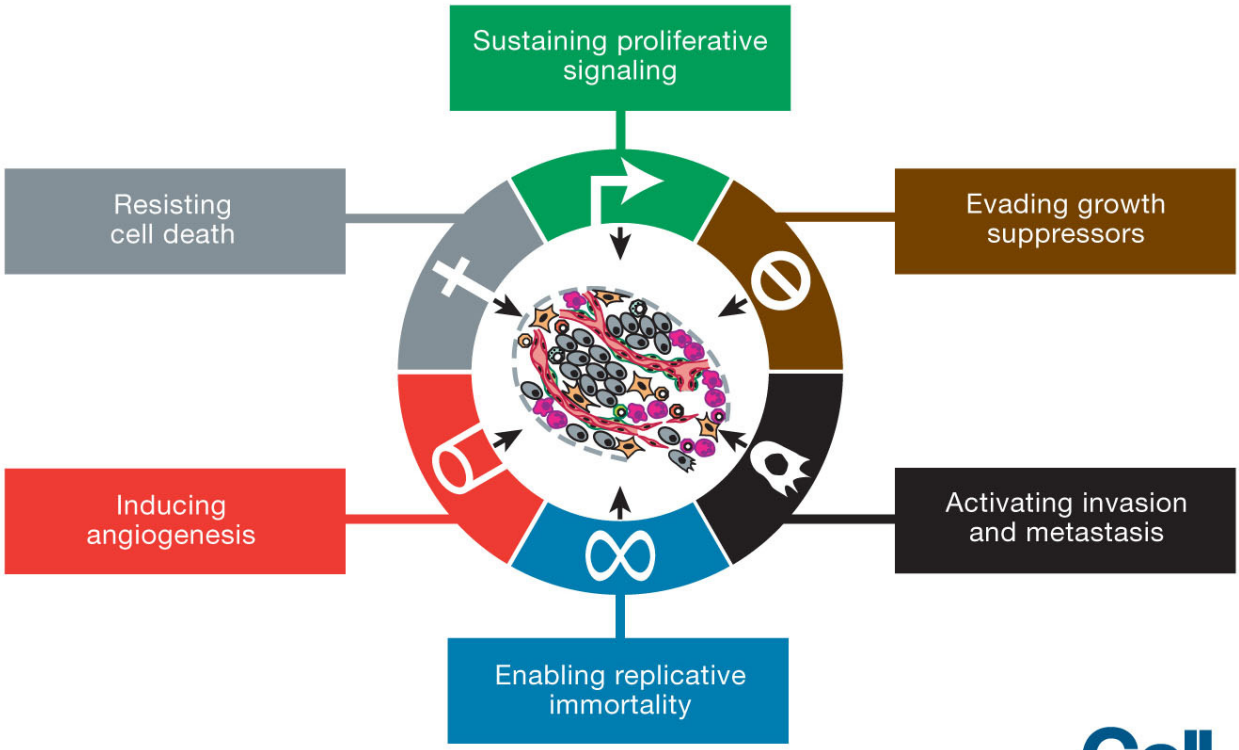
Brown CJ et al. Awakening guardian angels: drugging the p53 pathway. Nature Rev Cancer 2009, 9: 862-873.

Zur Hausen H: Papillomaviruses and cancer: from basic studies to clinical application. Nature Rev Cancer 2002, 2: 342-350

Case study 2. 'Hallmarks of Cancer'

Barbara, a biology teacher, discovered a lump in her breast when taking a shower. The lump felt different to the normal lumpiness she was used to feeling in her breast. She immediately called her general practitioner (GP). Fortunately, he could see her the same day and then made an appointment at the mammography department of a large regional hospital. "Do you think it could be cancer?" Some relatives on her mother's side of the family died of various types of cancer. And aren't some types of cancer hereditary? What is cancer exactly? Pressing questions she is asking her daughter.

You might think that all they do is grow quickly, but that is not the case. There are things like fibroids, rapidly growing tissues that are not malignant. Barbara remembers a diagram from the internet when she googled for hallmarks of cancer. The figure summarized a number of features that a cancer cell has to have. When she examines the diagram closely, is seems as if the cancerous cell is no longer responding appropriately to signals from its environment. It keeps on growing, even without growth factors and apparently does not start apoptosis. How did that work - signal transduction and apoptosis? Moreover, it would appear that the tumor cell is able to make blood vessels grow and that the cells can migrate into surrounding tissues or even wander around the whole body. Where had she seen this before? Finally, she is astonished at the fact that tumor cells can keep on dividing. She had only just been learning the chromosomes get shorter at the ends each time the cell divides and that this is the reason why cells are only able to divide a limited number of times. So what is going on with the tumor cells?



