THEME [HEALTH.2010.1.2-1]
[Tools for the identification and the detection of biomarkers in clinical samples and patients. FP7-HEALTH-2010-two-stage.]

Grant agreement for: Collaborative project*

Annex I - "Description of Work"

Project acronym: ImagInt

Project full title: " HER Imaging and Molecular Interaction Mapping in Breast Cancer "

Grant agreement no: 259881

Date of last change: 2011-02-16

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Part A

Workplan Tables

A1:
Project summary

Project Number [1]	259881	Project Acronym [2]	ImagInt

One form per project

General information			
Project title [3]	HER Imaging and Molecular Interaction Mapping in Breast Cancer		
Starting date [4]	The first day of the month after the signature by the Commission		
Duration in months [5]	48		
Call (part) identifier [6]	FP7-HEALTH-2010-two-stage		
Activity code(s) most relevant to your topic [7]	HEALTH.2010.1.2-1: Tools for the identification and the detection of biomarkers in clinical samples and patients. FP7-HEALTH-2010-two-stage.		
Free keywords [8]		bio-imaging, HER1, HER2, HER3, HER4, FRET , biomarkers, DARPIns, FLIM, CTCs, drug resistance, miRNA	

Abstract [9]

The aim is to develop tools for imaging and characterising protein/protein and protein/RNA interactions in cancer using Designed Ankyrin Repeat Proteins (DARPins). DARPins are small, ultrahighly stable, antibody-like proteins that bind specific targets with high affinity in monovalent form and are readily engineered for site-specific chemical modification. The exemplar protein family will be EGFR, with focus on HER2-mediated processes in cancer.

1. EGFR-reactive DARPins will be used to characterise HER2 homo- and hetero-dimers using 4 novel technologies: Single Molecule Fluorescence, Proximity Ligation, super-resolution microscopy and FRET/FLIM. The collected data will be analysed with information on clinical outcome to determine which HER2 interactions are associated with resistance to HER2 targeted treatments.
2. Protein/RNA complexes will be isolated and characterised. These complexes may be new biomarkers for breast cancer and their characterisation is aimed at elucidating mechanisms of transcriptional regulation in response to anti-HER2 treatment.
3. Protein networks associated with EGFR signalling by imaging clusters of at 50-100 different proteins in a single cell or tissue section. This will be achieved with a robot, using large dye-conjugated tag libraries, and automatically bleaching a dye after imaging and re-labelling with another.
4. Whole body imaging (Phase I/II) clinical trial will use radiolabelled anti-HER2 DARPins to improve specificity and sensitivity of quantitative PET/SPECT/CT. The trial aims to image HER2 positive metastatic cancer and provide circulating tumour cells (CTCs) and biopsies for more detailed analysis.
5. Multivariate data obtained by the new technologies will be analysed with a range of bioinformatic tools, including artificial neural network methods, to determine novel biomarkers that aim to classify breast cancer patients at an individualised level.

The outcome is to increase the tool panel of clinicians.

A2:
List of Beneficiaries

Project Number [1]	259881	Project Acronym [2]	ImagInt

List of Beneficiaries

No	Name	Short name	Country	Project entry month[10]	Project exit month
1	UNIVERSITY COLLEGE LONDON	UCL	United Kingdom	1	48
2	UNIVERSITAET ZUERICH	UZH	Switzerland	1	48
3	KING'S COLLEGE LONDON	KCL	United Kingdom	1	48
4	TOPOSNOMOS LTD	TNL	United Kingdom	1	48
5	MAX PLANCK GESELLSCHAFT ZUR FOERDERUNG DER WISSENSCHAFTEN E.V.	MPG	Germany	1	48
6	UPPSALA UNIVERSITET	UU	Sweden	1	48
7	Innovative Technologies in Biological Systems	INO	Spain	1	48
8	FIRALIS S.A.S.	FLS	France	1	48
9	MEDIPOLIS GMP OY	M-GMP	Finland	1	48
10	NOVAMEN SAS	ACIES-P2R	France	1	48

A3:
Budget Breakdown

Project Number [1]	259881	Project Acronym [2]	ImagInt

One Form per Project

Participant number in this project [11]	Participant short name	Fund. % [12]	Ind. costs [13]	Estimated eligible costs (whole duration of the project)					Total receipts	Requested EU contribution
				RTD / Innovation (A)	Demonstration (B)	Management (C)	Other (D)	Total A+B+C+D		
1	UCL	75.0	T	1,569,145.60	0.00	86,601.60	75,057.60	1,730,804.80	0.00	1,338,517.00
2	UZH	75.0	T	1,369,164.80	0.00	3,000.00	27,200.00	1,399,364.80	0.00	1,057,073.00
3	KCL	75.0	T	721,868.80	0.00	2,500.00	24,848.00	749,216.80	0.00	568,749.00
4	TNL	75.0	S	579,273.00	0.00	1,000.00	0.00	580,273.00	0.00	435,454.75
5	MPG	75.0	S	517,200.00	0.00	2,000.00	4,400.00	523,600.00	0.00	394,300.00
6	UU	75.0	T	707,200.00	0.00	2,500.00	19,280.00	728,980.00	0.00	552,180.00
7	INO	75.0	T	528,600.00	0.00	2,000.00	24,400.00	555,000.00	0.00	422,850.00
8	FLS	75.0	T	486,259.20	0.00	2,500.00	26,000.00	514,759.20	0.00	393,194.00
9	M-GMP	50.0	F	622,800.00	0.00	2,500.00	14,000.00	639,300.00	0.00	327,900.00
10	ACIES-P2R	75.0	A	0.00	0.00	173,368.00	20,004.00	193,372.00	0.00	193,372.00
Total				7,101,511.40	0.00	277,969.60	235,189.60	7,614,670.60	0.00	5,683,589.75

Note that the budget mentioned in this table is the total budget requested by the Beneficiary and associated Third Parties.

*** The following funding schemes are distinguished**

Collaborative Project (if a distinction is made in the call please state which type of Collaborative project is referred to: (i) Small of medium-scale focused research project, (ii) Large-scale integrating project, (iii) Project targeted to special groups such as SMEs and other smaller actors), Network of Excellence, Coordination Action, Support Action.

1. Project number

The project number has been assigned by the Commission as the unique identifier for your project, and it cannot be changed. The project number **should appear on each page of the grant agreement preparation documents** to prevent errors during its handling.

2. Project acronym

Use the project acronym as indicated in the submitted proposal. It cannot be changed, unless agreed during the negotiations. The same acronym **should appear on each page of the grant agreement preparation documents** to prevent errors during its handling.

3. Project title

Use the title (preferably no longer than 200 characters) as indicated in the submitted proposal. Minor corrections are possible if agreed during the preparation of the grant agreement.

4. Starting date

Unless a specific (fixed) starting date is duly justified and agreed upon during the preparation of the Grant Agreement, the project will start on the first day of the month following the entry info force of the Grant Agreement (NB : entry into force = signature by the Commission). Please note that if a fixed starting date is used, you will be required to provide a detailed justification on a separate note.

5. Duration

Insert the duration of the project in full months.

6. Call (part) identifier

The Call (part) identifier is the reference number given in the call or part of the call you were addressing, as indicated in the publication of the call in the Official Journal of the European Union. You have to use the identifier given by the Commission in the letter inviting to prepare the grant agreement.

7. Activity code

Select the activity code from the drop-down menu.

8. Free keywords

Use the free keywords from your original proposal; changes and additions are possible.

9. Abstract

10. The month at which the participant joined the consortium, month 1 marking the start date of the project, and all other start dates being relative to this start date.

11. The number allocated by the Consortium to the participant for this project.

12. Include the funding % for RTD/Innovation – either 50% or 75%

13. Indirect cost model

A: Actual Costs
S: Actual Costs Simplified Method
T: Transitional Flat rate
F :Flat Rate

Workplan Tables

Project number

259881

Project title

ImagInt—HER Imaging and Molecular Interaction Mapping in Breast Cancer

Call (part) identifier

FP7-HEALTH-2010-two-stage

Funding scheme

Collaborative project

WT1
List of work packages

Project Number [1]	259881	Project Acronym [2]	ImagInt

LIST OF WORK PACKAGES (WP)

WP Number [53]	WP Title	Type of activity [54]	Lead beneficiary number [55]	Person-months [56]	Start month [57]	End month [58]
WP 1	Generation and development of DARPins as tools for quantitative imaging and detection of interacting	RTD	2	121.00	1	48
WP 2	Tools for a multivariate tumour invasion signature	RTD	3	121.00	1	48
WP 3	Developing methods for isolation characterization of protein/RNA complexes from clinical tissues	RTD	7	107.00	1	46
WP 4	Development of radiolabelling and software for quantum imaging	RTD	6	112.00	1	42
WP 5	Phase I trial to assess safety and efficacy of quantitative imaging biomarkers in patients	RTD	1	87.00	1	42
WP 6	Data management, integrative Bayesian analysis of data derived from preclinical and clinical studies	RTD	3	109.00	1	48
WP 7	Dissemination, IPR and ethical issues	OTHER	1	25.50	1	48
WP 8	Management	MGT	1	15.00	1	48
			Total	697.50		

WT2:
List of Deliverables

Project Number [1]	259881	Project Acronym [2]	ImagInt

List of Deliverables - to be submitted for review to EC

Delive-rable Number [61]	Deliverable Title	WP number [53]	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
D1.1	High affinity DARPins to EGF-R, HER2, HER3, HER4	1	2	50.00	R	CO	25
D1.2	Measurement of HER pairings in relation to tumour outcome	1	2	71.00	R	CO	46
D2.3	Nanoscopy imaging of HER2 and related proteins	2	5	25.00	R	CO	36
D2.4	Methods for FRET/FLIM-based HER2/HER3 dimerisation and optical imaging of Raf-Rok□ pathways	2	3	24.00	R	CO	12
D2.5	Identification of HER2-associated toponome protein clusters	2	4	26.00	R	CO	16
D2.6	Imaging-based metastatic signature established and compared with FDG and/or FLT PET-CT	2	3	23.00	R	PU	36
D2.7	Full Toponomic analysis of breast cancer	2	4	23.00	R	CO	46
D3.8	Analysis of Ago proteins in P-bodies and establishment of RISC-miRNA technologies	3	7	52.00	R	CO	28
D3.9	Application of RISC-miRNA technologies to	3	7	55.00	R	CO	46

WT2:
List of Deliverables

Delive-rable Number [61]	Deliverable Title	WP number [53]	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
	breast cancer tissues						
D4.10	DARPins radiolabelled and tested in in vitro and in vivo	4	6	42.00	R	CO	18
D4.11	Development and evaluation of SPECT/PET imaging	4	1	70.00	R	CO	42
D5.12	GMP DARPin procedures, copies of ethical approval and clinical trials protocol	5	1	45.00	R	CO	18
D5.13	Data from HER2 imaging clinical trial	5	1	42.00	R	CO	42
D6.14	Establishment and analysis of database	6	3	58.00	R	CO	18
D6.15	Biomarker analysis and protocol for quantifying relations between data sets	6	8	51.00	R	PU	42
D7.16	Establish communication, IP and ethics stratergies	7	1	19.50	R	PP	24
D7.17	Project workshop	7	1	6.00	O	PU	48
D8.18	Project Management Manual	8	10	0.50	R	CO	3
D8.19	First Periodic Report	8	1	4.50	R	PU	12
D8.20	Second Periodic Report	8	1	4.50	R	CO	30
D8.21	Final Report	8	1	5.50	R	CO	48
			Total	697.50			

WT3:
Work package description

Project Number [1]	259881	Project Acronym [2]	ImagInt

One form per Work Package

Work package number [53]	WP1	Type of activity [54]	RTD
Work package title	Generation and development of DARPins as tools for quantitative imaging and detection of interacting		
Start month	1		
End month	48		
Lead beneficiary number [55]	2		

Objectives

- To develop additional specific DARPins for the EGFR family
- To establish rigorous understanding of the EGF-family receptor pairs and oligomers formed i) Using single molecule methodology; ii) Using nanoscopy

To convert this knowledge into the development of tools for diagnostics i) Using more robust technology such as proximity ligation ii) Using real tumor tissue and ultimately in vivo

Description of work and role of partners

Description of work (possibly broken down into tasks), and role of participants

Task 1.1: Generation of additional DARPins M1-M24
Partners involved: UZH, INO
While the key the anti-HER2 DARPin G3, to be developed in WP4 and WP5 for a cGMP manufacture and a clinical trial, already exists, such that these WPs can start immediately, additional DARPins need to be developed for WP2 and WP3.
Very high affinity DARPins have already been generated against EGFR, HER2 and HER4, but the work against HER3 has started only recently and will be pursued in this WP1. Using new cell panning strategies high affinity DARPins against this target, as well as additional picomolar binders against all 4 family members will be generated. It is important to have access to a range of high affinity reagents, as a very clear improvement of signal detecting HER2 in microarrays of paraffin-embedded tissue sections was seen with picomolar DARPins (Theurillat et al., manuscript submitted), reaching better discrimination level than an FDA approved antibody (same signal but less background), and with greatly improved flexibility for future applications and formats because of the recombinant nature. The reason why affinity is crucial is that, in order to reach high specificity, very stringent washing of the tissue microarrays has to be carried out, and for weakly expressed targets (such as, e.g., HER4) detection would become difficult.
A number of novel whole cell panning strategies will be used to be able to generate DARPins that recognized HER3 on cells. Two novel technologies will be used on HER3, as pure HER3 is rather aggregation-prone.
A variation of the pathfinder/proximol technology using fusions and coupling of the ligand to horse-radish peroxidase or to the receptor itself is being carried out (Boersma et al., unpublished results). By using biotin-tyramide labeling of receptors, this will, allow a covalent coupling and thus detection of phages binding on the desired target HER3, as opposed to other targets on the cells. By using these different approaches to "label" HER3 in situ, it is expected that a number of different epitopes will be targeted on HER3.
One of the major challenges in whole cell panning is binding to other epitopes on other proteins of the cell. The most obvious approach, to use the same cell type without the protein of interest overexpressed as a competitor, is not very effective, since it is not possible to carry this out with a large enough excess of competitor. For this reason, the use of membrane vesicles of the competitor cells (obtained by nitrogen cavitation) is being developed, as this allows a much greater excess of competitor, better separation from the cells of interest, and thus a much better approach to focusing on the target of interest
As an even more powerful approach (because of the greater library size possible and the built-in evolution) is to use whole cell panning in the context of ribosome display. This is currently under development at UZH and will be used on HER3.

Additionally, domains of HER3 will be expressed to focus the binders to particular domains. A refolding from E. coli inclusion bodies has recently already been achieved (Richter et al., unpublished work), such that there is already HER3 target available.
It should be noted that, for all of the ErbB family, both DARPins which do not influence signaling and dimerization are very valuable (as silent observers of the single molecule studies) (WP1, WP2), as well as those which prevent it as building blocks for future therapeutics (WP4, WP5), and this is an important reason to select for additional binders next to the ones that are already available.
To better elucidate the role of microRNAs in different forms of cancer (WP3), it is necessary to develop a powerful tool for their characterization. The prerequisite is to be able to quantitate the amount of the RNA-induced silencing complex (RISC), as well as to identify the bound RNAs. This in turn requires to be able to isolate the complex in the absence of any tagged components since the method must work in tumor tissues as well, and not only in model systems.
For this reason DARPins will be also developed against the key protein component of RISC, Argonaute protein (Ago). Besides Ago enzyme, eight different enzymes (FMRP, FXR1, FXR2, Dicer, Gemin-3, Gemin-4, MOV10, TNRC6B and PRMT5) have been identified as components of RISC involved in the recognition of target mRNA by the miRNA. INO will prepare functional, pure Ago protein in both biotinylated and non-biotinylated form (mg amount) and deliver it to UZH (M6). UZH will then select DARPins against Ago and any other proteins of the RISC complex which INO is able to provide in pure biotinylated form.
The selection of DARPins has the advantages over traditional monoclonal antibodies that the recombinant nature of the DARPins allows them to be directly labelled, by fluorescence, biotin, isotopes for clear identification of novel microRNAs. Since DARPins can be expressed within the cell, they could also be used to allow complex formation in situ, which can be isolated via the tagged (e.g. biotinylated) DARPin.

Task 1.2. Single molecule detection of homo- and heterodimeric pairs of the EGF-R superfamily M1-M48
Partners involved: UZH, MPG
By using monovalent DARPins with very high affinity, the movement of all members of the EGFR family can be directly tracked. This provides an enormous advantage over the use of recombinant GFP fusion proteins of the HER family members: First, the expression level of GFP fusions in the recombinant system would dictate the result and thus be meaningless for real cancer cells, and second, the interactions of HER with intracellular signal transduction components may significantly change their dimerization behavior. Thus, our strategy aims to use unmodified HER proteins in “real” tumor cells. DARPins will be expressed carrying either a unique cysteine or they will be selectively labelled at the N-terminal amino group by methods previously developed at UZH and shown to be very robust. The key strategy is to use aliquots of the same DARPin labelled with different dyes, which will report on stable homodimers by coincidence detection at the single molecule level. The labelling of different DARPins with the same strategy will provide information on heterodimer formation, eventually leading to a quantitative picture of homo- and heterodimeric assemblies as well as cluster formation. Labelling strategies will be mutually exchanged with MPG and KCL.

Task 1.3. Nanoscopy of the HER superfamily M1-M18
Partners involved: MPG, UZH
In classical immunochemistry, a primary and a secondary antibody of the IgG type is used (each of a size of 10 nm), and thereby the observed structure is increased by 30-40 nm. Therefore, the current labelling agents, i.e. antibodies, limit the attainable resolution in nanoscopy. In other words, the full potential of the nanoscopic methods (established at MPG) can only be exploited with smaller and less flexible labelling agents.

Using directly labelled DARPins (about 3 nm total), prepared in a similar way as in task 1.2, potentially enabling a much more detailed sub-cellular mapping of HER2 and other proteins, notably other members of the EGFR family, in the plasma membrane. The use of DARPins could potentially open up a resolution regime that with classical IgG-labeled samples is too large for FRET and too small for nanoscopy with classical IgG-labelled samples and thus by using DARPins provide new information on the behavior of the members of the ErbB family. Utilizing DARPins, we will establish labeling and imaging protocols using well characterized cultivated (breast cancer) cell lines. To this end, several fixation protocols including formaldehyde fixation and high pressure freezing will be evaluated for their use in combination with DARPins. These protocols will be used to image the nanoscopic distribution of HER proteins first in cultivated cells and subsequently (in WP2) in tissue samples.

Task 1.4. Proximity ligation and bispecific DARPins for use in tissue samples M1-M48
Partners involved: UZH, UCL, KCL

The methods developed in tasks 1.2 and 1.3 will help define exactly and quantitatively the homo- and heterodimerization behavior of the ErbB family members. However, these methods are too sophisticated to carry out in a routine setting and not suited for typical paraffin-embedded patient samples. The main function of tasks 1.2 and 1.3 is, therefore, to define the critical and decisive complexes that correlate with a particular growth activation. The purpose of the current task is then to find a simpler and more robust way of detecting the disease causing HER2 complexes. This will be carried out in two different ways, which both could potentially be developed into tools for determining a cancer phenotype.
(1) Proximity ligation assays will be used, where an oligonucleotide is fused to the DARPin to serve as a hybridization probe for bridging primers to amplify the signal if — and only if — the two probes are adjacent on the cell. The methods will be established at UZH, using tumor samples provided by UCL and KCL. Importantly, the proximity ligation is being established at UZH through an ongoing collaboration with UU).
There are three established ways how to label DARPins at a defined position. (i) with an engineered unique cystein (a very robust method) (ii) with reaction conditions strongly favouring the N-terminus over internal lysine amino groups (established at UZH) and (iii) with a fused engineered O6-alkylguanine-DNA- alkyltransferase ("Snap tag") and using suitably derivatized oligonucleotides (established at UZH).
(2) Experiments in paraffin-embedded patient samples for HER2 staining with DARPins have previously shown the enormous importance of affinity of these monovalent reagents (work carried out at UZH). This makes it very practical to create bispecific reagents for the very heterodimers deemed important from the results of task 1.2 and 1.3, as well as for WP2. This tailor-made creation of bi-specific reagents is straightforward with DARPins but would be extremely difficult with standard IgGs.

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	UCL	2.00
2	UZH	96.00
5	MPG	18.00
7	INO	5.00
	Total	121.00

List of deliverables

Delive-rable Number [61]	Deliverable Title	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
D1.1	High affinity DARPins to EGF-R, HER2, HER3, HER4	2	50.00	R	CO	25
D1.2	Measurement of HER pairings in relation to tumour outcome	2	71.00	R	CO	46
		Total	121.00			

Description of deliverables

D1.1) High affinity DARPins to EGF-R, HER2, HER3, HER4: [month 25]

D1.2) Measurement of HER pairings in relation to tumour outcome: [month 46]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead beneficiary number	Delivery date from Annex I [60]	Comments
MS1	Successful selection of DARPins to all 4 members of the HER family	2	24	Means of verification: FACS
MS2	Proximity ligation detects HER pairs in tumour cells	2	36	Means of verification: Assay
MS3	Combined TIS and FRET/FLIM analyses of archived cancer tissues	3	36	Means of verification: Steering committee decision
MS4	Combined medical imaging and tissue imaging-based diagnostics	3	36	Means of verification: Steering committee decision
MS5	Production of GMP anti-HER2 DARPins in compliance with EMEA cGMP regulations	1	18	Mean of verification: Product evaluation in respect to set product release criteria, validated during steering Committee

WT3:
Work package description

Project Number [1]	259881	Project Acronym [2]	ImagInt

One form per Work Package

Work package number [53]	WP2	Type of activity [54]	RTD
Work package title	Tools for a multivariate tumour invasion signature		
Start month	1		
End month	48		
Lead beneficiary number [55]	3		

Objectives

- To extend the recently established FRET/FLIM-based multivariate image signature that can predict metastatic relapse, by incorporating HER2 protein interaction and signalling events.
- To utilise DARPins to determine the distribution and clustering of HER2 on cultured breast cancer cells and clinical tissues with a resolution of better than 50 nm using STED nanoscopy.
- To apply toponome imaging system (TIS) to identify combinatorial molecular phenotypes (CMPs) that are linked to HER2 signalling
- To utilise the CMPs obtained to design high resolution fluorescence imaging assays based on FRET/FLIM and nanoscopy techniques.
- To correlate and integrate the functional response of HER2 protein interactions in tissues with that measured by FDG/FLT PET imaging, in a neoadjuvant setting

Description of work and role of partners

Task 2.1. Establishing a multiparametric tissue imaging-based metastatic signature M1-M33
Partners involved: KCL (Ng)
Building on KCL recent success in deriving a FRET/FLIM-derived biomarker that can classify individual cancer patients for prognostic assessment (Fig.4), KCL will validate and further improve on these tissue-based imaging biomarkers by incorporating two other major pathways, namely, A) the HER2/HER3 heterodimer and associated protein network (subtask 2.1.1); and B) Raf-1 which is a serine/threonine kinase which can link HER signalling to MEK/ERK signalling pathway. The concentration and activity of Raf-1 in cancer cells both contribute to resistance to chemotherapeutics such as doxorubicin (145). In addition, in EGF-stimulated MDA-MB-468 cells, Raf-1 has been shown by the Ng laboratory to associate with and hence inhibit the pro-apoptotic action of the Rho effector kinase, Rok☐ (146). For the Raf-Rok☐ pathway we will perform the following tissue-based optical imaging assays that are established in the Ng laboratory: i) Raf-1 protein expression; ii) the concentration of GTP-bound form of RhoA. Besides being modulated by Raf-1, Rok☐ is known to be a downstream effector of RhoA. The assay will be based on the binding of a recombinant Rho-binding domain (RBD) of Rhotekin (GFP-tagged) to FFPE tissues to reveal Rho-GTP (147). The concentrations of both Raf-1and GTP-RhoA will then be correlated with the surrogate markers of Rok☐ activity in patient tissues, as measured by iii) the amount of ERM (ezrin-radixin-moesin) phosphorylated at the conserved threonine residue in the COOH-terminus; and iv) the amount of phosphor-Ser 3 cofilin (148). Apart from archived breast cancer tissues (for deriving tools for prognostication), KCL will apply their technologies and tools to breast cancer tissues obtained from major European prospective studies (e.g. Neo-ALTTO, Neo-Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization, as agreed by Professor José Baselga).
Subtask 2.1.1. Dimerisation assay of endogenous HER2/HER3 M1-M18
To set up, optimise and perform a FRET/FLIM-based endogenous HER2/HER3 dimerisation assay using a donor fluorophore-labelled HER2 DARPin in combination with an acceptor fluorophore-anti-HER3 antibody on i) breast cell line models and ii) archived breast cancer tissues.
Subtask 2.1.2. Measurements of Raf-Rok☐ pathway by optical imaging M1-M12
To apply tissue-based optical imaging assays for measuring the Raf-Rok☐ pathway, to archived breast cancer tissues.
Subtask 2.1.3. Improvement of multivariate tumour invasion signature M12-M30

To incorporate the HER2/HER3 interaction and Raf-Rok□ assays into the existing ezrin/PKC-based multivariate tumour invasion signature plus validation using the same cohort of archived breast cancer tissues, and the Neo-ALTTO pre- and post-neoadjuvant samples.

Task 2.2. Toponomics M1-M48
Partner involved: TNL
Informative tag libraries for TIS have been built in Schubert's lab since 1990. Most of these tags are directed against cell surface molecules. In the present project we will start with the library we used in our recent work on prostate cancers (55), then extend this library by the one previously published (52), with an eventual aim of extending this library to several hundred cell surface molecules. The rational is that (i) it is clear that different cell types often use the same molecules at the cell surface, but in a cell type specific way, by generating cell type and disease specific new orders by them: specific protein clusters and specific spatial arrangements of these clusters (metaphor: different toponome sentences). Finding these cell type and disease specific orders for e.g. HER2+ve cancer cells will be the first essential step towards new diagnostic and therapeutic approaches.
Subtask 2.1. Creation of a tag library M1-M8
First, the informative tag libraries that have already been established and examined earlier for cell type specific cell surface and intracellular toponomes (more than 100 tags) will be calibrated for breast cancer cells related to tag concentration, and tag position in the library by random positional permutation as described (52).

Subtask 2.2. Exploration of HER2-associated protein network architectures and their protein cluster hierarchies M9-M24
The calibrated library will be used to map the cell surface toponome of breast cancer cells by inclusion of HER2-binding tags (several monoclonal antibodies recognizing different domains of HER2). The resulting Toponome data sets will be analysed for their hierarchical properties using MoPPi software (above) to find the protein clusters and their lead proteins, leading to clear cut information, whether HER2 itself is a lead protein, or, associated with other higher order lead proteins exerting control over HER2. The software presently used routinely for the analysis of toponome data is termed Modular Processing Pipeline (MoPPi) (55).

Subtask 2.2.3 Creation of a breast cancer database M20-M48
On the basis of identified HER2 Toponome a breast cancer toponome data base will be constructed that contains image information on the location of the corresponding subcellular toponomes and the hierarchies of proteins and their topology to be explorable interactively by users.

Task 2.3. Design of new FRET/FLIM assays based on toponomics M24-M33
Partners involved: KCL, TNL
The resulting HER2 toponome will contain information on lead proteins associated with HER2. These identified proteins will be delivered to WP2 FRET/FLIM specialists so that they can study the possible direct interaction of these candidate proteins with HER2 in all molecular and functional detail.

Task 2.4. Nanoscopy M19-M48
Partner involved: MPG
Nanoscopy is a highly dynamic research field of light microscopy that overcomes the resolution limit of light (51, 149,150). STED nanoscopy (50, 151) will be used to map the distribution and clustering of HER2 and related proteins in intact cells on the nanoscale. Protein distributions in this size regime have until now not been exploited as a potential prognostic tool.
Subtask 2.4.1. Optimisation of nanoscale imaging M19-M24
To set up, optimise and perform imaging with sub-50 nm resolution on different cultured breast cancer cell line models delivered by the partners using DARPins and antibodies against HER2 and related proteins.
Subtask 2.4.2. Characterization of instrumental parameters and potential limitations M19-M30
Set up nanoscopy on patient breast cancer tissues to enable the analysis of the distribution and clustering of HER2 and related proteins on tissues. Detailed characterization of the use of DARPins for imaging on tissues. Detailed characterization of the instrumental parameters required for nanoscopy on breast cancer tissues and analysis of the potential limitations.
Subtask 2.4.3. Distribution and clustering of HER2 on nanoscale M24-M48
Systematic imaging of the distribution of HER2 and other proteins defined by Task 2.2 on cultured cells or patient tissue using DARPins and antibodies to provide a statistically significant readout of their distribution and clustering on the nanoscale. The data will be made available to the partners.

Task 2.5. Combination of medical imaging (PET) and tissue imaging-based diagnostics M1-M36
Partner involved: KCL, TNL, MPG

Whole body imaging (e.g. PET-CT) allows whole body localisation and quantification of contrast agents (e.g. fluorodeoxyglucose, 18F-FDG and fluorothymidine, 18FLT) uptake but suffers from a lower sensitivity and spatial resolution in comparison with optical imaging. We will combine the high resolution tissue imaging techniques, offering molecular markers, with clinical bioimaging methods; a combination which will be truly complementary. It is expected that an integrated analysis of the datasets obtained for each patient will allow us to derive a new generation of biomarkers that can predict response to molecule-targeted treatments and have the potential for clinical validation and exploitation. We will establish a phase II neoadjuvant study on ☐ 40 HER2-positive patients with locally advanced breast cancers. Patients will receive sequential taxane / trastuzumab x 4 cycles followed by EC (epirubicin, cyclophosphamide) x 4 cycles. Recent studies have identified that a threshold of 45% decrease in SUV (Standardized Uptake Value) identified responders in ☐73% patients (152). In this proposed study, primary disease will be examined with FLT and FDG to assess the in vivo response characteristics. Tissue biopsies before and surgically obtained tissues (at 8 weeks) after neoadjuvant treatment will be analysed using the FRET/FLIM metastatic signature.

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	UCL	5.00
2	UZH	3.00
3	KCL	43.00
4	TNL	40.00
5	MPG	30.00
	Total	121.00

List of deliverables

Delive-rable Number [61]	Deliverable Title	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
D2.3	Nanoscopy imaging of HER2 and related proteins	5	25.00	R	CO	36
D2.4	Methods for FRET/FLIM-based HER2/HER3 dimerisation and optical imaging of Raf-Rok☐ pathways	3	24.00	R	CO	12
D2.5	Identification of HER2-associated toponome protein clusters	4	26.00	R	CO	16
D2.6	Imaging-based metastatic signature established and compared with FDG and/or FLT PET-CT	3	23.00	R	PU	36
D2.7	Full Toponomic analysis of breast cancer	4	23.00	R	CO	46
		Total	121.00			

Description of deliverables

D2.3) Nanoscopy imaging of HER2 and related proteins: [month 36]

D2.4) Methods for FRET/FLIM-based HER2/HER3 dimerisation and optical imaging of Raf-Rok☐ pathways: [month 12]

D2.5) Identification of HER2-associated toponome protein clusters: [month 16]

D2.6) Imaging-based metastatic signature established and compared with FDG and/or FLT PET-CT: [month 36]

D2.7) Full Toponomic analysis of breast cancer: [month 46]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS3	Combined TIS and FRET/FLIM analyses of archived cancer tissues	3	36	Means of verification: Steering committee decision
MS4	Combined medical imaging and tissue imaging-based diagnostics	3	36	Means of verification: Steering committee decision
MS6	Successful completion of Phase I/II clinical	1	42	Means of verification: Clinical Trial report validated

WT3:
Work package description

Project Number [1]	259881	Project Acronym [2]	ImagInt

One form per Work Package

Work package number [53]	WP3	Type of activity [54]	RTD
Work package title	Developing methods for isolation characterization of protein/RNA complexes from clinical tissues		
Start month	1		
End month	46		
Lead beneficiary number [55]	7		

Objectives

- To use anti-Ago DARPins as tools for IP of RISC and visualisation of P-bodies
- To develop robust and cost effective tools for IP/sequence technology of interacting miR/mRNA/proteins in RISC
- To test the tools for biomarker potential by application to pre and post trastuzumab-treated BT474 xenografts and uman tissues.

Description of work and role of partners

Task 3.1. Isolation of RNA/protein complexes using anti-Ago DARPin in the INNOPROT technology M1-M10
Partners involved: INO, UZH, UCL
This task is divided into 2 subtasks.

Subtask 3.1.1. Synthesis of Ago protein common region and anti-Ago DARPins M1-M6
Partners involved: INO, UZH
The Ago protein family is well conserved protein family part of RISC complex constituted by 4 members. INO will design the common region to the Ago proteins and produce the proteins that will be used by UZH to select anti-Age DARPIns.

Subtask 3.1.3. Use of anti-Ago DARPin to pull down the RNA/protein complex M6-M10
Partners involved: INO, UCL
The cells cultured in 6 well plates will be washed with cold PBS three times and lysed with 0.5 ml of Innoprot buffer at 4ºC. This buffer mimics in vivo conditions that allow to maintain the RNA-protein complexesand permit to lysate the cell membranes In parallel, the anti-Ago DARPins will be incubated with Dynabeads for 1 hour at room temperature to form the Dyanbed-anti-Ago DARPin complex (catching complex). The catching complex will be incubated with the cell lysis mixture at 4º C overnight to consolidate the union between catching complex and RISC/miR complex. The whole complex will be pulled down by a high speed centrifugation step under conditions that stabilise and maintain unity of the catching complex and their captured proteins. Then the Dynabeads-Ago complex union will be broken using a elution buffer and the miRs, mRNA will be characterized by deep sequencing (SOLEXA® Technology) at UCL and the proteins by MALDI-TOFF (AB SCIEX TOFF/TOFF 5800). The parameters measured will be: (i) miR, (ii) the targeted mRNA, (iii) the proteins forming the RISC complex, and (iv) the amount of the proteins which are forming the RISC.

Task 3.2. Isolation of RNA/complexes using Ago-2 tagged technology M1-M12
Partners involved: INO, UCL
Ago-2 protein will be cloned followed with a small tag such as Strep. The Ago-2 protein is the most common Ago member of the Ago family and more active which is the responsible of the mRNA targeting and catalytic process. The Ago-2 protein will be cloned in two different manners: (i) native form and (ii) catalytic region truncated form. The Ago-2 protein has a very high catalytic capacity and in order to avoid possible RNA degradation in the complex a catalytic domain will be truncated. This manipulation of the native structure could be critical. For this reason, an innate structure also will be cloned and both forms will be used and compared.
Then the two tagged Ago-2 sequences will be transfected in BT474 HER2+ breast cancer cell lines. The procedure is the traditional method of antibiotic cell selection (154), after transfection, and following screening of well transfected region and viability assay.

The cells will be cultured and lysed with 0.5 ml of Innoprot buffer at 4ºC. The lysed extract will be passed through biotin column to catch the micro RNA/ RISC complexes. Then the complexes will be eluted and the extracts will be analyzed. The RNA (miR and mRNA) will be deep sequenced (SOLEXA ® Technology) and the protein will be characterized by MALDI-TOFF (AB SCIEX TOFF/TOFF 5800). The measured parameters will be similar to that in the previous task.

Task 3.3. CLIP technology to isolate the miR/RISC complex M1-M10
Partners involved: INO, UZH, UCL
The DARPins developed in the task 1.1 and task 1.2 will be used to synthesize anti-Ago DARPins (M1-M6) that will be used for CLIP technology to isolate miR/RISC complexes isolation (M6-M10) (81). Therefore, the cells will be cultured in 6 well plates and irradiated to consolidate the RNA/protein unions. Then the cells will be lysed. In parallel the anti-Argo DARPins will be incubated with Dynabeds. The Ago-Dynabeds complex will be incubated with the lysed extract to catch the miR/RISC complex. After the incubation the complexes will be isolated by high speed centrifugation step. Then the complexes will be eluted and the miRs and mRNA will be sequenced by deep sequencing and the proteins will be analysed by mass spectrometry.

Task 3.4. Comparison of t technologies M12-M16
Partners involved: INO, FLS, KCL
Different technologies will be tested and data will be compared using the combinatorial analysis of FLS-KCL developed analysis technologies to obtain the most efficient one.

The differences between three technologies are the following:
In the first technology the small size of the DARPins allows the attachment of more molecule to the beads than can be achived with antibody.

In the second technology it could be possible to clone an Ago protein with the catalytic region truncated, by this way the Ago-RNA in the RISC complex would be more stable and catalyze less RNA breakdown.

In both technologies we use Innoprot buffer that mimic the in vivo conditions and permit avoid the irradiation step to crosslink the RNA/protein unions. The crosslinking process is performed by an irradiation step that can affect the different molecules of the cell. This irradiation step is necessary in the third technology and INO aim to avoid it with the first option.

Task 3.5. P-bodies study during RISC formation complex. M1-M10
Partners involved: INO, UZH

Partners involved: INO,KCL, FLS
The labelled anti-Ago will be used to determine the location of the complexes in the cell. The labelled anti-Ago DARPin will be used to determine the co-localization of the RISC complexes with the P-bodies proper protein GW182 labelled with another fluorochrome. INO will be used its own automated image platform (Pathway BD 855) that allows to work with confocal microscopy. The number and size of P-bodies containing Ago like RISC member will be measured and the data will be analysed to check if it could be a biomarker.

Task 3.6. Transfer the microRNA/mRNA/protein isolation and characterization technology for the antiHER2 treatment cases M16-M24
Partners involved: INO, UCL
In a first step, the BT4T4 HER2+ positive cells will be treated and non-treated in xenografts with trastuzumab like antiHER2 therapy by INO (M1-M6). This is a contrasted model for the breast cancer antiHER2 responsiveness treatment. Then, the technology developed during task 3.1, 3.2, 3.3 and 3.4 will be applied to isolate and identify the deregulated RNA and proteins in the case of antiHER2+ treatment by INO (M18-M24).

Task 3.7. Transfer the RISC location technology to antiHER2 treated cells M11-M14
Partners involved: INO
Similar steps used for task 3.6 will be applied for transferring the RISC location technology to antiHER2 treated cells. In a first step, the BT4T4 HER2+ positive cells will be treated and non- treated with trastuzumab like anti HER2 therapy (M11-M12). This is a model for e breast cancer antiHER2 responsiveness treatment. In a second step, RISC location technology will be applied to antiHER2 treated cells based on the achievement of task 3.5 to isolate and identify the deregulated RNA and proteins in the case of antiHER2+ treatment (M12-M14).

Task 3.8. The new biomarkers finding process by antiHER2 responsiveness therapies M24-M30
Partners involved: INO, FLS, UCL, TNL

The data obtained during task 6 and 7 and in the toponomic study will be analysed using the combinatorial analysis technology developed by FIR and KCL (Coolen group) in the WP6. The inputs for the study will be: (i) identified miRs (ii) amount of identified miRs, (iii) identified mRNAs (iv) amount of identified mRNAs (v) protein identified in the RISC complex (vi) amount of protein identified in the RISC complex, (vii) Ago location (viii) protein deregulated identified in the WP2.