Hanahan and Weinberg, 2011

Recommended literature

Papers

Douglas Hanahan, Robert A. Weinberg. Hallmarks of Cancer. Cell 2000, 100: 57-70

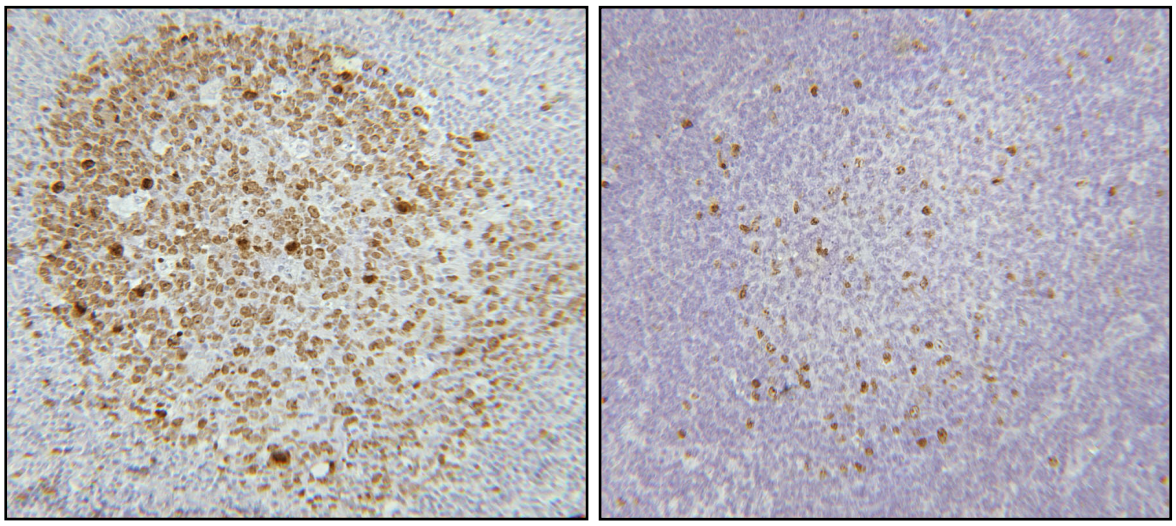
Douglas Hanahan, Robert A. Weinberg. Hallmarks of Cancer: The Next Generation. Cell 2011, 144: 646-674.

Case study 3. 'Apoptosis and Leukemia'

On one of the wards of the Department of Haematology there are two patients diagnosed with non-Hodgkin lymphoma (NHL). One of the patients is diagnosed with "diffuse large B-cell lymphoma" (DLBCL) and the other patient with a "low grade follicular lymphoma".

In the pathology report a high rate of proliferation is mentioned for the DLBCL as was determined immunohistochemically by staining a biopsy with the Ki67 antibody. Virtually 100% of the cells stained positive. In the follicular lymphoma however only few cells are positive (see figure). When stained for Bcl2 the situation seems to be the reverse, showing intense staining in the case of the "low grade follicular lymphoma".

Further analysis showed that the Bcl6 gene was mutated in case of DLBCL and a specific t14/t16 translocation was mentioned in case of the "low grade follicular lymphoma"



Mib1 (Ki67) proliferation staining of DLBCL (left) and follicular lymphoma (right)

Recommended literature

Papers

Baliga & Kumar: Role of Bcl-2 family of protein in malignancy. Hematological Oncology 20, 63-74, 2002

Delbridge, A R. D. Grabow, S, Strasser A and Vaux D.L. Thirty years of BCL-2: translating cell death discoveries into novel cancer therapies Nature Reviews Cancer 16,99–109 (2016)

Case study 4. 'Gleevec' Inhibition of protein kinase signalling *From the clinic to basic research and back.*

In a broadcasted documentary on developments in medical biotechnology, a couple of clinicians discuss the impact of basic and translational molecular genetics and cell biological research on the diagnosis, prognosis and treatment of human diseases, in particular cancer. With respect to the new developments in genomic research and its potential for cancer diagnosis and treatment, the opinions of the clinicians were divided. "This is the hundredth time that one thinks molecular research will revolutionize cancer diagnosis, but tell me, in how many cases do we actually use molecular markers and drugs resulting from these investigations to help us further doing our job?", a specialist put forward cynically. This opinion, however, was contradicted by most other clinicians and two successful cases of anti-cancer therapy were further highlighted in the documentary:

The philadelphia chromosome

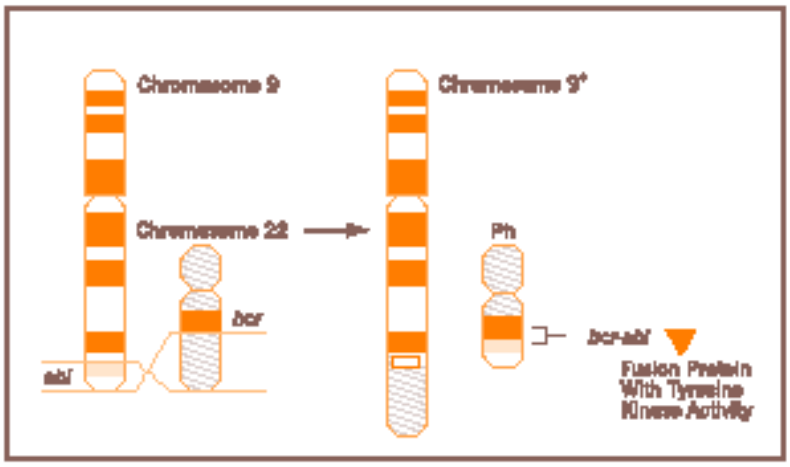
In 95% of patients with CML, a special form of leukemia, high white blood cell counts are found. Cytogenetic examination of bone marrow cells of these patients reveals proof for the presence of chromosomal alterations, particularly on chromosomes 9 and 22 (see figure below). As a consequence of this genetic alteration one of the cellular signal transduction pathways is disturbed. One of the newly developed treatments directly interferes with this pathway leading to a recovery of normal white blood cell counts.

Tastuzumab: anti-HER-2/neu-targeted therapy in breast cancer

Her-2/ neu gene amplification identifies a biologically unique subset of aggressive breast tumors that are sensitive to growth inhibition and apoptosis induced by anti-HER-2/neu-targeted therapies. The first HER-2/neu-targeted approach to reach the clinic was trastuzumab, a humanized monoclonal antibody directed against the extracellular domain of the HER-2/neu protein.

(a)

(b)



CML karyotype (a) and Philadelphia Chromosome (b)

Recommended literature

Papers

Ellen Weisberg et al. Second generation inhibitors of BCRABL for the treatment of imatinib resistant chronic myeloid leukaemia. Nature Rev Cancer 2007, 7: 345-356.

Ruibao Ren. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leykemaemia. Nature Rev Cancer 2005, 5: 172-183.

Ross JS1, Fletcher JA, Linette GP, Stec J, Clark E, Ayers M, Symmans WF, Pusztai L, Bloom KJ. The Her-2/neu gene and protein in breast cancer 2003: biomarker and target of therapy. Oncologist. 2003;8(4):307-25.

Van 't Veer LJ et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 415, 530-536, 2002

Tony Shen,et al. Clinical applications of next generation sequencing in cancer: from panels, to exomes, to genomes. Front Genet. 2015; 6: 215.

Case study 5. 'Tolerance: instruction and selection'

Today we are visiting a work shop of the Immunology course for students in the bachelor BioMedical Sciences (BMS)

The topic is 'T-cell tolerance', or in other words, how are T cells prevented from responding to and fighting our own tissue molecules. The case concerns a patient with an autoimmune disease. In the patient's blood, in contrast to blood from healthy persons, T cells were detected that reacted with a variety of 'self-antigens'.

The BMS students just had looked up some information and report that mutations in the AIRE gene can be a cause of autoimmunity. You, as an UCM student, remember from previous classes that in the development of T-cells in the thymus the process of 'central tolerance' occurs. But again, how exactly did this work? The students talk about the importance of thymus gene named AIRE and show a cartoon about the thymus from the internet, but you do not really understand it.

Questions that come up in your mind are:

- Which process do the light blue lines represent?
- Which process do the pink lines represent?
- What happens at the light blue table?
- What happens at the pink table?
- Why do we see happy and unhappy faces?

Case study 6. 'Multiple Sclerosis'

Jill is a 21-year spirited young woman. She is studying medicine and enjoys the life of a student. However, lately she is tired more than usual. Since a few days, she has vague complaints of her right leg. She decides to make an appointment with the doctor. The doctor remembers that Jill visited him a year ago with problems with her vision and pain in her left arm, which, however, naturally disappeared. He now decides to send her to a neurologist. The neurologist wants to test several aspects. A MRI and EEG *are made, also a lumbar puncture is taken and some tests for eye and nerve function are done . After two weeks the complaints this time did not disappear but became even worse,. Jill's mother accompanies her to hear about the results.*

The neurologist tells Jill that his suspicions have been confirmed. There are visible spots in the scan in both the neck and the brains (see figure1), and the lumbar puncture showed oligo clonal bands. Jill has multiple sclerosis, or in other words MS! Jill and her mother are rather shocked by the message. An aunt of her mother had suffered from this disease and Jill still remembers very well how she physically deteriorated in a few years and ended up in a wheelchair. Jill is too confused to follow the rest of the conversation.