Task 3.9 Transfer the technology to the human samples M31-M40
Partners involved: INO, KCL, UCL, FLS
The final task is focused on transferring the technology developed through the WP3 to the human samples. The human frozen samples will be provided by UCL and KCL. The human frozen tumour samples will be dissected to obtain the tumour cells using laser capture microscope (Zeiss Palm microbeam form UCL). Then, the in situ hybridization for colocalization of P-bodies with Ago proteins will be performed in these samples and whole the data will be analysed with the technology developed by FLS and KCL.

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	UCL	4.00
2	UZH	3.00
4	TNL	4.00
7	INO	36.00
8	FLS	60.00
	Total	107.00

List of deliverables

Delive-rable Number [61]	Deliverable Title	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
D3.8	Analysis of Ago proteins in P-bodies and establishment of RISC-miRNA technologies	7	52.00	R	CO	28
D3.9	Application of RISC-miRNA technologies to breast cancer tissues	7	55.00	R	CO	46
		Total	107.00			

Description of deliverables

D3.8) Analysis of Ago proteins in P-bodies and establishment of RISC-miRNA technologies: [month 28]

D3.9) Application of RISC-miRNA technologies to breast cancer tissues: [month 46]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments

WT3:
Work package description

Project Number [1]	259881	Project Acronym [2]	ImagInt

One form per Work Package

Work package number [53]	WP4	Type of activity [54]	RTD
Work package title	Development of radiolabelling and software for quantum imaging		
Start month	1		
End month	42		
Lead beneficiary number [55]	6		

Objectives

- Optimization of chemical procedures for purification of radionuclides
- Optimization of radiolabelling technology for DARPins.
- Validation of DARPin imaging agents in vitro in spheroids, human tissue and xenograft mice models
- Optimization of conditions for DARPin imaging of HER2 by concentration dependent studies of 67Ga and 68Ga using SPECT and PET. Validated imaging agents will be used to monitor Herceptin treatment together with WP3.
- Development of QSPECT for the clinical trial in WP5

Description of work and role of partners

Task 4.1. Further development of purification procedures for radionuclides M1-M15
Partners involved: UU, UCL, GEHC
Protocols for the purification of Ga3+ and In3+ will be developed by UU/GEHC using 68Ga from a 68Ge generator and commercially available 67Ga and 111In. The purification will be based on further optimization of conditions for anion chromatography with regards to Cl- concentration and pH. It has been shown by Långström that dissolution of the radioactive product from a 68Ge-generator in 5 M HCl leads to complexation of Ga3+ to form the anionic 68Ga(Cl)4- that can be trapped on an anion exchange resin, whereas several other cationic contaminants do not form anionic complexes to any great extent, and are separated. (WP4_1) Fe3+ still remains a problem due to the similarity of its complexation chemistry to that of Ga3+. Complexation by Cl- is concentration as well as pH dependent and the anionic chromatography will be optimized by variation of salt and pH followed by the determination of the specific radioactivity (SRA). Optimization of salt and pH will be attempted also for the removal of Fe3+, but in addition the use of reduction agents to generate Fe2+ will be tested. Release from the anionic resin provides for high concentration of Ga3+. The protocols developed for 68Ga will be applied to the purification of commercially available 67Ga, and validated by determination of SRA. Protocols for the purification of In3+ will be developed in parallel by UU/GEHC using broadly the same strategy and commercially available In111. The technology platform for labelling will be delivered to UCL.

Task 4.2. Development and biological validation of protocols for labelling technology M1-M15
Partners involved: UU, UZH, UCL, GEHC

Subtask 4.2.1. Development of protocols for the conjugation of DOTA chelators to DARPins M1-M15
Protocols for the conjugation of DOTA chelators to DARPins will be developed by UU. DARPins with a single Cys residue introduced at the N- or C- terminus by site-directed mutagenesis will be delivered by UZH (WP1) for development of coupling conditions. DOTA chelators equipped with PEG-type spacers and maleimide end groups that react specifically with thiols will be purchased from commercial suppliers. The DOTA derivative will be reacted with the DARPin in aqueous buffer to form a DARPin-DOTA conjugate, and the reaction will be monitored my MALDI-TOF MS to identify reaction conditions where the reaction is quantitative or near quantitative with regards to DARPin, since the separation of DARPin from labelled DARPin is expected to be more difficult than the separation of labelled DARPin from DOTA-PEG-maleimide reagent. Care will be taken to find commercially available reagents with high purity to ensure that the reaction will be reproducible. The reaction solvent, the reaction time and the concentrations of DARPin and reagents will be varied to find optimum conditions. Purification will be carried out by reversed phase HPLC and the identity of the reaction product will be established by MALDI-TOF MS. When the protocol has been developed a DARPin that specifically binds HER2

will be labelled and delivered to (UCL) for determination of affinity for HER2 by Biacore. A spacer length will be chosen that has negligible effect on affinity. The final protocol will be delivered to UCL.

Subtask 4.2.2. Optimisation of the incorporation of radionuclides M6-M15
The incorporation of radionuclides will be optimized by UU using microwave heating for fast incorporation of Ga3+ into the DOTA chelate, conjugated to a DARPin provided by UZH. It has been shown by Långström that the amount of chelator to be labelled can be reduced when microwave heating is employed and that it is likely that the SRA is increased. (102,103) DARPins are extremely stable proteins and can sustain conventional heating to 85oC for 20-30 min and even those that denature will reversibly refold upon cooling. It is expected that DARPins will be amenable to microwave assisted heating for the time required to achieve 90% labelling with radionuclide. Reaction conditions for the incorporation will be optimized with regards to temperature, reaction volume and heating time and yield and SRA after labelling determined. Protocols for radiolabelling of DOTA conjugated DARPins will be delivered to UCL, where also the affinity for HER2 of the labelled DARPin will be determined using Biacore. The incorporation of a Ga3+ will charge neutralize the DOTA and may have a pronounced effect on the interactions of the DOTA group.

Subtask 4.2.3. Validation DARPins radiolabelling M12-M15
Validation of DARPin characteristics will be performed by UU/GEHC in spheroids, human tissue and xenograft mice models. Radiolabelled DARPin will be injected several times and the biodistribution recorded, using 68Ga and animal PET. The use of PET is justified by the accessibility of a 68Ge generator in house, that will considerably simplify all procedures related to radiolabelling. Results from validation will be transferred to UCL as supporting information for labelling protocols.

Task 4.3 Development of improved QSPECT imaging for 67Ga/111In M1-M18.
Partners involved UCL, GEHC
Monte Carlo models of data acquisition for 67Ga and 111In for the General Electric (GE) Discovery MN/CT 670 gamma camera and appropriate collimator will be developed and verified with list-mode data from the camera. This model will be used for determination of optimal spectrum settings for acquisition windows and parameters for TEW scatter compensation for QSPECT. In collaboration with GE, using their OSEM reconstruction software and Evolution toolkit for resolution recovery a QSPECT image reconstruction protocol and set of reconstruction parameters will be developed. This method will be validated in a series of radioactive phantom experiments. Standard operating procedures for data acquisition and image reconstruction in the DARPin clinical trial will be written determination of optimal spectrum settings for acquisition windows and parameters for TEW scatter compensation for QSPECT. In collaboration with GE, using their OSEM reconstruction software [ref] and Evolution toolkit for resolution recovery a QSPECT image reconstruction protocol and set of reconstruction parameters will be developed. This method will be validated in a serie of radioactive phantom experiments. Standard operating procedures for data acquisition and image reconstruction in the DARPin clinical trial will be written.

Task 4.4 Optimization of DARPin imaging agents in spheroids and mice M16-M42
Partners involved: UU,GEHC, UZH, UCL

Concentration dependence and kinetics of signal intensity and localization will be determined in spheroids of appropriate cell lines, human tumour tissue and xenografts in mice in order to identify under what conditions the best sensitivity for HER2 receptor imaging is obtained. High affinity does not necessarily correlate with long residence time and radiotracer concentration will influence not only receptor population but also competing biological processes. Dual PET/SPECT/CT imaging with the PET tracer 68Ga and the SPECT tracer 67Ga will be carried out in order to follow the time course of binding with high signal to noise as a function of concentration. It is expected that the SRA achieved will allow us to measure at very low concentration and identify conditions that provide the best signal to noise for imaging using DARPin imaging agents for HER2. The necessity for multiple measurements to obtain good statistical accuracy and reproducibility makes this task labour intensive and time consuming. It is therefore necessary to allow this task to run in parallel with clinical trials based on SPECT/CT. However, the outcome of the work will make it possible ultimately to decide on the optimum strategy for HER2 labelling using DARPins and is thus an important deliverable of WP4. In order to compare the performance of the small DARPins to antibody technology even smaller peptides with an affinity for HER2 will be radiolabelled and evaluated with regards to affinity and residence times. The imaging agents will be validated for monitoring of HER2 expression under Herceptin treatment in collaboration with WP3.(98,99,105) and efficacy measurements validated in combinations with golden standards like 18FDG.

WT3:
Work package description

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	UCL	5.00
2	UZH	12.00
6	UU	90.00
9	M-GMP	5.00
	Total	112.00

List of deliverables

Delive-rable Number [61]	Deliverable Title	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
D4.10	DARPins radiolabelled and tested in in vitro and in vivo	6	42.00	R	CO	18
D4.11	Development and evaluation of SPECT/PET imaging	1	70.00	R	CO	42
		Total	112.00			

Description of deliverables

D4.10) DARPins radiolabelled and tested in in vitro and in vivo: [month 18]

D4.11) Development and evaluation of SPECT/PET imaging: [month 42]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS6	Successful completion of Phase I/II clinical	1	42	Means of verification: Clinical Trial report validated

WT3:
Work package description

Project Number [1]	259881	Project Acronym [2]	ImagInt

One form per Work Package

Work package number [53]	WP5	Type of activity [54]	RTD
Work package title	Phase I trial to assess safety and efficacy of quantitative imaging biomarkers in patients		
Start month	1		
End month	42		
Lead beneficiary number [55]	1		

Objectives

• To perform GMP manufacture and product analysis of an anti-HER2 DARPin with a Cys tag for chelate attachment
• To establish the safety of DARPins in man
• To test the potential of radiolabelled DARPins for detecting HER2+ve tumours in patients using Quantitative Single Photon Emission Computed Tomography (QSPECT) imaging
• To collect and characterise circulating tumour cells (CTCs) from patients with breast cancer.

Description of work and role of partners

Task 5.1: Manufacture and analysis of GMP DARPin M1-M18
Partners involved: M-GMP, UZH, UCL, UU/GE, KCL
This task is divided into 3 subtasks.

Subtask 5.1.1: Development of GMP process M1 – M6
Process development will be performed by M-GMP using E. coli GMP seed lot of the cys-tagged anti-HER2 DARPin that will be generated and validated by UCL prior to the start of the project. The process will be based on established E. coli expression protocols provided on M1 by UZH. A robust fermentation method will be developed by performing a series of bench scale studies using certified raw materials. Once a process is established, 3 bench scale batches will be produced to show consistency and robustness of the fermentation method. For downstream processing, a purification strategy will be developed to optimise recovery of the product and remove impurities while maintaining product stability. To this effect, clarification studies will be investigated to maximise cell debris removal. Affinity chromatography will be developed for product capture. Size exclusion chromatography/endotoxin removal will be investigated for final product polishing. Once established, the upstream and downstream procedures will be scaled up as pre-GMP manufacturing processes, 3 x 15L production runs will be performed to allow process performance to be characterized and to set parameters for GMP manufacture/release criteria. The purified products from the development process will be tested for purity and identity (SDS-PAGE, mass spectrometry), stability (in different buffers and temperatures; -80°C, -20°C, 2-8°C, 25°C, 37°C) antigen binding (BiaCore), size exclusion chromatography (purity and aggregation) and N-terminal sequencing (physicochemical). The products generated during M2-M6 will be transferred to UU for development of radiolabelling technology.

Subtask 5.1.2 GMP production of cys-tagged anti-HER2 DARPin M6-M12
3 x 15L GMP production runs (fermentation/purification) will be performed by M-GMP using the processes developed in subtask 5.1.1. Standard product release criteria – e.g. sterility, purity, endotoxin, will be applied and biological release criteria (e.g. antigen binding) will be established with M-GMP, UCL and UU based on tests developed in subtask 5.1.1 Standard operating procedures (SOPs) will be generated for these tests. The product will be vialled (250 x 5mg aliquots). 30 vials will be used for toxicity testing and stability, 120 x 5mg aliquots will be transferred to UCL for Phase I/II trial studies and analysis. 20 x 5mg aliquots will be transferred to UU for development of radiolabelling technology.

Subtask 5.1.3. Analytical characterization, stability and toxicity test M12-M18
Analytical characterization studies, stability and toxicity testing will be performed on the vialed product in line with regulatory guidelines. Routine product quality control tests will be developed from the SOPs generated in

Task 1.2 to establish biological activity, purity and quantity. Qualification reports will be generated. Preclinical safety evaluations will be conducted in compliance with ICH guidelines S6 (Preclinical Safety Evaluation of Biotechnology-Derived Products) and S9 (Nonclinical Evaluation for Anticancer Pharmaceuticals). Where appropriate advice will be sort from the MHRA on the suitability of animal model(s), given the nature of the compound and its target/mechanism of action.

Task 5.2. Collection and Storage and CTCs M1-M42
Partners involved: UCL

Subtask 5.2.1 Ethical Approval M1-6
Ethical approval will be obtained using IRAS (Integrated Research Application System) to perform the Phase I/II trial and collect CTCs which will be stored at the Royal Free Hospital Tissue Bank. CTCs will be analysed with tools developed in WP1, WP2 and WP3 to detect interacting molecules.

Subtask 5.2.2. Circulating tumour cell CTCs M12-M42
The circulating tumour cells (CTC) population will be investigated using a CellSearch™ System (Veridex Ltd), which is FDA approved to enumerate CTCs and in the EU approved under the In Vitro Diagnostic Medical Devices Directive (IVD Directive 98/79/EC). In a semi automatic manner CTC are enumerated from peripheral blood, using an antibody to the epithelial cell adhesion molecule (EpCAM) in an immunomagnetic capture process. The instrument has three defined channels to establish true CTCs, which are defined as: cytokeratin positive (channel 1), nucleated cells (channel 2) and CD45 negative (channel 3, to exclude EpCAM cross reactive leukocytes). Channel 4 is user defined and the IMAGINT consortium will exploit this to detect specific HER family members and or member pairs. In addition, capured cells will be analysd with the CellSearch Profile Kit and, after capture will be prepared with OCT medium (embedding gel for cutting frozen tissue sections), snap-frozen in iso-pentane (cooled in liquid Nitrogen) and stored at -80C in the UCL biobank. When required, frozen pellets of OCT (containing CTCs) will be cut as 5-10μm cryostat sections and used for application of IMAGINT tools to study: HER dimers, RISC componants and molecular networks (Toponome). Note: Contamination of the CTC sample with a few leukocytes can be easily distinguished from CTCs by using a pan-leukocyte marker.

Task 5.3: Phase I/II M12-M48

Partners involved: UCL

Subtask 5.3.1. Development and submission of the Phase I/II protocol M12-M18
A Phase I/II protocol and patient information sheet will be developed and submitted for MHRA, ARSAC, R&D approval. Ethical approval and sponsorship will be applied for and obtained to allow recruitment of patients with metastatic breast cancer to enter the trial defined by the protocol. Data from the mouse imaging and biodistribution work obtained from WP4 will be used to design the protocol for a Phase I/II trial of anti-HER2 DARPin imaging. The preclinical tissue biodistribution and PET/SPECT-CT will inform a model of the biodistribution time course in man. This model will be used to estimate the cumulated activity per Bq injected (“residence time”) for 111In and 68Ga, for whole body, kidney and any other organs identified as being of interest for radiation dosimetry in the preclinical work. The Olinda dosimetry package will be used to predict the radiation doses for patients entering the clinical trial. This data will form the basis for an application for ARSAC approval for the trial. The trial protocol will define gamma camera acquisition of data in dynamic, whole body and SPECT modes as appropriate. Initially this will be decided from the preclinical clearance data; once initial data in man has been collected the types and timings of data collection in the trial will be reassessed and revised if appropriate. The resolution recovery software developed in WP4 will be employed in the processing of the acquired data to provide time course data for the DARPins and images of anti-HER2 DARPin distribution. Distribution images will be combined with co-registered CT images display and assessment of diagnostic utility.

Subtask 5.3.2. Validation of ELISA assay M12-M18
A validated ELISA assay will be established to measure any pre-existing human anti DARPin antibodies (HADA) or HADA produced in response to treatment.

Subtask 5.3.3. Immunoassay and imaging trial M18-M42
Patients with HER 2 +ve metastatic breast cancer assessable by conventional imaging (MRI or CT) with performance status 0/1, and satisfactory organ function will be invited to enter the study. HER2+ status will be confirmed on the primary breast tumour by FISH (HER2+ = gene amplification >2.2). Serum HER2 ECD levels will be determined using an immunoassay (Bayer Healthcare ADVIA Centaur® test station). The assay is based

on a sandwich chemiluminescence immunoassay using two monoclonal antibodies directed against the ECD of the HER2 antigen method and is FDA approved for follow-up and monitoring of patients with metastatic breast cancer. Patients with a history of atopic asthma/eczema, human antiDARPin antibody (HADA), or a positive response to an intradermal injection of a test dose of DARPin will be excluded. The anti-HER2 DARPIn will be radiolabelled with 111In using methods developed by UU/GEHC (WP 4). The radiolabelled DARPin will be administered i.v. and in vivo distribution will be measured using QSPECT combined with 16 slice computed tomography (CT) using software developed by GEHC/UCL in (WP 4). Q-SPECT will be performed at 4 time points over 6 days. The trial (M18 - M42) will determine the toxicity, immunogenicity, pharmacokinetics and recommended dose (threshold for detection by Q-SPECT) of the radiolabelled DARPin. The starting dose will be based on NOAEL (no observable adverse effect level) using data from preclinical studies and dose escalation will be informed by pharmacokinetic data and performed according to a standard 3+3 dose escalation strategy. To measure immunogenicity of the new imaging agent, HADA assays will be performed on blood samples taken 7, 14 and 28 days post treatment. It is estimated that 20 patients will be studied in the Phase I/II trial.

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	UCL	72.00
6	UU	6.00
9	M-GMP	9.00
	Total	87.00

List of deliverables

Delive-rable Number [61]	Deliverable Title	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
D5.12	GMP DARPin procedures, copies of ethical approval and clinical trials protocol	1	45.00	R	CO	18
D5.13	Data from HER2 imaging clinical trial	1	42.00	R	CO	42
		Total	87.00			

Description of deliverables

D5.12) GMP DARPin procedures, copies of ethical approval and clinical trials protocol: [month 18]

D5.13) Data from HER2 imaging clinical trial: [month 42]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS5	Production of GMP anti-HER2 DARPins in compliance with EMEA cGMP regulations	1	18	Mean of verification: Product evaluation in respect to set product release criteria,

WT3:
Work package description

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead beneficiary number	Delivery date from Annex I [60]	Comments
				validated during steering Committee
MS6	Successful completion of Phase I/II clinical	1	42	Means of verification: Clinical Trial report validated

WT3:
Work package description

Project Number [1]	259881	Project Acronym [2]	ImagInt

One form per Work Package

Work package number [53]	WP6	Type of activity [54]	RTD
Work package title	Data management, integrative Bayesian analysis of data derived from preclinical and clinical studies		
Start month	1		
End month	48		
Lead beneficiary number [55]	3		

Objectives

- To quantify the clinical prediction potential of the extended FRET/FLIM-based multivariate image signature, incorporating HER2 protein interactions and signalling events, as generated within WP2, using conventional Bayesian binary classifiers and self-organizing maps.
- To develop and implement numerically an integrated mathematical method for nonlinear Bayesian pattern detection (with built-in dimensionality selection of latent variable spaces), visualization, and Bayesian prediction.
- To quantify the clinical prediction potential of the extended FRET/FLIM-based multivariate image signature, incorporating HER2 protein interactions and signalling events, as generated within WP2, using the newly developed integrated mathematical and computational tool.
- To develop rational methods for predicting candidate biomarkers from toponome (protein and microRNA) and protein interaction data on HER2-related pathways, collected within WP2 and WP3, based on new distance measures for nodes in graphs that take account of functionally relevant path multiplicities.
- Investigate statistical regularities between molecular biomarker and PET data before and after Trastuzumab and chemotherapy treatment, as generated within WP2, using the newly developed integrated mathematical and computational tool.
- Investigate statistical regularities and intrinsic dimensionality of signals in terms of `latent variables' for all clinico-pathological data, tissue-based toponome cluster and molecular interaction data, and functional imaging data such as FDG/FLT, produced within Imagint, using the newly developed integrated mathematical and computational tool.
- To create a database for results which facilitates integration of the various elements of this proposal and which facilitates data sharing within the framework of ELIXIR, the EBI and international data resources

Description of work and role of partners

Task 6.1: Construct and manage a database for IMAGINT's biomedical data M1-M48
Partners involved: KCL, FLS
We will create a flexible data base for the storage of IMAGINT's accumulating biomedical data. It will be managed using the TRAC project management system. The TRAC system (www.trac.edgewall.org) allows for remote data access, either via a web browser or a terminal, and features authentication, revision control and time management.

Task 6.2. Construct a generalized version of the BGVLM model with latent variables, tailored to the analysis of data in biomarker discovery M1-M12
Partners involved: KCL, FLS
Starting from the recently proposed Bayesian Latent Variable Gaussian Process method (163,164), we construct a general mathematical method with which both to quantify the structure in different noisy biological or clinical data streams and to predict probabilistically from these streams variables related to disease progression and treatment response. The proposed formalism involves so-called `latent' variables, which can be interpreted as generalizations of the two abstract map coordinates in self-organizing maps; here, however, the number of required latent variables is not imposed beforehand but decided on the basis of the regularities of the input signals. Although a Gaussian process description lies at the core of the method (which allows for transparent parameter determination and regression), the Gaussian variables are subjected to nonlinear transformations. In the context of the present problem we will allow for Gaussian mixtures, to represent more faithfully the

discrete nature of some of the biomarkers to be used. We will pay significant attention to the probabilistic interpretation of the theory, so that in future it can be regarded as a generalization of the more conventional medial statistics methods (such as Cox regression), and be accepted by medical statisticians. We will also perform information-theoretic analysis of the new method, in order to interpret its outcomes (e.g. the extent to which different biomarker sets contain similar information) in terms of clear measures (e.g. bits). The end result will be a mathematically sound and practical statistical framework with which to detect the intrinsic dimensionality of candidate biomarkers, to quantify how much information they share, to quantify the information they contain on disease progression and treatment response, and to carry out the disease progression and treatment response predictions optimally and with precise measures of associate uncertainty. This first mathematical task will be mainly carried out by Coolen and co-workers.

Task 6.3. Numerical implementation of the generalized version of the BGVLM model M6-M18
Partners involved: FLS
The new comprehensive mathematical formalism developed under Task 6.21 will be implemented in user-friendly software. This task will combine the mathematical know-how of Coolen and coworkers in the field of advanced scientific c-programming with the extensive knowledge within FLS of biomedical software standards. Upon generation of the new software, FLS will carry out extensive testing on controlled synthetic data sets and benchmark the new tool against existing (commercial and open-source) software tools for biomedical data analysis.

Task 6.4. Application of existing Bayesian analysis tools to extended FRET/FLIM signatures
M12-M15
Partners involved: KCL, FLS
WP2 will produce extended FRET/FLIM-based multivariate image signatures, incorporating HER2 protein interactions and signalling events. We judge it prudent to first analyse these novel signatures with more conventional tools that have been used in the recent past (159) in a similar but more limited context. The present task will consist of applying conventional Bayesian binary classifiers and self-organizing maps, which can be compared to the results of [AC 4] and thereby serve as a valuable consistency test (since the new signatures contain those used in (159) as a subset, the observed prediction performance of the extended signature must be at least equal to that in (159).

Task 6.5. Application of generalized BGVLM method to extended FRET/FLIM signatures M15-M18
Partners involved: KCL, FLS
The extended FRET/FLIM-based multivariate image signatures, incorporating HER2 protein interactions and signalling events, will next be analysed with the new comprehensive mathematical formalism developed under Task 6.1. This would generate the most rigorous quantification of the prediction potential of the WP2 image signatures, including a ranking of biomarkers within the multivariate signature, detection of possible relations between the biomarkers, and detection of possible cooperativity between biomarkers in generating statistically significant predictions. Comparison with the results of Task 6.3 will furthermore provide a valuable comparative test of the potential and limitations of the new formalism, in comparison with previous methods, in a fully realistic biomarker setting (as opposed to in a context of synthetic data).

Task 6.6. Predicting candidate biomarkers from toponome (protein and microRNA) and protein interaction data M18-M24
Partners involved: FLS, KCL, TNL
Rational methods will be developed for identifying critical nodes in signalling networks, based on new `effective' distance measures for network nodes that take account of functionally relevant path multiplicities. The `effective' distances between any two nodes will be calculated analytically (as opposed to via numerical simulation) by carrying out appropriate weighted sums over all paths connecting the nodes (as opposed to relying on the shortest path only). Pilot studies have shown such calculations to be feasible. If used in subsequent clustering protocols and integrated with functional connectivity information (165), the effective distances give more detailed and biologically meaningful predictions of functional network modules and hence of functionally critical nodes. The mathematical tools obtained will be used to predict candidate biomarkers from toponome (protein and microRNA) and protein interaction data on HER2-related pathways, as collected within WP2 and WP3.

Task 6.7. Quantify anti-HER2 treatment effects on biomarker signatures M25-M28
Partners involved: FLS, KCL
The new comprehensive mathematical formalism developed under Task 6.1 will be used to investigate statistical regularities and statistically significant deviations between molecular biomarker and PET data before and after combined Trastuzumab-chemotherapy treatment, as generated within WP2. If statistically significant regularities

and deviations are detected, the results of our analytical tool will (in close collaboration with WP2 and WP3) be translated into suggested biomarker signatures from which to predict neoadjuvant treatment response and efficacy at the individual patient level.

Task 6.8. Quantify intrinsic dimensionality and cross-prediction of disjunct data sets M28-42M36
Partners involved: FLS, KCL, UCL,
We will use the new comprehensive mathematical formalism developed under Task 6.1 to
(a) calculate the statistical regularities and intrinsic dimensionalities of all clinico-pathological data, tissue-based toponome clusters and molecular interaction data, and functional imaging data such as FDG/FLT, as generated within Imagint,
and
(b) calculate for those data sets that relate to the same archive tissue material (i.e. toponome, microRNA and protein interaction data of WP2) quantitative estimates of the amount of information which the distinct data sources have in common, via comparison of the intrinsic dimensionalities of joint signatures to the sum of the dimensionalities of the individual signatures. The result will provide insight into how the distinct sources of data are combined optimally.

Task 6.9. Use of Standards and Construction of a Database for deposition of results and data sharing and dissemination M28-M48
Partners involved: FLS, KCL
ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines (GMP. GCLP and GCP) will be used for the preparation and conduct of clinical investigations.
Data from the experiments in IMAGINT will be collected with appropriate metadata using standards defined for the areas concerned such as those for protein structure and interactions encompassed within UniProt (www.uniprot.org) or for images within caImage https://cabig.nci.nih.gov/tools/caIMAGE. Appropriate databases will be identified using the ONIX search engine of the UK National Cancer Research Institute. Linkage between different types of data will be structured use Guidelines for Information about Therapy Expeiments (GIATE) www.genscript.com/giate-viewer. This is based on the use of concepts from the NCI Thesaurus and common data elements from the caDSR (https://cabig.nci.nih.gov/concepts/caDSR/). GIATE is constructed with a UML model which forms the basis for a database in which the diverse information acquired in IMAGINT can be recorded using compatible metadata. This facilitates the integration of data and the dissemination of data which can be identified by searches from outside using the well established concepts and common data elements specified. The system can also be adapted for access via the semantic web Resource Description Framework (RDF) World Wide Web Consortium (W3C) specifications.

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	UCL	5.00
3	KCL	45.00
4	TNL	2.00
5	MPG	6.00
7	INO	3.00
8	FLS	48.00
	Total	109.00

WT3:
Work package description

List of deliverables

Delive-rable Number [61]	Deliverable Title	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
D6.14	Establishment and analysis of database	3	58.00	R	CO	18
D6.15	Biomarker analysis and protocol for quantifying relations between data sets	8	51.00	R	PU	42
		Total	109.00			

Description of deliverables

D6.14) Establishment and analysis of database: [month 18]

D6.15) Biomarker analysis and protocol for quantifying relations between data sets: [month 42]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS3	Combined TIS and FRET/FLIM analyses of archived cancer tissues	3	36	Means of verification: Steering committee decision
MS4	Combined medical imaging and tissue imaging-based diagnostics	3	36	Means of verification: Steering committee decision

WT3:
Work package description

Project Number [1]	259881	Project Acronym [2]	ImagInt

One form per Work Package

Work package number [53]	WP7	Type of activity [54]	OTHER
Work package title	Dissemination, IPR and ethical issues		
Start month	1		
End month	48		
Lead beneficiary number [55]	1		

Objectives

- Disseminate and promote IMAGINT results to the scientific community
- Disseminate information on project outcomes to the IMAGINT stakeholders and to the general public via an IMAGINT website
- Reinforce communication of IMAGINT findings
- Define procedures for handling the industrial property rights and the related patent rights as managed by the IPC / Create an IPR database for results and knowledge generated from the research activities.
- Organize IMAGINT knowledge dissemination workshop
- To ensure that i) all experiments performed in the project comply with national and European rules; ii) all ethical issues arising from the IMAGINT research are properly addressed.
- To ensure that IMAGINT clinical trials are conducted in accordance with the regulatory, ethical and quality requirements.

Description of work and role of partners

Task 7.1: Project communication and dissemination M1-M48
Partners involved: UCL, all partners
IMAGINT non-proprietary foreground will be shared with scientists worldwide via presentations in international scientific conferences and publications in international peer-reviewed journals.
The planned activities include:
• Creating a project website for IMAGINT with newsletters, information on progress of the research activities, meeting reports and novel data or publications from the group members. There will also be a useful information section that contains a list of publications, conference proceedings etc relevant to the project. An open section describing our aims and major achievements will be in the public domain. Links from this website will advertise related conferences or events where IMAGINT representatives will be available to discuss the project.
• developing online tools (email discussion forums and a partner portal) for collaborative work by project partners
• an annual IMAGINT newsletter that will be published on the website
To ensure efficient communication among partners and with the European community, internal communication between partners will be promoted by implementing a collaborative portal accessible only to authorised members. Online tools will be developed for collaborative work of project partners. External communication between the project and the outside world will be ensured by the development of harmonised and shared communication tools (logo, electronic presentations and leaflet) to ensure a striking and common project promotion.
The transparency and accessibility of the project to the general public is of great importance, and this task will ensure the public is informed of the aims, developments and outcome of the IMAGINT project as well as its contribution to the achievement of the EC health objectives and its impact on the quality of life. Publications in non-specific journals will be prepared to attract maximum attention on the benefits of cellular therapeutics. Furthermore, researchers involved in IMAGINT will participate at the national level in interviews for the general audience and general-interest events in their areas. Public discussion forum on the IMAGINT website will also ensure that there is an explicit way for members of the public to access the outcomes of the project and to provide their inputs in their areas of concern.

Task 7.2: IPR/Knowledge management of IMAGINT M1-M48
Partners involved: UCL

Many scientific discoveries and innovations will derive from the IMAGINT Project.
Industrial property monitoring and patent survey is an indispensable prerequisite to ensure that the research is driven into the right direction.
The consortium will create an intellectual property knowledge base, which will guide the consortium in its choice of research and achieve operational freedom in the most relevant areas. A special effort is planned in order to identify quickly any element in the project that is susceptible to IMAGINT protection and to take the necessary measures for securing property and insuring the final exploitation of deliverables by partners.
An Intellectual Property Committee (IPC) will be implemented at the beginning of the project. It will be formed by IP specialists to advice IMAGINT consortium members. According to the consortium agreement, it will determine the ownership of intellectual and industrial property. This committee will also assist partner in negotiating on joint ownership where applicable, check if access rights are granted, commission market feasibility studies, file patents and seek other legal protection of proprietary material. The IPC will regularly advice and participate in the discussions with the Steering Committee where it will periodically determine the overall exploitation strategy of IMAGINT potential development.

An exploitation manager, a research technician (from UCL), shall be appointed at the beginning of the project to deal with all Intellectual Property Rights issues. He will also be responsible for the IPR knowledge base update, the IPR protection linked to any innovative technology proposed by the project as well as for the relationships with the Project Ethics Committee. He will be in charge of a follow-up of partner requests concerning the use of results and will transfer accordingly these requests to the Steering Committee IMAGINT steering committee and/or IPC.

Intellectual property generated under this programme would be exploited by the relevant owners and in compliance with the consortium agreement.
All partners have technology transfer offices well versed in patent protection.

Task 7.3. Translation of IMAGINT findings into clinical tools M1-M48
Partners involved: UCL, all partners
The translation of the IMAGINT findings will obviously be dependent upon the results of the project. However it is likely that the findings will be extremely positive in terms of non-invasive tools in diagnostics of breast cancer and the monitoring of therapeutic efficiency. Therefore it is the intention of IMAGINT consortium to propose these tools for future clinical applications.

Task 7.4. European/international IMAGINT workshop to communicate findings of IMAGINT to clinicians and scientists M40-M48
Partners involved: UZH, UCL, and all Partners
IMAGINT consortium will use the invitations to one or several conferences dealing with antibodies and breast cancer campaign at the beginning of project to communicate the findings of IMAGINT to a broad audience composed of patient association, clinicians and scientists
Communication of the findings will be done through the following channels:
Working Group Position Papers
Media Events and press releases
Dissemination of results at international scientific meetings where many of the Consortium members are invited (often Key) speakers and/or on advisory committees. For example the IBC International Conference on Recombinant Antibodies which takes place annually in Europe.
Publications in high impact factor scientific journals.
GMP processes will be made widely accessible to the scientific community and contribute to developing standards and regulations in the area.
We will have a commitment to Data Sharing. Data standards for the pre-clinical and clinical studies are being established under the auspices of the UK National Cancer Research Institute (NCRI) Informatics Initiative (http://www.cancerinformatics.org.uk/) which is in strategic partnership with the European Bioinformatics Institute.

Task 7.5. Set-up the Project Ethics Committee M1-M4
Partners involved: UCL, KCL; UZH, all partners (where appropriate)
The Project Ethics Committee (PEC) will be set up at the beginning of the project and made up of a representative from each partner (with ethical background) and external experts. It will be coordinated by the “research technician (defined in Task 7.2) The PEC will have the task of providing ethical guidance to the consortium as well as ensuring that all experimental work is carried out within national and European

regulations. The PEC will ensure that no experimental studies foreseen by any partner will start before prior approval of all relevant local ethics committees. In particular, the role of PEC will be to:
Remind the researchers of the main ethical practices to be complied with on the project
Advise the Steering Committee on potential ethical problems and on appropriate ethical procedures.
Contribute to ethical standards, should any be developed within the project.
Contribute towards establishing and maintaining researchers' awareness on relevant ethical issues.
Furthermore, all protocols regarding animal experimentation will be reviewed and implemented with the help of the PEC in order to promote and apply the "3 Rs" concept (reduction, refinement and replacement) with the aim of finding alternatives to animal experimentations.
PEC will meet at least once a year and upon request.

Task 7.6. Follow-up of the ethical issues related to the project M4-M48
Partners involved: UCL, KCL, all partners (when appropriate)
This task will ensure that all ethical and regulatory issues associated with the nature and outcomes of the project will be properly identified and managed. Its objectives will be to:
ensure that all experiments are complying with national and European rules
anticipate any ethical issues and apply corrective measure via the PEC;
protect human health and environment by applying the precautionary principle (COM (2000) 1 final)
raise partner awareness and sensitivity to ethical issues
Communicate all ethical issues to IMAGINT steering committee
ensure that trial results will be publicly disseminated
This task aims at ensuring that, during the entire duration of the IMAGINT Project; the existing ethical rules are met, checking that no experimental studies foreseen by any partner will start before the prior approval of all relevant local ethics committee.
At the project start, in order to meet the requirements of the Ethical Report for IMAGINT, the consortium will collect the following pre-existing documents pertaining to already on-going activities:
Copies of the necessary local/national approvals by the relevant competent ethical and legal bodies for all partners;
Informed consent forms used by all partners dealing with human beings and biological samples;
Data storage and protection policy to be used within the project. This first report on Ethical Issues in IMAGINT will then be updated every year according to the progress of the research, and these updates will be collected in the IMAGINT Periodical Report on Ethical issues.

More specifically, a Research Technician (UCL) would be responsible for the co-ordination of research ethics applications, inter-institutional contracts (intellectual property and material transfer) and transportation of samples between the IMAGINT partners.

Before any tissues can be accessed from either UCL or GST tissue bank there will need to be either a Research Ethics Committee or Tissue Bank Access application. For the number of groups involved (INO, TNL, MPG) this could mean multiple applications. Whilst material transfer agreement would be incorporated into a REC application, a separate MTA is required before releasing tissue from a tissue bank. The latter requires corporate level agreement rather than local investigators. The proposed technician could co-ordinate and monitor progress of these ethical/legal requirements facilitating timely access to materials.

The Research Technician could also co-ordinate the REC application associated with the proposed phase II neoadjuvant/PET imaging trial. All tissue handling elements, including provision of storage materials, protocols, transportation etc would be handled directly by the technician. They would also have direct involvement during the trial to ensure appropriate pre- and post-treatment tissue collection by liaising with radiologists and pathologists.
Finally, to access translational clinical trial tissue collections (eg neo-ALTO, Lapatinib) application needs to be made to the Chief Investigator or Trial Management team as well as to a REC, which would also be in the remit of this technician.
Completion of ethics and contractual requirements for another establishment to use tissue from a Bank takes 3 to 6 months per request. From the information it's not clear what the least number of applications will be required but these will be phased through year 1 and early year 2. Requests to the Guy's Tissue Bank (eg Work Package 3 requirement for archived material) will require case selection, tissue preparation, documentation and dispatch (year 1). Likewise, there is a need for the proposed technician to identify suitable cases, prepare and transport frozen materials to IMAGINT partners to validate the technology (M1-M24).

The Research Technician would play a pivotal role in co-ordinating and monitoring the ethical and legal aspects of this international collaboration to ensure firstly the multiple studies are not delayed because of process and secondly in the timely preparation and transfer of tissues.

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	UCL	10.00
2	UZH	3.00
3	KCL	1.00
4	TNL	2.00
5	MPG	0.50
6	UU	1.00
7	INO	3.00
8	FLS	3.00
9	M-GMP	1.00
10	ACIES-P2R	1.00
	Total	25.50

List of deliverables

Deliverable Number [61]	Deliverable Title	Lead beneficiary number	Estimated indicative person-months	Nature [62]	Dissemination level [63]	Delivery date [64]
D7.16	Establish communication, IP and ethics stratergies	1	19.50	R	PP	24
D7.17	Project workshop	1	6.00	O	PU	48
		Total	25.50			

Description of deliverables

D7.16) Establish communication, IP and ethics stratergies: [month 24]

D7.17) Project workshop: [month 48]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead beneficiary number	Delivery date from Annex I [60]	Comments
MS6	Successful completion of Phase I/II clinical	1	42	Means of verification: Clinical Trial report validated

WT3:
Work package description

Project Number [1]	259881	Project Acronym [2]	ImagInt

One form per Work Package

Work package number [53]	WP8	Type of activity [54]	MGT
Work package title	Management		
Start month	1		
End month	48		
Lead beneficiary number [55]	1		

Objectives

• Deploy and implement management best practices
• Monitor progress and coordinate the different tasks within WPs
• Ensure that the work and tasks are completed on time, within the allocated budget and according to high quality standards
• Ensure that reporting is performed on a regular basis, in the most efficient and pragmatic way, according to the EC guidelines
• Provide IMAGINT consortium members with all important and relevant information that can influence the project's outcomes
• Ensure that all budgetary actions are performed correctly and according to the rules and regulations established by the EC and the consortium agreement, ensuring that the received funds are correctly distributed and accounted for
• Liaise with the European Commission
• Ensure that gender equality issues are adequately addressed

Description of work and role of partners

Task 8.1: IMAGINT Strategic Chairmanship M1-M48
Partners involved: UCL and IMAGINT Steering Committee members
The strategic management activities aim at defining, planning, applying, coordinating, leading and facilitating human, financial and technological resources in order to reach a goal and to achieve clearly defined objectives. Management of excellence encompasses how the mission and vision of the project will be defined, developed and facilitated by the leaders through the governing bodies (Steering Committee, Executive Management Committee and Project Coordinator). During the entire project duration, the Steering Committee and the Coordinator will be in charge of ensuring the adherence to rules established in the Grant Agreement and the Consortium Agreement (such as the roles and voting rights within the SC, the rules for distributing the advance payment, etc.)
Furthermore, they will take opportunity of the bi-annual Steering Committee meetings to:
- Discuss the work in progress
- Study the difficulties/deviations encountered and decide about an action plan
- Inform partners on overall financial situation and give recommendations
- Ensure an open flow of information within the project
- Validate the visible outputs (breakthroughs, deliverables, presentation materials, etc.) and milestones completion of the project
- Define the priority of action(s) and next step(s) of the project
- Decide policy and strategic orientations of the project.
- Obviously, other meetings could be organised at any time if requested by the project.

Task 8.2: IMAGINT Operational Management M1-M48
Partners involved: UCL, ACIES/P2R
This task includes the development and implementation of the IMAGINT management manual which establish the decision-making rules and procedures to be followed during the Project. The procedures will define the following processes:

- Management of project integration: this procedure contains the project management plan, the interfaces' management, the analysis of advancement and the conclusion of the project
- Management of the content: this step consists in the conception and definition of the content, the definition of research activities and the control of project evolution.
- Management of the timing of work activities, scheduling, planning, and reporting activities.
- Management of costs, including cost estimation, budgeting, and cost effectiveness.
- Management of resources, including scheduling, assignment and inspection.
- Management of communication, both internally through consortium interfaces and externally.
- Management of purchases, including the organisation of subcontracted work.
- Management of risks via identification, evaluation and control of risks.
- Quality management: surveying of quality indicators throughout the project will allow the periodic improvement of the functioning of the project.

Task 8.3: Progress Coordination and Monitoring M1-M48
Partners involved: UCL, ACIES/P2R
Once the management system is in place, UZH, assisted by the management partner ACIES/P2R will ensure its smooth operation and adjust it as necessary according to the PDCA continuous improvement principle (Plan, Do, Check, Act). The Coordinator and partners will refer to the management system and procedures throughout the project. The Coordinator will manage the interfaces between the different modules as well as the preparation and dissemination of the project reports using information provided by partners. The Coordinator will also ensure communication with the EC, by transferring the reports and informing the EC of any major issues/modifications of the work plan.

Task 8.4: Gender issues M1-M48
Partners involved: UCL, ACIES/P2R
Women are equally represented among the researchers taking part in the IMAGINT project. Nevertheless, gender equality issues need to be carefully and objectively monitored to avoid any deviation. Where successes are noted, with a number of senior positions occupied by women, their active role should serve to stimulate other women to enter this type of research and take responsibilities. Where gender balance is not yet equal, this should be made explicit to enable steps towards the target of equality to be taken into account. This task will therefore gather data on the current state of equality in the consortium. It will also gather information about the equal opportunities policies of each partner institution and will ensure partners are aware of actions taking place in this field, for example by the European Platform of Women Scientists (www.epws.org). A set of gender indicators will be produced in order to measure gender equality in the IMAGINT research fields and will be updated regularly.

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	UCL	5.00
10	ACIES-P2R	10.00
	Total	15.00

List of deliverables

Delive-rable Number [61]	Deliverable Title	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
D8.18	Project Management Manual	10	0.50	R	CO	3
D8.19	First Periodic Report	1	4.50	R	PU	12
D8.20	Second Periodic Report	1	4.50	R	CO	30
D8.21	Final Report	1	5.50	R	CO	48

WT3:
Work package description

List of deliverables

Delive-rable Number [61]	Deliverable Title	Lead benefi-ciary number	Estimated indicative person-months	Nature [62]	Dissemi-nation level [63]	Delivery date [64]
		Total	15.00			

Description of deliverables

D8.18) Project Management Manual: [month 3]

D8.19) First Periodic Report: [month 12]

D8.20) Second Periodic Report: [month 30]

D8.21) Final Report: [month 48]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments

WT4:
List of Milestones

Project Number [1]	259881	Project Acronym [2]	ImagInt

List and Schedule of Milestones

Milestone number [59]	Milestone name	WP number [53]	Lead beneficiary number	Delivery date from Annex I [60]	Comments
MS1	Successful selection of DARPins to all 4 members of the HER family	WP1	2	24	Means of verification: FACS
MS2	Proximity ligation detects HER pairs in tumour cells	WP1	2	36	Means of verification: Assay
MS3	Combined TIS and FRET/FLIM analyses of archived cancer tissues	WP1, WP2, WP6	3	36	Means of verification: Steering committee decision
MS4	Combined medical imaging and tissue imaging-based diagnostics	WP1, WP2, WP6	3	36	Means of verification: Steering committee decision
MS5	Production of GMP anti-HER2 DARPins in compliance with EMEA cGMP regulations	WP1, WP5	1	18	Mean of verification: Product evaluation in respect to set product release criteria, validated during steering Committee
MS6	Successful completion of Phase I/II clinical	WP2, WP4, WP5, WP7	1	42	Means of verification: Clinical Trial report validated

WT5:
Tentative schedule of Project Reviews

Project Number [1]	259881	Project Acronym [2]	ImagInt

Tentative schedule of Project Reviews

Review number [65]	Tentative timing	Planned venue of review	Comments, if any
RV 1	24	Brussels	

WT6:
Project Effort by Beneficiary and Work Package

Project Number [1]	259881	Project Acronym [2]	ImagInt

Indicative efforts (man-months) per Beneficiary per Work Package

Beneficiary number and short-name	WP 1	WP 2	WP 3	WP 4	WP 5	WP 6	WP 7	WP 8	Total per Beneficiary
1 - UCL	2.00	5.00	4.00	5.00	72.00	5.00	10.00	5.00	108.00
2 - UZH	96.00	3.00	3.00	12.00	0.00	0.00	3.00	0.00	117.00
3 - KCL	0.00	43.00	0.00	0.00	0.00	45.00	1.00	0.00	89.00
4 - TNL	0.00	40.00	4.00	0.00	0.00	2.00	2.00	0.00	48.00
5 - MPG	18.00	30.00	0.00	0.00	0.00	6.00	0.50	0.00	54.50
6 - UU	0.00	0.00	0.00	90.00	6.00	0.00	1.00	0.00	97.00
7 - INO	5.00	0.00	36.00	0.00	0.00	3.00	3.00	0.00	47.00
8 - FLS	0.00	0.00	60.00	0.00	0.00	48.00	3.00	0.00	111.00
9 - M-GMP	0.00	0.00	0.00	5.00	9.00	0.00	1.00	0.00	15.00
10 - ACIES-P2R	0.00	0.00	0.00	0.00	0.00	0.00	1.00	10.00	11.00
Total	121.00	121.00	107.00	112.00	87.00	109.00	25.50	15.00	697.50

WT7:
Project Effort by Activity type per Beneficiary

Project Number [1]	259881	Project Acronym [2]	ImagInt

Indicative efforts per Activity Type per Beneficiary

Activity type	Part. 1 UCL	Part. 2 UZH	Part. 3 KCL	Part. 4 TNL	Part. 5 MPG	Part. 6 UU	Part. 7 INO	Part. 8 FLS	Part. 9 M-GMP	Part. 10 ACIES-P	Total
1. RTD/Innovation activities											
WP 1	2.00	96.00	0.00	0.00	18.00	0.00	5.00	0.00	0.00	0.00	121.00
WP 2	5.00	3.00	43.00	40.00	30.00	0.00	0.00	0.00	0.00	0.00	121.00
WP 3	4.00	3.00	0.00	4.00	0.00	0.00	36.00	60.00	0.00	0.00	107.00
WP 4	5.00	12.00	0.00	0.00	0.00	90.00	0.00	0.00	5.00	0.00	112.00
WP 5	72.00	0.00	0.00	0.00	0.00	6.00	0.00	0.00	9.00	0.00	87.00
WP 6	5.00	0.00	45.00	2.00	6.00	0.00	3.00	48.00	0.00	0.00	109.00
Total Research	93.00	114.00	88.00	46.00	54.00	96.00	44.00	108.00	14.00	0.00	657.00
2. Demonstration activities											
Total Demo	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3. Consortium Management activities											
WP 8	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	15.00
Total Management	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	15.00
4. Other activities											
WP 7	10.00	3.00	1.00	2.00	0.50	1.00	3.00	3.00	1.00	1.00	25.50
Total other	10.00	3.00	1.00	2.00	0.50	1.00	3.00	3.00	1.00	1.00	25.50
Total	108.00	117.00	89.00	48.00	54.50	97.00	47.00	111.00	15.00	11.00	697.50

WT8:
Project Effort and costs

Project Number [1]	259881	Project Acronym [2]	ImagInt

Project efforts and costs

Benefi-ciary number	Beneficiary short name	Estimated eligible costs (whole duration of the project)						Total receipts (€)	Requested EU contribution (€)
		Effort (PM)	Personnel costs (€)	Subcontracting (€)	Other Direct costs (€)	Indirect costs OR lump sum, flat-rate or scale-of-unit (€)	Total costs		
1	UCL	108.00	869,717.00	3,000.00	210,161.00	647,926.80	1,730,804.80	0.00	1,338,517.00
2	UZH	117.00	646,528.00	3,000.00	226,200.00	523,636.80	1,399,364.80	0.00	1,057,073.00
3	KCL	89.00	368,008.00	2,500.00	98,690.00	280,018.80	749,216.80	0.00	568,749.00
4	TNL	48.00	21,955.00	1,000.00	250,000.00	307,318.00	580,273.00	0.00	435,454.75
5	MPG	54.50	169,833.00	2,000.00	114,000.00	237,767.00	523,600.00	0.00	394,300.00
6	UU	97.00	344,000.00	2,500.00	110,050.00	272,430.00	728,980.00	0.00	552,180.00
7	INO	47.00	251,000.00	11,000.00	89,000.00	204,000.00	555,000.00	0.00	422,850.00
8	FLS	111.00	269,912.00	4,500.00	49,000.00	191,347.20	514,759.20	0.00	393,194.00
9	M-GMP	15.00	522,000.00	4,500.00	7,000.00	105,800.00	639,300.00	0.00	327,900.00
10	ACIES-P2R	11.00	108,000.00	0.00	8,000.00	77,372.00	193,372.00	0.00	193,372.00
Total		697.50	3,570,953.00	34,000.00	1,162,101.00	2,847,616.60	7,614,670.60	0.00	5,683,589.75

1. Project number

The project number has been assigned by the Commission as the unique identifier for your project. It cannot be changed. The project number **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

2. Project acronym

Use the project acronym as given in the submitted proposal. It cannot be changed unless agreed so during the negotiations. The same acronym **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

53. Work Package number

Work package number: WP1, WP2, WP3, ..., WPn

54. Type of activity

For all FP7 projects each work package must relate to one (and only one) of the following possible types of activity (only if applicable for the chosen funding scheme – must correspond to the GPF Form Ax.v):

• **RTD/INNO =** Research and technological development including scientific coordination - applicable for Collaborative Projects and Networks of Excellence

• **DEM =** Demonstration - applicable for collaborative projects and Research for the Benefit of Specific Groups

• **MGT =** Management of the consortium - applicable for all funding schemes

• **OTHER =** Other specific activities, applicable for all funding schemes

• **COORD =** Coordination activities – applicable only for CAs

• **SUPP =** Support activities – applicable only for SAs

55. Lead beneficiary number

Number of the beneficiary leading the work in this work package.

56. Person-months per work package

The total number of person-months allocated to each work package.

57. Start month

Relative start date for the work in the specific work packages, month 1 marking the start date of the project, and all other start dates being relative to this start date.

58. End month

Relative end date, month 1 marking the start date of the project, and all end dates being relative to this start date.

59. Milestone number

Milestone number:MS1, MS2, …, MSn

60. Delivery date for Milestone

Month in which the milestone will be achieved. Month 1 marking the start date of the project, and all delivery dates being relative to this start date.

61. Deliverable number

Deliverable numbers in order of delivery dates: D1 – Dn

62. Nature

Please indicate the nature of the deliverable using one of the following codes

R = Report, **P** = Prototype, **D** = Demonstrator, **O** = Other

63. Dissemination level

Please indicate the dissemination level using one of the following codes:

• **PU =** Public

• **PP =** Restricted to other programme participants (including the Commission Services)

• **RE =** Restricted to a group specified by the consortium (including the Commission Services)

• **CO =** Confidential, only for members of the consortium (including the Commission Services)

• **Restreint UE =** Classified with the classification level "Restreint UE" according to Commission Decision 2001/844 and amendments

• **Confidentiel UE =** Classified with the mention of the classification level "Confidentiel UE" according to Commission Decision 2001/844 and amendments

• **Secret UE =** Classified with the mention of the classification level "Secret UE" according to Commission Decision 2001/844 and amendments

64. Delivery date for Deliverable

Month in which the deliverables will be available. Month 1 marking the start date of the project, and all delivery dates being relative to this start date

65. Review number

Review number: RV1, RV2, ..., RVn

66. Tentative timing of reviews

Month after which the review will take place. Month 1 marking the start date of the project, and all delivery dates being relative to this start date.

67. Person-months per Deliverable

The total number of person-month allocated to each deliverable.

Type of funding scheme

Collaborative Project

(Small or medium-scale focused research project)

HEALTH.2010.1.2-1

Annex I – PART B of the "Description of Work"

Proposal full title: ImagInt—HER Imaging and Molecular Interaction Mapping in Breast Cancer

Project acronym: ***IMAGINT***
Proposal no: **259881**

Date of preparation of Annex I (latest version): 4th August 2010

Date of approval of Annex I by Commission: (to be completed by Commission)

TABLE OF CONTENTS

B.1. CONCEPT AND OBJECTIVES, PROGRESS BEYOND THE STATE OF THE ART, S/T METHODOLOGY AND WORK PLAN

B.1.1. Concept and project objectives

IMAGINT is a multidisciplinary project that aims to develop a range of new **tools** for **imag**ing the human epidermal growth factor receptor (HER) family of tyrosine kinase cell surface receptors and their **int**eractions in breast cancer.

Context of the project

The incidence of **breast cancer** in Europe is > 430,000 per annum (1). In this **chronic disease,** individuals whose tumours show **increased levels** of the **HER family** member, **HER2**, are known to have more aggressive cancers and **high mortality** (2-4). HER2 is a **potent oncoprotein** with a central role in the development and maintenance of breast cancer (2-4) Consistent with this pivotal function, HER2 has become an **established target** for breast cancer treatment, both with antibodies and small molecules. For instance, trastuzumab (Herceptin), a humanized monoclonal antibody against the extracellular domain of HER2 is standard of care in adjuvant treatment and first line treatment of metastatic disease. Another example, Lapatinib, a small molecule tyrosine kinase inhibitor, has proven activity in trastuzumab resistant disease (5-12). These current antiHER2 treatments are effective but tumours still progress due to innate or acquired resistance and there is urgent **need for new HER2-related biomarkers to guide treatment regimes and for prediction, diagnosis, monitoring and prognosis of disease**. Furthermore, innovative new targeted agents for HER2 positive (HER2+ve) disease are in development. Here informative biomarkers are required to help determine optimal duration and sequencing of treatment to maintain HER2 suppression.

IMAGINT will address the urgent need for developing new robust tools to identify, characterise measure and image a wide range of potential new biomarkers for breast cancer. Because of its central role, IMAGINT will focus on tools to dissect HER2-related events. Once developed for breast cancer, the tools will find wide application for other cancers in which HER2 is clinically relevant, for example, gastric cancer, another chronic disease and the world's second leading cause of cancer death (13,14). To achieve its goal, IMAGINT will use the properties of Designed Ankyrin Repeat Proteins (DARPins) which are antibody-like proteins based on human protein scaffolds but 10 times smaller than antibodies. DARPins are highly stable and bind specific targets with high affinity in monovalent form; they are also readily engineered for site-specific chemical modification to create specific marker-tags and thus allow for the creation of tools that would be difficult or impossible to create from antibodies. IMAGINT will generate DARPin-based tools to address the following 4 areas:

- Tools for detection, isolation and functional characterisation of complexes of interacting molecules for diagnostic purposes
- Development of new quantitative imaging biomarkers for monitoring therapeutic effects and safety in chronic diseases
- High throughput molecular diagnostic imaging
- Development and implementation of quantum imaging of X-rays/γ-rays for diagnosis

Concepts of the project

Despite the pivotal role of the HER2 in breast and other cancers, the current cutting-edge tools and technologies used in biomarker studies of HER2 related events are confronted by many limitations for early diagnostic, prognostic and monitoring of disease. There are no established tools for whole body quantitative clinical imaging to assess the extent and location of HER2+ve metastases, or for reliably measuring the wide range of specific HER dimers that occur in human tissues. Furthermore, the RNA/protein interactions that control behaviour of HER2+ve cancer in response to treatment are not elucidated and the topographical protein clusters linked to HER2 signalling are not known. This information cannot be obtained by gene expression microarray studies. Moreover, there is the need for advanced mathematical and computational tools to enable integration of the different types of biomarker information in a holistic manner. As a result of the current tools' limitations, the segregation of the breast cancer disease into more specific subsets is not complete. Nonetheless, a cost

effective approach of the disease in which cancer detection, diagnosis and treatment are tailored to each individual molecular profile is essential. IMAGINT will address these issues and develop innovative **DARPin-based** tools that will enable researchers to discover new biomarkers – such as HER/HER protein interactions, RNA/protein interactions and cellular clusters, metastatic signatures imaging agents and software tools - that will enable stratification and monitoring of breast cancer. The information obtained with these biomarkers will lead to better treatment decisions for excising therapies will assist in the development of new drugs.

Concepts behind characterizing and quantifying the new biomarkers:
IMAGINT will use the DARPins to develop tools for different applications based on current scientific understanding of HER2-related cancer processes and aimed to address areas of unmet need.

- **HER/HER protein interactions**: The HER family of proteins are cell surface tyrosine kinase receptors that are involved in regulation of cellular proliferation and survival. HER2 - the focus of IMAGINT - is activated by a variety of mechanisms, including overexpression. However, HER2 does not work in isolation but functions as homodimer, or a shared co-receptor with other members of the family. There are 4 members of the family; HER1 (EGFR), HER2, HER3 and HER4 (15) and their dysregulation is related to a large fraction of human cancers (15). HER2 is the preferred dimerisation partner of all family members (16) but the family can undergo a wide variety of homo- and heterodimeric associations, depending on their expression rates, stimulation by external or autocrine ligands, receptor mutations and changes in the molecules interacting on the cytoplasmic side (15). **Therefore, the measurement of homo- and heterodimeric association, as well as potential clustering of receptors or receptor pairs could provide the scientific basis of stratifying tumours and provide the basis for developing next-generation tools.** HER2 plays a central role and current research indicates that specific HER dimers will be more important and informative biomarkers than HER2 alone. For example, HER3 has been found to preferentially interact with HER2 (16) and the HER2/HER3 heterodimer is purported to be a particularly potent mitogenic and oncogenic unit for a variety of cancers including breast cancer (17). Additionally, there is accumulating evidence that two *different* complexes of HER2 and HER3 exist, one with the liganded form of HER3, the other with the non-liganded form of HER3, and these are formed as a function of HER2 level (18). While **signaling** in both cases appears to occur mainly through HER3 phosphorylation, the two different complexes have an important therapeutic consequence: only the second one reacts to trastuzumab. HER2 can also be partnered with HER1, depending on the expression level and on particular mutations in the HER1 gene, which can have important consequences for signaling and response to anti-HER2 therapeutics (19). HER2/HER4 interactions are also of interest for IMAGINT. It has been shown that expression of HER4 is linked to increased sensitivity to antiHER2 treatment with traztuzmab in patients with breast cancer (20) and it has also been speculated that the signaling between HER2 and HER4 could mediate cardiotoxic effects. Clearly, if confirmed, these findings would have significant consequences for the further development of safe antiHER2 therapeutics. In summary, **there is increasing evidence that the measurement and understanding of the real transmembrane complexes of HER2 present in particular tumours will be of great benefit for a rational development of therapy.** There is urgent need for **robust tools** to measure these different HER dimers in **human tissues**. As biomarkers, the specific dimers could provide **prognostic information** on disease outcome and **diagnostic information** on disease stage and response to therapy. This will allow the segregation of disease into more specific sub-sets and a personalised therapy could be designed for specific dimer types.

- **Molecular networks:**

  Unravelling the complexity of the **interacting molecules** can reveal **undiscovered biomarkers** or **diagnostic signatures.** This is important because the therapeutic responsiveness of cancers to HER-targeted therapies does not necessarily correlate simply with the receptor levels (21). For instance, when trastuzumab is given as a single agent for first-line treatment of HER2-overexpressing metastatic breast cancer, it is associated only with a 40% objective response rate (22). In addition to primary resistance, HER2+ve breast cancer patients who initially respond can subsequently progress/relapse on trastuzumab treatment (acquired resistance). Also, the incidence of mutations (such as AKT1 and PTEN) in breast tumours is typically low (23) and there may not be complete penetrance. Studying protein function (in terms of activity and spatiotemporal location) will give a

broader patient base to integrate into a **diagnostic signature**, regardless of whether patients have germline or somatic mutations. The **special protein clusters** linked to HER2 signalling are not detected by gene expression microarray studies but hundreds of molecules can be **visualised** simultaneously on cancer cells by **high throughput robotic handing of fluorescent probes** to provide **detailed cellular images** of co-localised molecules. When the information is combined with disease outcome it could provide **undiscovered new clinically-relevant biomarkers**. There is need to develop imaging and mathematical tools to identify these clusters and link them to disease status.

RNA/protein interactions:. Although the signalling pathways following HER activation are well defined and their potential as targets investigated (24, 25), RNA/protein interactions controlling these pathways are not yet elucidated. These **complexes of interacting molecules** could be an **untapped source of new biomarkers for diagnostic purposes** and tools are required for their detection, isolation and functional characterisation and identification.

- **Addressing metastases**: Most deaths from cancer are not directly due to the primary tumour but as a consequence of disseminated metastases (1). Many patients diagnosed with early breast cancer can expect to survive, due in part to the widespread adoption of adjuvant systemic therapy to combat micrometastatic disease. Some patients derive little benefit from systemic treatment, however; either because their tumours metastasize despite receiving chemotherapy, or because their tumours would never have metastasized. There remains a critical need for **diagnostic tools** to identify those individuals who are at high risk of metastatic recurrence and **rationally select therapy** to which they will respond. IMAGINT aims to develop practical and informatics tools to achieve this.
- **Imaging HER2 positive (HER2+ve) disease:** Despite the pivotal role of HER2 in diagnosis and treatment of cancer, there are still no established tools for **quantitative clinical imaging** of the extent and location of metastatic disease. There is need to develop these tools because **non-invasive** whole body quantum imaging of HER2+ve metastatic cancer in patients could provide important clinical diagnostic information by early detection of sub-clinical HER2+ve disease, optimal management of current anti-HER2 therapies and response assessment of novel therapeutics.

- **Systems biology:** The new types of biomarker described above will each provide individually important and different parts of information and understanding about HER2 and its role in driving malignancy. However, increased knowledge and greater understanding can be achieved by integrating the different levels of information in a holistic manner, with each other, with public databases and with information on clinical outcome. Achieving this requires building an efficient integrated database for the accumulating biomarker data and clinical data, and the development of new mathematical and computational tools with which to bring together the various sources and quantify their (combined) predictive potential.

Scientific and technological objectives of IMAGINT

The overall objective of IMAGINT is to use a multidisciplinary approach to develop and bring together a range of innovative new tools that will increase understanding of HER2-related malignancy processes in patients and ultimately provide robust new biomarkers. The new tools should raise the EU to the forefront in the development of biomarkers for breast cancer and other HER2-driven malignancies.

IMAGINT will develop DARPins for new technologies that can be used to identify biomarkers:

Four cutting-edge technologies using DARPins will be developed to **measure directly interacting proteins**: (i) Single Molecule Fluorescence (SMF), (ii) Far-field sub-diffraction resolution microscopy (Nanoscopy), (iii) Fluorescence energy transfer (FRET)/ fluorescence life time imaging (FLIM) and (iv) Proximity Ligation (PrL). The new technologies will be applied to characterise HER2 homo- and heterodimer interactions thanks to a range of fluorophore-tagged and DNA-tagged DARPins reactive with HER1, HER2, HER3 and HER4.

Fluorophore-tagged DARPins will be developed to map **topological protein networks** associated with HER signalling. This will be achieved using the robotic Toponome Imaging System (TIS). Once

toponomic lead proteins are identified, their specific interactions will be studied in greater resolution using the above 4 cutting-edge technologies.
A new technology will be developed to exploit DARPins as a tool to isolate **protein/RNA complexes** in RNA-induced Silencing Complexes (RISC) in breast cancer cells, xenografts and clinical tissues.
Radiolabelled anti-HER2 DARPins will be developed, in combination with software, to **improve quantum imaging** of HER2+ve cancer metastases in patients. This will be tested in a (Phase I/II) clinical trial using Single Photon Emission Computed Tomography (SPECT) combined with 16 slice computed tomography (CT) but the tools developed will be also applicable to or Positron emission Tomography (PET).
Innovative mathematic and computational models will be developed as tools for **information technologies to analyse the multivariate data** obtained with the new DARPin-based tools. The information will be integrated and interrogated with preclinical and clinical information to determine how HER2 interactions, HER2-related biological mechanisms and HER2 imaging can be used as biomarkers for improved clinical care of patients.
The new tools will be developed at **Molecular**, **Cellular**, **Tissue**, **Whole Body** and **Systems level** as outlined in figure 10**.**

IMAGINT specific scientific and technological objectives are listed below:

- Develop new **high affinity** DARPins as **tools** to specifically detect and isolate individual members of the HER family: HER1, HER2, HER3, HER4

- Utilise DARPins as **robust tools** to determine **protein/protein complexes** of HER family dimers, initially in cultured breast cancer cells and subsequently in clinical tissues, using four complementary fluorescence imaging technologies

- Extend and improve **sensitivity** of a recently established FRET/FLIM-based multivariate image signature of **clinical tissues** to predict breast cancer disease and to correlate with FDG/FLT PET imaging of the same patients

- Obtain **detailed cellular images** of co-localised of molecules on a large scale of hundreds of molecules simultaneously using **robotic handling of fluorescent probes** as a tool to provide topographical protein clusters and discover new biomarkers

- Develop DARPins as tools to isolate **RNA/protein complexes** and evaluate their potential as biomarkers and a source of clinically relevant microRNA

- Develop radiochemistry and software tools to **increase sensitivity** of **quantitative clinical** SPECT /PET imaging

- Perform a Phase I/II clinical trial to test safety of DARPins in humans and the potential of radiolabelled DARPins to **quantitatively image** HER2+ve metastases **in patients** with advanced breast cancer

- Create a database of results in a format that is compatible with European initiatives such as the European Bioinformatics Initiative and the concepts of the European Life Sciences Infrastructure for Biological Information (ELIXIR)

- Develop numerical implementation of an integrated mathematical formalism for nonlinear Bayesian pattern detection, visualization, and Bayesian prediction as **new informatic tools** for the analysis and integration of diverse biomedical data sources

- Apply new and validated bioinfomatic tools to integrate diverse data and provide a **model systems tool** for obtaining new biomarkers

- Conduct all steps in compliance with regulatory procedures for EU **ethical standards** and **quality assurance** (GLP, GMP and GCP).

- Use recognised data standards to facilitate data sharing within our consortium and with the international research community

B.1.2. Progress beyond the state-of-the-art

State-of-the-art of IMAGINT

WP1 - DARPIns

To achieve its ambitious aims, IMAGINT will employ the many remarkable properties of a new class of binding and targeting agents known as DARPins. These are small, designer proteins of the 'ankyrin repeat' type. Ankyrin repeat proteins occur naturally in many species, including humans, and are implicated in a number of biological processes, such as cell cycle control, transcriptional regulation, innate immunity, vesicular trafficking, cell differentiation, apoptosis, or cellular scaffolding. The evolutionary success of members of this protein family originates from their ability to specifically bind to virtually any target protein by simple adaptation of their molecular surface and by displaying highly variable residues throughout the protein. Furthermore, the modularity of ankyrin repeat domains enables surface evolution by duplications, deletions or shuffling of the repeats within repeat domains. Hence, the ankyrin repeat domain fold is a very versatile scaffold for the evolutionary generation of protein domains displaying specific binding surfaces.

UZH has developed a strategy to build combinatorial libraries of repeat proteins by extracting sequence and structural information from compatible natural repeats to design a consensus amino acid sequence motif encoding self-compatible repeat modules (26). Such a designed repeat sequence motif comprises fixed and variable positions. The fixed positions mainly reflect conserved framework positions of the compatible natural repeats, while the six variable positions per repeat mainly reflect non-conserved surface-exposed residues that can be potentially engaged in interactions with the target (Figure 1).

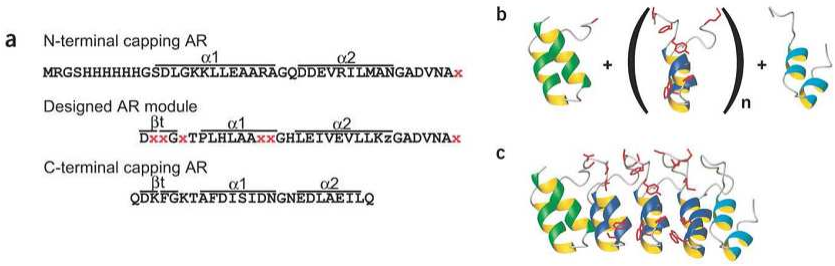


Figure 1: DARPin library (a) sequences of the capping repeats and the internal, randomized repeats, (b) structure of the single repeats, (c) structure of the complete protein. Note that this is a single contiguous polypeptide chain, in which the repeats rigidly stack on each other (Adapted from Ref 26)

UZH have constructed DARPin libraries with theoretical diversities of $5.2 \cdot 10^{15}$ or $3.8 \cdot 10^{23}$ for two-module or tree-module binders, respectively. The actual diversity, given by the number of molecules present in ribosome display, is about 10^{12}. By using only few rounds of ribosome display (27, 28) or SRP phage display (29,30), UZH have already isolated specific DARPin binders against a number of diverse proteins (31-34) indicating that DARPin libraries are highly valuable source for novel binding molecules suitable for biotechnological and biomedical applications.

DARPins have some unique properties which make them especially suitable for the IMAGINT: (i) They can be easily chemically derivatized in a very defined manner, as they have no cysteine, and can therefore provided with a unique cysteine residue for chemical labelling, without any change in their high expression yield, and without complications of additional disulfides. (ii) Because of their robust

folding, they can be fused to a wide range of additional domains, including autofluorescent proteins such as GFP, and domains for rapid covalent coupling, such as the SNAP tag. (iii) Because of their small size, they can be used to detect proximity of receptors at much higher spatial resolution than whole IgGs. (iv) They can be conveniently fused to other DARPins, thereby creating bispecific binders.

Most important for IMAGINT, UZH have binders to all members of the HER receptor family (29, 35, 36) except HER3, which will be generated as part of WP1. Binders to HER2 have been successfully tested on whole cells (35, 36) in paraffinated patient tissues to predict HER2 amplification status (35) and in vivo in mice bearing human tumour xenografts (37). The structure and size of a monomeric and dimeric DARPin in relation to the HER dimer and an IgG is shown in Figure 2.

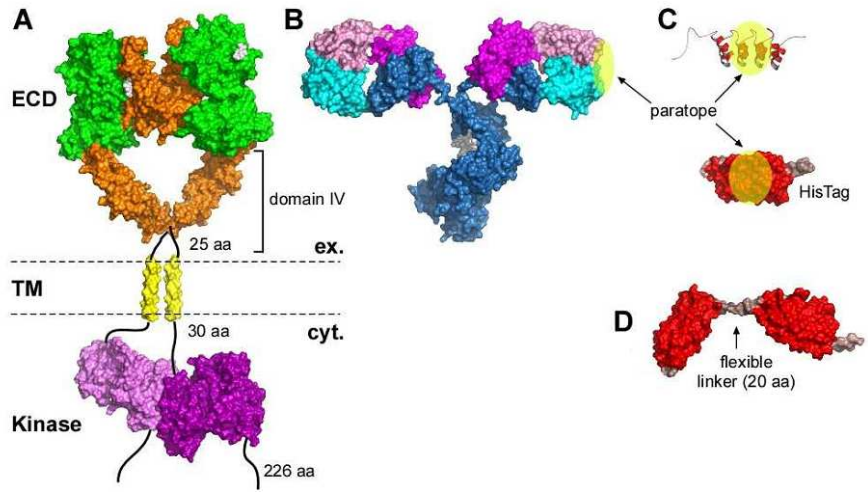


Figure 2. Size comparison of the HER2 receptor with a DARPin and an antibody of the IgG type, all drawn to scale. (**A**) **HER2 dimer**, with extracellular (ECD), transmembrane (TM), kinase domain, and C-terminal unstructured region of 226 aa (**B**) Bivalent **IgG** (150 kDa), with the paratope overlaid in yellow. (**C**) DARPin structure shown as a ribbon model (above) and a space-filling model (below). Note that the size of a DARPin is only 1/10 of the IgG (14.5 kDa), but the size of its paratope (overlaid in yellow) is comparable to that of an antibody. The unstructured C-terminal His Tag for purification (right) is indicated. (**D**) Bivalent or **bispecific DARPin** with a flexible linker of 20 aa, allowing the DARPin domains bind independently. Fluorescent dyes (for single molecule fluorescence spectroscopy, FRET, or nanoscopy) or chelators for radionuclides or oligonucleotides (for proximity ligation) can be conveniently attached at either flexible end of the DARPin (**C**) or (**D**), where a unique cysteine can be placed, without interfering with **target binding**, which involves the central portion (orange overlay in **C**).

Taken together, the small size, high affinity, availability, stability and ease of production make DARPIns an ideal foundation to develop and deliver the range on new tools required for IMAGINT.

WP1 - Proximity ligation

One of the first technologies that will be developed using DARPins is a method for detecting specific HER dimers by virtue of their closeness or proximity. The method, known as Proximity Ligation enables sensitive detection of specific protein monomers, homo-or heterodimers. (38). IMAGINT proximity probes will be monovalent DARPins containing oligonucleotide extensions designed to bind pairwise to target proteins and to form amplifiable oligonucleotide sequences by ligation when brought in proximity (Figure 3a). IMAGINT will use two strategies. First, a direct chemical coupling using an engineered single cysteine and an NH2-derivatized oligonucleotide. This has the advantage of being very small and therefore **very conclusively reporting on close interactions**. Second, a fusion of a DARPin to the SNAP tag (39), a modified form of O(6)-methylguanine-DNA methyltransferase, which can be covalently labelled by simple mixing with suitably modified oligonucleotide derivatives.

IMAGINT will use Proximity Ligation to quantify receptor homo- and heterodimers being formed on the surface of cancer cells. For this purpose, UZH is currently collaborating with the laboratory of Ulf Landegren, Uppsala, Sweden, that has pioneered the methodology of Proximity Ligation (38). Pilot experiments with SNAP-tagged DARPins have shown success in detecting HER2 dimers (Figure 3).