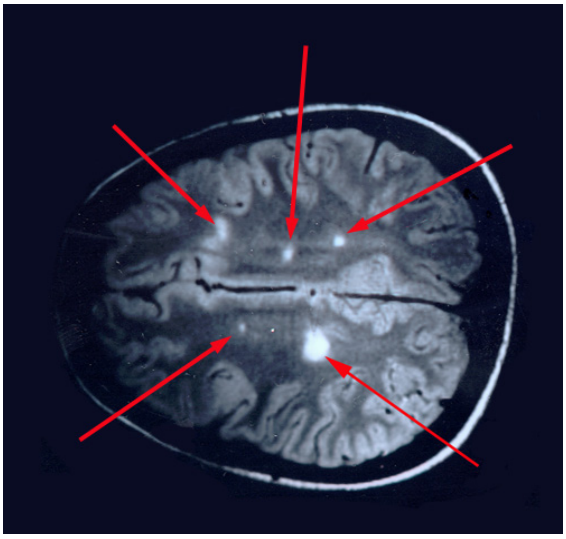


Figure 1: MRI of the brains

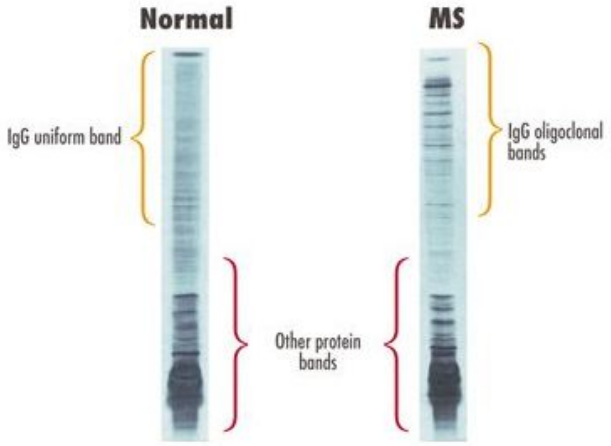


Figure 2: Oligo clonal bands in lumbar fluid

One day later she is hospitalized in order to undergo an infusion therapy with methylprednisolone. Three days, later Jill experiences improvement and she is allowed to go home. The neurologist wants to see her back in three months to discuss further treatment. Meanwhile, Jill still has a bunch of questions:

- Is she genetically burdened to develop MS, her mother's aunt , after all, also had MS, and what if she wants children in the future, will they also get MS? Are there tests for this?
- She can remember a newspaper article about the beneficial effects of vitamin D. Could she have been prevented from getting MS by using vitamin D supplementation? Or could it help her now?
- Is it possible that the neurologist has made the wrong diagnosis? She has learned during her training that her symptoms fit in many other diseases. And what about the test that she had undergone, are the results unequivocal?

- What are the choices regarding therapy? And by the way, she had the idea that MS was an incurable disease and that the damage could not be repaired. However, with the current trends in research, e.g., in the field of stem cell therapy, it that may that there is still hope!

Jill diggs into scientific literature on MS to get answers to her questions. During her next visit to the neurologist prescribes her Rebif.

Case study 7. 'Too much Pizza'

At the reunion of the alumni society of Maastricht University this year, 2 classmates, Bob and Ronald, who graduated together in 1988 run in to each other. Bob seems to have gained quite some weight. During the years at the university he developed a liking for burgers, pizza and French fries, resulting in a Body Mass Index (BMI) of around 35. Bob tells Ronald that he has just been diagnosed with Type 2 Diabetes based a blood test. His blood pressure was also too high. The doctor warned him about the possible complications of this condition and urged him to lose weight in addition to conventional treatment. He is quite surprised since he did not notice anything of his condition. It makes him wonder whether it is that serious. He should go back next week for some kind of test called a "glucose tolerance test" to confirm diagnosis. Ronald replies that his sister has diabetes for many years, ever since she was a child. This type of diabetes was called juvenile or Insulin-Dependent-Diabetes-mellitus (IDDM). From that time on she got insulin injections, but now she has an automatic insulin pump, making life much easier. Bob was surprised that his doctor did not suggest this.

Below are the blood test results of Bob.