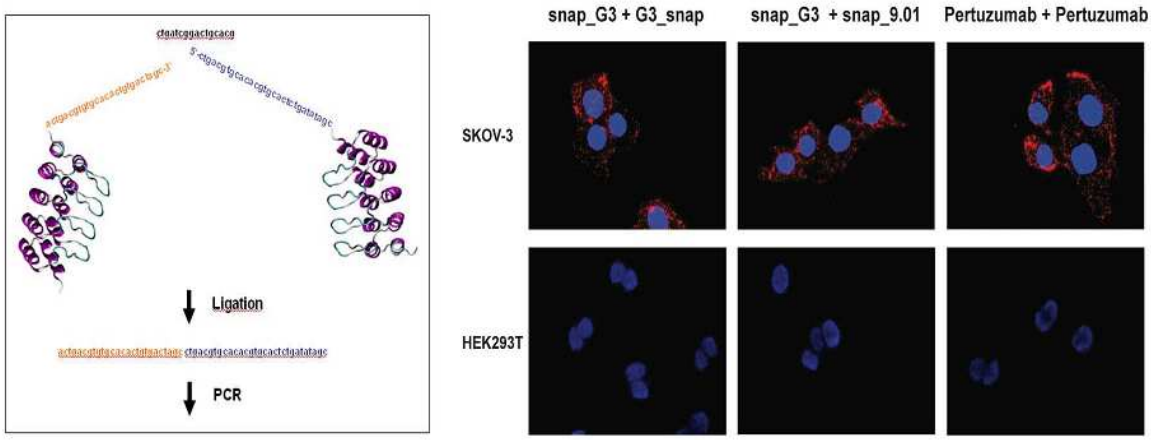


Figure 3 a) Principle and Work-flow of Proximity Ligation. b) In situ PLA detection of HER2-dimers using DARPins in HER2-overexpressing human ovarian carcinoma cell line SKOV-3 cells. Two HER2 specific DARPins, G3 and 9.01, were employed to show HER2 dimer interactions in EtOH-fixed cells. Pertuzumab, a commercial anti-HER2 antibody was used as a positive control. Human embryonic kidney cell line, HEK293T cells were used as negative control. HER2 was visualized using hybridization probes labelled with Alexa 555 (red). Cell nuclei are counterstained with DAPI (blue). (Plückthun and Landegren, unpublished)

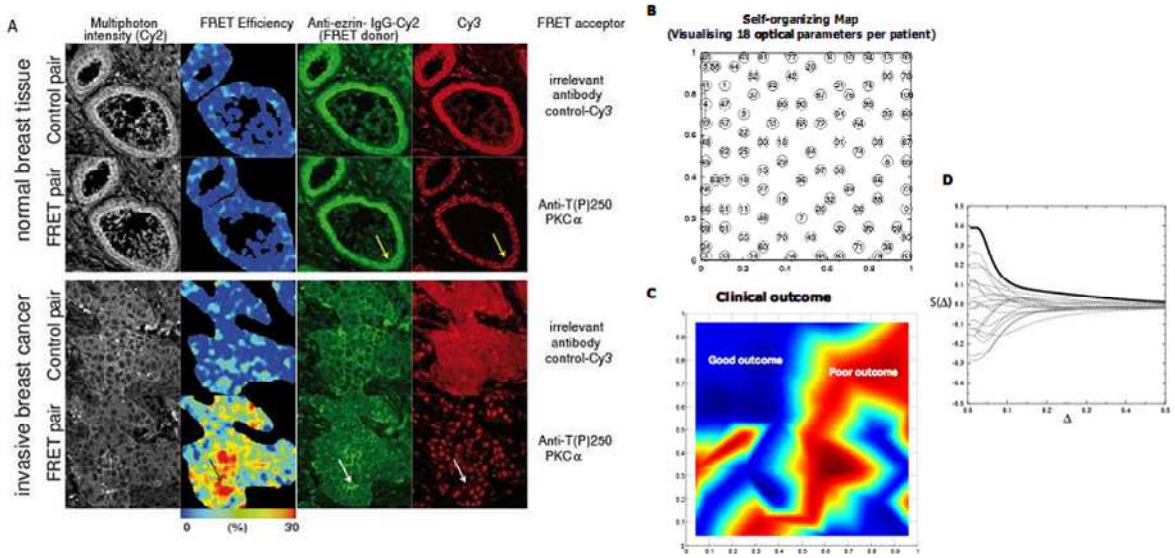
When established, the **Proximity Ligation** test can be performed on clinical samples to report which HER pairings are occurring in which tumours, or which parts of an individual tumour, and enable the specific HER pairs to be evaluated as biomarkers.

WP2 - Tools for a multivariate tumour invasion signature

A major goal of IMAGINT is to develop tools that will enable clinicians to make more informed decisions about which treatments to use for patients with early metastases. This is important because certain therapies are effective in some patients but not in others and doctors need a molecular 'fingerprint' - from the primary tumour – that will inform them on the best way to treat potential secondaries. KCL have begun to address this using a derived a multivariate tumour invasion signature 'fingerprint' that can classify breast cancer patients at an individualized level, by the macromolecular interaction between activated protein kinase C (PKC) and ezrin imaging in breast cancer tissue cores from 173 patients, (Figure 4) Ezrin is of importance to IMAGINT since it regulates the linkage of the HER receptor signalling to the cytoskeleton. ERM family proteins (ezrin, radixin, and moesin) represent a class of proteins that can provide the direct linkage between the cytoskeleton and integral membrane proteins (40). The activation of ezrin in particular has long been linked to the signalling of the epidermal growth factor receptor (EGFR/HER) receptor family (41). Ng originally demonstrated, in a breast cancer cell line, that the promigratory function of ezrin is dependent on a protein complex between activated protein kinase C (PKC) and ezrin (42); subsequently others have shown this protein interaction is not cancer type-specific (43). Using automated Förster resonance energy transfer (FRET)/fluorescence lifetime imaging microscopy (FLIM) analysis that is firmly established in the Ng lab (44,45), this protein-protein interaction was assessed at cellular resolution, together with various image analysis algorithms that quantify molecular events that occur as a consequence of the interaction. Together these make up a protein imaging-based signature that correlates with the likelihood of metastasis. On the basis of the vast amount of literature highlighting the importance of this class of membrane-cytoskeletal linkers, the metastatic signature (Figure 4, comprised of 18 optical

imaging parameters) should be applicable to many cancer types. In addition, the Ng laboratory has recently published the FRET/FLIM image of the HER2/HER3 heterodimer (46) which has been purported to be a potent mitogenic and oncogenic unit for breast tumours (17). Precisely how the HER2/HER3 dimer may signal to ezrin which is a highly connected node in the human protein interaction network will be studied in more detail in the proposed IMAGINT program using the new DARPIn-based tools.

Figure 4 A, The direct interaction between endogenous ezrin and activated PKCα has been demonstrated by multiphoton FRET/FLIM, in patient-derived cancer tissues that have been labelled



with Cy2-labelled anti-ezrin-antibody (2H3) and Cy3 labelled anti-T(P)250-PKCα antibody. Two examples (normal breast tissues vs. invasive breast cancers) are shown. A detailed description of the derivation of FRET efficiency in this figure can be found in (46). B, A self-organizing map (SOM), an artificial neural network that is trained using unsupervised learning to produce a two dimensional, discretized representation (map), was employed to characterise the inter-patient differences according to 18 optical imaging parameters (comprised of the quantitative measurement of the ezrin–PKC Cα interaction; analyses of ezrin localisation (at membrane/cytoskeleton junction) and phosphorylation). C, Clinical outcome plot that directly corresponds to the individual position in B. D, Neighbourhood distance (Δ) in the map measures the dis-similarity of the optical parameters between patients and S(Δ) is the average correlation of clinical outcomes for patients at distance Δ in the map. Apart from the true data set (bold solid line), 20 randomisations (light broken lines), in which the clinical outcome values are shuffled each time, are also shown. The probability of obtaining S(0)=0.39 (true data) from the random outputs is 0.007546.

It is envisaged that the 'fingerprint' obtained by adding the anti-HER DARPin FRET/FLIM results to those previously discovered will be more much better able to help clinicians decide which treatment will be best for an individual patient.

WP2 - Nanoscale distribution of proteins: Stimulated Emission Depletion (STED) nanoscopy is a technique that uses the non-linear de-excitation of fluorescent dyes to overcome the resolution limit imposed by diffraction with standard confocal laser scanning microscopes and conventional far-field optical microscopes (47-51). Until the last few years research on nanoscopy was mainly physics driven and only very recently STED microscopy has proved to be sufficiently mature to be used to address clinical questions. Now nanoscopy enables for the first time to systematically image the distribution of proteins on the 50 nm scale in chemically fixed but otherwise intact cells. The improved resolution obtained with nanoscopy is illustrated in Figure 5. In combination with the small size of DARPins, STED nanoscopy will enable the systematic mapping of the distribution of HER2 and associated proteins at the nanoscale, possibly revealing new information on the **distribution of**

these proteins under different conditions, which were just not accessible previously. Of particular interest will be the clustering of these receptors, which is extremely difficult to access by any other technique.

The use of STED-Nanoscopy in IMAGINT will add strength to the predictive and diagnostic and tumour 'fingerprint' and aid clinical decision making.

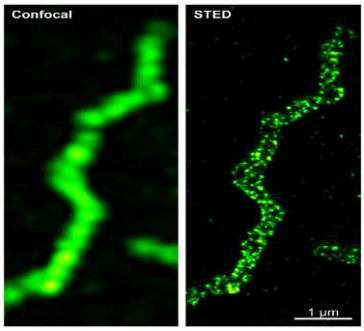


Figure 5. STED-Nanoscopy enables a superior optical resolution compared to conventional state-of-the-art fluorescence microscopy. Displayed are mitochondria of a human cell line stained for the mitochondrial protein Tom20. This protein accumulates in specific regions of the mitochondrial outer membrane. Left: image recorded with conventional laser scanning fluorescence microscopy. Right: image recorded with STED nanoscopy. Only STED reveals the clustering of Tom20 (Image, Jacobs unpublished).

WP 2 - Spatial structure of interacting proteins by TIS (Toponome Imaging system):

Central to the analysis of HER2-associated protein networks will be the use of a breakthrough *technology* termed Toponomic Analysis (52) that directly maps the spatio-temporal togetherness of proteins in a cell and in a tissue (52,53). Toponomic analysis permits the tag library-based robotically controlled co-localization of more than 100 proteins in single cells or tissue sections, thereby providing insight into the higher order combinatorial organisation of proteomes *in situ*. Toponomic analyses are based on the robot technology known as multi-epitope-ligand cartography (MELC)/toponome imaging system (TIS), which breaks the limits of simultaneous detection of different chromophores given by their spectral overlap in fluorescence microscopy (52,53). The procedure relies on fixation of samples and on dye-conjugated tag libraries. (e.g. antibodies, lectins etc). It runs repetitive cycles of tag-dye incubation, imaging and bleaching resulting in the simultaneous visualization of thousands of protein clusters interlocked as a network in the identical cell and tissue structure (Toponome).

Protein location images generated by dozens to hundreds of TIS cycles in the identical tissue section/visual field are aligned and then analyzed for presence of protein signal combinations per pixel/voxel (protein clusters) by using a specific software termed MoPPI (modular processing pipeline).This software generates a new type of high dimensional data space, which is solely based on structure-bound protein networks (toponome data), topologically composed of interlocked protein clusters, formally described as combinatorial molecular phenotypes (CMPs) (52,53). Briefly, each signal within a CMP is mapped as tag present (=1) or tag absent (=0), depending on whether the value for the fluorescence signal is above or below this threshold, respectively (=1 bit information per protein at a pixel/voxel). CMPs assembled as a group will have unique features as defined by the assembly's lead proteins (L=1), absent proteins (A = anticolocated, 0) and wild card proteins (W = variably occurrence of a protein 0 and 1 in given CMPs of a CMP group).

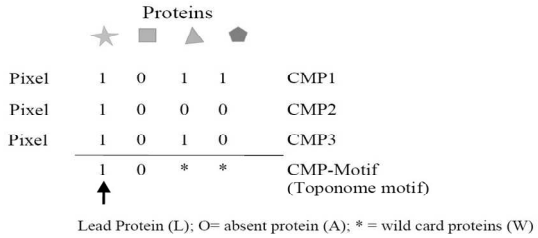


Figure 6. Illustration of the topological hierarchies of proteins within the toponome. L, lead protein (common to all CMPs of a CMP motif); A, absent protein (absent in all CMPs of a CMP motif); W, wild card proteins (proteins that are variably associated with the (L) and the (A) proteins of a CMP motif) (54).

We define such CMP groups as a CMP motif (Figure 6), denoting a given functional region of a cell or tissue. By using a three symbol code (LAW, Figure 6) (54) differences of cell states and cell types can be readily identified in studies comparing large experiments, or, diseases with normal conditions. Most

importantly, lead proteins detected de novo by the MoPPI approach, have proven to be key target molecules exerting control over whole protein networks - a finding that has led to effective blockade of tumour cell polarization and migration/metastasis (52). This approach proved to be a powerful way of identifying the cellular and tissue protein networks in healthy and diseased states. For the first time, we intend to apply this approach in this project for the identification of HER-2 related protein network structures.

TIS is the first technology capable of resolving the spatial structure of large molecular systems, which are composed of thousands of protein clusters interlocked as a network *in situ* (a single tissue section, cell or many cells simultaneously). An example of a 3D TIS image, obtained with a single human hepatocyte, is shown in Figure 7. TIS has proven to solve key problems in biology by (i) identifying protein networks directly in a cell or one single tissue section with subcellular resolution (ii) identifying lead(hub) proteins exerting control over such networks, (iii) blocking tumour cell polarization by inhibiting detected lead (hub) proteins. So far, toponome studies have been able to find specific signatures for virtually all cancerous cells studied *in situ* (55), and a similar predictive power for tumour cell polarization protein networks has been substantiated *in vitro* (52). On the basis of these experiences toponome fingerprinting in breast cancer cells is likely to find predictive toponome biomarkers, protein network structures and lead (hub) proteins for diagnostic and clinical validation. One important aspect of TIS is that - once a lead protein complex is found by TIS - this complex can be further validated by high throughput fluorescence microscopy involving approximately 100 clinical samples per week. TNL holds the I.P. for this technology, and it will be able to translate such results technology patents and partnering for **disease monitoring in clinical trials**.

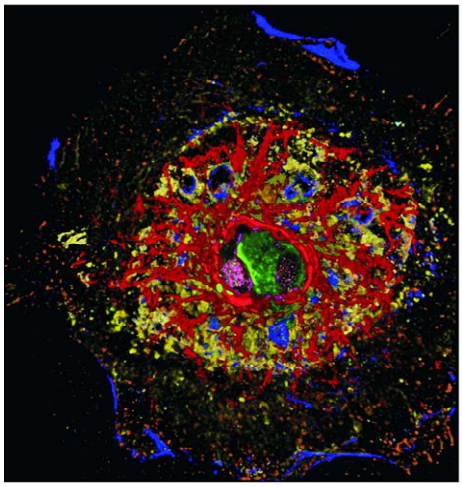


Figure 7. Toponome image of a single human hepatocyte showing 3D visualisation of more then 7,000 protein clusters (different colours). Unlike traditional fluorescence microscopy, which can detect only a few fluorescent tags on one sample simultaneously, TIS labels a sample with a 2 tagged antibodies and, after recording the image, TIS bleaches the tags. Then, without moving the sample or objective lens, TIS repeats the process with a new set of tagged antibodies or other specific binders. In this way, TIS can visualise clusters of 100 different proteins. The process is achieved by robotic high throughput application of tagged antibodies or other specific binders. The clusters of proteins revealed are known as combinatorial molecular phenotypes (CMPs) and are given false colours to allow 3D visualisation of their interactions and their position in cells. (52).

By applying the new DARPin tools for use in the toponome system, IMAGINT will make two further substantial improvements to the development of TIS for cancer treatment. First, it will add completely new and exciting information to the cancer 'fingerprint' and help individualised patient care. Second it will lead to finding new biomarkers and revealing previously unknown targets.

WP2 - FDG/FLT PET-CT imaging and correlation with biological pathway activities in breast cancer

IMAGINT will the compare the tumour fingerprint results obtained from tissue imaging with tumour response measured by state of the art imaging methods that have been applied to study treatment response in the clinic. In breast cancer two tracers have been used to demonstrate response in vivo, namely, ^{18}fluorothymidine (^{18}FLT) and ^{18}fluorodeoxyglucose (FDG). Both tracers have limitations in response assessment and their predictive role in outcome. ^{18}FLT is a potential marker of tumour proliferation. There is high thymidine kinase activity during S phase of the cell cycle and a far lower level during G_0/G_1 phase. A high correlation with other surrogates of cell proliferation have been demonstrated e.g. Ki67 (56). Measurements using FLT may predict response as early as 1 week after treatment (57). FDG data in a large clinical study measuring response in breast cancer has shown that

a 45% reduction in the uptake of FDG is predictive of histopathologic response. However, the accuracy of prediction is only ~70 % (58). It is likely that the proposed combination of tissue-derived molecular imaging parameters, in combination with the clinical imaging markers in this work package, will have a greater ability to predict outcome and select patients early in chemotherapy who will do well and allow migration of patients with poorer outcome to different therapy. The assessment of patients with the relatively more established clinical imaging markers will allow relationships with the biological pathways (from tissue imaging) to be explored and relate these to early tumour response measured histopathologically.

WP3 - Developing methods for isolation characterization of interacting protein/RNA complexes:

IMAGINT will also use DARPins to develop tools that can provide information beyond that possible by the study of proteins/protein interactions. To achieve this, the consortium will extend to the relatively unexplored field of studying the interactions of proteins with RNA. Until recently, the major role of RNA in cancer was considered to be passive; essentially mediating a transfer of information from DNA (the genetic code) to proteins (the executive arm). However, discoveries of new regulatory non-protein-coding RNAs (ncRNAs), and their novel functions (e.g. 59) have revealed that RNA has many different and exciting cellular roles and that these can become perturbed in cancer.

One important class of the ncRNAs that illustrate to importance of the ncRNA is the microRNA (miRNA) family. These are short ncRNAs that are initially transcribed from intergenic or intronic sequences as long precursors and subsequently processed by endonucleases (Drosha and Dicer) into sequences of 20–24 nucleotides (60-62). Over 700 miRNAs have been discovered (64-66) and it has been shown that they are important gene regulators (67) that control gene expression at the post-transcriptional level, usually by down regulation (67). There is a wealth of increasing evidence that specific miRNA are associated with cancer and can provide useful biomarkers. For example, a signature of as few as 200 miRNAs may be sufficient for cancer classification (67) and the expression profiles of miRNAs can be used to identify the origin of poorly differentiated cancer (67-69). In breast cancer various miRNAs have been identified (70-72) of which some are specifically circulating miRNA and could have diagnostic or prognostic value (73).

The interest and obvious importance of miRNA has led to development of established and commercially available tools for miRNA analysis that will no doubt facilitate worldwide investigation of the biomarker potential of miRNA and is likely to lead to routine application of miRNA tests in the near future. The next stage in finding ncRNA biomarkers is to develop **tools to elucidate the mode of action and the cell pathways that are controlled by miRNA** and this is the challenge that will be taken on by IMAGINT. The first task is to find which the mRNA targets are for the miRNA involved in HER2-driven breast cancer. This is not a trivial task because miRNAs are in most cases poorly homologous to their mRNA targets. Thus each miRNA can target multiple mRNAs, and an mRNA may contain target sites for multiple miRNAs. Various algorithms exist for prediction of miRNA targets (74,75) and are being continuously optimised. However they tend to predict a high number of targets (several hundreds in many cases) for each miRNA (74,75) and these must each be validated experimentally.

The way that IMAGINT will approach the task is to develop new DARPIns that can be used to purify miRNA/mRNA pairs that are actively engaged in the cancer cells. This occurs in special cytoplasmic complexes known as RNA-induced silencing complexes (RISC). In RISC, miRNA become combined with an Argonaute (Ago) proteins, of which there are 4 commonly expressed in mammalian cells (76,77) and it is here where miRNA regulation of mRNA targets and subsequent mRNA degradation in thought to occur (78) (79) (80). In the cell, RISC accumulate in cytoplasmic foci known as processing bodies (P- bodies). The cellular pattern of P-bodies can change depending on cell activity and status (40) and it is possible that this pattern could provide useful biomarker information. RISC also provide a source of biologically relevant mRNA/miRNA pairs because it is the miRNA loaded into RISC that actively interferes with the translation of select mRNAs by hybridizing to complementary target sites and either cleaving the mRNA or blocking its translation, depending on the degree of homology between the miRNA and its target mRNA (82,83). There is need for new tools to

isolate and characterise these components. Researchers have used various means to achieve this. For example, Ago-containing RISC has been pulled down with Anti-Ago antibodies and their miRNAs and mRNAs identified using various procedures (84-86, 87, 88). The results have been subjected to bioinformatic analysis to characterise the miRNA/mRNA pairs. Another approach has been to search for mRNAs associated with a specific miRNA of interest, achieved by introduction of biotinylated synthetic miRNAs into cells, followed by a pull-down on streptavidin beads (89, 90, 91). This has helped identify new targets for miRNAs but tends to suffer from high background which may be addressed by employing a two-step procedure in which the mRNA/miRNA complex is first pulled down with anti-FLAG antibodies and then purified on streptavidin beads (92).

In IMAGINT, the consortium will generate new tools for purifying and characterising RISC. This will be achieved by generating a panel of anti-Ago DARPins which are tagged for biotinylation and will be used or capture RISC from cancer cells. The strengths of INO, a small SME specialising in RNA/protein purifications will facilitate the anti-Ago DARPins to be developed as robust tools. INO also have the technology to study P-body cellular distribution with anti-Ago DARPins and to characterise the protein component of the purified RISC. UCL have the deep sequencing technology necessary for analysis of the miRNA/mRNA components. Bioinformatic tools to analyse the data will be developed and applied in WP6. As an exciting bonus, P-bodies miRNA or mRNA biomarkers discovered in WP3 will be tested in the toponome system to further elucidate their positional role in the cellular networks. By application of these combined tools to characterise RISC on cells, xenografts and patient tumours with known clinical outcome, **IMAGINT will move beyond the confines of protein-coding genes** and develop tools for **biomarkers** that will address the growing body of opinion that an understanding protein/RNA interactions is **necessary for a comprehensive understanding** of cancer.

WP4/WP5 – Developing and using DARPins as tools for medical imaging

IMAGINT will also exploit the properties of DARPins to fulfil the need for more specific and sensitive tools for medical imaging. The consortium believe that a safe, sensitive and specific method for *in vivo* imaging of HER2+ breast cancer will provide vitally important clinical information: (i) by early detection of small volume HER2+ disease, (ii) by non-invasive monitoring of disease and optimal delivery of current anti-HER2 therapies and (iii) to aid development of the best treatment regimes for experimental therapeutics, e.g. Hsp90 inhibitors (93). HER2 appears to be a suitable target for imaging purposes: 111In-trastuzumab 89Zr-trastuzumab have shown HER2-specific tumour uptake and ability to detect metastases in pre-clinical studies (94) and 111In-trastuzumab has been used for imaging breast cancer in the clinic, with promising detection of new tumour lesions in 13 of 15 patients (95). These studies indicate that the clinical imaging of HER2+ve metastases in feasible and can provide useful information. Pre-clinical studies with anti-HER2 DARPins (37) and other small anti-HER proteins (96,97) also indicate that small proteins will be far superior to whole antibodies for imaging (98). Encouragingly HER2 imaging has also been reported to measure changes in HER2 expression *in vivo* in response to therapeutic interventions (98) and may be an earlier predictor of tumour response to therapy than ^{18}F-FDG PET (99) and a more accurate predictor of subsequent tumour growth inhibition (99). These pre-clinical studies indicate that robust and sensitive HER2 imaging agents could provide extremely valuable clinical biomarker tools. DARPins, due to their size, stability, ease of manufacture, and potential for site-specific radiolabelling are poised to meet the need for HER2+ve imaging agents for metastatic breast cancer.

UZH have generated a variety of HER2 targeted DARPins and mapped their HER binding in relation to trastuzumab (36). One DARPin, G3, binds with high (picomolar) affinity to HER2 (36). Importantly, although the G3 DARPin binds to the same domain as trastuzumab (domain 4), it has a different and non-overlapping epitope, which will facilitate its use for imaging even when patients are receiving trastuzumab treatment. Furthermore, it has been chosen for imaging because experimental evidence indicates that it has no effect on HER2 signalling, although it binds effectively and specifically to HER2: experiments at UZH have found no evidence of G3 influencing cell viability,

cell cycle, or phosphorylation status of key signalling molecules or receptor shedding. Using a biologically inert but effective imaging DARPin will facilitate the safest possible first-in-human testing of radiolabelled antiHER DARPins.

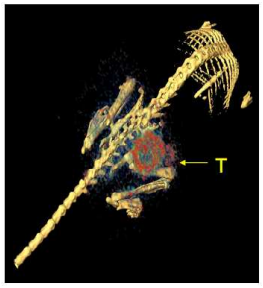


Figure 8. SPECT/CT image of a SKOV3ip tumour (T) on the right hind leg of a mouse 24 h post i.v. inj. of $^{99m}Tc(CO)_3$ labelled G3 DARPin.

When expressed in *E. coli* and labelled with technetium ($^{99m}Tc(CO)_3$), the G3 DARPIn has a half-life of ~ 3 min in mice and localises effectively in HER2+ human SK-OV-ip3 xenografts with values of 11%ID/g after 4 h, 8%ID/g after 24 h and 4.6%ID/g after 48 h. This gave rise to tumour:blood ratios in the range of 50:1 - 60:1 (37) and excellent SPECT imaging as illustrated in Figure 8 that illustrates the undoubted imaging potential of DARPins.

IMAGINT will now combine the favourable properties of Cys-tagged anti-HER2 G3 DARPIn with the radiochemistry and pre-clinical image expertise of UU and the clinical imaging/first-in-man trials expertise UCL to translate this pre-clinical potential into a new tool for quantitive image and monitoring of therapy.

WP4 - Improving sensitivity of Image detection - Radiochemistry and pre-clinical localisation studies

In order to improve and optimise the performance of medical imaging, IMAGINT will develop innovative, GMP compliant, radiolabelling technology for site specific labelling of DARPins and new software for sensitive quantitative imaging of the localised DARPins in patients. The improvements will be developed to improve medical imaging with SPECT/CT and PET/CT, which offer complementary advantages. SPECT/CT is preferred as long as tumour localization via receptor binding might be slow due to the longer half lives of suitable isotopes, 111In (2.85 days) and 67Ga (3.3 days) in comparison to that of 68Ga (68 min) or 110In (69 min). PET/CT provides higher sensitivity if kinetics of uptake is rapid since better signal to noise will be obtained from a nuclide with short half life. Development of DARPin imaging agents requires labelling protocols for maximum performance in terms of specific radioactivity. The development of radiolabelling technology for both Ga^{3+} and In^{3+} is justified by the fact that they have closely similar complexation chemistry and that a relatively modest extra work effort will provide radiolabelling protocols for both. The risk to the project is reduced in the process since it would be possible to select one or the other depending on the preclinical validation.

The radiochemistry research in WP4 will focus on developing robust methods for obtaining the most pure radiolabelled DARPins. Chemical impurities in the form of stable +3 cations will reduce the specific radioactivity achievable in labelling and can be reduced, using for example anion exchange columns in combination with complexation and variation in pH. (100) DOTA chelating agents have high affinity for In3+ (log K=23.9) as well as for Ga3+ (log K = 21.5) and are suitable for site-specific protein labelling when equipped with spacer and functional group that can react with specific residues on the protein surface (101). The maleimide group is especially useful since it reacts exclusively with thiols, the side chains of cysteine residues. In a protein with a single cysteine residue the coupling becomes specific and well-defined. The DARPins contain no cysteine residues and by site-directed mutagenesis a unique cysteine residue can be introduced in the desired position, enabling the incorporation of a DOTA chelate at virtually any position. The incorporation of a metal ion into a DOTA chelator is slow at room temperature but considerably more efficient at elevated temperatures and the use of DARPins will allow labelling to take place at high temperatures because DARPins are

extremely stable proteins and will **undergo heating to 85°C for 20-30 min.** without adverse effects on protein structure. Microwave heating is a technology that is advantageous for radiochemistry because it heats solutions uniformly and rapidly, in contrast to conventional heating where substantial temperature gradients arise. Reactions under microwave control show great promise in the labelling of model peptides especially dealing with short-lived radionuclides when synthesis time may be advantageous and have been shown to give higher specific radioactivity than conventional heating (102,103).

The development of robust and efficient methods for radiolabelling has the objective to obtain unprecedented specific radioactivity for optimal imaging using DARPins (104). The technology developed in IMAGINT will allow ^{67}Ga-anti-HER DARPin to be unequivocally tested for sensitivity and specificity of imaging in comparison to FDG/FLT-PET, using the same mice and the same tumours. It will also allow simultaneous labelling of DARPIns with ^{68}Ga and ^{67}Ga for PET/SPECT/CT which will provide tools to monitor the time course of receptor binding. Since the dissociation constant K_D (the "affinity") is the ratio between the rate constants k_{off} and k_{on} and tight binding can be due to fast binding and fast dissociation as well as to slow binding and slow dissociation, it is not possible to conclude without experimental data whether the best sensitivity is obtained at a short time after injection of after a long time after injection. In addition, biological processes such as binding and transport are concentration dependent which makes it necessary also to vary the concentration of the imaging agent and the specific radioactivity (SRA) to identify conditions under which HER2 is occupied to the largest extent in comparison to "non-specific" binding. The dependence on concentration in combination with results from binding kinetics will ultimately provide the best signal to noise and thus the best conditions for imaging of the HER2 receptor. Also the biological half life of the DARPin under imaging conditions needs to be established. These studies will be conducted in parallel with the WP5 Phase I/II clinical trial and will benefit from bedside-to-bench information. The ultimate aim is to aim to provide the most optimal radiolabelled DARPin (for SPECT and PET) for use in the next stage in development of this important tool. WP4 will validate the use of radiolabelled DARPins for monitoring Herceptin treatment in spheroids and xenograft mice models (98, 99, 105).

In addition to developing the imaging agent, WP4 will involve the development of improved imaging methods for Quantitative SPECT (Q-SPECT). It is important that high quality SPECT imaging is available due to the much wider availability of SPECT technology and thus the eventual DARPin imaging agent will be readily available to a greater patient population. The tools for developing higher resolution and more quantitatively accurate SPECT have been developed by GE Medical. In conjunction with GE Medical Systems Israel Ltd we will develop QSPECT for ^{111}In and ^{67}Ga so that either could be employed depending on the availability of GMP radionuclides at the time of deployment.

WP5 - DARPins in the clinic

The Targeting and Imaging Group at UCL have an established track record for bench-bedside development of antibody-based therapeutics (106-111) and clinical molecular imaging with radionuclide labelled antibodies (110-115) (e.g. Figure 9).

We will now apply this technology to molecular imaging of HER2 with DARPins in HER2+ve breast cancer where we have established clinical practice and expertise (116-118)

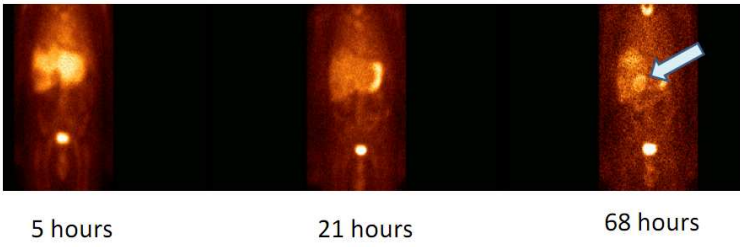


Figure 9. Planar SPECT images at three time points following injection of 131I-A5B7 (anti-CEA MAB) in a patient with liver metastasis from colorectal cancer. At the early time point the antibody is cleared through the liver however at 68 hours there is retention in the tumour (arrowed). (114).

DARPins may also be advantageous in regard to circulating antigen, which is sometimes viewed as troublesome for imaging purposes, although in the experience of UCL, circulating antigen has not been a problem for diagnostic or therapeutic use of anti-CEA, either pre clinically or clinically. Nevertheless, the smaller size of DARPIns than antibodies will allow approximately 10x more HER2 binding molecule/mg of DARPin protein than /mg of antibody protein. Thus, even taking high estimates of 100 ng/ml circulating ECD HER2 extra cellular domain (ECD; MW 110kD) in patients who had raised (>15ng/ml) ECD pre-treatment [120] as the basis for calculations, and assuming 5l of blood in a "standard woman" (scaled from Cember [121]) giving a total circulating HER3 ECD of 500 µg, a 1:1 binding molecular equivalence will occur at ~70µg circulating DARPin (MW 15kD). Although the DARPins can be expected to migrate into the extravascular spaces and clear rapidly via the kidney, 1 mg administered DARPin is expected to provide sufficient DARPin excess to saturate circulating HER2 and allow clinical imaging of tumour.

WP5 - Circulating tumour cells (CTC)

IMAGINT will also explore the possibility of applying its tools to study and analysis of **Circulating Tumour Cells** (**CTCs**) an exciting new and **non-invasive** source of clinical tissue that can be a biomarker merely by their presence, or can be used for 'fingerprinting'. CTCs are shed into circulation, from either primary or metastatic tumours at an early stage in breast cancer progression, which is followed by metastasis (122,123). CTCs have been observed in the blood of patients with all major carcinomas including breast cancer but not in healthy volunteers (124). The significance of CTCs has been assessed in various trials and shown to be of clinical significance; i.e. prognostic relevance and predictive of overall survival (125,126,127,128). Although the incidence of CTCs (ratio to leukocytes) can be low, CTCs are readily detected based on a combination of an enrichment step paired with a subsequent detection step (129,130). The potential of CTCs is reflected in the fast number of techniques currently explored to isolate these cells. The enrichment stage can be based on cell morphology. For example a filtration technique has been described based on the larger size (>8 um) of CTCs relative to non-cancerous cells (131). Also the higher cell density of CTCs can be exploited for density gradient enrichment (Oncoquick, www.greinerbioone.com; 131,132). Alternatively, enrichment can be performed by immunomagnetic separation in a (semi)-automatic manner. In this case a magnetically labelled antibody is used. The Magnetic Activated Cell Sorting system from Miltenyi (MACS; www.miltenyibiotec.com) uses this principle. For example, a positive selection based on an antibody to the epithelial cell adhesion molecule (EpCAM) is magnetically labelled to allow for a positive selection of CTCs. If desired, the enriched pool can subsequently be probed with another magnetically labelled antibody. Similarly, the Veridex CellSearchTM technology (Johnson&Johnson; www.veridex.com) fully automatically magnetically enriches the CTC pool based on EpCAM. Alternative immunomagnetic separation platforms are under development, which aim to increase purity of the CTC preparation.

At UCL we are already using the Veridex CellSearchTM technology to detect synaptophysin positive neuroendocrine cells in patients with midgut carcinoid and are therefore familiar with developing novel applications. In IMAGINT we will use the CellSearch machine, available in the Cancer Institute to enumerate HER2 positive CTCs in patients participating in the anti HER2 DARPin clinical trial and we will compare the detection of HER2 positive disease with these 2 tools. Moreover we will develop methods to analyse paring of HER family receptors in CTCs and subject CTCs to full toponomics

analysis. It is expected that the powerful new information obtained with CTCs will substantially refine the prognostication that is currently available.

WP6 - Data analysis and prediction

IMAGINT will produce large amounts of diverse biomedical data, which have to be integrated and analyzed in order for the consortium to identify the most powerful (combined) cancer biomarkers related to the HER2 pathways. This requires as a first step the creation and maintenance of an efficient, robust and flexible database. IMAGINT diversity of data types and characteristics places nontrivial requirements on data analysis tools. First, they should be of sufficient complexity to detect nonlinear patterns; there is no reason for biomarker regularities to be linear. Second, IMAGINT's applications will run on multi-dimensional data sets with 200 patients (on archive tissue) or less. These conditions bring the danger of `over-fitting' (detecting spurious patterns), which becomes a serious issue even for simple linear separators roughly when the intrinsic dimensionality of our data approaches or exceeds N/2, where N is the number of patients (133). To avoid overfitting our tools must be Bayesian in origin, complementing predictions with reliability estimates without ad-hoc regularization. Third, since all our biomarkers represent partial windows on the cellular signalling system, we require methods that allow for so-called `hidden' or `latent' variables. Standard medical statistical packages tend to implement versions of Cox regression (134,135), regarding biomarkers as so-called covariates and calculating their most likely impact weights to explain observed cancer recurrence. Cox regression involves unwelcome assumptions: first, patients are taken to differ only in their biomarker values (no `hidden variables'); second, the `proportional hazards' assumption implies that disease severity depends monotonically on a single quantity which depends linearly on the biomarker values (the prediction task is linearly separable). Moreover, Cox-type regression cannot detect nonlinear regularities amongst biomarkers. The second family of available tools originates from the machine learning community, with a prominent role played by kernel methods such as support vector machines (SVM (136,137)). Standard machine learning software tends to be either Bayesian but based on linear separation, or (as in SVM or self-organizing maps (137)) capable of non-linear separation but non-Bayesian. All available tools meet at most two of our requirements; there is no data processing and prediction tool yet that can deal with nonlinear separations, is Bayesian in nature (to guard against overfitting) and allows for hidden or latent variables.

WP6 - Derivation of biomarkers from toponome and protein interaction data.
This task requires identifying critical nodes in signalling networks. In the past such identification would have been done on the basis solely of topological features of the network considered. Moreover, the topological information used involved primitive measures of the `distance' between pairs of nodes (e.g. shortest path length). There is now increasing awareness that more sophisticated approaches should be followed, that aimed at identifying critical nodes of networks on the basis of their functionality, as opposed to on the basis of distance properties only; so far only tentative results in this direction have been published (138,139). IMAGINT will go beyond these studies and develop new mathematical protocols for the identification of candidate biomarkers from toponome and protein interaction data that build on more advanced representations of node distances (by summing over multiple paths between nodes) and of node relevance (by analysis of their impact on signalling dynamics).

Data will be deposited in recognised databases to optimise their availability for use by other researchers.

Progress beyond the state-of-the-art

Current status	Progress beyond the state-of-the-art
Breast cancer imaging is performed by CT and FDG/PET. This **lacks specificity for HER2**. Antibodies can be used for imaging but do not have favourable PK. Preclinical data indicate that small molecules will be superior Unreliable non-invasive methods to image HER2+ve metastases in clinical practice.	IMAGINT will develop and clinically evaluate high affinity anti-HER2 DARPins for imaging HER2+ve metastases in patient with breast cancer. Tools should provide sensitive and specific **quantum imaging techniques for** HER+ve breast cancer metastases based on DARPins
Routine measurement of HER2 on primary tumour biopsies by IHC (protein) and FISH (gene copy number). **Test does not distinguish** between the different HER Homodimers and hetrodimers	IMAGINT will develop **robust tools** to **image** and measure specific HER heterodimers in **clinical tissues** and relate their presence to clinical outcome. Potential of **new diagnostic/prognostic biomarkers** in addition to greater scientific understanding
Available technology to study protein-protein interactions: FRET/ FLIM **Unavailable** comparison of technologies and system biology approach to model protein-protein interactions with molecular interactions	Development of a **multidisciplinary approach** combining FRET/FLIM with bioinformatics, network modelling and biochemical techniques to monitor **interacting proteins**, biochemical events and therefore protein function *in situ*, in normal and tumour cells both in space and time. Data to be used to generate a **molecular interaction**-based, multivariate signature for cancer **diagnosis** and **prognosis**.
Nanoscopy: a cutting edge technology overcoming the resolution limit of light No previous combination of DARPins with nanoscopy to optimise optical resolution	Innovative combination of immunochemistry approaches (DARPins) utilized in combination with nanoscopy to rely on using antibodies which increase the observed structure by 30-40 nm. The small size of DARPins could allow a **higher optical resolution** in nanoscopy and potentially open a resolution regime. New information on the **behaviour of HER2** and its **interacting partners on the nanoscale**.
Toponome imaging system: a cutting edge technology to identify specific protein cluster networks on a large scale of proteins (more than 100 simultaneously) in a single cell or tissue section	**Greater resolution** of TIS system by using DARPins. Creation of a toponome **signature for breast cancer** Greater **understanding of cellular networks** that drive breast cancer **Discovery of new therapeutic targets**
Only **molecular diagnostics** based on genetic and genomic developed for clinical use are available Lack of sensitivity of this type of diagnostic	IMAGINT will develop new tools to measure **clinically important molecular networks** to complement and extend current signatures and **Discovery of new biomarkers**

Current status	Progress beyond the state-of-the-art
The current RNA/protein complex isolation technologies are based on antibodies and multiple denaturating steps (cross-linking) The RNA/protein interactions occur in RISC in cytoplasmic clusters known as P-bodies. Cytoplasmic pattern of P-bodies could have biomarker potential	Use of DARPins, and related detection technologies, **as tools** to efficiently isolate the RNA/protein complexes *in vitro* conditions and from *ex vivo* without denaturing the complex. **New biomarkers** obtained from characterising RISC **New biomarkers** from imaging cellular location of RISC This technology will be transferred to clinical samples.
Limited labelling protocols for radiochemistry in terms of selectivity obtained in reactions between large proteins and bifunctional coupling reagents and due to poor tolerance of proteins to heating.	Use of innovative purifying and concentrating process based on 68Ga and microwave to prepare cysteine-tagged DARPins containing appropriate chelates for radiolabelling with high specific activity for **increased sensitivity of diagnostic imaging**.
Limited Bioinformatics methods based on Bayesian approaches with regard to the detection of nonlinear patterns, or based on kernel methods which suffer from overfitting. Limitation of both methods with respect to the probabilistic integration of qualitatively distinct data sources.	Development of **new mathematically framework** built on Bayesian latent variable models for integrating distinct data sources, for detecting (possibly nonlinear) probabilistic patterns in such data, and for quantifying their predictive potential.
CTC population is **approved as biomarker** by FDA and the EU for breast cancer. The corresponding detection methods are approved as well.	Methods will be developed to isolate CTCs from the peripheral blood of patients and **to characterise their signature** using the most effective new DARPin based tools developed in IMAGINT. This tool will substantially improve the level of **diagnostic and prognostic information** obtained from CTCs and provide a new standard of **non-invasive biomarker** for clinical care.