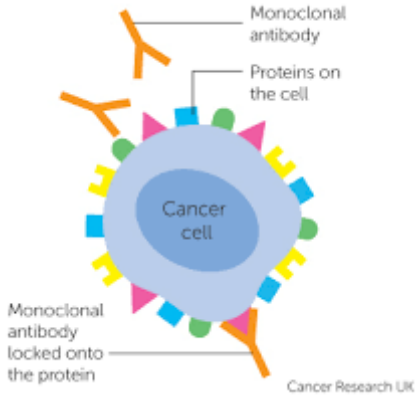
Marker	Level	Normal Range
Fasting glucose	7,5	4,0 - 6,4 mmol/L
Albumin	45	35 - 55 g/L
HbA1c	7	4 - 6%
Triglycerides	4,3	1,3 - 2,7 g/L
Total Cholesterol	7,5	4 - 6,4 mmol/L
LDL cholesterol	6,4	2 - 3,4 mmol/L
HDL cholesterol	1,1	0,9 – 2 mmol/L
LDL/HDL	5,8	< 5

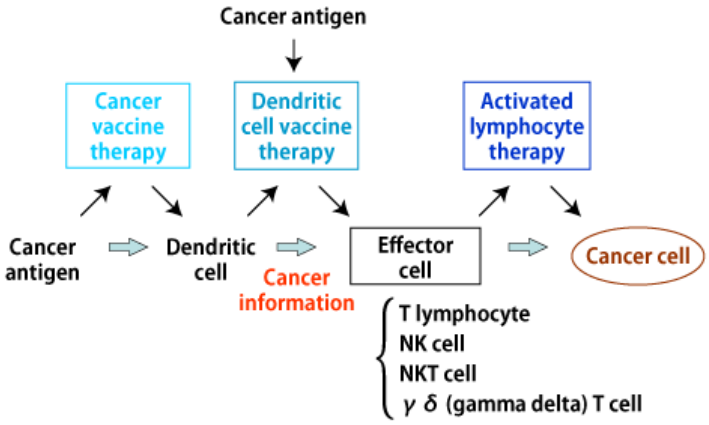
Case study 8. New therapies for cancer: Immunotherapy

Oncologist Brenda Vandenbilt today has seen two patients with cancer, for whom she has significant concerns. Mr. Harmsen is diagnosed with prostate cancer and Ms. Tan with breast cancer. Normally common but also highly treatable cancers, with more than 85% survival rate after 5 years. Nevertheless, this means that she will lose within five years 15% of these patients. She is afraid that this will apply to her two patients. She wishes that we were 20 years or more ahead, with new cancer therapies available, therapies which are at this moment still experimental. She then could have offered more hope not only to her 2, but to all of her cancer patients.

Coming home in the evening Brenda discusses the issue of new therapies with her daughter Sandra, who is a student in Biomedical Sciences. Sandra brings up that she heard about hopeful results of immunotherapy in treatment of cancers. 'I just had a lecture about antibody and cell therapy this morning' she mentions. 'It indeed sounds futuristic, but antibodies and immune cells such as dendritic cells, cytotoxic T-cells and NK cells are currently under research and the results are promising. Look, here are some slides from the lecture of this morning' she enthusiastically explains.

'Who knows, this may be a breakthrough in cancer therapy, I really want to know more about it' Sandra's mother says.





Workshops, Practicals and Journal Club

Workshop/Practical. 'Visualisation: From theory to practice'

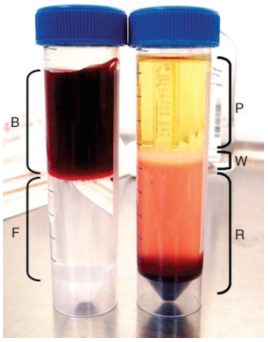
Pathology is a medical discipline that is primarily involved in the examination of organs, tissues, and bodily fluids in order to make a diagnosis of disease. In pathology, the causes, mechanisms and extent of disease are examined using surgically removed bodily specimens or sometimes of the whole body (autopsy). In addition to making a diagnosis these investigations might reveal the presence of specific molecules that can be used to guide clinicians in targeted therapy.

During the workshop students will be introduced to aspects of routine pathology and its role in diagnosing cancer. Students will examine tissue sections that have been in which specific targets have been stained.

Workshop Flow Cytometry

Introduction

Peripheral blood mononuclear cells (PBMC) are white blood cells (leukocytes) with a round nucleus, like monocytes, lymphocytes and NK cells. These blood cells constitute a critical component of the immune system and thus play a central role in the defence against microbes. Many scientists conducting research in the fields of immunology (including auto-immune disorders), infectious disease, haematological malignancies, vaccine development, transplant immunology, and high-throughput screening are frequent users of PBMC. PBMC isolation is often an important first step followed by further analyses like immune cell phenotyping. A widely used technique to extract PBMC from whole blood is density-centrifugation using Ficoll, a hydrophilic polysaccharide. Ficoll (F) is normally placed at the bottom of a conical tube, and blood (B) is then carefully layered on top of the Ficoll (Figure, left). After being centrifuged, the following layers will be visible in the conical tube, from top to bottom (Figure, right): plasma and other constituents (P), a layer of mono-nuclear cells (W; PBMC/MNC), Ficoll (R), and erythrocytes & granulocytes (R) which should be present in pellet form. This separation allows easy harvest of PBMC.



In the practical introduction lecture, you will learn more about monoclonal antibodies, their production and their use in phenotyping. The use of fluorescently labelled antibodies for *Fluorescence activated cell sorting* (FACS) will be discussed in more detail.

Fluorescently labelled antibodies and FACS can be used to determine the composition of the leukocyte population in the blood and to further identify and characterize the lymphocyte population. FACS on blood cells can be performed on whole blood (after lysis of the erythrocytes) or on isolated PBMC. Obviously, the source of blood cells (whole blood or PBMC) can influence the results of the FACS analysis.

For this practical, the instructors have isolated PBMC, using ficoll density centrifugation. Moreover, they determined the yield of the isolated cells. Next, a leukocyte and lymphocyte characterisation was performed by the practical supervisors by analyzing the expression of cell-specific molecules (CD 'cluster of designation') by FACS. The expression level of CD66b (granulocytes), CD14 (monocytes), CD19 (B cells), CD3 (T cells), CD4 (helper T cells) and CD8 (cytotoxic T cells) was measured both in whole blood and PBMC of the same subject.

Below you can find the protocol of the procedure that was followed. During the practical, FACS results will come available during the practical for analysis and interpretation. Based on the protocol and the results from FACS, you will complete the report.

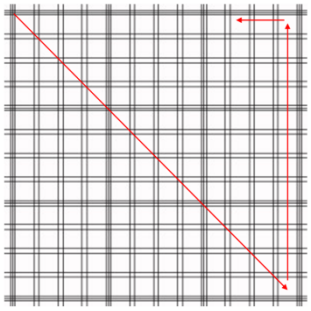
Protocol PBMC isolation and FACS

A. PBMC isolation

- Transfer 5 ml blood ***carefully*** with a Pasteur pipet to the tube containing 5 ml Ficoll → make sure that you put the blood on top of the Ficoll (don't mix them!)
- Carefully take this 15-ml tube to the centrifuge
- Centrifuge: 25 min; 800 G; room temperature, **without brake**
- After centrifugation: check for a layer of cells on top of the Ficoll!!
- Carefully remove the mononuclear cells (interphase) with a pasteur pipet and put them in a new 15 mL tube. Don't take along Ficoll.
- Bring the cell suspension up to a total volume of 13 ml by adding PBS to wash the cells
- Centrifuge: 6 min; 420 G; room temperature
- After centrifugation: check for a cell pellet at the bottom of the tube!!
- Remove the supernatant carefully with a pasteur pipet
- Resuspend the cells in 500 µL of PBS

B. Cell quantification

- Dilute the cell suspension with Türk solution: 20 µl cell suspension in 60 µl türk (residual RBC will be lysed)
- clean slide and cover-glass carefully with Kleenex-paper
- put the cover glass under the clamp of the counter plate
- fill one side of the counting chamber under the cover-glass with a single smooth flow of suspension using a micropipet (avoid air bubbles)
- count cells in 25 small squares under a microscope (objective 40 x). Count cells which touch the upper and left border but not those which touch the lower and right borders



Calculation:

The size of a Bürker counter chamber is 0.2 mm x 0.2 mm. The depth is 0.1 mm. 25 squares together have a volume of 25 x 0.2 x 0.2 x 0.1 = 0.1 mm^3, corresponding to 0.1 µl.

The number of cells per ml can then be calculated: number of counted cells (in 0.1 µl) x 10.000 (conversion factor to 1000 µl) x dilution factor

C. Analysis and interpretation of FACS data

FACS staining *whole blood*

1. Add the desired antibody cocktail to 50 µl of human blood and mix the blood with the antibodies by vortexing (briefly)
2. Incubate the samples for 15 minutes at room temperature in the dark
3. Add 2 ml of lysis buffer to each tube and vortex briefly
4. Incubate the samples for 10 minutes at room temperature in the dark.
5. Centrifuge: 5 minutes at 1400 rpm.
6. Remove the supernatant by decantation. The cells remain in the tube with a small volume of buffer. Vortex briefly to resuspend the cells.
7. Add 2 ml of 1x PBS and vortex briefly.
8. Centrifuge: 5 minutes at 1200 rpm.
9. Remove the supernatant as described under 6.
10. Vortex briefly to resuspend cells. Add 250 µl of FACS-buffer and mix by vortexing.
11. The cells are ready for flow cytometry

FACS staining *PBMC*

1. Bring the PBMC suspension in a concentration of 1 million cells per ml
2. Take 100ul of cell suspension and add the desired antibody cocktail. Mix by vortexing (briefly).
3. Incubate the samples for 15 minutes at room temperature in the dark
4. Add 2 ml of 1x PBS and vortex briefly
5. Centrifuge: 5 minutes at 1200 rpm.
6. Remove the supernatant by decantation. The cells remain in the tube with a small volume of buffer.
7. Vortex briefly to resuspend the cells. Add 250 µl of FACS-buffer and mix by vortexing.
8. The cells are ready for flow cytometry

Shortly after the staining, the samples have been acquired on a flow cytometer and the data have been analyzed using FACS Diva software. A print-out of the data will become available during this practical.