B.1.3. S/T methodology and associated work plan

.1.3.1 Overall strategy and general description

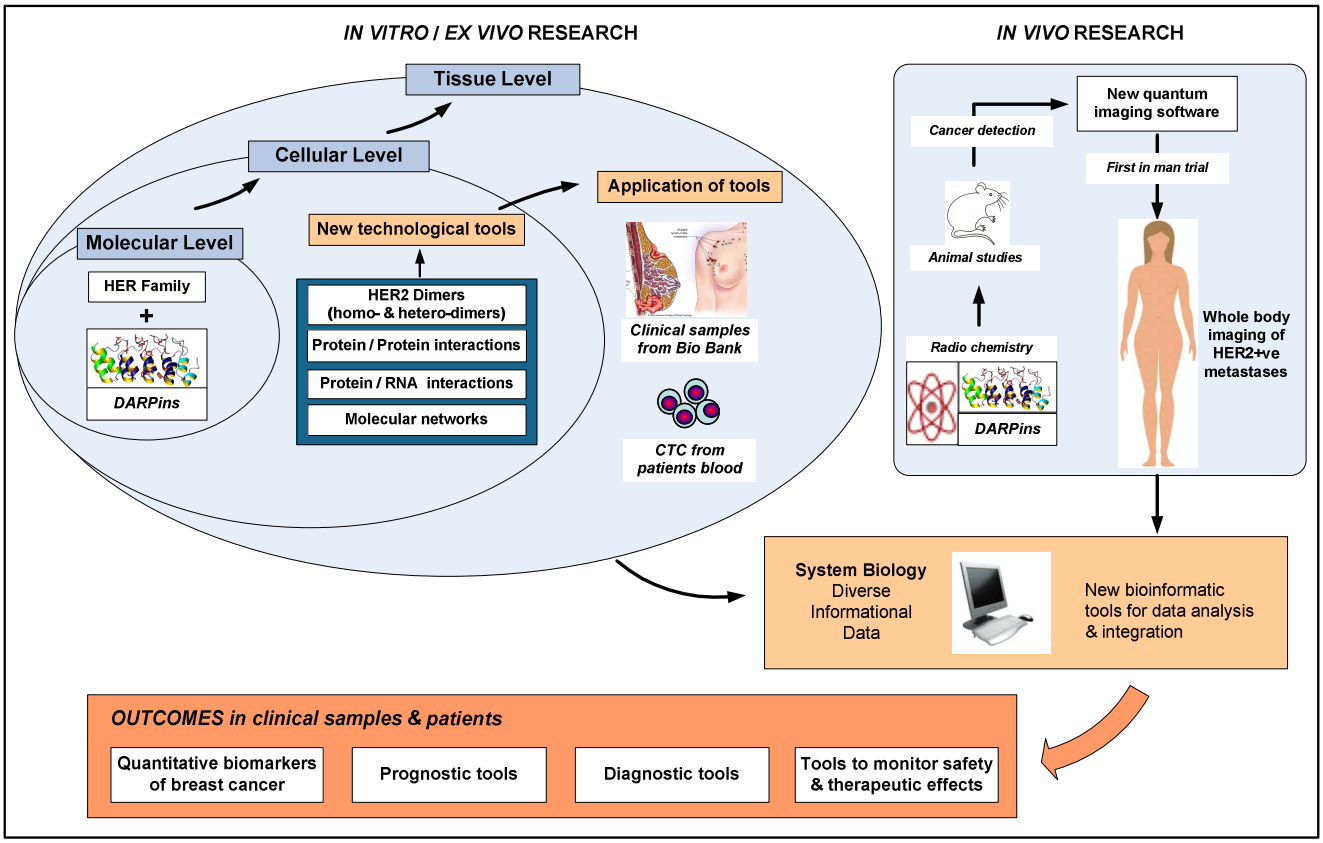
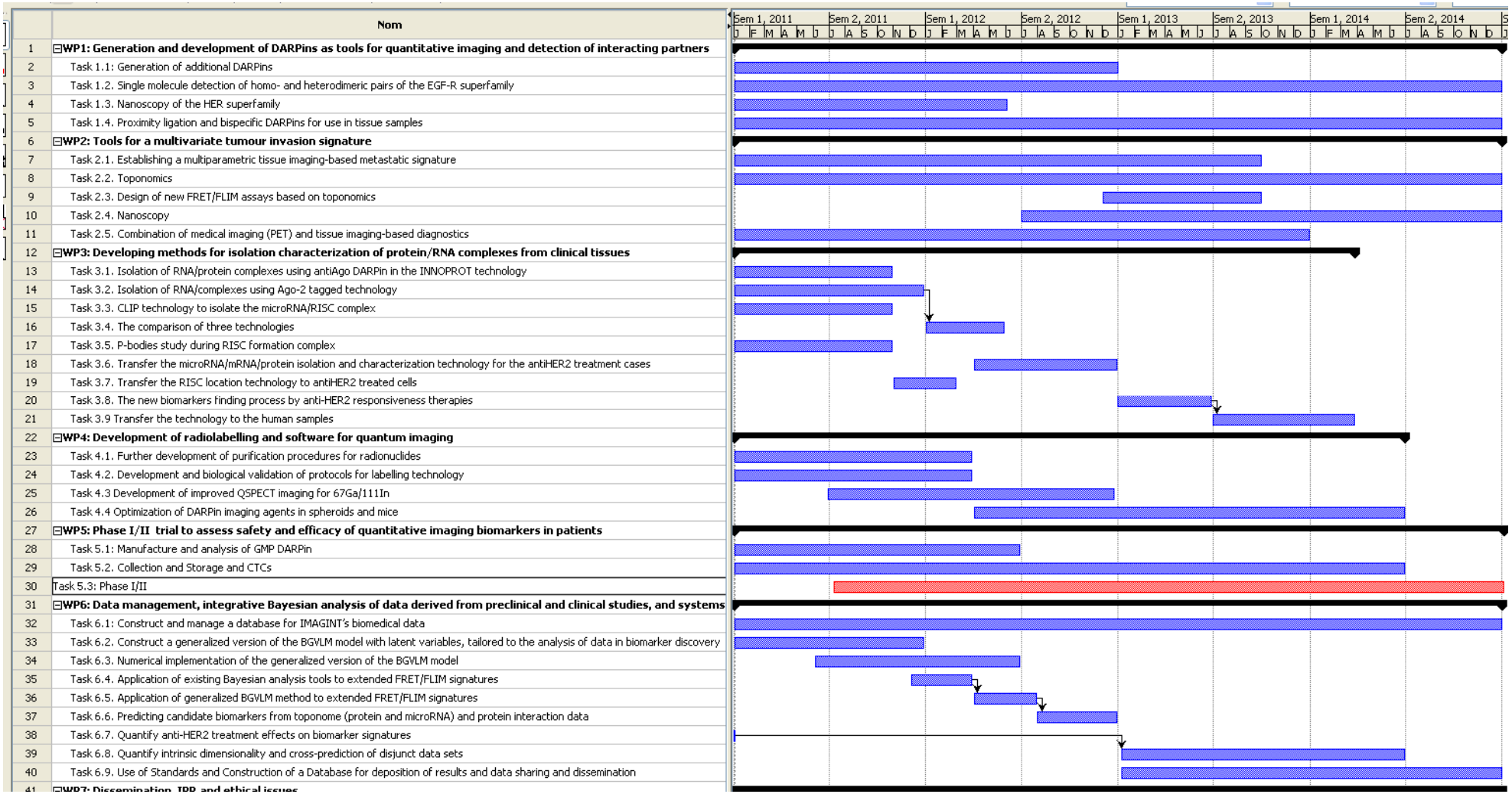


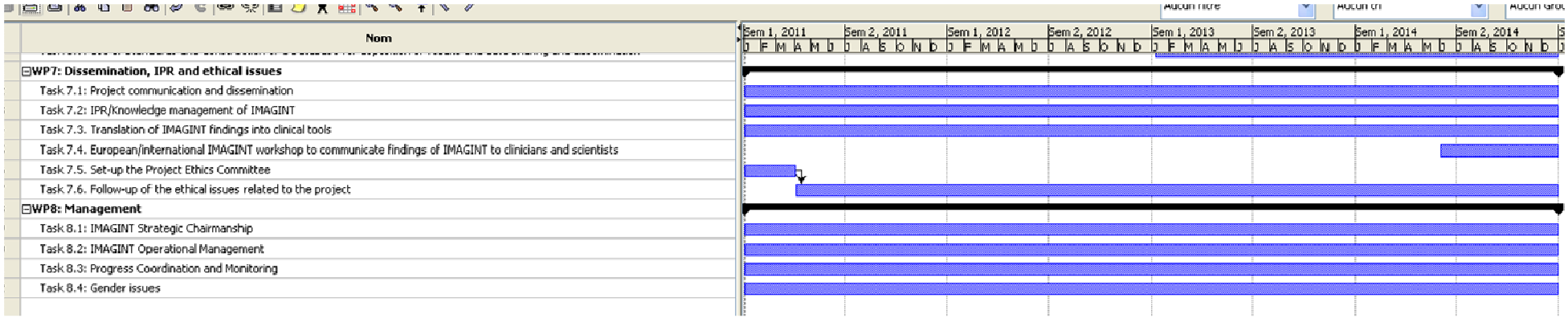
Figure 10: Synopsis of IMAGINT

WP1 will generate and optimise the targeted DARPins in order to enable to WP2, 3 and 4 to develop tools to (i) measure directly interacting proteins, (ii) map topological protein networks, (iii) isolate protein/RNA complexes (WP3) and (iv) improve quantum imaging (WP4). WP5 will provide clinical samples to WP2, 3 and 4, to achieve some of their goals. It will implement quantum imaging methods from preclinical (WP4) trial to a first-in-man trial (WP5) using cGMP-produced DARPins (WP1 and 5). Multivariate data generated in WP1, 2, 3, and 5 will be integratively analysed in WP6. Moreover, all these RTD WP will be interconnected with transversal activities (ethics/IP/dissemination WP7, management WP8) and the exploitation of results will be explored to transfer the knowledge to benefit patients.

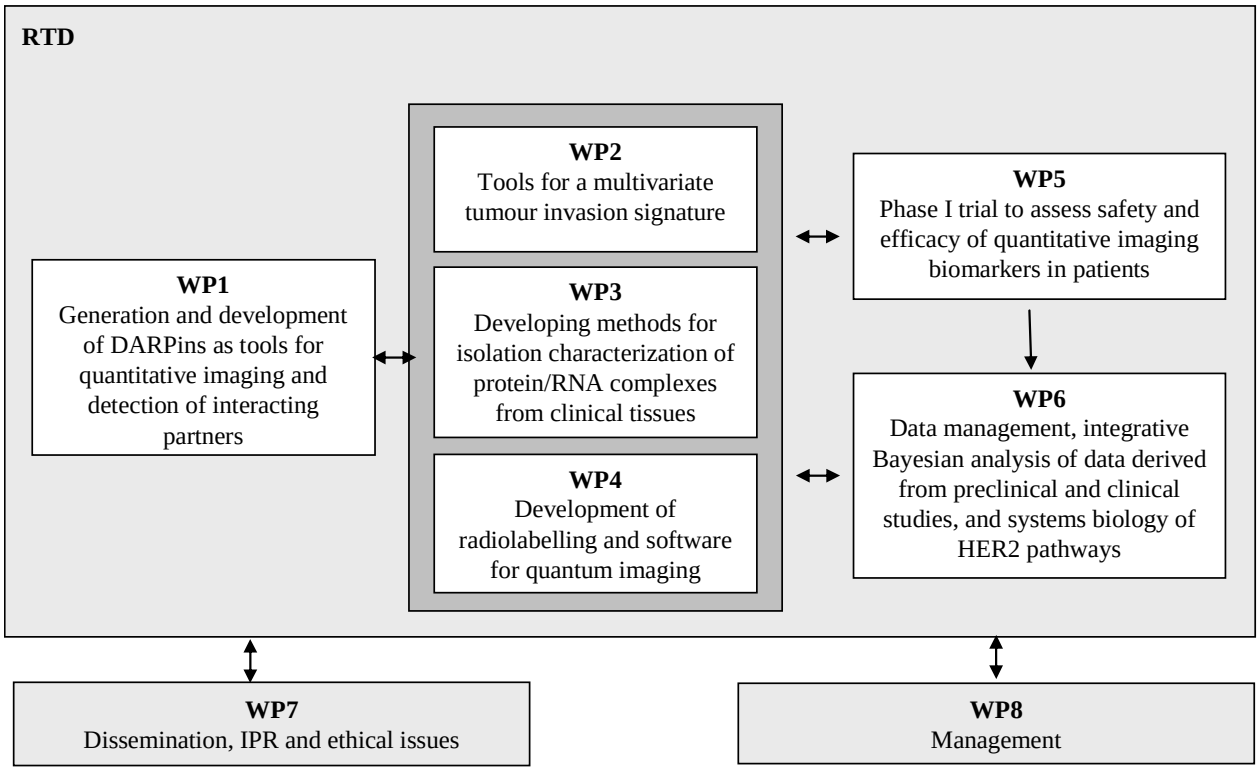
IMAGINT will develop the HER2 imaging and interaction mapping tools using breast cancer models but this will not limit the utility of the tools. Once developed for breast cancer the tools will have wide application for other cancers in which HER2 is clinically relevant and provides a potential target in a definable sub-set of patients. For example, gastric cancer, which is the world's second leading cause of cancer death. Another example is pancreatic cancer, a devastating disease, resistant to conventional therapies and a major cause of cancer death, accounting for over 60,000 deaths in a single year in the EU. HER2 has also been reported as a potential target in wide range of other cancers including ovarian cancer, non-small-cell lung cancer and prostate cancer. Cell lines, xenograft models and clinical tissues are available from the majority of these cancer types in the consortium. If opportunity arises, these will be included in the IMAGINT project, at appropriate points, where their addition will provide added value or useful controls, without loss of focus on our goals.

.1.3.2 Timing of Work Packages and their components (GANTT Chart)




Nom
Sem 1, 2011
Sem 2, 2011
Sem 1, 2012
Sem 2, 2012
Sem 1, 2013
Sem 2, 2013
Sem 1, 2014
Sem 2, 2014
WP7: Dissemination, IPR and ethical issues
Task 7.1: Project communication and dissemination
Task 7.2: IPR/Knowledge management of IMAGINT
Task 7.3. Translation of IMAGINT findings into clinical tools
Task 7.4. European/international IMAGINT workshop to communicate findings of IMAGINT to clinicians and scientists
Task 7.5. Set-up the Project Ethics Committee
Task 7.6. Follow-up of the ethical issues related to the project
WP8: Management
Task 8.1: IMAGINT Strategic Chairmanship
Task 8.2: IMAGINT Operational Management
Task 8.3: Progress Coordination and Monitoring
Task 8.4: Gender issues

Interdependencies between components (PERT Diagram)



Risk management associated to RTD WPs

Description of significant risks	Contingency plans
WP1	
Interaction of HER family members may be complex and not easily quantifiable by tractable mathematical models. E.g. there are probably two different HER2/HER3 complexes (with and without ligand), whose abundance depends on HER2 levels. HER2/EGFR pairing may depend on EGFR mutations.	For classifying patient samples, establish only the key diagnostic pairs (e.g. sum of all HER2/HER3) for a particular tumour phenotype.
Selected DARPins against HER3 may be of insufficient affinity	Affinity mature HER3 binding DARPins, using error-prone PCR and off-rate selection
Sufficient quantities of biotinylated Argonaute protein cannot be prepared by INO	Selection will instead be carried out against other members of the RISC complex
WP2	
For the HER2/HER3 dimerisation (by FRET/FLIM) assay using fluorescently-labelled HER2 DARPin, there is a risk that the DARPin may prove to be difficult to optimise due to the need to perform single cysteine labelling and the need to separate labelled and unlabelled DARPins (which are small) and free dye.	To send co-workers to UZH to progress the DARPin labelling or to set up the FRET/FLIM in parallel using fluorescently labelled anti-HER2 antibodies which are available in the Ng laboratory.
For the analysis of the distribution and clustering of HER2 in cultured cells there is a risk that the DARPins may prove to be difficult to be used for this application, even though no reason is currently apparent.	To use antibodies which are commercially available. This would reduce the attainable resolution to about ~ 50- 60 nm, which would still represent a significant improvement over the current state of the art and could potentially be used as a prognostic tool.

TIS measuring (Toponomics) procedures might be hampered by insufficient quality of the tissues provided by third party´s labs: only one instead of several visual fields per tissue section might show the quality needed	Compensation can be achieved by increasing the throughput by max factor 5: five times more tissue sections to be measured
For nanoscopy there is the risk that the breast cancer tissues cannot be imaged due to auto fluorescence or other unfavourable properties.	Concentration on cultured breast cancer cell lines. The number of analysed cell lines would be strongly increased to systematically determine the differences of protein distributions and to determine the potential of this readout as a prognostic tool.
WP3	
The RNA/Ago protein does not purify using INO technology because the Ago protein used to select anti-Ago DARPIns does not contain the appropriate epitopes for efficient capture of RISC.	Two approaches will be taken in parallel: One is to redesign the DARPIns against another protein sequence of the Ago protein. The other is to readapt the INO technology to commercial anti-Ago antibodies.
CLIP technology does not readily perform the with the anti-Ago DARPIns	Optimisation of the CLIP conditions using available antibodies will be developed, running in parallel with the generation of pure recombinant Ago for DARPin generation. Therefore the conditions will be established beforehand to give understanding of the technology. Furthermore, if there are unforeseen problems with DARPin CLIP, the antibody system will provide a backup means to identify relevant miRNA whist the DARPIn system is being optimised.
WP4	
DARPins radiolabelled using DOTA as chelator may be unstable in vitro e.g. to challenge with competing agents (such as other chelators), or in vivo, due to the complexity of biological systems. This may influence the biodistribution of the radiolabelled DARPin.	With regards to In-labelling the consortium will explore the use DTPA in addition to DOTA. With regards to the 68Ga-labelling NOTA will be investigated as well as DOTA. Other chelators and linkage methods will also be explored in the unlikely event that the first contingency does not meet with success.
WP5	
Unexpected problems in adapting the established E. coli laboratory production methods for DARPin expression to GMP compliant protocols.	Use an alternative expression system. Yeast *P. pastoris* is established as a GMP expression platform at UCL and has been successfully applied to DARPins in a laboratory setting using GMP protocols.
Insufficient DARPin uptake by tumour for diagnostic utility in the initial trial protocol	Escalate DARPin injected amount
Ethical Approval for clinical trial not granted.	We will respond to the comments of the ethical review committee to ensure approval. A Research Ethics Committee (REC) is required to give an ethical opinion within 60 calendar days of the receipt of a valid application. Where the REC considers that further information is required in order to give an opinion, the REC may make one request in writing for further information. The period of 60 days will be suspended pending receipt of this information.

Cancer Research UK do not fund sponsor or fund the clinical trial through the New Agents committee. The risk is not possible to rate because the committee is independent and decisions are made by an on a case-by-case-basis. However, the proposed trial fits the remit of the committee and 7/8 previous applications from the oncology group have been funded and carried through.	The advantage of Cancer Research UK sponsorship is that, if successful, the Charity also provides trial management through its internal resources. However, if Cancer Research UK does not sponsor the clinical trial we will request sponsorship from UCL who sponsor academic trials led by UCL investigators and have previously sponsored trials by Dr Tim Meyer. Dr Meyer has made preliminary contact with UCL and confirmed that trial management can be provided within the exisiting infrastructure of the UCL Cancer Trials Centre, the UCL Experimental Cancer Medicine Centre and the CRUK Imaging Centre.
High kidney uptake of DARPin	Assess tumour radiation dose; if significant amend protocol to allow pre dose with kidney protective agent.
WP6	
Availability of biomedical data required for Analytical tasks is delayed	Tasks involving data collection and mathematical modelling will be prioritised until data is available

B.2. IMPLEMENTATION

B.2.1. Management structure and procedures

Overall description

The IMAGINT consortium includes 10 partners from 7 European Member States and one associated country: Switzerland. The IMAGINT Consortium will implement and maintain all along the 48-month duration **a high-quality management** in order to permanently supervise the work performed and to ensure the achievement of project objectives on time and on budget.
IMAGINT is structured around six technical Work Packages (WP1 to WP6) which directly meet the project goals. One cross-cutting WP is set up to address knowledge dissemination, IPR and ethical issues (WP7). These WPs are sustained by WP8 which is fully dedicated to Project and Quality Management. Project management ranges from gathering intelligence to exploiting the generated results, through planning the research project, executing the project and correcting any deviation from planned actions. It obviously includes quality management and reporting to the EC. Project Management is therefore needed **both at strategic level and on a day-to-day basis.**
The very ambitious technical goals of IMAGINT, as well as the diversity of partners' backgrounds require a highly structured and result-oriented management system, which objectives are:
To effectively enable progress in the project by assessing, anticipating and managing all types of risk;
To trigger and maintain the project's momentum: build an effective and collaborative project team, where individuals co-operate and are motivated to achieve the project goals and deliverables;
To generate the project added value: exploit to the full the knowledge and capabilities of people working in the consortium teams, and increment results into the deliverables.
To guarantee the project quality: plan, implement and control quality from work-plan processes to deliverables

Management structure
The management structure is thus divided into <u>two levels:</u>

- Day-to-day Management that consists in operational management.
- Strategic Management that consists in formulating, selecting, prioritizing and evaluating cross-functional decisions and strategies that will enable the consortium to meet the project objectives.

Day-to-day Management
The day-to-day management will be supervised by the Project Coordinator: Kerry Chester has unanimously been appointed as Project Coordinator by all partners. She will be assisted by a Management Team, a system biology team (WP 6), a scientific advisory board, an IMAGINT steering committee, and ultimately by WP leaders in the day-to-day management project activities.

<u>The Coordinator's responsibilities</u> are:

- To liaise with the EC on behalf of the Consortium
- To be responsible for the achievements of the project in line with the Grant Agreement, on time and within budget.
- To act as the chairperson of the IMAGINT steering committee to ensure the coherence between decision-making bodies
- To mediate disputes within the consortium
- To keep contact with the scientific advisory board

She will have overall responsibility for delivering the project and for managing risk and change in an effective manner. The Coordinator will ensure that the IMAGINT project is able to meet and successfully pass periodic reviews as required by the European Commission procedures. The Coordinator will also act as the primary conduit between the IMAGINT consortium and the European Commission, as well as the IMAGINT steering committee, and the advisory board. The coordinator will head the Executive Management Committee. The Coordinator will formally chair the IMAGINT Steering Committee.
The coordinator will be supported by a Management Team. The Management Team is responsible for the day-to-day management, planning and monitoring. It performs the actions necessary to coordinate the different Work-Packages and ensures their progress. The Management Team is composed of resources from the Coordinator's own organisation and by the management-specialist partner, ACIES/P2R.

ACIES is "Recognised for Excellence in Europe" by the European Foundation for Quality Management since 2002. ACIES/P2R was Winner of the French Quality Award in 2004. ACIES/P2R will apply its expertise and quality management systems to provide guidance, management assistance and training when necessary to the members of the consortium.
ACIES/P2R has a proven track record in the management of complex and large-scale European research projects. ACIES/P2R manages projects with duration from 2 to 5 years, made up of 5 to 35 European partners and with budgets ranging from 2.5 to 50 millions Euros. ACIES/P2R is currently managing more than 25 FP6 and FP7 projects in the Health, Environment, ICT, Transport and Energy themes.

The Management Team role consists in:

- Assisting the Steering Committee (SC) in effective implementation of the work program
- Managing administrative, legal, financial and all non-technical aspects of the project
- Coordinating the release of all the deliverables and periodic reporting to the European Commission in due time
- Is responsible for the bottom-up and top-down communication within the consortium and the SC members of partner organisations involved in the work program
- Identifying issues and delays and reporting to the Steering Committee
- Collecting and collating the progress reports and resource expenditures
- Sending reports to the European Commission
- Providing guidance on administrative and financial issues

WP Leaders responsibilities

All Scientific and Technical activities will be coordinated and managed at WP level by Work Package Leaders (WPL). WPL were appointed by the members of the WP to lead the activities within a specific WP.

WP nb	WP Leader	WP nb	WP Leader
WP1	UZH	WP5	UCL
WP2	KCL	WP6	FLS, KCL
WP3	INO	WP7	UCL
WP4	UU	WP8	UCL

WP Leaders will:

- Coordinate and supervise the Scientific and Technical WPs they lead (including planning, monitoring and reporting of activities)
- Be responsible of the content of the released deliverables
- Organise, when needed, special Quality Assurance and risk-assessment meetings to determine suitable corrective measures
- Ensure that milestones and deliverables are fulfilled and delivered in due time.
- Provide feedback to the management team on any delays or issues regarding the achievement of WP objectives.

WP meetings will take place at least every six months and as often as required. They will be conducted in person or via phone/video conference). Written minutes will be released after each meeting and send to all WP participants.

Strategic Management

The strategic management level includes **two governance bodies**:

- The Steering Committee IMAGINT Steering Committee
- The Executive Management Committee chaired by the Project Coordinator (Kerry Chester).
- It will be assisted by three advisory committees and boards:
- The Scientific Advisory Board (SAB)
- The Project Ethics Committee (PEC)
- The Intellectual Property Committee (IPC)

The Steering Committee (SC) is the ultimate decision-making level within the project that makes sure to represent the interest of all partners. It is chaired by the Project Coordinator.

The Steering Committee (SC) role consists in:

- Revising annual scientific objectives, policy and strategic orientations of the project in accordance with the rules of the Grant Agreement and the project work program
- Overseeing the progress of the project towards its objectives, deliverables and milestones
- Ensuring proper administrative, legal and financial operations (including decision on the distribution of the EC pre-financing / interim payment)
- Deciding on the changes within the consortium such as the inclusion/ exclusion of partners, the funding redistribution, any corrective measures, modifications or suspension of the research program

The Executive management Committee (EMC) represents the functional decision level within the project management organisation. It is composed of the Project Coordinator, the Work Package (WP) Leaders and the Management Team.

EMC role consists in:

- Ensuring proper implementation of Steering Committee's decisions
- Monitoring the effective and efficient implementation of the project
- Initiating and supervising all operational aspects including technical (deliverables, milestones), financial (audit certificate, cost statements), legal (Grant Agreement, consortium agreement, IP rules) and communication (workflow, reporting) aspects
- Informing the Steering Committee on the progress of the project
- Proposing any adjustments on the budget allocation and/or on research objectives to the Steering Committee when required.

Advisory Committee meetings will take place at least once a year and as often as requested by the IMAGINT project. These meetings will aim at evaluating the work performed as well as the results obtained on specific aspects relevant to the committee (Commitment letters are available in Annex 6.3).

Scientific Advisory Board (SAB)				
▪ Advise the Coordinator and the Steering Committee on project orientations and/or any relevant specific issues ▪ Perform market, science and technological studies to keep the consortium aware of opportunities and threats ▪ Make proposals and transmit any information relevant to the project				
Org. Name	**Country**	**Contact**	**Position**	**Expertise**
Vall d'Hebron Institut d'Oncologia (VHIO)	SP	**Prof. José BASELGA**	Director	Oncology, clinical breast cancer, translational and early clinical research. HER family signalling and downstream molecules as targets for breast cancer therapy. Clinical development of antiHER targeted therapies
Fox Chase Cancer Center	USA	**Prof. Greg ADAMS**	Head of Antibody target group	Engineered antibody fragments, Antibody based HER2 targeting and ImmunoPET imaging
Centre for Molecular Oncology and Imaging, Institute of Cancer Barts and The London School of Medicine	UK	**Prof. Stephen MATHER**	Professor of Radiopharmacy	UK's most experienced Radiopharmaceutical Scientist with >20 years track record heading the ICRF/CR-UK Nuclear Medicine Research Laboratory. His group are world leaders in the field of antibody radiolabeling

Project Ethical Committee (PEC)				
▪ Remind the researchers of the main ethical practices to be followed on the project ▪ Advise the Steering Committee on potential ethical problems and on appropriate ethical procedures ▪ Contribute to ethical standards, shall any be developed within the project ▪ Contribute toward establishing and maintaining researchers' awareness on relevant ethical issues				
Org. Name	**Country**	**Contact**	**Position**	**Expertise**
Breast Unit at The Royal Marsden	UK	**Dr Charles SWANTON**	Consultant Oncologist	Dr Swanton is a specialist in translational research and personalised medicine. He has a specialist interest in chemotherapy drug resistance and the development of new therapies for patients with drug-resistant breast cancer. His work involves both basic molecular research and its application to breast cancer and early phase clinical trials.
Cancer Research UK	UK	**Dr Rob WILLIAMS**	Head of Nonclinical Operations & Chief Development Scientist	Dr Williams Heads the pre-clinical development work at the Cancer Research UK Drug Development Office. He has expertise in all aspects of pre-clinical testing to support exploratory clinical trials In particular for IMAGINT, Dr Williams will provide external advice on ethical issues associated with use of pre-clinical animal models.

Intellectual Property Committee (IPC)
Composed of IPR experts from partner's organisations ▪ Assist partners in identifying foreground requiring protection and/or dissemination ▪ Provide advice on: foreground ownership, management of joint ownership, access rights granting, the freedom to operate, patentability, the choice between patent and other protections ▪ Adapt the project exploitation plan taking into account the new industrial and market opportunities ▪ Update the project background ▪ Monitor and approve the dissemination issues proposed by partners

The IPC will check if publication does not contain any confidential information belonging to other partners. The specific process for obtaining the position of the IPC will be detailed in the Consortium Agreement.
The other main role of the IPC will be to decide on property and to negotiate on projects results property assignment.

Competencies and Responsibilities of the coordinating organisation and coordinator

Prof. Dr. Kerry Chester, the Project Coordinator of IMAGINT has extensive expertise in antibody targeting of cancer and **an international expert on recombinant antibodies**. Kerry Chester leads the antibody engineering research at the UCL Cancer Institute. She has directed work resulting in design and production of antibody-based biotherapeutics for cancer imaging and therapy for over a decade. Her team produced the first singe chain Fv to enter clinical imaging trials and she has a sustained record of generating antibody-based therapies for clinical use. For breast cancer expertise, Professor Chester works closely with Dr Alison Jones, Consultant Oncologist with special interest in HER2 expressing breast cancer and for Phase I/II trials in collaboration with Consultant Oncologist, Dr Tim Meyer. Prof. Dr. Kerry Chester received the prestigious international ISOBM Abbott Award (2003) for 'outstanding contributions in cancer research'. She is the first, second, or senior

author of peer-reviewed publications, and reviews, chapters, and editorials and has generated four patents on antibody therapeutics.

Management procedures

Strategic management procedures

Decision-making process
The aim of the consortium is to achieve consensus on the project issues and make all decisions within the consortium democratically. The crucial strategic decisions related to the evolution of the consortium (entry of a new party, withdrawal of a party, change of the coordinator) or to the suspension of the project will be made by unanimous vote whereas all other decisions within the consortium will always be made by a qualified majority of two-third (2/3) of the partners' vote.

Resolution of conflicts
Disputes among the Beneficiaries regarding workflow, on time delivery of results, dissemination of information among the consortium, IPR issues, etc. will be managed according to a pre-defined scale of interventions laid down in the Consortium Agreement. At the first level, it shall be assumed that those involved in a dispute shall attempt to resolve the matter to their mutual satisfaction. If this is not possible, the dispute shall be referred in the first instance to the Project Co-ordinator, who shall keep a record of all interactions with all parties concerning the matter. The Co-ordinator will act as a mediator and attempt to resolve the dispute to the satisfaction of all, and in the best interests of the Project.

If, in the Co-ordinator's judgement, a dispute between partners is negatively impacting the Project, or appears likely to continue to a point that it will do so, the dispute shall then be considered to be a conflict. The Co-ordinator shall then send a request to all members of the Steering Committee that a Conflict Mediation Committee be formed. The Steering Committee shall be a committee with one representative from each Beneficiary. This Conflict Mediation Committee shall comprise of three nominated representatives from three different partners, the constitution of which shall be decided by the Steering Committee. The nomination process shall not require an extraordinary meeting of the Steering Committee, and shall be discussed and approved by electronic means. The Committee should be in a position to act in as impartial a manner as possible, and will investigate the conflict and attempt to come to a mutually acceptable resolution within six weeks of its formation.

If the Conflict Resolution Committee fails in its task, or in the case that the original dispute concerns a decision or action made either by the Steering Committee, Executive Management Committee or Project Co-ordinator that falls within the scope of their responsibilities as laid down in the Consortium Agreement, the above process shall not be adhered to, and the matter shall be referred directly to the Steering Committee. The opinion of the Conflict Mediation Committee, if applicable, will be submitted in writing, and the conflict will then be resolved through a Decision of the Steering Committee, following the usual process set down in the Consortium Agreement. The Conflict will then be deemed to be resolved, and the parties shall abide by the decision.

Those involved will, however, retain the right to refer the matter to be finally settled under the Rules of Arbitration of the International Chamber of Commerce, by one or more arbitrators appointed in accordance with the rules. The award of the arbitration will be final and binding upon those involved.

Risk management
Risk management is a structured approach to manage uncertainty through risk assessment, contingency plans, and mitigation of risk using managerial resources. Risk management will be given great consideration during the project lifetime. The different processes related to risks are the following: (i) Risk identification (using expertise and data from previous experiences / projects). Foreseeable risks were identified by the consortium during the elaboration of the project and are stated in section 1.3; (ii) Risk treatment: plans to face identified risks and/or alternative plans to limit their impact will be drawn up. Contingency plans are already described in section 1.3. A careful follow-up of risks is one of the main roles of the Steering Committee.

Milestones
The control of the achievement of Milestones will be linked to Risk Management. Indeed, the Milestones table is an assessment tool enabling a monitoring of risk and anticipation of corrective measures. Milestones of the project have been defined in accordance with identified risks. The concerned WP leader(s), assisted by partners involved in the corresponding WP, will be in charge of monitoring their milestones and to report to the Coordinator who will act as the risks manager. The Coordinator will report to the SC who will recommend the continuation or implementation of contingency plans.

Communication flow
Communication across the management structure is critical to the success of the project. The Management Team will ensure that any relevant information and knowledge efficiently circulate between partners. Furthermore, to promote the project and its achievements within and outside the consortium, communication tools such as a public website, scientific and technical papers, seminars, conferences and flyers will be implemented and targeted towards various audiences.

Day-to-day management procedures: Monitoring and progress reporting

Internal reporting
ACIES/P2R, the Management Partner, will design and disseminate within the consortium a Project Management Handbook consisting in the main processes and templates essential to the efficient monitoring and reporting activities of the project.
Every six months, each **Work Package Leader** will **submit** to the Coordinator a consolidated progress report on different aspects of the project. This report will include information about the technical progress, results obtained (i.e. deliverables), the compliance with the work programme and all the relevant information at management level (resources, costs, delays, etc.). The Project Coordinator and the Management Partner will consolidate the information and finalise the report.

Contractual reporting
At the end of each period, the reports listed hereafter will be prepared by the WP Leaders and the relevant partners. The Management Team will finalise a consolidated version of these reports and will ensure that they are complete and consistent with the Grant Agreement before submitting them to the European Commission:

- **Periodic Report** (every 18 months) will contain an overview of the activities carried out during the reporting period including the following: a publishable summary, the description of the progress in relation to the project objectives, the milestones achievements, and the deliverables set for the period, the management activities developed over the period, any problems encountered as well as the corrective actions taken. It will also include a detailed justification of the costs incurred and of the resources deployed by each partner.
- **Final report** will be delivered at the end of the project. It will contain a synthesis of the main work conducted and the results obtained as well as the status of the costs incurred by the consortium throughout the whole duration of IMAGINT.

Plan for using and disseminating foreground, including health economic impact and target groups for the results of the research, will also be delivered at the end of the project. It will explain the potential application of the breakthroughs as well as a program describing how the results will be exploited and disseminated to end users, the scientific community and the general public. The plan will consist of two different parts: one is made available in the public domain, demonstrating the added-value and positive impact of the project on the European Community, the other one, specifying the exploitable foreground and its plans for exploitation, be kept confidential by the European Commission.

Project reviews
Technical reviews may be initiated by the EC at any time during the project lifetime. The aim of this review is to assess the work carried out under the project over a certain period and obtain recommendations from the EC. The reviewer(s) will be given full access to all relevant documents (Annex I, periodic reports, deliverables…). They will provide an assessment of the project based on the written material and information provided at a review meeting. Final recommendations and/or reorientations of the project will be given by the EC.

Consortium Agreement

The Consortium Agreement, under a DESCA model, is complementary to the Grant Agreement and will take into account the individual aspects of the consortium and procedures specific to the project. It will provide the legal basis for collaboration within the consortium, listing and defining partners' responsibilities and commitments as well as the IP management rules. All partners will sign it before the project starts.
The way in which the IP will be treated in the Consortium Agreement is outlined in section 3.2.2 below.

Quality assurance

Throughout the project duration, the quality of the results achieved will be continuously monitored to ensure that IMAGINT meets the quality standards. Quality monitoring will be performed by the "degree of excellence" management process and will be implemented within the first month of the project. Quality assurance will aim at ensuring the progress of the project towards its objective and the quality of deliverables. In IMAGINT, where all activities, milestones and deliverables and related budget are described at the beginning of the project, the assurance that the project is heading towards the right direction arises from the comparison of the progress and costs to the initial plans. Any deviation will be analysed and treated. This activity is the main role of the Steering Committee, and the internal reporting process will provide accurate information to help the Steering Committee to carry it out.