D. Report

The reporting of this practical consists of answering a series of questions related to the operations performed, the FACS protocols used and obtained FACS data. This questionnaire will be distributed during the practical and the answers should be discussed with the practical supervisors at the end of the practical.

Report Flow Cytometry

Name + ID nummer 1: ……

Name + ID nummer 2: ……

Name + ID nummer 3: …… ……

Questions about the flow cytometry staining protocols

- Read the staining protocol of whole blood carefully. Describe in your own words the three most important steps of this protocol and explain the goal of each step.
  ……
  ……
  ……
  ……
  ……...
- What is the most important difference between the staining protocol of whole blood and the staining protocol of PBMC? Why do the protocols differ at this step?
  ……
  ……
  ……
  ……
  ……
- We want to discriminate 5 leukocyte populations (granulocytes, monocytes, B cells, T helper cells and cytotoxic T cells in the same sample. This is possible by using a combination of 6 antibodies that are a) specific for a leukocyte subset and b) each are labeled with a different fluorochrome. Our FACS machine has 3 excitation lasers (488nm, 633nm, 405nm). Chose from the list below 6 fluorochromes that are compatible based on their excitation and emission properties.

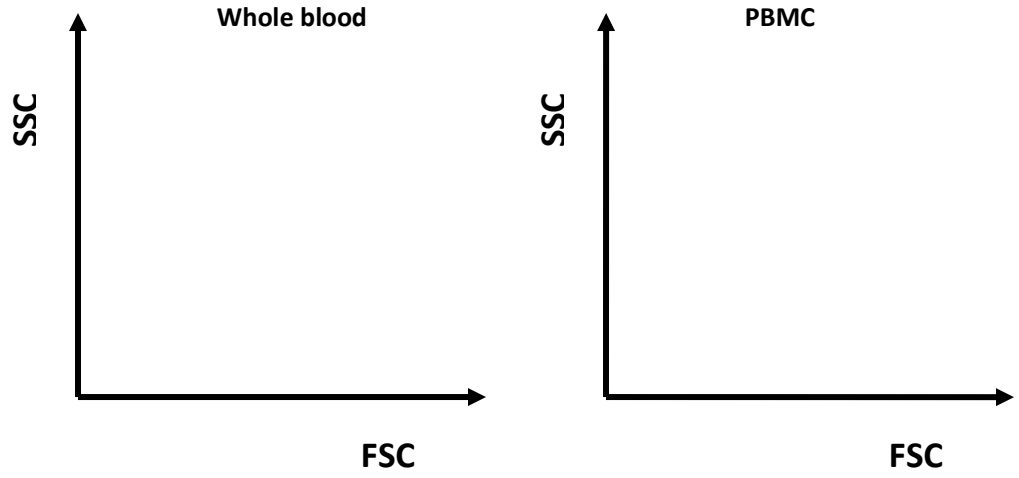
  1. …….
  2. …….
  3. …….
  4. …….
  5. …….
  6. …….

Fluorochrome	Fluorescence Emission Color	Ex-Max (nm)	Excitation Laser Line (nm)*	Em-Max (nm)
BD Horizon™ V450		404	405	448
Pacific Blue™		401	405	452
BD Horizon™ V500		415	405	500
AmCyan		457	405	491
Alexa Fluor® 488		495	488	519
FITC		494	488	519
PE		496, 564	488, 532, 561	578
BD Horizon™ PE-CF594		496, 564	488, 532, 561	612
APC†		650	633, 635, 640	660
Alexa Fluor® 647		650	633, 635, 640	668
PE-Cy™5†		496, 564	488, 532, 561	667
PerCP		482	488, 532	678
PerCP-Cy™5.5		482	488, 532	695
Alexa Fluor® 700		696	633, 635, 640	719
PE-Cy™7		496, 564	488, 532, 561	785
APC-Cy7		650	633, 635, 640	785
BD APC-H7		650	633, 635, 640	785

<u>Questions about data interpretation</u>

Ask the data sheets with the flow cytometry results from the staining of whole blood and PBMC. Compare the results and answer the questions below.

- What leukcocyte populations are present (and detectable by FACS) in the whole blood staining compared to PBMC?
    i. Whole blood: ……
    ……
    ii. PBMC:……
    ……
- These cells can be distinguished based on their size and granularity. These parameters can be assessed by measuring forward (FSC) and side (SSC) scattering of light. Draw in the FSC/SSC plots below the major leukocyte populations in whole blood (left) and PBMC (right)



- What is ‘gating’ when do you use ‘gating’?.....