B.2.2. Beneficiaries

PARTNER 1 - UCL
Organisation description
General description: UCL/UCLH is one of Europe's leading health research multidisciplinary universities and is one of the largest and most productive centers of Biomedical Science in the EU. **The UCL Cancer Institute** which opened in 2007 includes clinicians, clinician-scientists and scientists, tackling all aspects of cancer research, making fundamental discoveries about molecular mechanisms leading to cancer and aiming to teach and train the next generation of cancer scientists and to conduct early phase and randomized clinical trials that will impact on patient care. The institute is equipped with the most modern facilities for microscopy, cell culture, molecular biology, cell biology, histology, bio-informatics, genomics, and clinical research. The Cancer Institute is closely integrated with UCLH, a leading cancer hospital housing a fully staffed Phase I Clinical Research Facility and an internationally regarded Department of Nuclear Medicine with a dedicated research SPECT/CT, on site radiopharmacy and suite of lead lined rooms. **Website:** http://www.ucl.ac.uk/cancer **Expertise:** The UCL Targeting and Imaging group has extensive bench-to-bedside expertise in pre-clinical and clinical cancer targeting, including first-in-man trials with radiolabelled antibodies, a MHRA licensed GMP facility and relevant regulatory knowledge. The UCL Viral Oncology Group has expertise in miRNA discovery and analysis. **Facilities:** The Institute offers an exceptional environment for clinical translation with a state-of-the-art facility for early phase clinical trials to ensure maximum patient safety and comfort. Other relevant equipment for IMAGINT include: CellSearch™ System (Veridex Ltd), for enumeration of CTCs. BiaCore, Next Generation Sequencing - Solexa, The Zeiss Palm MicrobeamTM system for laser capture microscopy, GIPZ shRNAmir lentiviral library for RNAi, fluorescence imaging and preclinical SPECT/CT and PET/CT**.** **Other EU Projects:** UCL is participating in 99 FP7 grants with a total research budget from the EU of €35.2 million.
Role in the project
UCL will lead the IMAGINT consortium and lead WP5. In addition UCL will perform 'Deep Sequencing' and laser capture microscopy for WP3 and collect CTCs for WP2.
Key personnel
Professor Kerry Chester (Coordinator) will be responsible for managing the consortia in compliance with the consortia agreement and with support of the management team. She will liaise with the different scientific and clinical participants. She has a proven track record of bench through to bedside development of recombinant therapeutics. **Dr Tim Meyer** will lead the Phase I/II trial, he is a Senior Lecturer and Consultant Oncologist, an expert in drug development and has an established track record in early phase clinical trials with a special interest in molecular targeting and imaging For breast cancer expertise. **Dr Alison Jones**, Consultant Oncologist with special interest in HER2-expressing breast cancer will provide breast cancer expertise. **Dr Berend Tolner** will oversee development of product release criteria and liaise with M-GMP; he has extensive expertise in GMP production of antibody-based molecules for clinical trials. **Professor Barbara Pedley** will oversee the animal work at UCL, she has extensive expertise in pre-clinical *in vivo* imaging. **Dr Alan Green**, Head of Imaging and Dosimetry will oversee the quality and data collection for patient SPECT imaging. **Dr Surinder Sharma** will oversee the GCP assay development for monitoring immune response to DARPIns. **Professor Richard Begent,** Consultant Physician will act in an advisory role. He is s a world leader in the field of targeted cancer therapy with special interest in data standards. He has been leading first-in–human trials for over 20 years. **Dr Dimitris Lagos,**co-Principal investigator of the Cancer Research UK Viral Oncology Group with expertise in miRNA, RNAi and RNA sequencing, will oversee the interaction with WP3.
Main publications and patents
Publications: Vigor K et al. (2010) Biomaterials 31:1307-15; Jones AL et al. (2009) Br J Cancer 100:684-92; Meyer T et al. (2009) Clin Cancer Res 15:4484-92; Dancey G et al. (2009) Targeted Oncol 4:201-17; Yong M et al. (2009) Protein Eng Des Sel 22:221-4; Green A et al. (2008) **Eur J Nucl Med Mol Imaging 35:393-406**; Kogelberg H et al. (2008) J Mol Biol 382:385-401; Tolner B et al. (2007) Eur J Cancer 43:2515-22**;** Kogelberg H et al. (2007) Glycobiol 17: 36-45; Tolner B et al. (2006) Nature Prot **1**:1006-21; Tolner B et al. (2006) Nature Prot 1:1213–22; Mayer A et al. (2006) Clin Cancer Res 12: 6509-16

PARTNER 2 - UZH

Organisation description

General description: The University of Zurich enjoys international renown as a place of education and research. Zurich's international reputation is based on groundbreaking research, particularly in molecular biology, brain research and anthropology, and on the work of the University Hospital and Veterinary Hospital. The Department of Biochemistry is committed to cutting-edge research and to offering an outstanding biochemistry curriculum compliant with international standards. Research is focused on the functional investigation of proteins, using biophysical, biochemical and structural approaches. Currently, the department houses twelve research groups and holds the leadership of the National Centre of Competence in Research Structural Biology. Several spin-off companies evolved from the department. **Website:** http://www.bioc.uzh.ch
Expertise: Dept. Biochemistry, University of Zürich have carried out pioneering work in the development of the recombinant antibody technology. Its contributions have included the development of the first bacterial expression system, design of the first fully synthetic antibody library, the development of ribosome display, and numerous contributions to protein engineering and evolution. An immunotoxin has progressed to late phase II clinical trial. More recently, the group developed DARPins as a new scaffold. The core expertise is protein engineering in all aspects, from computer design, molecular biology, directed evolution, biophysical characterization, cell culture and tumor targeting in mice. **Facilities:** The lab is fully equipped for protein characterization (CD, UV-Vis, Fluorescence, FT-IR, MALS, QELS, stopped flow, BIACORE, Bioveris), including HT equipment, BIOROBOT 8000, all molecular biology, cell culture, prokaryotic fermentation equipment. The department has x-ray crystallography, NMR and MS, as well as an animal facility. State-of-the-art single molecule fluorescence equipment is available within the department in the group of Prof. Ben Schuler.
Other European projects: PROTAFFIN (Project no.241481) - AFFINITY PROTEOME (Contract no. 222635) - PROTEOME BINDERS (Contract no. 026008 (RICA)) - PCUBE (Contract no. 227764)

Role in the project

The task of UZH will be to select DARPins against members of the EGFR family, to characterize them for their binding properties and specificities using assays described in the WP, engineer them for particular detection methods and provide them to partners for different tests.

Key personnel

Prof. Andreas Plückthun is a Professor of Biochemistry, who specializes in Protein Engineering; he is author of over 300 publications, which have been cited over 14,000 times, h-index 69. He was elected member of the German Academy of Science (Leopoldina) and EMBO. Inventor on 26 patent families, he co-founded the Biotech company Morphosys AG (Munich, Germany, 300 employees) and the Biotech company Molecular Partners AG (Zurich, Switzerland, 30 employees). He has been invited to over 150 plenary lectures at international conferences of which about 20 were denoted "keynote lectures". He has served, over the years, on the editorial board of about 20 journals. **Annemarie Honegger** is a senior research associate, with unique knowledge in protein modeling and computer aided protein design and thus a true asset to any project dealing with the creation of new proteins. She has written over 40 publications which have been cited 2500 times. **Ykelien Boersma** is a postdoctoral fellow with great experience in phage display of DARPins, whole cell selections, and the investigation of signaling. She is driving the single-molecule fluorescence studies of DARPins. **Christian Jost** is a PhD student with great experience in DARPin engineering, notably a range of bispecific formats and their effect on signaling. He is directly involved in the proximity ligation studies and expert in fluorescent labelling of DARPins.

Main publications and patents

Publications: Steiner, D., Forrer, P. and Plückthun, A. (2008). *J. Mol. Biol.* **382**, 1211-1227. / Zahnd, C., Wyler, E., Schwenk, J. M., Steiner, D., Lawrence, M. C., McKern, N. M., Pecorari, F., Ward, C. W., Joos, T. O. and Plückthun, A. (2007). *J. Mol. Biol.* **369**, 1015-1028. / Zahnd, C., Amstutz, P. and Plückthun, A. (2007).. *Nat. Methods* **4**, 269-279. / Zahnd, C., Pécorari, F., Straumann, N., Wyler, E. and Plückthun, A. (2006). *J. Biol. Chem.* **281**, 35167-35175. / Binz, H. K., Amstutz, P., Kohl, A., Stumpp, M. T., Briand, C., Forrer, P., Grütter, M. G. and Plückthun, A. (2004). *Nat. Biotechnol.* **22**, 575-582.
Patents: Steiner D., Forrer, P., Stumpp, M.T. and Plückthun, A., WO 2007/006665 (filed July 8, 2005) / Stumpp, M.T., Forrer, P., Binz, H.K., and Plückthun, A., WO 2002/20565 (filed Sept 10, 2001) / Parmeggiano, F., Pellarin, R., Larsen, A. P., Varadamsetty, G., Stumpp, M.T., and Plückthun, A., Designed Armadillo Repeat Proteins, WO2009/040338 (filed Sept 24, 2007) / Plückthun, A. and Hanes, J., Novel WO98/48008 (filed April 23, 1997)

PARTNER 3 – KCL – ST THOMAS' HOSPITAL

Organisation description

General description: King's College London is one of England's oldest and most prestigious university institutions: a multi-faculty research-led university college which is ranked as one of the world's top 25 universities.

The clinical PET imaging centre at St Thomas' Hospital is jointly owned by the medical school and the NHS Foundation Trust. The Centre is staffed appropriately with dedicated radiographers, technologists, physicists, radiochemists and cyclotron engineers. The clinicians and the scientists are employed as academics with the medical school. The unit has established the network of PET centres in the UK to work to the same quality assurance and quality control methods and this network is now an NCRI network. The same QA/QC methods have now been established for an international study of response measurement in lymphoma. The group publish widely in physics, oncology and imaging journals.

Expertise: The core expertise is in the application of PET/CT imaging to staging and response assessment of tumours. As a part of the comprehensive cancer imaging centre we link our expertise with our clinicians and other scientists to develop multimodality imaging methods for this purpose. The team has experience in quantitative PET imaging and modelling of new tracers in human research. The team also has experience of the manufacture of new tracers and is currently taking three tracers through an investigational medicinal product license application with the MHRA.

Facilities: Facilities include a cyclotron and radiochemistry facility for production of routine clinical and research radiotracers in addition to a scanning facility with 2 GE PET/CT scanners – one Discovery ST 4 slice CT and a Discovery VCT 64slice CT. The Centre presently performs ~ 3,500 PET/CT scans per annum. The majority of the scans are in oncology. Imaging sciences also has a nano PETCT system and an animal SPECT system.

Other EU Projects:

euHeart, 224495; Sublima; HyperImage

Role in the project

Responsible for neoadjuvant FLT/FDG PETCT imaging in WP2 and will contribute data to integrated analysis in WP6

Key personnel

Michael O'Doherty. Senior lecturer in imaging sciences. Experience in PETCT imaging in oncology with particular interest in diagnosis, staging and response monitoring of cancer as well as the use of novel radiotracers in oncology. Experience in IMP application process. Established criteria for QA/QC of Pet research in multicentre trials. **Paul Marsden**. Reader in Imaging Sciences with expertise is in the area of PET methodology, in particular instrumentation development, data acquisition and data analysis for multi-modality imaging systems. He is the National Cancer Research Institute Technology lead for PET research in the UK. He is the Scientific advisor for the national and international PET clinical trials and has a major interest in PETMRI. **Sally Barrington**. Consultant in PETCT and Nuclear Medicine with expertise in clinical PET research with oncology and runs national and international clinical trials involving PET. She has 19 years experience in PET with a variety of PET tracers that have been introduced into clinical imaging over this time. **Margaret Cooper**. Post doctoral fellow. Expertise as a radiochemist, radiopharmacist and a Qualified Person. Essential person for taking products through the IMP process. **Marcel Cleij**. Research radiochemist who has manufactured FLT and will produced FLT for research.

Main publications and patents

Publications:

Sutcliffe-Goulden J, **et al** Rapid solid phase synthesis and biodistribution of 18F labelled linear RGD containing peptides. Eur J Nucl Med 2002; 29: 754 – 759.

Mikhaeel NG, et al. FDG-PET after two to three cycles of chemotherapy predicts progression-free and overall survival in high-grade non-Hodgkin lymphoma. Ann Oncol. 2005 Sep;16(9):1514-1523.

Schleyer PJ et al. Retrospective data-driven respiratory gating for PET/CT. Phys Med Biol. 2009; 54(7): 1935-50.

Warbey VS et al. [(18)F]FDG PET/CT in the diagnosis of malignant peripheral nerve sheath tumours in neurofibromatosis type-1. Eur J Nucl Med Mol Imaging. 2009;36(5):751-7

PARTNER 3 – KCL – RICHARD DIMBLEBY LABORATORY OF CANCER RESEARCH

Organisation description

General description: King's College London is one of England's oldest and most prestigious university institutions ranked as one of the world's top 25 universities. The Richard Dimbleby Laboratory of Cancer Research (RDLCR) is directed by Prof. Tony Ng and belongs to both the Randall Division of Cell & Molecular Biophysics and Division of Cancer Studies. As such, the Dimbleby Laboratory is well placed to bring together research scientists, who are experts in their own individual disciplines such as optical physics (Dr Simon Ameer-Beg), molecular and cell biology (**Dr Melanie Keppler**), tumour pathology and clinical cancer treatment; working collaboratively to develop a variety of new technologies in the field of cancer cell and tissue imaging which researchers hope will help evaluate patients' disease progression. **Website:** http://www.dimblebycancercare.org/ **Expertise:** The RDLCR has a unique mix of training/expertise in Medicine, Immunology, Cancer cell biology (particular focus on the mechanisms of cancer cell migration), Biochemistry (study of signal transduction in cancer cells) and Optical Imaging and Cell Biophysics. Apart from Biology, Optical Physics and Medicine (esp. Breast cancers), expertise in Chemistry, Mathematics, Breast Pathology, Bioinformatics. PET/MR physics, etc is available within the KCL-UCL Comprehensive Cancer Imaging Centre, which Prof. Ng coordinates.

Facilities:

	Facility	Pre-clinical	Clinical
1	Imaging * – PET	Small animal PET-CT (nanoPET) and Small animal SPECT-CT (nanoSPECT)	2 PET-CTs
2	Imaging * – MRI	9.4T MRI, 3T clinical MRI with preclinical imaging coil	1.5 T and 3T clinical MRI with 32 channel technology
3	Imaging * – Other	For animal imaging: Multiphoton FLIM, endoscopic confocal, In-house PET-MR	

Other European projects: Growthstop. (FP6 - 037731)

Role in the project

Coordinator of WP2 and will contribute towards WP6 (integrated analysis work package).

Key personnel

Prof Tony Ng and others pioneered the application of fluorescence lifetime imaging microscopy (FLIM) to monitor proteomic changes such as phosphorylation, ubiquitination, sumoylation and protein-protein interactions in live and fixed cells, as well as archived pathological material. T Ng currently leads the Imaging sub-section of the KCL Experimental Cancer Centre awarded by Cancer Research UK/DoH. He is the Coordinator and Principal Investigator establishing a KCL-UCL Comprehensive Cancer Imaging Centre. Through these centres and initiatives, he is actively engaged in translating basic research to cancer biomarker discovery and patient benefits.
Dr Ameer-Beg is a non-clinical physicist lecturer within the Richard Dimbleby department of cancer research. His research is to develop optical instruments to address fundamental biological questions regarding the dynamic interaction of protein partners within the cellular membrane, furthering our understanding of cell signalling dynamics and control. **Dr Cheryl Gillett** is the Manager for the Guy's breast tissue bank (formalin fixed, paraffin-wax bank: 7,140 primary tumours, frozen tissue bank: 2,500 primary tumours) that have continued to accrue since the 1970's. **Dr Paul Ellis** is a senior oncology clinician in the Breast Unit at Guy's & St Thomas' Hospital Foundation Trust.

Main publications and patents

Publications: Ng T, Squire A, Hansra G, Bornancin F, Prevostel C, Hanby A, Harris W, Barnes D, Schmidt S, Mellor H, Bastiaens PIH, Parker PJ. Imaging protein kinase Ca activation in cells. Science (Washington, DC) (1999) 283: 2085-2089. / N. Anilkumar, M. Parsons, R. Monk, T. **Ng*** and J.C. **Adams***.Joint corresponding: Ng and J.C. Adams. Interaction of fascin and protein kinase Ca: a novel regulatory intersection in cell adhesion and motility. EMBO J. 22, 5390-5402 (2003). / Makrogianneli, K., Carlin, L.M., Keppler, M.D., Matthews, D.R., Ofo, E., Coolen, A., Ameer-Beg, S.M., Barber, P.R., Vojnovic, B. and **Ng, T.** (2009) Integrating receptor signal inputs that influence small Rho GTPase activation dynamics at the immunological synapse. Mol Cell Biol, 29, 2997-3006. / Morris, J.R.*, C. Boutell*, M. **Keppler***. Densham, D. Weekes, A. Alamshah, L. Butler, Y. Galanty, L. Pangon, T. Kiuchi, **T. Ng**, and E. Solomon. 2009. * Joint first authors. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress, Nature 462; 886-90.

PARTNER 3 – KCL - DISORDERED SYSTEMS GROUP
Organisation description
General description: King's College London is one of England's most prestigious universities: a multi-faculty research-led institution, ranked as one of the world's top 25 universities. Its Department of Mathematics has about faculty members and received an excellent rating in the UK's recent Research Assessment Exercise. The Disordered Systems Group of the Department specializes in the development and application of advanced mathematical techniques to complex problems in biomedicine, physics and informatics. **Website:** www.kcl.ac.uk/schools/pse/maths **Expertise:** The members of the Disordered Systems Group have excellent track records in the mathematical theory of Bayesian and neural information processing systems and machine learning and in the mathematical analysis of complex and inhomogeneous many-variable systems. Furthermore they have a significant track record of successful multi-disciplinary projects at the interface between applied mathematics and the biomedical sciences, including the analysis of cellular signalling pathways, protein-protein interaction networks, gene regulation networks, and the identification of biomarkers. **Facilities:** The partner (Coolen) and his coworkers will have access to several computing clusters in the College, such as the UNIX clusters of the Department of Mathematics and of the Center for Bioinformatics. **Other European projects:**
Role in the project
Professor Coolen's group will contribute to WP6, the integrative analysis of multidimensional data derived from preclinical and clinical studies and systems biology. They will focus on the integration of machine learning methods (e.g. kernel methods and self-organizing maps) with parameter and model selection in Bayesian latent variable models, in order to achieve improved but mathematically rigorous and stable detection and prediction protocols, that can be integrated with conventional medical regression techniques.
Key personnel
Prof. ACC (Ton) Coolen: Professor of Applied Mathematics with specialization in the mathematical analysis of complex physical and biomedical systems, including protein interaction networks and (Bayesian, neural, or information-theoretic) biomedical data analysis and parameter extraction. He has published more than 100 research papers and two books
Main publications and patents
Publications: Coolen ACC et al. (2005) **Theory of Neural Information Processing Systems (Oxford University Press)**, Bianconi G et al. (2008) **Phys. Rev. E78, 016114**, Rabello S et al. (2008) **J. Phys. A41, 285004**, Makrogianneli K et al. (2009) **Mol. Cell Biol. 29, 2997-06, Annibale A et al (2009) J. Phys. A42, 485001** **Specific awards:** Awarded a Springboard Fellowship (2007-2009) by the Engineering and Physical Sciences Research Council (UK)

PARTNER 4 - TNL

Organisation description

General description: ToposNomos Ltd (TNL), is an innovative provider and consultant partner for applications of toponomics in the life sciences. The company is equipped with exclusive I.P. for the whole field of toponomics. TNL is the world leader in this field. TNL provides the robot Toponome Imaging technology MELC/TIS as well as training in toponomics for research institutions and industry partners. One ongoing TNL activity is foundation and running toponome research centers world wide.

Website: http://www.toposnomos.com/

Expertise: TNL runs several reference labs (see below) involved in the human toponome project aiming at mapping and deciphering the protein network code in health and disease. The specific scientific competence of TNL is (a) engineering and running Toponome Imaging Systems (company feature in Nature Methods - 4, (2007). iii-iv), (ii) training scientists in using TIS imaging robots and (iii) consulting toponome researchers in analyzing toponome data sets by using unique software.

TIS is unparalleled by the following features: (i) it enables researchers for the first time to microscopically resolve the unique spatial structures of molecular systems in situ contaning up to several hundred thousand protein clusters, by applying a "power of combinatorial molecular discrimination" (PCMD) of more than 2^{100} per data point in a single cell, or, collections of thousands of cells, or, in a singe tissue section, simultaneously with high lateral subcelluar resolution; (ii) reveals the topological and functional hierarchies of (interacting) proteins; (iii) identifies protein networks directly in cells and tissues, and (iv) has proven to solve key problems in biology (publications, below). One prominent expertise of TNL and associated researchers is to identify lead (hub) proteins in tumour cells that exert control over disease-specific protein networks. Blockade of such lead(hub) proteins have been shown to interfere with tumour cell polarization/metastasis (Nat Biotech, below).

Facilities: TNL has access to the established toponome centers at (i) university of Magdeburg, Germany, (ii) university of Frankfurt, Germany; and (iii) university of Warwick, UK. In the present project specific tasks will be performed at the center, above (i).

Role in the project

TNL has a specific task in the project. TNL provides the TIS technology, tag libraries and Toponome data analysis for the consortium in order to (I) find and analyse the cellular protein networks associated with HER2 in breast cancer cells, (ii) deliver the corresponding protein network codes to the consortium, (iii) deliver the corresponding lead proteins to all other imaging techniques for further detailed analyses (e.g. in WP2)

Key personnel

Walter Schubert, M.D. Professor for Toponomics, he is inventor of the TIS technology and author of more than 100 publications and many international patents (12 families), he is member of the editorial board of Cytometry A, he is Chief Representative of the firm ToposNomos Ltd. and head of the Molecular Pattern Recognition Research group, Unv. of Magdeburg, Germany, he has launched the human toponome project and is visiting professor at the Max-Planck-CAS PICB Shanghai, China. ISAC best paper award 2008 for the three-symbol code of organized proteomes. **Andreas Krusche**, Dipl. Ing. is a specialized toponome TIS robot engineer and is expert in constructing TIS robots as well a in programming TIS protocols for specific tasks and calibrating TIS tag libraries. **Anne Gieseler**, Dipl. Biol. is specialized in Toponome Biology, she has profound experience in tissue and cell preparation for TIS measuring procedures. **Reyk Hillert**, Dipl Ing is specialized in Computer Visualistics and Toponome data analysis. He is specialized in the use and applications of our in-house software MoPPI (Modular Processing Pipeline) for Toponome data analysis.

Main publications and patents

Publications: Schubert, W (2003) ***Adv. Biochem. Eng. Biotechnol.* 83, 189 – 209**; Schubert W et al (2006) **Nat. Biotechnol 24, 1270-78** (with front cover toponome image)**;** Friedenberger M & Schubert W et al**.** (2007) **Nat. Protoc 2, 2285-2294** (with front cover toponome image); Schubert W (2007) **Cytometry A. 71(6):352-60;** Schubert W et al. (2008) ***Exp Rev Proteomics* 2008, 5, 361 – 369;** Bode et al. (2008) ***Proteomics* 8, 1170 – 1178;** Schubert W et al. (2008) **Biochim Biophys Acta. 1783(11):2080-8;** Schubert W et al. (2009) **J Proteome Res. 8(6), 2696-707.**

Patents: Schubert, W.: Automated Determining & Measuring Device & Method. US-Patent 6,150,173 (2000); EP 97 107 943.9-2204 (0810428); Publication Number WO/2007/104486; Priority date 13.03.2006; Publication date 20.09.2007; International application No. PCT/EP2007/002069; Internatl. Filing date 09.03.2007; Ref No PCT156656.

PARTNER 5 - MPG

Organisation description

General description: The Max Planck Institute for Biophysical Chemistry MPIbpc currently encompasses eleven departments plus numerous independent research groups. With a total work force of over 700 individuals – among them approximately 400 scientists – it is one of the largest institutes of the Max Planck Society. With more than 250 publications per year in high ranking journals and a total of 121 patents from a budget of 30 Mio. Euro, the institute has been recognized "successful and world-class" by the Scientific Advisory Board. As the only truly multidisciplinary Max Planck Institute, the research areas include physics, spectroscopy, imaging, protein chemistry, structural biology, cell- and developmental biology, and neuroscience. **Expertise:** The Department of NanoBiophotonics (Director: Prof. Stefan W. Hell) has pioneered the development and application of sub-diffraction resolution microscopy (nanoscopy). This department is the world-leading research group in STED-nanoscopy. The pioneering work of this department has been acknowledged by numerous prizes and distinctions to Prof. Hell. The Jakobs lab, which is part of the Department of NanoBiophotonics, has been among the first to utilize these technologies for cell biology. The lab has extensive experience in the adaptation and use of different labelling techniques, including classical immunofluorescence, fluorescent proteins, FlAsH and others. A major research focus of the lab is the application of nanoscopy to the analysis of sub-cellular protein distributions and dynamics not accessible by conventionally approaches. **http://www.mitoweb.de**
Facilities: The Department is equipped with state-of-the-art microscopy, including STED microscopes. The microscopy is in part world-wide unique. The research group Jakobs has full access to the microscopic equipment and infrastructure of the department. Furthermore, the Jakobs lab is fully equipped for molecular and cell biology including mammalian cell culture. **Other European projects:** FLUODIAMON (FP7 – 201837) – FLUOROMAG (FP6 -37465)

Role in the project

The task of the Jakobs lab at the Max Planck Institute for Biophysical Chemistry will be to investigate the conditions required to utilize DARPins provided by UZH for nanoscopy on cells. Their properties will be compared to antibodies when used for conventional staining. The image data obtained with the DARPins on the sub-cellular distributions of various members of the EGFR HER family of surface receptors on samples provided by the partners will be shared with the other partners.

Key personnel

Dr. Stefan Jakobs is heading the research group 'Mitochondrial Structure and Dynamics' at the Max-Planck-Institute for Biophysical Chemistry. He got is habilitation in cell biology/botany from the University of Göttingen. He has 10 years experience in nanoscopy and published more than 40 original articles using different forms of high resolution fluorescence microscopy. **Dr. Christian Wurm** studied biology at the University of Darmstadt. In 2004, he joined the RG Mitochondrial Structure and Dynamics in the Department of NanoBiophotonics and graduated in 2008 at the University of Heidelberg. Currently, he is working as a postdoctoral fellow in Stefan Jakobs group at the MPIbpc. **Stefan Stoldt** studied biology at the University of Göttingen. In 2005, he joined the RG Mitochondrial Structure and Dynamics, where he is currently carrying out his PhD thesis. **Prof. Stefan Hell** is director at the MPI for Biophysical Chemistry and has pioneered the investigation of new approaches to overcome the diffraction barrier in far-field fluorescence microscopy. He has been awarded numerous national and international prizes including the Karl-Heinz-Beckurts-award (2002), the Innovation Award of the German Federal President (2006), the Gottfried-Wilhelm-Leibnitz-Prize (2008) and the Otto-Hahn-Prize (2009).

Main publications and patents

Publications: Andresen et al., M., Stiel, A.C., Fölling, J., Wenzel, D., Schönle, A., Egner, A., Eggeling, C., Hell, S.W., and Jakobs, S. (2008). Novel photoswitchable fluorescent proteins enable monochromatic multilabel imaging and dual color fluorescence nanoscopy; **Nature Biotech. 26, 1035-1040;** / Schmidt, R. et al., Wurm, C.A., Jakobs, S., Engelhardt, J., Egner, A., and Hell, S.W. (2008). Spherical nanosized focal spot unravels the interior of cells). **Nature Methods, 5(6), 539-549;** . / Stiel, A.C.. et. al. , Andresen, M., Bock, H., Hilbert, M., Schilde, J., Schönle, A., Eggeling, C., Egner, A., Hell, S.W., and Jakobs, S. (2008)) . Generation of monomeric reversibly switchable red fluorescent proteins for far-field fluorescence nanoscopy. **Biophys. J. 95, 2989-2997**; Fölling, J. et al (2008) **Nature Methods *5*, 943-945** / Andresen, M., et al. Stiel, A.C., Trowitzsch, S., Weber, G., Eggeling, C., Wahl, M.C., Hell, S.W., and Jakobs, S. (2007). Structural basis for reversible photoswitching in Dronpa. **Proc. Natl. Acad. Sci. USA *104*, 2471-2476 /** Willig, K.I.. et al. , Kellner, R.R., Medda, R., Hein, B.,

Jakobs, S., and Hell, S.W. (2006) Nanoscale resolution in GFP based microscopy.

PARTNER 6 – UU/GEHC
Organisation description
Uppsala university enjoys a worldwide reputation for excellence in research and teaching with e.g. six Nobel Prize winners in chemistry, physics and medicine. Uppsala is ranked number 75 in the world by Time Higher Education and number 75 by Shanghai Jiao Tong university. The university has nine faculties and 40000 students. The biomedical center (BMC) offers world class infrastructure for research that ranges from research on small molecules to the study of whole organisms. **Website:** www.uu.se **Expertise: The department of biochemistry and organic chemistry** combines cutting edge expertise in small molecule design and synthesis, including method development as well as applications, with expertise in protein and peptide separation, structure, function and functionalization. The expertise in PET tracer chemistry is at the international forefront and has been leading the Uppsala PET research for several decades and has strong relations with preclinical and clinical scientist at the Medical, Pharmaceutical faculties including a strong collaborative network with US, Japan and South America. The department is embedded in the BMC infrastructure and enjoys the participation in several centers of excellence with highly prominent research groups in microbiology, pharmacology, materials science, structural biology as well as with clinicians from several disciplines. Several companies have been spun off from research in the department. **Facilities:** Except for access to good organic chemistry labs, there is also a very good PET centre facility with good opportunity for labelling chemistry and dedicated lab for biology and pharmacology research using short-lived radionuclides. There is also a well established PET centre for preclinical and clinical research. **Other EU Projects:** most relevant to the current proposal (FP7): AVERT-IT; ENGAGE; LUPA; DIAPREPP; ULTRA; AffinityProteome; AngioScaff; MONAMI; PROTSIGN
Role in the project
UU/GEHC will coordinate WP4 and develop purification methods for radionuclides as well as protein labelling protocols for DARPins on platforms which can be distributed to other partners. UU/GEHC will use PET/SPECT/CT to characterize DARPins as imaging agents in vitro and in vivo, to optimize imaging conditions.
Key personnel
Prof. Bengt Långström has long experience in PET tracer chemistry and applications resulting in 350 papers in chemistry journals and another 470 papers in the area of life science and medicine. When heading the Uppsala university PET Centre Bengt Långström was PI for more than 100 clinical trials in man using PET tracers. In 2008 he was invited to be chairman of the RIKEN Molecular Imaging Scientific Board and to be a member of the RIKEN Scientific advisory Board, in recognition of his outstanding achievements. **Prof. Lars Baltzer** has long experience in protein and polypeptide design including the development of conjugation and labelling techniques for the incorporation of small organic molecules as well as chelators for metal ions such as radionuclides, into folded polypeptides. He has close to 100 papers in chemistry journals. He has been the founder/cofounder of 3 companies and now serves as the Managing Director and CSO of the Biotech company Modpro AB. **Dr Azita Monazzam**, Ph D in chemistry 2008, postdoc GEHC 2008, specializes in tracer validation using translational radiotechnology from specific cellines, human tissue to in vivo animal models.
Main publications and patents
Publications: Andersson T et al. (2005) ***Chem. Biol.*, 12, 1245-1252** ; Baltzer L (2007) ***Topics in Current Chemistry*, 277, 89 – 106** ; Aili D et al (2009) ***Small. 5, 2445-2452***; Velikyan I et al (2008) ***Nuclear medicine and biology 35, 529-536.*** ; Blom E et al (2009) ***Bioconjugate chemistry 20, 1146-1151*** ; Bergstrom M et al (2003) ***Eur J Clin Pharmacol 59, 357-366.*** **Patents:** Baltzer L et al. –WO 03/080653; Novel Polypeptide Scaffolds and Uses Thereof, Baltzer L et al–WO 97/43302; Acyl Transfer with Stabilized Transition Complex Using Catalyst with Catalytic Imidazole (e.g. Histidine) Function; Langstrom B et al WO 2009102378 A2 20090820 Purification of 68Ge/68Ga generator eluate from Fe(III) intended to improve specific radioactivity of 68Ga-based radiopharmaceuticals,; Velikyan I et al WO 2008026051 A2 20080306, 68Ga-labeling of a free and macromolecule-conjugated macrocyclic chelator at ambient temperature. Velikyan I et al, WO 2004089517 A1 20041021, Method of obtaining gallium-68 and use thereof and device for carrying out the method; Velikyan I et al WO 2004089425 A1 20041021 Microwave method for preparing radiolabelled gallium complexes.

PARTNER 7 - INO
Organisation description
General description: Innoprot, a spin-off from the University of the Basque Country, is provider of *Cell-Based Assay* Kits for drug discovery & development procedures through DNA recombinant technology, RNAi & protein interactions. Innoprot has got to lead the Spanish national market in cell-based assays kits and molecular biology related services, and at the moment it is introducing in European, American and Asian markets. **Website:** www.innoprot.com **Expertise:** Innoprot has a special department of molecular biology integrated in the research and development team composed by 2 Ph D senior scientists in biochemistry and 2 technicians with long time experience in DNA recombinant, RNA and protein interaction technology. **Facilities:** Innoprot has a specific laboratory of molecular biology with specific devices such as: thermocyclers, complete protein isolation systems, gel documentation platform including analysis software or automated fluorescence image platform (Pathway 855 Becton Dickinson-Hamilton Robot- Atto Vission image software Becton Dickinson) ; with safety requirements for this kind of works and the hygienic standards to work with human tissue samples. Innoprot installations are equipped to work with RNA and proteins which requires specific laboratories (P2).
Role in the project
The task of INNOPROT in the project is to isolate and identify the interactomes protein/protein and RNA/Protein and identify specific miRNA implicated in the anti HER2 therapies. INNOPROT is the leader of WP3 and its team has the specific skills to work with RNA-protein interaction, isolation and characterization of miRNA.
Key personnel
Dr. Iker Badiola. PhD in Biology (2006). On going publications in Cancer Research and oral communications in International congresses such as AACR (Los Angeles 2007), TUMIC (Florence 2006), ISCHSS (Nigata 2006).Best Young Investigator Award ISCHSS (Nigata 2006). Founder of Innoprot company in 2008. **Dr. Patricia Villacé**. PhD in Biochemistry (2004). She did the postdoc in the Biophisic Unit in the CSIC (Bilbao, Spain) and Scripps Research Institute (San Diego, USA). International publication in protein and RNA technologies. Founder of Innoprot company in 2008.
Main publications and patents
Publications: Badiola I, Basaldua I, Arteta B, Vidal-Vanaclocha F, Olaso E. Role of Tyrosine Kinase Receptors in Stellate Cell Activation During Liver Metastasis. *The International cancer microenvironment society*. March. 2007 / Bernbom N, Licth TR, Brogren CH, Jelle B, Badiola I, Vogenssen FK, Norrung B. Effects of Lactococcus lactis on composition of intestinal microbiota: role of nisin. *Appl Environ Microbiol*. 2006 Jan;72(1):239-44. / Badiola I, Olaso E, Vidal-Vanaclocha F. Discoidin Domain Receptor 2 deficiency predisposes hepatic tissue to colon carcinoma metastasis (in progress) / Villacé P., Marión RM., Ortín J. The composición of Staufen-containing RNA granules from human cells indicates their role in the regulated transport and translation of messenger RNAs.: *Nucleic Acids Research*, 2004, Vol 32, Nº 8 1-10 / Etxeberria1 A, Rodriguez-Alfaro JA, Aivar1 P, Alaimo A, Villacé P, Gómez-Posada JC, Santana-Castro I, Areso P , Villarroel A. Calmodulin regulates the trafficking of KCNQ2 channels *FASEB J.* 2008 Apr;22(4):1135-43 **Specific awards:** 2009 Entrepreneurs Bizkaia ekinez award. Iker Badiola-Patricia Villacé 2008 Toribio Echeverria Award in Technological Area.Iker Badiola-Patricia Villacé 2006 Iker Badiola. Young Investigator Award ISHSC 2006 Niigata (Japan).

PARTNER 8 - FLS
Organisation description
General description: Firalis is a life sciences company with leading expertise in biomarkers (BM) development for translational applications. Through its large European network of clinical centers of excellence, Firalis qualifies candidate biomarkers of clinical and pharmaceutical interest. As the coordinator of SAFE-T public consortium (The first IMI-JU project launched), Firalis is enjoying a leader position in translational biomarkers field in Europe. **Website:** www.firalis.com **Expertise:** Firalis has academic and global Pharma talents in BM R&D and develops proprietary biomarkers for translational applications, while in particular; integrative data analysis is a core expertise of Firalis. Prof H. Firat established and led the global integrative data analysis sections in Novartis AG during 5 years. P. Thomann, a senior scientist and expert statistician, has worked many years for big pharma and CROs before leading the Integrative Data-analysis section of Firalis. **Facilities:** Firalis has its own biomarker assay development, validation and testing laboratories and a data analysis team with broad pre-clinical and clinical expertise. Furthermore, as the major contributor to SAFE-T, it has access to specific software for statistics, laboratory facilities and to specific biobanks. French Establishment of Blood (EFS) is the biggest public structure on blood and its products in France. Firalis has common biobanking and laboratory facilities with EFS certified as ISO 9001. **Other European projects:** SAFE-T Project (G.A. #115 003) **Standardisation:** Firalis is also leading activities of the EU project to establish a scientific generic process for the qualification of translational Safety biomarkers together with public, EFPIA partners and EMEA. The process report will be released as a reference guideline.
Role in the project
Firalis will lead WP6 Data analysis. Firalis scientific team, composed of high level specialists, has solid experience in integrative data analysis. Firalis will provide statistics-based evaluations & evidences via integrative data analysis of data generated within the IMAGENT consortium projects. Firalis is also leading the activities on Data Analysis & Project Database within SAFE-T project, to provide statistics-based integrative evaluations and evidences for supporting biomarker qualifications.
Key personnel
Prof. Hueseyin Firat: Pediatrician, practiced as an associate professor in Paris Uni. hospitals. After PhD of Immunology at Pasteur Institute, accredited as research director responsible of the Immunology lab. at Généthon. Joined Novartis in 2002, he created and headed the global data analysis sections worldwide, served as senior BM Expert and was member of the innovation team. His research activities generated several patented discoveries. **Dr. Peter Thomann**: Expert clinical statistician with more than 20 years of experience (big Pharma and international CROs) in statistical analysis of complex clinical data and pharmacokinetics. **Dr. Dorina Bratfalean:** PhD. At Firalis, she is responsible for integrative BM analysis and R&D for validation of candidate BMs for pre- and clinical applications.
Main publications and patents
Publications: *Vidal I et al. (2008)* **Cell Transplant. 17(5), 507-24**; *Hartmann B et al. (2006*) **Inflamm Res. 55(8), 322-34;** *Hernandez J et al. (2002)* **Proc Natl Acad Sci USA 99(19), 12275-80.** Thomann P et al. (1994) Eur J of Clin Pharmacol 47(1) 14-17; Merz M et al (1998), J Clin Pharmacol 1998; 38:1144-1150; Henrik Agersø et al (2002) J. Clin Pharmacol 42, 1262-1268; Brennscheidt U et al (2007) Arzneimittelforschung. 57(2) 106-11. **Patents:** Firat H, Boisclair J, Grenet O, Perentes E, Schumaher M, "Biomarkers for cardiovascular side-effects induced by inhibitor compounds of COX-2" EP1910825 (publication: 14.09.2006) **Specific awards:**French Ministry of Research & Higher Education, national competition for innovative companies, Creation-Development category, Winner in 2009

PARTNER 9 – M-GMP

Organisation description

General description:
Medipolis GMP Oy is Finland based process development and contract manufacturing SME company for production of Biopharmaceuticals. Medipolis GMP Works on projects in Biopharmaceuticals, technology development and cGMP production of biologics for Phase I to Phase III and commercial batches.

Website: www.medipolisgmp.com

Expertise:
Since 2001, Medipolis GMP has initiated process development and technology optimization program belonging to Medipolis GMP. This is in addition to the contract manufacturing of biologics undertaken by the company. Medipolis GMP has successfully optimized technology and developed a human vaccine which is now going ahead for cGMP production.

Facilities:
Medipolis GMP was established in 2001 and company´s cGMP facility was validated in 2003. Medipolis GMP since 2003 has done contract manufacturing for clients in USA and Europe. The product manufactured by Medipolis GMP has been successfully used in Phase II clinical trials by European clients.

Role in the project

Medipolis GMP is looking to participate in the project for process development and optimization and cGMP production of the biomarker molecules. The process optimization of fermentation and downstream processing will be done for better yield of the bio-molecules.

Key personnel

Dr. Ashesh Kumar- Director- Biopharmaceuticals- Dr. Kumar has more than 15 years of biopharmaceutical process development and cGMP experience and worked on product development currently in commercial use. Dr. Kumar is a Ph.D. in Biotechnology from Indian Institute of Roorkee, India. He currently leads the team at Medipolis GMP bringing excellent knowledge base from Asia and his European experience.

Seppo Turunen- Seppo Turunen has more than 25 years of industry experience and is a trained biotechnology professional. He is Head of Regulatory and ´Quality Assurance at Medipolis GMP.

Sirkka Aho is the Chief Technical Officer at Medipolis GMP. She is trained process engineer and has a very rich experience of technical developments for biopharmaceuticals.

Jyrki Perttunen- is the Fermentation technology expert at Medipolis GMP and is a process engineer by profession. Medipolis GMP has developed excellent process optimization and increased process yield on many projects by his very vast experience in the area.

PARTNER 10 – ACIES/P2R
Organisation description
General description: ACIES is a French SME, founded in 1990. Its activities cover the promotion, organisation and management of Research and Innovation in Europe. The activities related to collaborative research in general and FP7 in particular, were grouped under the same umbrella, «PROJETS ET RÉSEAUX DE RECHERCHE» (P2R), which was created in March 2009 under the same holding company than ACIES. ACIES/P2R team members advise and assist players worldwide and from all sectors, in funding strategy, management and the promotion of their R&D programs at economic, scientific and societal level. ACIES/P2R provides a unique combination of multidisciplinary skills in scientific, technological, economic, financial, marketing, fiscal, legal and managerial fields. **Website**: www.acies.fr **Expertise:** ACIES/P2R has a proven track record in the design, implementation and the management of complex and large European research programs. Our team manages projects with a duration from 2 to 5 years, made up of 5 to 35 European partners and budgets ranging from €2.5 to €50 millions. On the basis of a proven management system and in-depth knowledge of the European context for collaborative research, ACIES/P2R capabilities are deployed in the management of research programs and networks. ACIES/P2R areas of intervention include all fields and themes of the COOPERATION, CAPACITIES and PEOPLE Programs. **Other European projects:** ACIES/P2R has contributed to the successful preparation of many proposals over the last 10 years. Today **ACIES/P2R is partner in and manages more than 25 FP projects** of which one counts a Network of Excellence (TREAT-NMD – FP6 036825), integrated large scale projects (PERSIST FP7 222878; SYBILLA – FP7 201106; SPIDIA – FP7 222916) and small to medium-size projects (THERADPOX – FP6 018700, CHILDHOPE – FP6 037381; BIO-NMD – FP7 241665).
Role in the project
ACIES/P2R will be involved in two WPs: "*Management*" (WP11) and "*Knowledge Dissemination*" (WP9). More specifically, ACIES/P2R will accompany the project coordinator in producing, monitoring, controlling, consolidating and submitting the project's deliverables and periodic reports as well as monitoring the project's progress in terms of quality, costs, schedule and risks, in accordance with EU regulations and the Grant Agreement. Through an evaluation process based on performance indicators, ACIES/P2R contributes to the continuous improvement of the project and to the achievement of its objectives on time and within budget. To facilitate and optimise this action, ACIES/P2R develops and implements communication and collaborative management tools that allow promoting research results and securing the exchange of information with partners
Key personnel
Laurence MAZURANOK Ir.: Agronomist (2001), phytotherapist (2005) and specialised in nutrition and health environment (2008). She has recognised experience in phytotherapy, food formulation (trainer in phytotherapy, invited in several international conferences in Thailand, Mexico, France, etc.). She has been working as a consultant for ACIES/P2R since 2008, assisting R&D players from all sectors of activity in designing and managing European projects and networks. **Marie-Laure MUIRAS:** MBA, PhD in molecular biology. She has a strong experience in R&D programs through public-private partnerships. Very familiar of the FP environment as she has personally contributed to it since FP4 as a researcher and later as Program manager and WP leader (Cooperation and Capacity program). She has worked at the DKFZ (German Cancer Research Centre- Heidelberg) and advised members of two renowned Research Institutes at the University of Newcastle, as a Technology Transfer Officer and a European Program Manager, before joining ACIES/P2R in 2009.
Main publications and patents
Publications ACIES/P2R: "A scientific approach to research and innovation governance", Medevielle JP and Courtot L." (2008), Proceedings of the Transport Research Arena Europe 2008 conference, Ljubljana, Slovenia, April 2008. Ed. Ales Znidaric, Slovenia. "Strategic Entrepreneurship and its relevance to a Research University", Muiras ML (2004) MBA thesis, Ed. Durham Business School, Durham, UK. **Specific awards:** Winner of the « French Quality Award », « SME-SMI » category (2004) (Mouvement Français pour la Qualité – MFQ)

B.2.3. Consortium as a whole

The consortium is designed to be multidisciplinary and perfectly complementary to fulfil the ambitious objectives of the project to develop imaging and fluorescent tools for the identification and detection of biomarkers, composed of protein-protein and RNA-protein complexes in clinical samples and patients. The consortium is well balanced between academics and private companies, mostly SMEs. All partners work at least with one another, which will help building an efficient consortium sharing common work practices and goals. All partners are worldwide experts in their fields.

Main skills	**Partners**
Quality Assurance for Phase I/II cancer trials A Quality system for production and testing of biotherapeutics Extensive expertise in cancer trials / Infrastructure for imaging trial shared with KCL	UCL
Characterisation of protein-protein interactions (FRET/FLIM) Infrastructure for imaging trial shared with UCL Multidisciplinary approach to understand the cancer metastatic cascade Signal transduction in cancer / Model selection for prognostic biomarkers Quality Assurance for Phase I/II cancer trials	KCL
Select DARPins to characterize them for their binding properties and specificities Engineer them for particular detection methods and provide them to partners for different tests Characterisation of protein-protein interactions (PrL) Quality Assurance for Phase I/II cancer trials	UZH
Systems biology of signalling in the proteome (mathematical analysis of complex networks)	FLS, KCL
Developing high resolution fluorescence imaging technology: Nanoscopy/STED-microscopy, Image analysis of STED-images. Cell labelling and immunochemistry	MPG
Characterization of protein clusters in situ (toponomes)	TNL
Isolation and characterization of RNA-protein interactions	INO
Development of radiotracer tracer chemistry and applications in Collaboration with Applied Science Lab, GE Healthcare	UU
Main areas of competence are in: Microbial fermentation, Small scale cGMP manufacturing, QA/QC, Regulatory issues, Process development and scale up	M-GMP
Management expertise, EU project specialists	P2R

The skills and expertise brought by partners answer perfectly to the needs of the project structure.

The first step in the project (WP1) is to generate and optimize DARPins. This will be easily achieved through the unique access to the DARPins granted by UZH and the expertise of MPG in the Development of sub-diffraction resolution microscopy / nanoscopy. Both partners will be supported by UCL, KCL and INO. The two formers will provide and validate tumour samples while the later will provide pure biotinyalted Ago Protein. Based on this first step, a parallel work will develop and improve tools and technologies for the identification and the detection of biomarkers in clinical samples and patients.

WP2 will develop high resolution fluorescence diagnostic imaging for detection and characterization of protein networks. The results of the WP will be obtained by the combination of expertise offered by the partners involved. KCL will contribute through its knowledge in Optical Imaging and Cell Biophysics and Nuclear Medicine (PET/SPECT). This knowledge will be complemented by the expertise in Nanoscopy / STED-microscopy, Image analysis of STED-images and cell labelling, immunocytochemistry provided by MPG. These imaging methods established by KCL and MPG will analyse the identification of HER2 associated protein networks (TNL).

The goal of WP3 is to develop methods for isolation and characterization of protein/RNA complexes from clinical tissues. The goal can only be achieved by the access to the isolation and identification of specific RNA/protein complexes techniques provided by INO. Access to breast cancer samples will be assured by the participation of UCL and KCL, while INO will cross validate WP3 results by using WP2 data provided by TNL and UZH will produce specific DARPins. The whole work will be supported by the expertise of UCL and FLS in biomedical data statistical study.

Radiolabelling and software for Quantum imaging will be developed in WP4. The core of the WP lays on the expertise of UU/GEHC in Radiotracer development, animal SPECT and PET. It will be complemented by the specific development of DARPINS provided by UZH and the knowledge of UU/GEHC and UCL in software development.

All results gained in WP2, WP3 and WP4 will be tested in WP5 through a phase I/II trial to assess safety and efficacy of tools and technologies. UCL and KCL being linked to hospital and having a recorded expertise in clinical trials will play a major role in this WP. Based on its skills in GMP production methods, M-GMP will define and implement the GMP production process, using the Protein expression methods provided by UZH. The clinical trial expertises will be combined to achieve evaluations on human samples for the outcomes of WP2 and 3 and on patients for the first-in-man trial of quantum imaging technologies developed in WP5.

The development work of the IMAGINT project will be supported by expertise in Mathematical methods and more specifically analysis of complex biological signalling networks (KCL) and biomedical data handling (FLS). These expertises will ensure that the issue of ‘over-fitting’ data will be dealt with, in an appropriate manner. In addition, they will provide rational methods for predicting clinical relevance of candidate biomarkers and the associate detection technologogies.

WP7 will be in charge of Dissemination, IPR and Ethical issue. The Ethical issue must be addressed according to International, EC and National regulations using expertise in these domains (UCL, KCL and UZH).

Being accustomed to publish in international peer reviewed journals and to present research results in international conference, all partners will disseminate the results of the project. The exploitation of results will be entrusted to the IPC (Intellectual Property Committee) which will be composed of IPR experts from each partner. In addition, the coordination of dissemination and exploitation activities will benefit of the expertise of UCL’s dedicated teams in these domains.

Management activities are gathered in WP8. These activities will be achieved thanks to the know-how of UCL in coordinating and managing ambitious research projects and to the expertise of ACIES/P2R in managing FP7 projects.

Sub-contrating

INO will wubcontract to CNB (Centro Nacional de Biotecnología, Madrid. http://www.cnb.csic.es/content/services/genomics/index.php?l=1) the study of the microRNA expression using their microRNA microarray system.
INO does not have the instrument to perform the microarray and will subcontract the study for an estimated amount of € 7 000.

Third Parties

No third parties are involved in the project

B.2.4. Resources to be committed

Overall description

All partners involved in the IMAGINT project aim at achieving a significant breakthrough in the field of tools for identification and the detection of biomarkers in clinical samples and patients. The excellence of each member in their respective field of expertise, the high level of competence of each key personnel involved, will guarantee the effective achievement of IMAGINT objectives. The resources and facilities that each organisation dedicates to the project further demonstrate their determination to successfully implement the tasks ascribed to this project.

All of the partners are in well-equipped laboratories of premier research institutions, and as highly successful scientists, have additional grants in adjacent fields, as well as funding from their home institutions. They are able, therefore, to mobilize significant additional resources for this project, beyond the actual direct project costs. This involves the participation of specialized personnel paid for by the home institution, the cost-free use of sophisticated instrumentation, and the synergy with other laboratory personnel working on related projects paid for from other sources. Therefore, the RTD funds for this project are leveraged, further increasing the impact of this proposal.

The total project effort amounts **697.5 Person Months**, out of 657 **PM** are fully dedicated to RTD (**R**esearch and **T**echnological **D**evelopment) activities, **15 PM** to MGT activities and **25.5 PM** dedicated to the OTHER activities (dissemination, IPR, ethics and gender aspects). Each work package is perfectly integrated in the whole IMAGINT work plan as described in section 1.3.
The resources were allocated among the different WPs according to an analysis detailing the importance that each WP should have in order to successfully achieve the project objectives.

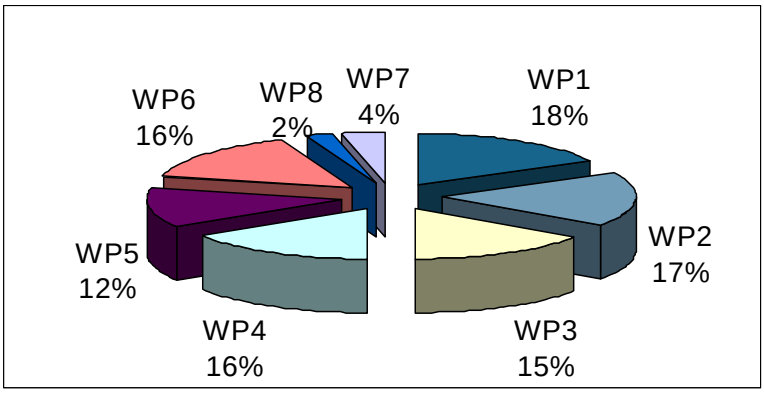


Figure 11 – Effort distribution per WP

The distribution of efforts shows that the **6 RTD work packages** (WP1 to WP6) represent **94 %** of the total of the efforts (see figure above), demonstrating its priority. Taking into account the overall resources mobilized by partners, it is obvious that the **investment will be higher** in terms of efforts than these mentioned figures.

The overall costs of the IMAGINT project is **€7.8m** and the corresponding requested EC contribution is **€5,683m**.

The distribution of the EC contribution per country is as detailed in the left figure. It shows the European dimension of the project and a strong commitment of all countries represented. The budget is quite well equilibrated between the participating countries. The grant dedicated to UK is explained by the high costs of clinical trial, defined as a first-in-man trial.

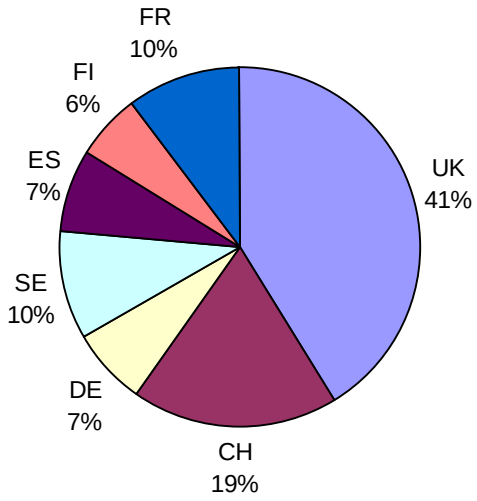


Figure 12: EC contribution per country

The EC contribution per activity describes the high investment in R&D activities (91%) coordinated by an efficient management (4%) and supported by activities addressing ethical issues and dealing with dissemination and exploitation of knowledge which are gathered within the OTHER activities categories (5%). These activities include final symposium, technology transfer dedicated to create clinically-relevant tools for prognostic, diagnostic and monitoring safety and therapeutic effects in breast cancer, but also that could be transferred to other cancer diseases.

It is highly interesting to see the high involvement of SMEs in this project (TNL, INO, FLS) for RTD activities, achieving nearly 25 % of the funding. This is perfectly in line with the new objectives of the European Union to involve always more SMEs in EU projects and the target of having 15% of the FP7 funding assigned to SME

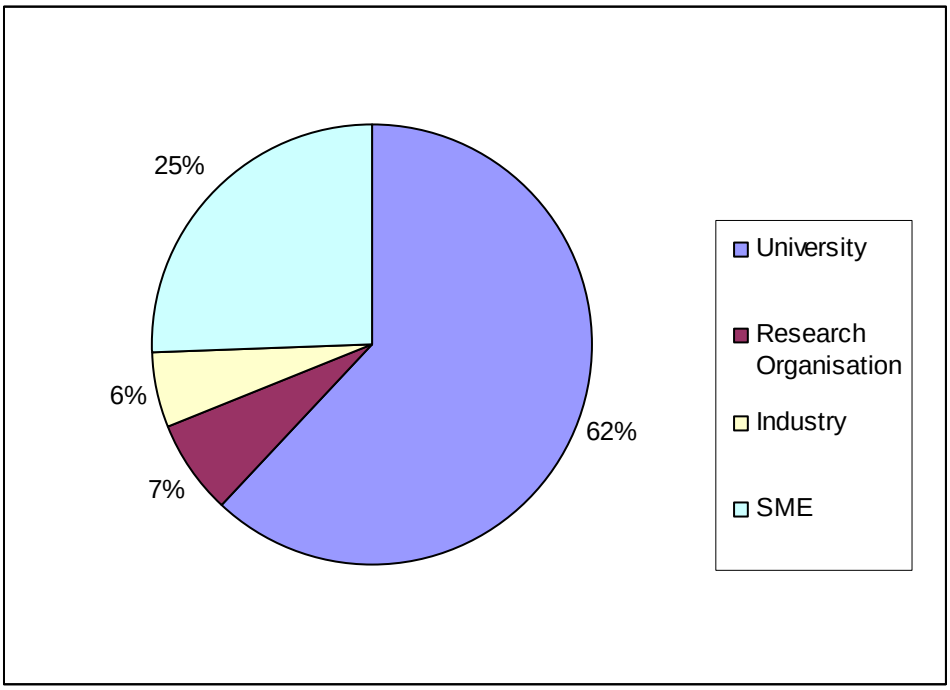


Figure 13: EC contribution per profile

Justification of equipment

Participant short name	Planned equipment	Costs associated
KCL	Chameleon Vision II laser system with integrated precompensation: 80MHz (Coherent UK Ltd) And Home built (in-house) Tissue microarray imaging platform	18387 €
MPG	Small optical parts for the adaptation of the STED-microscope including specific mirrors and optical filters	4000 €
INO	Automated fluorescent imaging platform (Hamilton robot-Pathway 855 Becton Dickinson)	41000 €

Sub-contracting

Participant # and short name	Planned subcontracting	Related WP	Costs associated
UCL	3 audit certificates	WP8	3 000
UZH	2 audit certificates	WP8	3 000
KCL	1 audit certificate	WP8	2 500
TNL	1 audit certificate	WP8	1 000
MPG	1 audit certificate	WP8	2 000
UU	1 audit certificate	WP8	2 500
INO	Database	WP3	7 000
	1 audit certificate	WP8	2 000
FLS	1 audit certificate	WP8	2 500
M-GMP	1 audit certificate	WP8	2 000

B.3. POTENTIAL IMPACT

IMAGINT is a 48-month collaborative project addressing the objectives of the HEALTH priority of the FP7 Cooperation programme, and in particular the area "detection, diagnosis and monitoring" applied to cancer. IMAGINT will develop tools combining bio-imaging and molecular testing biomarkers from RNA, DNA and/or protein and their complexes as mentioned in **HEALTH.2010.1.2.1**. Thus, it is expected to have impacts in terms of science, health, health economics, society and European competitiveness. In addition, IMAGINT will contribute to the **translational approach** from laboratory discovery to clinical tools and the systems biology methods. The data generated will be shared with the research community through a system of standards and a database which is compatible with FP7 capacities infrastructure. The ambitious goal of IMAGINT requires a European consortium bringing together top-level experts from public and private organizations to cover all the fields required for the development of safe, efficient **non-invasive tools** for the identification and the detection of biomarkers in clinical samples and patients.

B.3.1. Strategic impact

Expected impacts listed in the work programme.

More specifically, and with respect to the Health European policy, IMAGINT will have tremendous impacts in terms of:

- **enhancing the research capacities at European level**, with the development of workflows, methods, and analytical tools that will facilitate multimodality biomarker analysis
- **Scientific breakthroughs**: IMAGINT is built around public-private collaborations which is to lead to the design of **cutting-edge technologies,** as well as the validation of **new biomarkers** in cancer, and new tools for the detection, isolation and functional characterization of complex and interacting bio-molecules for diagnosis and prognosis purposes
- **Strengthening the competitiveness of European industry,** by providing a science and technology basis to **develop new methods – including cGMP protocols -** and **design new techniques** for the fast growing world market of biotechnology. Moreover, due to the **multidisciplinary and transversal approach** of IMAGINT, the **European SMEs** involved in the project will develop 4 cutting edge technologies using DARPins to measure directly interacting proteins. New fluorophore-tagged DARPins will be developed to map topological protein networks associated with HER signaling. IMAGINT will also develop a new technology to isolate protein/RNA complexes in RNA, innovative mathematics and computational models, and new tools at molecular, cellular, tissue, whole body and system level.
- **Societal impacts: increasing employment and local conditions, improving health and quality of life** by proposing the use of new diagnosis, prognosis and therapeutic agents to **treat a major diseases** such as cancer
- **European standards and policies:** this project represents a real opportunity to ensure complementary and coherence with health **European strategies and initiatives** such as the **Innovative Medicines initiative (IMI**).
- **Structuring the ERA:** IMAGINT will also greatly contribute as it aims at **creating structured networks** and **supporting durable interactions between projects in the area of cancer.**

Relevance to the European and International priorities

IMAGINT project is relevant to several policies. In the Second Programme of Community Action in the Field of Health 2007-2013, cancer was one of the eight leading causes of mortality and morbidity highlighted by the WHO European Region. Moreover, in the communication of the EC, the medicine of tomorrow should be "safe, innovative and accessible" for patients based a personalized medicine (166). The outcomes of the project will reinforce the competitiveness of Europe in the biotechnology domain, one of the leading European sectors. In addition, IMAGINT will contribute to the objectives mentioned in the priorities of Innovative Medicines Initiative (IMI) (167) and to the objectives of the European partnership Against Cancer for the period 2009-2013 (168).

Technology and Innovation impacts

Innovation impacts are expected to be outstanding. IMAGINT will develop **new technologies** opening the way for new applications. Thus, the clinical tool panel for diagnostic and treatment monitoring purposes will be more suitable for the use in personalised medicine. Moreover, these methods could be **transferred to other cancer diseases**. The development of these new technologies will be based on the discovery of new biomarkers

(identification, isolation and functional characterisation of complexes of interacting molecules). Both new technologies and new biomarkers will lead to patenting. Transfer of knowledge as well as patenting will be carefully dealt with by the IPC (Industrial Property Committee) and ILC (Industrial Liaison Council) as explained in section 2.1.

Outputs	Outcomes: Short-term Impacts	Long-Term Impacts
A novel combined in vivo/ in vitro biomarker	Knowledge about the correlation between tumours proliferation/metabolic processes and molecular interactions within tumours	The combination of both information (imaging data pertaining to the same cancer patients but at different length scales) will improve the accuracy of predicting therapeutic outcome and hence contribute towards personalisation of cancer therapies
Development of robust tools to measure the different HER dimers in human tissues	Measurement and understanding of the real complexes of HER2 present in particular tumours	Great benefit to a rational development of safer breast cancer therapy
Development of practical and informatics diagnostic tools	Identification of individuals who are at risk of metastatic recurrence	Rational selection of therapy
Development of imaging and mathematical tools to identify the protein clusters	Study of protein function in terms of activity and spatiotemporal location	Broader patient base to integrate into a diagnostic signature
Development of cutting edge tools and technology using DARPins for the detection, isolation and functional characterisation identification of complexes of interacting molecules (RNA/protein)	Exploration of an untapped source of new biomarkers	Development of innovative diagnostic methods
Development of robust tools for quantitative clinical imaging of the extent and location of metastatic disease	Provision of important clinical diagnostic information allowing for optimal management of current anti-HER2 therapies	Will allow for response assessment of novel therapeutics

Scientific impacts

The IMAGINT project will enhance the Knowledge and the State-of-the-Art

Following a **systems biology methodology,** new types of biomarker identified and characterised in IMAGINT will each provide individually important and different pieces of information and understanding about HER2 and its role in driving malignancy. Moreover, increased knowledge and greater understanding will be achieved by **integrating** the different levels of information in a **holistic manner**, with each other, with public databases and with information on clinical outcome. There is need for **new information technologies** that provide mathematical and computational tools to achieve it. This will be one of the crucial contribution and impact of IMAGINT.

The IMAGINT project will provide responses to scientific needs to a great extent and will be advancing the scientific and technological frontiers in many disciplines as IMAGINT will develop DARPIns for new technologies that can be used to identify biomarkers:

- IMAGINT will develop a new method based on DARPins to address the fundamental challenge of the association of membrane receptors to form homodimers, heterodimers and larger aggregates. Therefore, the IMAGINT cutting-edge methodology will allow investigating other receptor equilibria, extending the scope of the IMAGINT methodology beyond the particular system of HER receptors.
- The further development of the technologies resulting from the IMAGINT project, will be of enormous general importance for science, and radiate far outside the particular problem of the HER receptor family.

- Using the HER system of receptors, which is still reasonably well characterized compared to many other receptor families, technologies on how to robustly detect receptor pairs in patient samples can be worked out, using fluorescence life time imaging and proximity ligation. Undoubtedly, in the IMAGINT project, much knowledge will be accumulated such that this knowledge can be transferred to other receptor pairs in the future.
- IMAGINT will collect data from the various technological areas and novel hypotheses, giving a unique archive of new approaches and new information relevant to the problem of cancer. Its importance will extend beyond breast cancer giving **new paradigms for investigation of cancer biology, imaging and therapy**.

Thus, the IMAGINT project is unique in starting from a clinical need (namely better tools for tumour diagnosis), identifying the bottleneck (namely the sensitive detection of the HER family with all their interactions) and then developing tools and scientific procedures, which will have an impact **far beyond** the actual HER family of receptors. From a basic science perspective, it is likely that from this work, a much deeper understanding of receptor interactions will emerge, and for the HER family, their relationship to tumour outcome in patients. The boundaries in some of the most advanced detection technologies will be pushed, which will be of very general utility in many fields of biological discovery.

The IMAGINT project will highly contribute to building and strengthening the European Research Area in the Health and Cancer areas
This project will provide complementary diagnostic tools and biomarkers for clinicians compared to previous EU projects as HYPERIMAGE, FLUODIAMON.

In addition, the partners of the IMAGINT project intend to create links with European Technology Platform such as IMI and NanoMedicine. They will also initiate relationships with European Network of Excellence related to the research topics of the project and in particular:

- EMIL (European Molecular Imaging Laboratories)
- TRANS-BIG (Translating molecular knowledge into early breast cancer management: building on the BIG (Breast International Group) network for improved treatment tailoring)
- DIMI (Diagnostic molecular imaging)

The IMAGINT project will enhance cooperation between different research groups to a large extent. It will greatly contribute to the foundation for future projects by enabling collaboration between scientists from many different disciplines, including economics (from SMEs and Institutes), industrial processes, biotechnology, computational medicine, and imaging. By addressing this full range of research areas, the IMAGINT project will enhance the interdisciplinary structure of the European Scientific Community.

The IMAGINT project will provide an outstanding tool for **international standardised exchange of data**. The database that will be created will be structured using internationally accepted data standards and a novel approach to integrating different types of data defined by the standards and a linked data model. This will optimise the ability to disseminate information to the research community as well as providing a model for other researchers to use for data deposition in a compatible form. The database will be made publicly available after securing any intellectual property and prospectively ensuring consent and confidentiality for clinical records (169). Its power for dissemination will be optimised because it can be searched using NCI Thesaurus concepts and NCI common data elements and will be amenable to searching through the semantic web. In the medium term, this efficient sharing data throughout Europe and beyond will accelerate the pace of research, minimise needless repetition of experiments and improve the cost-effectiveness of research. Systems biology analyses of data generated will be facilitated by access to the orderly data sets which can be readily integrated across different technological areas.

European dimension of the project
Breast cancer has a huge incidence in Europe, with more than 430,000 new cases per annum (1). Research has shown that human epidermal growth factor receptor 2 (HER2) is a potent oncoprotein and it has become an established target for breast cancer treatment. There is urgent need for new HER2-related biomarkers to guide treatment regimes and for prediction, diagnosis, monitoring and prognosis of disease. IMAGINT will develop tools to find these new biomarkers for breast cancer. Once developed for breast cancer, the tools could have

wide application for other cancers in which overexpression of members of HER family proteins is clinically relevant; for example, gastric cancer, another **chronic disease** and the world's second leading cause of cancer death (13,14).

The aim of IMAGINT is to develop a structured approach at a European level. It will bring together resources that are not available in any single country. It will increase the understanding of complexes of interacting molecules involved in breast cancer and more generally in cancer development. It will address comprehensively new avenues for cure and lead to personalized therapy by means of more targeted diagnosis and prognostic technologies and biomarkers. The IMAGINT project will thus provide important tools for translational research in cancer, including the following aspects: systems biology, cell therapy, diagnosis, monitoring and prognosis. This will contribute towards structuring EU efforts and better understanding of cancer mechanisms, and biomarkers discovery.

The ambitious goal of the IMAGINT project requires an international consortium, thus bringing together recognised experts from international public organisations and European private industries to cover all disciplinary areas required for an optimal and reproducible research and development of new technologies, methods and biomarkers. In particular, the innovative nature of the IMAGINT project requires a multidisciplinary approach combining the expertise listed above.

IMAGINT implements a relevant integrated and translational approach which involves the most awarded European experts in the needed fields (publishing in the best scientific journals, already participating in other EU research projects, international projects and in drug discovery developments in their respected industrial applications) bringing complementary knowledge and strengthening excellence.

Health impacts and societal impacts

Approximately 22 million people are living worldwide with cancer and nearly 7 million people die from it. Cancer prevalence is a statistic of primary interest in public health because it identifies the level of burden of this disease on the population and health care system. More than 10 million new cases of cancer appear worldwide each year, and this number is expected to increase by 2.4% by 2020 to 14 million each year. The most common cancers globally include lung, breast, colon/rectum, stomach, liver, prostate, cervical, oesophageal, and bladder. Breast cancer is the second most common malignancy affecting women, causing each year in the EU 88 000 deaths among women aged 35-60, i.e. women with important professional, family and societal responsibilities. IMAGINT will provide tools for non-invasive detection and diagnosis of the disease, prognosis and monitoring. These tools will enable to gain new insights into the metabolism of breast cancer, and putative biomarkers such as the HER family, and ultimately to design rational and personalized treatments.

In the **healthcare** domain, the impacts of IMAGINT will be widespread from new biomarkers through molecular discoveries, to new patient treatment methods. Metastatic breast cancer is usually diagnosed by a combination of clinical and radiological findings. With IMAGINT project, the new tools (imaging and molecular testing markers) developed will concomitantly:

- permit the identification and detection of biomarkers in clinical samples and patients validated in larger prospective studies and characterize molecular pathways
- ensure an earlier detection of metastatic breast cancer and increase by more than 10% early diagnosis improving even more the prognosis
- avoid some biopsies associated with negative outcomes: pre-procedure anxiety, post-procedural pain
- optimise the monitoring of therapeutic agents for a safer and more efficient use and also quicker results
- allow identification of resistance to anticancer treatments earlier than can be done clinically
- eliminate morbidity due to unnecessary treatments
- give patients a realistic expectancy of treatment response.

In summary, IMAGINT project will demonstrate the following concept: the targeted oncology is to select the "right patient for the right drug at precisely the right point in their cancer journey" to avoid unnecessary side effects and improve treatment outcomes at efficient costs.

The societal consequences of IMAGINT have to be approached by the **life quality improvement**. Thanks to an earlier detection, diagnosis and monitoring of breast cancer, surgeons will be able to preserve most of the breast tissue by performing a lumpectomy, where only the tumour and some surroundings tissue are removed. Thus, the breast structure could be preserved as much as possible and will limit the common woman depression. Safer and more efficient tools developed for identification and detection of disease biomarkers can reduce even more

the hospitalization rate thus reducing the treatment costs and increase survival prognosis maintaining societal relationships and ensures a better quality of life.

In addition to benefit to patients with breast cancer, the tools developed in IMAGINT would also benefit to other HER2+ve cancers, including gastric cancer (second leading cause of cancer death) against which antiHER2 drugs such as trastuzumab are currently being trialled. Therefore, developing new tools for detection, diagnosis and monitoring the disease could prove very beneficial to the **reduction of cancer deaths**, owing in particular to earlier diagnosis and detection (166). For example, new diagnosis tools and surgical technologies have permitted the **decrease of the hospitalization rate** for breast cancer by 34 percent between 1997 and 2004 (Source: Agency for Healthcare Research and Quality).

Economic impacts

IMAGINT will enhance **European competitiveness**. The European pharmaceutical and biotechnology sectors have emerged as the top R&D investor, accounting for 19.2 % of the European R&D industrial effort (EC JRC Scoreboard 2008). These sectors will be reinforced by IMAGINT project with the development of bio-imaging and molecular biomarkers.

IMAGINT will contribute in providing new technologies for biomarker identification and detection. On a global scale, the *in vitro* diagnostic (IVD) market is worth $ 37.5 billion (€ 27 Bn) in 2007 growing with a CAGR of 9% with an estimate of 69.5 Bn in 2014 (Source: Strategic Opportunities & Directions in Molecular Diagnostics by TSG, 2009). This project will contribute to the EU competitiveness in this fast growing market. In particular, it will meet the EU objectives in terms of SME participation. In addition, the involvement of Uppsala University as academic partner together with Uppsala Applied Science Lab, GE Healthcare located in EU will contribute to boost competitiveness of European companies and reinforce their world leader position in the Biomarker market.

In the domain of **health economics**, IMAGINT will dramatically help reducing the cancer treatment costs.

Since treatment costs are considerably lower when a tumour is discovered at an early stage, early diagnosis and screening programs have economic value. Screening can be accomplished by self-examination or by a health care provider, and by mammography (early detection improved by 15 to 35 percent). Cost-effectiveness studies have estimated the cost of screening at between $13,200 (approx. € 9,400) and $28,000 (approx. € 20,000) per year of life saved. The National Cancer Institute reports that screening mammography every one to two years reduces breast cancer deaths by a third or more for women 50 and older. However, published cost-effectiveness studies have challenged the return on health care budget invested for frequent mammograms. The ratios from several studies indicate the cost effectiveness of an annual mammography to be from $62,000 (approx. 44,300€) to $190,000 (approx. €135,700) per life-year for women aged 40-49 and $17,000 (approx. 12,100 €) to $110,000 (approx. 78,600 €) for women aged 50-65. The cost effectiveness of a mammography every three years for women age 50-65 was determined to be $2,700 (approx. 1,900 €) per life year in another study.

With this background, the sensitive and robust diagnosis tools and biomarkers developed in IMAGINT will definitely decrease screening costs and increase its efficiency. IMAGINT imaging and molecular testing markers will contribute to decreasing the cost of false positive test results (indicating a woman has a disease when she really hasn't). There is evidence that an upfront investment in IMAGINT that will develop more accurate and less costly diagnosis tools can pay off. Early diagnosis using IMAGINT tools and biomarkers (and consequently early treatment) will prevent some hospitalizations. Considering that hospital expenses for breast cancer treatment totalled more than $1.6 billion (approx. € 1.1 billion) in 2003, even a 5% reduction in the expenses radically influence on the hospitalization costs.

In addition the IVD's share in the global Healthcare spending will remain strongly limited as shown in the figure below. This demonstrates that the estimated gain will remain on a long-term perspective.

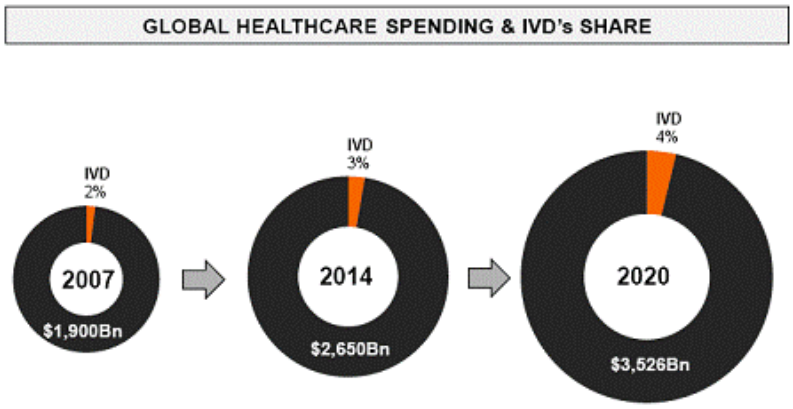


Figure 14: Global healthcare spending and IVD share, by The Sharma Group and TSG Partners, 2009

The IMAGINT project will permit the selection of the most promising clinically-relevant biomarkers and the related technologies, meaning not only a sensitive diagnostics but also an earlier diagnostics. Thus, this project will contribute to optimise IVD costs in the global health expenses.

Beside diagnosis costs, IMAGINT outputs will also greatly reduce treatment costs, as the project will lead to a fine segmentation of the breast cancer in specific subsets and therefore propose rational and personalized therapies. As a matter of fact, treatment may require hospitalisation, physician services, medication, nursing, home care, home healthcare and emergency department visits. A retrospective cohort study of women with disseminated breast cancer in Sweden included 53 patients with a total mean cost of € 93,700. Drugs and hospitalisations were the largest single cost sources. HER2-positive patients had slightly higher mean costs (€ 123,300), while triple negative patients had lower mean costs (€ 70,600). The IMAGINT project will have a critical impact in discriminating the more appropriate therapy, and/or treatment continuation or termination in patients who no longer require it. For instance, showing the inappropriateness to give four cycles of taxane-containing chemotherapy in 30% of patients with metastatic breast cancer would reduce the average lifetime cost per patient by £3200 (approx. € 3,600) (170).