……

… .....

Different leukocyte subsets can thus be distinguished based on differences in size and granularity. In addition, a more in depth separation can be done by measuring the expression of specific proteins on the cell surface using fluorescently labelled antibodies.

- What is the fraction of cells (expressed as % of all leukocytes) of granulocytes, monocytes, B- and T-cells in whole blood and in PBMC? (See result sheet)

Leukocyte subset	Can be recognized by the expression of	Whole blood	PBMC
		% of leukocytes	% of leukocytes
Granulocytes		…	…
Monocytes		…	…
B cells		…	…
T cells		…	…

- Explain the differences you found between whole blood and PBMC

……
……
……
……
……
……
…….....

- What is the fraction of cells (expressed as % of all lymphocytes) T-cells, T helper cells and cytotoxic T cells in whole blood and in PBMC? (See result sheet)

Leukocyt subset	Can be recognized by the expression of	Whole blood	PBMC
		% of lymphocytes	% of lymphocytes
T cellen		…	…
CD4+ T cells		…	…
CD8+ T cells		…	…

- Explain the differences you found between whole blood and PBMC

……
……
……
……
……

- helper en cytotoxic T cells both express CD3. Which of these cell types expresses the highest amount, based on the FACS data?

.....
.....……
……...

Question about cell quantification

- Carefully read the protocol. At the end, you have a cell suspension of 500µl containing the isolated PBMCs. Proceed with cell quantification

Amount of PBMC in 25 squares (counted 2x): ……../……, average:…….

Concentration = …… cells / ml

Total number of PBMC isolated from 5 ml blood: ……

Workshop. Journal Club

Scientific findings are reported to the community by publishing the results in scientific journals. This is very important part of the scientific progress. To ensure the quality of the scientific article, these papers are first assessed for their quality and scientific merit before publication. Generally, this is performed by a process called “peer review”, i.e. the work is critically read (“reviewed”) by fellow scientists in the field (“peers”). This process tries to ensure that manuscripts are clear, the findings are original, the data are generated by appropriate methods, statistical analysis is rigorous and that the conclusions of the article are backed up by the data shown.

A journal club is a session where a paper is chosen to discuss in group. The goal is to critically analyse the presented results in detail as well as to perform your own review of the paper. The goal is not only to gain understanding in the way the authors performed their research, how they performed measurements and how they presented the data. Moreover, it is a way to better understand the process of scientific writing.

An introduction lecture about the topic of the journal club paper and how to prepare and participate in the journal club session will be given.

Lectures

Lectures

Lecture 'Introduction to the course and to Oncology' (B. Schutte)
The lecture will provide information about the course and an overview of the past and current developments in oncology. The emphasis is on the role of disciplines like molecular cell biology, genetics and pathology in providing a scaffold for diagnosis and modern treatment modalities.

Lecture 'HPV and its role in cervical cancer' (T. Hopman)
This lecture addresses the role of the HPV virus in the carcinogenesis of cervical cancer.

Lecture 'Introduction to the workshop 1: Visualisation of processes in cancer' ('T. Hopman en J. Cleutjens)
An overview will be presented of the role that the disciplines molecular cell biology, genetics and pathology are playing in the diagnosis and treatment of cancer. Emphasis will be on the techniques that are currently used in routine pathology. It is an introduction to the workshop 'Visualization of processes in cancer'.

Lecture 'Introduction to Immunology' (K.Wouters)
To understand inflammatory diseases one has to know how the immune system functions. This lecture summarizes the immune components and immune mechanisms involved in the immune response.

Lecture 'Introduction to the Journal club' (K. Wouters)
The lecture informs you about the research topic of the Journal club and how to prepare for and execute the Journal club session.

Lecture 'Tolerance' (W. Germeraad)
Immunological tolerance is a requirement for appropriate functioning of the immune system. The mechanisms of lymphocyte tolerance, and in particular T-cell tolerance for 'self ' (auto) antigens will be discussed.

Lecture 'Multiple Sclerosis' (J. Damoiseaux)
Multiple sclerosis (MS) is a devastating neurological autoimmune disease that may bring young adults suffering from slightly impaired mobility in a time span of years to severe impaired mobility ending in a wheel chair. In the lecture the ins and outs of MS, including immunological mechanisms and approaches for prevention and therapy will be discussed.

Lecture 'Research'
This lecture will introduce the ins and outs of a research project. The topic is the role of inflammation in obesity and associated diseases.

Lecture 'Immunotherapy' (Lotte Wieten)
This lecture will address the various forms of Immunotherapy.

Deadlines

Handing in the report on an Immunological topic.
The deadline for handing in the hardcopy version and for uploading on ELEUM is: Tuesday May 31st, 2018