In addition, the **indirect economic benefits** of IMAGINT project will be to render cancer treatment more affordable to patients and acceptable for healthcare commissioners.

IMAGINT will exploit project results after the end of the funding; therefore, the project will allow for a huge leverage effect of the funding allocated to project participants. The project will generate patents, products, processes and services, and will have an important impact on employment and job creation. Moreover, the participants will be in the position to disseminate their findings to the relevant stakeholders.

Direct Economic Benefits
● Increased competitiveness of Europe in biotechnology ● Increased competitiveness of the European Healthcare systems ● Increased competitiveness of EU industries and SMEs ● Commercialisation of new biomarkers: ● Economic added value and jobs creation
Indirect Economic Benefits
● Increased knowledge basis and reduced time to market for new technologies ● Proposition of new alternative treatments ● Development of less invasive diagnosis and prognosis methods ● Minimisation of health costs ● Personalised medicine ● Increased quality of life for cancer patients

B.3.2. Plan for the use and dissemination of foreground

Industrial dissemination and exploitation plan

An exploitation manager**,** representing the interest of all partners, shall be appointed at the beginning of the project to deal with all Intellectual Property Rights issues. He/she will also be responsible for the IPR knowledge base update, the IPR protection linked to any innovative technology proposed by the project as well as for the relationships with the Project Ethics Committee. He/she will be in charge of a follow-up of partner requests concerning the use of results and will transfer accordingly these requests to the IMAGINT Governing Board and/or IPC. Intellectual property generated under this programme would be exploited by the relevant owners and in compliance with the consortium agreement. For example, UCL protects ideas, processes and novel therapeutic products through patenting, and in the case of computer programmes (copyright), in conjunction with Cancer Research UK (CR-UK) Technologies (CRT). In addition, UCL Business will provide professional and coherent support for academic/industry collaborations.

There are several levels at which the project results can and will be commercialized. This will be also facilitated by some of the team members who have, next to their primary academic appointments, close industry connections (e.g. Andreas Plückthun is a cofounder of Molecular Partners AG (MPAG), who are commercializing the DARPin technology). Out of economic considerations, MPAG are only developing therapeutic products internally at this point, and also the choice of targets is exclusively governed by economic considerations. However, MPAG are very interested to retain the option to in-license at the end of the project a mature set of novel tools and fully support the idea that this is best developed in this high-caliber and high-profile academic consortium with the interdisciplinary expertise required for such an ambitious undertaking.

One level is the Ga-67/68 labelling technology, which will be further developed within this project. One of the visions is to develop labelling procedures that are fully compatible with GMP and can routinely be used in a hospital setting. The Phase I imaging trial (WP5) would be an ideal proof of concept in man, which would put Europe in front in this technology, having the possibility to make it truly accessible to hospitals all across Europe.

The second level, with a slightly longer horizon, is the imaging reagent itself, which potentially could be produced and commercially developed.

At each Governing Board meeting, the IMAGINT consortium will review the target objectives and raise community awareness in biomarkers and cancer. The SME partners will be responsible to further develop the new products, encouraging the interest of large industries through the ILC. However, the information communicated shall always be controlled by the **IPC** (Intellectual Property Committee) before publication or diffusion, so that no sensitive information provided by partners is disclosed. Within the Plan for using and disseminating the foreground, the consortium will define a “roadmap” to the industrial development of new findings. Main project results will be identified regarding their scientific impacts, technological impacts, health impacts, economic and industrial perspectives, as well as community added value.

The economic growth that could be generated by the project results will be evaluated in terms of market conditions. The exploitation routes outlined in this section should be further detailed and refined during the progress of the project, based on new market studies, results from ongoing research work and new partnerships.

At the present time, the expected Foreground and foreseen ownership are detailed in the table below. The table will be updated annually within the annual Plan for Using and Dissemination knowledge.

Expected foreground IP	**Foreseen Ownership**	**Timing for use**	**Protection needed**
the Ga-67/68 labelling technology	UU	Mid-term	IP
The imaging reagent	GE Healthcare UZH, with exclusive licensing to Molecular Partners AG	Long-term	IP
DARPin pairs for screening	UZH	Long-term	IP

The protection of foreground IP is another key element of the Plan for Using Foreground IP and the time frame for its use. Beneficiaries are aware of the necessity to protect their foreground. Depending on the foreground IP,

the type of protection will be decided. It will be up to the beneficiary that has generated the knowledge to find out if it can be the subject of IP rights protection. Most likely, a patent or a utility model will be used for the Foreground IP developed within the IMAGINT project. Alternatively, the Foreground IP may be kept secret if this is more profitable to the owner.

Dissemination strategy

IMAGINT strategy is to spread the excellence and disseminate the knowledge within and outside the consortium. Nevertheless, and as a prerequisite to maximise economic impacts aforementioned, project results will be protected in view of their **industrial development** to increase European industry competitiveness with the promotion of the partnering SMEs' activities. In addition, IMAGINT has already anticipated the settlement of an **Industrial Liaison Council (ILC)** that will be composed of industrials. The Industrial Liaison Council will: (i) provide an industrial insight into the project as well as commercial advice and relationship networks; (ii) facilitate the transfer of knowledge between academia and industry; (iii) propose innovative solutions/technologies from the industry that could improve the research project and/or solve the difficulties encountered by partners. At the time this project proposal is released, GE HEALTHCARE and BAYER have already confirmed their participation in the ILC. The confirmation of NOVARTIS, ROCHE and AstraZeneca is expected soon. Letters of intent from ILC members are available in Annex 6.3.

Internal dissemination

The internal communication process aims at keeping all partners informed of the project status and documents issued in order to ensure the synergy of the cooperation. The Governing Board (GB) meetings and WP meetings will play a crucial role in that respect. All relevant information will be sent to the Project Coordinator for dissemination to the relevant partners.

External dissemination

Disseminating the project approaches and results will be done through concrete actions aiming at creating synergies with a large number of stakeholders in the areas of cancer treatment, imaging technologies, biomarkers and biotechnologies. A set of actions will be developed: **local and regional actions** involving **citizens** towards target groups (patient organisations, regional healthcare authorities, business developers) with a view to disseminating best practices and methods; **national initiatives** in order to link IMAGINT with national health care **policies** and events relating to cancer **European groups** that are interested in developing complementary diagnosis and prognostic technologies and methods for breast cancer or other cancers types can be integrated in the IMAGINT ILC. In addition, dissemination activities will pay specific attention to adequately promote **synergies between private and public sectors**. The project research will provide opportunities for publications in high-level international "peer-reviewed" journals. For public communication purposes, **non sensitive information** will be shared on the public website.

Scientific community: Selected information from IMAGINT developments and results will be made available to the international scientific community. IMAGINT includes the creation of a **website** which will be a key vector for dissemination activities. This website will interface with the international scientific community, giving researchers the opportunity to communicate freely and increase their mobility and interaction with other project-related topics. In addition, **scientific presentations** will be given by IMAGINT partners at specific conferences on the fields addressed by IMAGINT including biological activities, for example on biomarkers discovery. As explained above in section 3.1.3.4, IMAGINT partners will also liaise with European platform Networks and projects. The following contributions are also envisioned in scientific journals:

Name of journal	*Targeted Audience of Journal*	*Result to be disseminated*
Journal of the National Cancer Institute	Oncologists, pathologists and cancer imagers	Combined biomarker based on PET and protein interaction imaging for clinical outcome prediction
Nature Biotechnology.	General biological and imaging physicist communities	FRET-FLIM validation of new interacting targets from Toponome-based protein cluster analyses in cancer tissues
Proc. Natl. Acad.	Scientists researchers	Single molecule

Sci. USA		investigation of receptor association
Journal of Clinical Oncology	Scientist researchers, clinicians	Imaging HER2+-ve breast cancer with DARPins

Industrial: Each Governing Board meeting will address the dissemination and exploitation strategy. Specific workshops will be organised in order to inform industrials of the knowledge generated by IMAGINT and of its latest results.
General public: The transparency and accessibility of the project to the general public is of great importance in order to ensure that EU citizens are informed of IMAGINT aims, developments and outcomes. Regular e-newsletters, as well as the project website and printed publications, will be made available to the public and especially to patient organisations. Public discussion forums on the website could also ensure a mean for public members to interact with scientists. The following actions are planned: (i) develop a communication plan, with a press release at project start, regular public website updates, (ii) publish articles in the non-specialist press about biomarkers, new diagnostic and prognostic methods, personalised medicine for breast cancer.

Management of intellectual property rights (IPR)

The terms of Intellectual Property Management will be specified in detail in the **Consortium Agreement** to be start before project start. They will be guided by Annex II of the FP7 Model contract.
The *general principles* relating to access rights are the following:
Access rights shall be granted to any of the other partners upon written request. The granting of access rights may be made conditional on the conclusion of specific agreements aimed at ensuring that they are only used for the intended purpose, and of appropriate undertakings as to confidentiality. Partners may also conclude agreements with the purpose of granting additional or more favourable access rights, including access rights to third parties, in particular to enterprises associated with the partner(s), or specifying the requirements applicable to access rights, but not restricting the latter.
The question of background exclusion is dealt with in a simple way. The participants have to define the "background needed" to set up the project and "where appropriate excludes specific background".
Joint ownership will be managed by specific agreements negotiated by involved partners. If partners can not reach an agreement, they will comply with the defaulting mechanism of the Consortium Agreement.
Where no joint ownership agreement has yet been concluded:

- each of the joint owners shall be entitled to Use their jointly owned Foreground on a royalty-free basis, and without requiring the prior consent of the other joint owner(s), and
- each of the joint owners shall be entitled to grant non-exclusive licenses to third parties, without any right to sub-license, subject to the following conditions:
    - at least 45 days prior notice must be given to the other joint owner(s); and
    - fair and reasonable compensation must be provided to the other joint owner(s).

Access rights for execution of the project are the following:
Partners shall enjoy access rights to the foreground and the background IPR, if that foreground or background IPR is needed to carry out their own work under that project. Access rights to foreground IPR shall be granted on a royalty-free basis. Access rights to background IPR shall be granted on a royalty-free basis.
Subject to its legitimate interests, the termination of the participation of a partner shall in no way affect its obligation to grant access rights to the other partners pursuant to the previous paragraph until the end of the project.

Access rights for use of foreground IPR are the following:

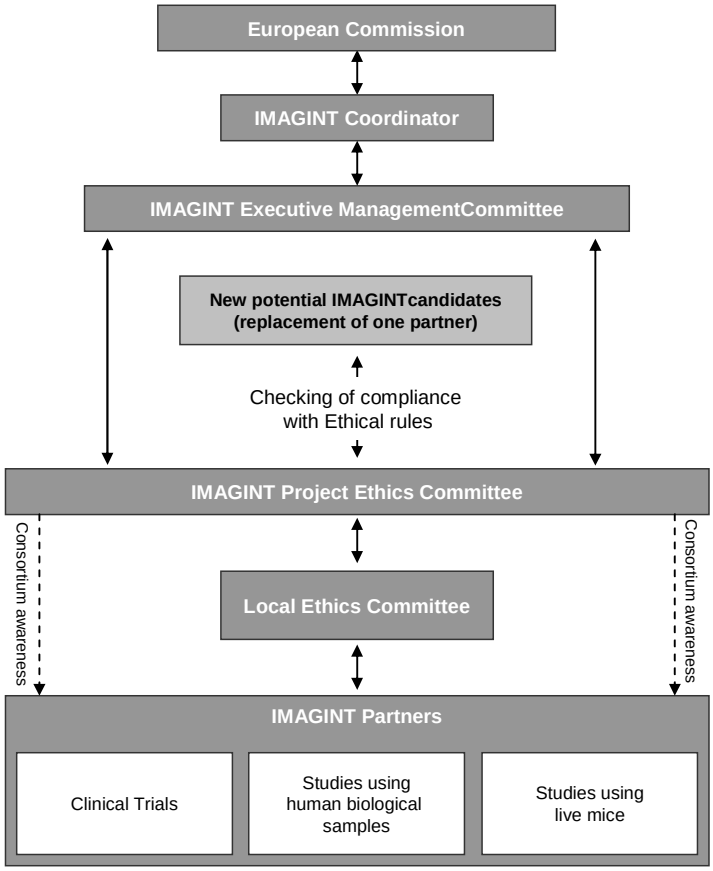
- Partners shall enjoy access rights to foreground and to background IPR, if that foreground or background IPR is needed to use their own knowledge. Access rights for use purposes have to be granted either under fair and reasonable conditions or royalty-free (participants may choose). The period during which access rights for use may be requested is reduced from 2 years to 1, unless the participants agree differently (i.e. shorter or longer period).
- Exclusive licensing is expressly accepted (both for foreground and background) but is conditional on all participants waiving their access rights to the specific resource and confirming in a writing document.

		Access rights to background IPR	Access rights to foreground IPR resulting from the project
For carrying out the project	Access	Yes if partner needs it to carry out its own work under the project	
	Terms	Royalty-free	
For use outside the project (exploitation or further research)	Access	Yes if partner needs it to carry out its own work to exploit the results of the project	
	Terms	Fair and reasonable conditions or royalty fees	

B.4. ETHICAL ISSUES

Research in the breast cancer raises a number of ethical issues. Consequently, the IMAGINT project dedicates a WP including 'Ethical aspects' and dissemination and IPR issues (WP7) to identify and manage potential ethical concerns associated with the nature and outcomes of the project. Its objectives will be to: i) ensure that all experiments comply with all applicable national and European rules; ii) anticipate any ethical problems; iii) protect human health and environment by applying the precautionary principle (COM (2000) 1 final); and iv) ensure dissemination of information to increase awareness.

This WP proposes the formation of a Project Ethical Committee (PEC), composed of experts in the field, who have already indicated an interest in participating. This Committee will be responsible for keeping the participants well-informed about new ethical regulations applying to IMAGINT. It will also ensure that the existing ethical rules are met, checking that no experimental studies planned by any partner will start before the prior approval of all relevant ethics committees. Lastly, the PEC will monitor the work performed by the Governing Board and advise it when necessary.



The IMAGINT consortium partners will follow the guidelines for the implementation of the 'Universal Declaration on the Human Genome and Human Rights' from 1999 including participation in the dissemination of knowledge to the public, and participation in public and political debates about ethical issues where reasonable. They will also apply several European and national laws and guidelines reported throughout the following paragraphs as well as those listed below under section 4.7.

There are no obvious risks associated with the proposed research. However, the IMAGINT consortium pays consideration to the ethical implications raised by the proposed project and will ensure that ethical issues are managed optimally. The current and specific ethical issues of IMAGINT and the way they are currently addressed are detailed in the paragraphs below.

IMAGINT involves research on human beings, human biological samples as well as research on mice. Each partner has specified whether his/her proposed research activity within the project involve these specific issues.

B.4.1. Conduction of clinical trials

A significant component of IMAGINT involves the performance of clinical trials to assess the safety and efficacy of novel complexes used for diagnosis and monitoring of therapeutic effects in breast cancer. The successful running of this study is essential in translating preclinical research into safe and efficient clinical tools.

Ethics and responsibility

Conduct of clinical trials is founded on the protection of human rights and the dignity of the human beings (Helsinki declaration, World Medical Association). Therefore, in the proposed clinical trials on human subjects, considerations related to the well-being of the human subject will take precedence over the interests of science and society accordingly to the statement of its Clause 5.

The protection of research subjects is safeguarded through risk assessment based on the results of pre-clinical and validation experiments prior to any clinical trial and consideration by ethics committees and Member State

competent authorities. Rules on the protection of personal data (Directive 2001/20/EC) will be also carefully applied. In addition, ICH Good Clinical Practice (GCP) is a set of internationally recognised ethical and scientific quality requirements, which must be observed for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects.
Compliance with this good practice guidance provides assurance that the rights, safety and well-being of trial subjects are protected, and that the results of the clinical trials are credible.

Standards for clinical trials are set out in:

- Clinical trials will be conducted in accordance with the World Medical Association Declaration of Helsinki
- International Conference on Harmonisation (ICH) Topic E6 (R1), Guideline for Good Clinical Practise (2002). CPMP/ICH/135/95.

World Health Organization, Guidelines for Good Clinical Practice for Trials on Pharmaceutical Products (1995)
"Oviedo Convention" - Council of Europe Convention on Human Rights and Biomedicine and it's additional protocols, particularly the Additional Protocol concerning Biomedical Research (January 2005) Strasbourg, 25.I.2005.

- Universal Declaration of Human Rights (1948)
- EU Clinical Trials Directive (2001/20/EC), EU Directive 2005/28/EC transposed into UK law as « The Medicines for Human Use (Clinical Trials) Regulation 2004, SI 2004/1031 & Amendment Regulations 2006, SI 2006/1928.
- CIOMS (Council for International Organizations of Medical Sciences) International Ethical Guidelines for Biomedical Research Involving Human Subjects (2002)
- Regulation 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 which came into force on 1st January 2009.
- ARSAC regulation Notes for Guidance on the Clinical Administration of Radiopharmaceuticals and use of sealed radioactive sources (2006).
- EU Tissue and Cells Directive (2004/23/EC): Setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells

Two clinical trials (CT1-2) will be conducted at 2 institutes in the same country (United Kingdom). The clinical trial (CT2) described in WP5 will be the first in man.
All approval process is planned and will be fully designed at the beginning of the project. UCL and KCL are internationally recognized and have acquired more than significant experience in conducting clinical trials in accordance with all applicable ethical rules, GCP, MHRA and ARSAC.

The well-being of all trial participants will be ensured and an adequate information communication system will be implemented. This information system will ensure that new safety findings are transmitted to all participating sites and that the integrity of the study design is not compromised.

Both trials will respect the European and UK ethical guidelines. A EUDRACT Number will be assigned to this clinical study prior to submitting the protocols to the regulatory agencies and to the Institutional Review Boards. During the trial, every year, a report will be submitted to the ethical committee as a follow-up of ethical regulations. At the end of this study, a final report detailing the clinical trial and its results will be sent to the national regulatory agencies and Ethics Committee, where applicable.

Information on the content, commencement and termination of a clinical trial will be available to the Member States where the trial takes place and all the other Member States will have access to the same information. A European database bringing together this information will therefore be set up, with due regard for the rules of confidentiality.

Pre-requisite for the proposed clinical trials

Pre-clinical studies in animal models
This proposed clinical trial (CT2) will be preceded by a preclinical investigation on animal models made in UU.

Animal studies will provide data on toxicity and pharmacokinetic which will inform the starting dose and dose escalation strategy.

Pharmacodynamics, Pharmacokinetics, Safety pharmacology, Toxicology

Predicting the potentially severe adverse reactions following use in human subjects involves the **identification of risk factors** including the mode of action, the nature of the target and the relevance of animal models. The starting dose and dose escalation strategy will be defined with reference to the EMEA Guidance (Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products Document Ref.EMEA/CHMP/SWP/28367/07). Recognising the limitations of animal pharmacokinetics we will perform real-time pharmacokinetics and make adjustments to dose escalation as indicated.

Manufacturing of radioactive tagged DARPins in imaging

In order to ensure the cGxP compliance, thorough knowledge of regulations governing the use of diagnostic tools for human use is required.

Commission Directive **2003/94/EC** (European GMP guide) lays down the principles and guidelines of Good Manufacturing Practice (GMP) in respect of Medicinal Products for human use and IMPs for human use. Any IMPs will be manufactured in accordance with the EU GMP guidance – Eudralex, the Rules Governing Medicinal Products in the European Union. Volume 4 – EU Guidelines for Good Manufacturing Practices for Medicinal Products for Human and Veterinary Use.

Furthermore, the authorities developing these rules and guarding their implementation are developing appropriate regulations to adequately control new imaging tools and biomarkers.

In order to find a path where the scientific inventions can smoothly be transferred to clinical applications that are in compliance with the GLP, GMP and GCP , **the different regulatory aspects will be coordinated and managed (WP7)**.

The requirements of the competent EU authorities, EMEA and ICH guidelines as well as specific national requirements will thus be ensured in compiling and submitting the appropriate clinical trial application including the **Investigational Medicinal Products Dossier** (IMPD) comprising preclinical, manufacturing, controls and clinical documentation).

Manufacturing of investigational medicinal products will be performed in **accredited GMP manufacturing facilities**. Where applicable, a manufacturing authorization from the competent national authority will be obtained. The investigational medicinal product will be certified and released for human use by a qualified person (QP) based on its compliance with product specific release criteria.

Medicinal therapeutic for use in CT:

For the radiation dose related to FDG and FLT PET:

FDG PET/CT scans. 400MBq of F-18 FDG will be injected and a PETCT scan performed from midbrain to mid thigh. The radiation dose from the FDG scan will be 8mSv and from the CT scan 10 mSv. Total 18 mSv for one scan and for the two scans in this study 36mSv.

FLT PET/CT. 250 MBq of FLT will be injected and a PETCT scan performed from midbrain to mid thigh. The radiation dose from the FLT will be 8 mSv and from the CT 10mSv. This will result in a total of 18mSv per scan. For the two scans in this study this will be 36 mSv.

The ethical issue therefore relates to the additional fatal cancer risk from exposure to radiation. This is a low risk compared to the lifetime risk of cancer in normal population.

Implementation of the proposed clinical trials

Centre facilities (UCL and KCL)

IMAGINT clinical trials will take place in UK, centres of clinical excellence and will be conducted by experienced investigators, who have acquired the necessary expertise in conducting early phase trials (i.e. phase I-II), and with medical support staff with appropriate level of training and previous experience in imaging.

The Trial Centres in United Kingdom involved in IMAGINT will be the following:

- **University College London** (Chief Investigator Dr Tim Meyer) The Cancer Research Targeting and Imaging Group at the UCL Cancer Institute have an established track record for drug development including radiopharmaceuticals. There is extensive infrastructure to support these studies including dedicated imaging facilities, a GCLP laboratory and dedicated and fully staffed drug development unit.
- **Kings College London** (Dr Paul Ellis)

Centres were selected based on academic excellence and relevant expertise. Centres are known personally to the Consortium coordinator.

Regulatory and Ethics Committee Approval

For this research clinical trial, ethical approval from local and/or national authorities is required for conducting the study. In order to obtain approval from the Ethical committees, investigators will provide a valid authorisation request, complying with the detailed guidelines on the application/format and documentation to be submitted in an application for an ethics committee opinion.

The study will only start after the written approval is received from the different organisation involved in the ethical process: ARSAC as there will be injection of radioactive substances, MHRA, an Institutional Review Board / Ethics Committee (IRB/EC), which operates according to ICH GCP Guidelines. The written approval and the names and qualifications of members of the IRB/EC must be made available to the sponsor before the study can start. The investigator is, together with the sponsor, responsible for submission to and communication with the IRB/EC.

In addition, the investigator should conduct the study in accordance with the protocol, the Declaration of Helsinki () and the ICH GCP Guidelines (CPMP/ICH/135/95). The investigator and the sponsor will sign the protocol and Clinical Trial Agreement to confirm this.

Study Documentation

An essential component of GCP is the **maintenance of complete study file documentation**. It is the responsibility of the investigator to ensure that the study centre file is maintained in accordance with Section 8 of the International Conference on Harmonization (ICH) Guideline for Good Clinical Practices (GCP). Additionally, during the course of the clinical trial, independent monitoring will be carried out by the trial Sponsor, to ensure that record keeping and reporting is in accordance with required standards of Good Clinical Practice (GCP).

The study documentation will include the following documents:

- Study protocol (and amendments)
- Investigators Brochure (IB) / Investigational Medicinal Product Dossier (IMPD)
- Subject information documents including Patient Information Sheet (PIS), Donor information Sheet (DIS) and Informed Consent Form (ICF)
- Trial specific case report form (CRFs) and IRAE report forms
- Investigator's Site File (ISF)
- Study medication documentation
- Clinical Trial Agreement (CTA)

In order to start the study, the investigator is required to have the following documentation available:

- Signed confidentiality agreement
- Signed investigator statement for this protocol (Investigator's Signature Page)
- Signed Clinical Trial Agreement (CTA)
- Submission letter to the IEC
- IEC approval letter, stating the sponsor's name, study number and investigational drugs, as well as all documents reviewed and should include a list of members present at the meeting
- Recent CVs of investigators and sub-investigators (signed and dated)
- Signature sheet, documenting delegation of tasks and names and initials of the investigator team
- Laboratory normal ranges and quality certificates and/or accreditations.

During the study, the sponsor should have collected:

- All study medications related completed documentations
- All left-over study medications

- All completed CRFs.

The compliance with these requirements may also be ascertained by independent Quality Assurance unit.

Quality Control and Quality Assurance

- **Monitoring:** the sponsor has ethical, legal and scientific obligations to carefully follow this study in a detailed and orderly manner in accordance with established research principles and FDA/EMEA regulations. As part of a concerted effort to fulfil these obligations (maintain current personal knowledge of the progress of the study), the sponsor monitors will visit the centre during the study in addition to maintaining frequent telephone and written communication.
- **Monitoring and communication of adverse events/reactions:** The trial design will provide a specific plan for monitoring for adverse events or adverse reactions. Expected events will be described in the protocol and this information will be used to assist Investigators in the appropriate reporting of events. These studies involve investigation of patients being treated with radioactive tagged DARPins to identify breast cancer metastases. Events may occur which meet the definition of serious and need a revision of the protocol to submit to the IRB/IEC.
- **Stopping rules and decision making:** Any safety reports received during the studies will be considered by **an independent Data and Safety Monitoring Committee.** The Committee will work to a Charter which will define stopping rules for each particular study. In addition, the Charter will stipulate the frequency of meeting to be held and document the working procedures of the Committee.
- **Reporting: Annual Progress Report.** The sponsor/investigator will submit a summary of the progress of the trial to the accredited IRB/IEC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.
- **Reporting: End of Study Report.** The investigator will notify the accredited Ethics Committee (EC) and the competent authorities involved of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. In case the study is ended prematurely, the investigator will notify the accredited EC and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited EC and the competent authority. In case the final study report will not be available within one year, another term should be defined including the reasons.
- **Auditing:** several audits will be conducted in the involved clinical centre(s) in order to ensure high quality standards for this centre. Audits will include, but are not limited to, drug supply, presence of required documents, the informed consent process, and comparison of CRFs with source documents. The investigator agrees to participate with audits conducted at a reasonable time in a reasonable manner.

  Regulatory authorities worldwide may also inspect the investigator during or after the study. The investigator should contact the sponsor immediately if this occurs, and must fully cooperate with the inspections conducted at a reasonable time in a reasonable manner.

Protection of clinical trial subjects and Patient Informed Consent

Protection of clinical trial subjects

- The proposed clinical trials will be conducted in compliances with Directive 2001/20/EC without prejudice to the national provisions on the protection of clinical trial subjects if they are more comprehensive than the provisions of this Directive.

The clinical trials can be undertaken since the **foreseeable risks and inconveniences** have already been weighed against the anticipated benefit for the individual trial subject and other present and future patients. However, the Sponsor will discuss this aspect in his clinical trial authorisation application and the clinical trial will be initiated only if the Ethics Committee and/or the competent authority come to the conclusion that the anticipated therapeutic and public health benefits justify the risks. Lastly, the clinical trial will be continued only if compliance with this requirement is permanently monitored and validated. Identified risks and discomfort involved in the participation of the study will be indicated in the patient information form.

The investigator is responsible for ensuring that the subjects (patient and donor) **fully understand the nature and purpose of the study**. Proposed trial subjects will consent in person or, when the person is not able to give informed consent: his legal representative, who has had the opportunity, in a prior interview with the

investigator or a member of the investigating team, to **understand** the objectives, possible risks, harms and inconveniences related to conduct of the trial as well as all the conditions under which it will be conducted, the purpose of collection and storage of data/biological material, methods and techniques used, measures taken to protect confidentiality, who will access to the data, duration of the storage. The subject will be also informed of his right **to withdraw** from the trial at any time. Indeed, the information will make clear that refusal to participate or withdrawal from the study at any stage of the trial will not induce any prejudice to the subject's subsequent care. Lastly, subjects will be allowed sufficient time to decide whether or not they wish to participate. Only after the respect of this entire process, the subject or, when the person is not able to give informed consent: his legal representative, will be asked to provide a **written Informed Consent**. More information on the mandatory content of the Informed Consent form will be detailed in its dedicated section.

- The rights of the subject to physical and mental integrity, to privacy and to the protection of the data concerning him will be safeguarded in accordance with Directive 95/46/EC.
- Provision has been made for **insurance or indemnity** to cover the liability of the investigator and sponsor. In accordance with ICH GCP Guidelines and applicable regulatory requirements, the sponsors will ensure that adequate patient insurance is in place at each clinical site for the period of the study. Therefore, the sponsors have contracted Insurance for individual patient's cover during the time of the study. Details on insurance will be part of the regulatory package for this study.
- Due to the nature of the clinical trial (Phase I-II), subjects will not receive any **financial reward or compensation**. The **medical care** given to, and medical decisions made on behalf of, subjects will be the **responsibility of an appropriately qualified doctor.** The subjects will be provided with a contact point where they may obtain further information related to the proposed clinical trial. Furthermore, if subjects comes from different cultural backgrounds both Informed Consent and assent should be obtained using independent mediators who understand the language, traditions, religion and other aspects of the social and cultural context.

Patient Informed consent

Subject information: The subjects will give their written informed consent before participating in any clinical trial or related procedure(s). The signed Informed Consents will be retained by the investigator and made available (for review only) to the study monitor, auditor and inspector. All information related to the proposed clinical trial (the objectives, possible risks, harms and inconveniences related to conduct of the trial as well as all the conditions under which it will be conducted, the purpose of collection and storage of data/biological material, methods and techniques used, measures taken to protect confidentiality, who will access to the data, duration of the storage,) will be provided to the subjects before their decision to participate or abstain from participation.

Therefore, the Informed Consent to be used in the proposed clinical trial will describe in the subject national language:

- Purpose and plan of the research
- Nature and extent of any procedures
- Possible risks and benefits
- Local ethics committee approval (if available at submission date)
- Right to withdraw consent at any time without discrimination or disadvantage
- Right and procedure to eliminate one sample from the bio bank at any time without discrimination or disadvantage
- Other medical alternatives
- Arrangements for responding to adverse events
- Confidentiality and privacy arrangements
- Arrangements for access to information
- Compensation arrangements
- Any foreseen future uses of the research results, data or materials
- Source of co-funding, if any, for the research
- Right to get communication of the results of the research
- Arrangements for taking care of the subjects after their participation has ended
- Name of the Principal Investigator of the clinical trial and of the physician responsible for enrolment and follow-up of the subject.
- Right to continue being followed-up by his/her own physician in addition to the clinical trial physicians

Furthermore, the investigator will pay attention to provide **adequate** and **understandable information** avoiding exhaustive and excessive amount of information to patients (information versus liability concerns of the sponsor). According to the conclusion of the conference EMEA-European commission on the « Clinical Trials Directive (Directive 2001/20/EC) and Perspectives for the Future » (2007), the investigator will commit to extensively communicate to the trial subject on **what happen at the end of the trial** (treatment, publication).

Clinical centres involved in IMAGINT have an extensive expertise in implementing and conducting clinical trials.

Follow-up of clinical trial subjects

Specific requirements for patient follow-up will be indicated in the individual study protocols. In general the protocols are designed to mirror standard treatment and monitoring practice for this patient group. Most studies will require **a six month follow-up period after the administration of radioactive substances**, but will continue to be under the care of the Investigator team for a considerable time thereafter.

During each subject visit to the hospital, a clinician participating in the study will record progress notes to document all significant observations.
At a minimum, these notes will contain:

- Documentation of the informed consent process, including any revised consents;
- Demographic data (date of birth, sex);
- Details related to the inclusion criteria;
- Medical history and physical examination details;
- The date of the visit and the corresponding Visit or Day in the study schedule;
- General subject status remarks, including any significant medical findings. The severity, frequency, and duration of any adverse experiences and the investigator's assessment of relationship to study drug must also be recorded;
- Any changes in concomitant medications or dosages;
- Results of relevant examinations;
- Laboratory print-outs;
- A general reference to the procedures completed;
- The signature (or initials) and date of all clinicians who made an entry in the progress notes.

In addition, any contact with subject via telephone or other means that provides significant clinical information will also be documented in the progress notes as described above.
Information from the study progress notes and other source documents will be promptly entered into the CRF for transmission to the sponsor. CRFs should be completed using a black ball-point pen.
Any changes to information in the study progress notes and other source documents will be initialled and dated on the day the change is made by a site study staff member authorized to make a change. Changes will be made by striking a single line through erroneous data, and clearly entering the correct data (eg, wrong data right data), together with the initials and date. If the reason for the change is not apparent, a brief explanation for the change will be written to the source documentation by the clinician.

Data protection and privacy

Confidentiality

During IMAGINT, all information collected as part of the studies will be treated confidentially. All study data related to the participants will be coded when registered.
The subject will be made aware of (and give consent to) the fact that monitors, auditors, the Ethics Committee and regulatory authorities will be granted direct access to the subjects medical records without violating subject confidentiality, and to the extent permitted by applicable regulations. The subject should be informed that by signing the Informed Consent form, the subject authorizes such access.
All information generated in this study will be considered highly confidential and will not be disclosed to any persons, not directly concerned with the study without written prior permission from the sponsor. However,

authorised regulatory officials and sponsor personnel (or their representatives) will be allowed full access to inspect and copy the records. All study drugs, subject bodily fluids, and/or other materials collected shall be used solely in accordance with this protocol, unless otherwise agreed to in writing by the sponsor.

Subjects will be identified only by unique subject numbers in CRFs. Their full names may, however, be made known to a regulatory agency or other authorized officials if necessary.

Data storage, management and handling will be protected in accordance with European Commission and national directives that are listed in section 4.7. Data will be collected and sent to the Data Management Centre by monitors. Additional data may be transferred in secure electronic format. Daily backups of the database allow recovery of the most recent data in case data are inadvertently lost. These backups are stored in a fireproof armoured filling cabinet. All databases are password protected.

Furthermore, subject privacy and protection of personal data will be safeguarded according to Directive 95/46/EEC dealing with:

- Coding, storage and protection of identity, biological material, recorded data: the storage of personalized identifiers besides a pseudo-anonymised coding of results.
- Right of the subject to get information on what data are recorded, how they are recorded, who will be responsible for keeping the data and who will have access to the data.

In publications with reference to this study, the subject's identity will remain confidential.

Regarding the transfer or disclose of data to third parties, only coded data are authorized to be communicated. That is to say that, all personal identifiers will be removed and data will be pseudo-anonymised before transfer. The identifiers above will be replaced by a unique identifier, which is separately and securely held by the principal investigator.

Records Retention at the Study Site

All patient records are entered in an **anonymous database** where they are numbered (pseudo-anonymised) and linked to their clinical and biological information. The database allows to link patient information along with the exact location where the sample is stored.

According to **FDA and EMEA regulations**, the investigator(s) participating in this clinical trial will maintain detailed clinical data as required by the EU Tissue and Cells Directive or **for a 15 years period, whichever is the longer,** starting from the official date of end of study communicated by the sponsor to the relevant authorities.

The investigator should not dispose of any records relevant to this study without either (1) written permission from the sponsor or (2) providing an opportunity for the sponsor to collect such records. The investigator, assisted by, will take responsibility for maintaining adequate and accurate electronic or hard copy source documents of all observations and data generated during this study, including the CRF data. Such documentation is subject to inspection by the sponsor and relevant regulatory agencies.

Furthermore, if the investigator withdraws from the study (i.e.: relocation, retirement), all study related records should be transferred to a mutually agreed upon designee. Notice of such transfer will be given to the sponsor in writing.

Lastly, the investigator will pay attention to clearly explain what happens to the data or samples at the end of the research period. Since the data/samples will be retained for further research, the Informed Consent will clearly mention this further use.

Protocol design

Sample Handling

The donation, procurement and testing of human tissues is performed according to Human Tissue Act 2004, Human Tissue Authority Code of Practice 8.

The acquisition, storage, export and disposal of human tissues (relevant material) will be carried out in accordance with the Human Tissue Act 2004 and the Human Tissue Authority Code of Practice 8: Import and export of human bodies, body parts and tissue.

All samples will be handled using a standard, written procedure to maximise tissue viability for subsequent procedures. A histopathological assessment of all tissues will be made to confirm the presence of malignant cells.

Participants will be asked to consent to, use any residual material for other research purposes.

All laboratories involved will store, use and dispose of human materials in accordance with the agreed study protocol, material transfer agreement and relevant other legislation or regulatory requirements as may be required in the Territory.

Recruitment and number of subjects for clinical trials:
All patients and donors considered for the clinical studies will have given written informed consent prior to any study-related procedures being performed. For the clinical study, **specific exclusion criteria** are detailed in the trial protocols below. The study will recruit mainly females (negative pregnancy test required) with risk of breast cancer and all patients will be scheduled to undergo an injection of radioactive tagged DARPins

For the first clinical trial (CT1), this is a dose escalation trial in which the starting dose will be determined by the animal toxicity and pharmacokinetics and the application of NOAEL (no observable adverse effect level). Dose escalation will be according to the standard 3+3 model with initial escalation informed by real-time pharmacokinetics in order to obtain the target AUC. Hence the number of patients treated will be minimised while ensuring patient safety. The total number of patients treated is likely to be less than 20.

For the Second clinical trial (CT2), the powering calculations are outlined below.

Protocol for the clinical trials

Protocols for studies included in IMAGINT will be designed by the Investigators in discussion with the Sponsor and Statisticians. Moreover, the standard components of these clinical trial protocols are deliverables of the IMAGINT and will be considered in WP2 and 5. All clinical trials will be conducted by the designated **Chief Investigators (CI)** with the assistance of institutional statisticians, experts in the field of clinical studies design. The CIs are:

CT1: Dr Tim Meyer

CT2: Dr Paul Ellis

They will be assisted by the IMAGINT core group.

Clinical Trial 1 (CT1)

Below is the synopsis of the protocol to be performed during IMAGINT (WP5) for CT1.

Protocol Summary	***CI:** Tim Meyer **- Sponsor:** UCL*
Investigative objective	Gallium 67/68 anti-HER2 DARPins
Primary endpoint	Toxicity
Secondary endpoints	Determine recommended dose (threshold for detection by SPECT/CT) Pharmacokinetic
Patient group	Patients with metastatic HER2 +ve breast cancer
Treatment arms	Single arm phase I dose escalation trial
Sample size	20 patients
Centre	**UCL**

Clinical Trial 2 (CT2)

Protocol Summary	***CI:** Dr Paul Ellis **- Sponsor:** KCL*
Investigative objective	Comparison of FRET/FLIM metastatic signature with FDG and/or FLT PET-CT data, as means of response assessment in a neo-adjuvant setting

Primary endpoint	assessment of in vivo response characteristics: FDG first (baseline and first cycle of therapy) and FLT second (baseline and second cycle of therapy) and vice versa
Secondary endpoints	Tissue biopsies before and surgically obtained tissues (at 8 weeks) after neo-adjuvant treatment, analysed using the FRET/FLIM metastatic signature.
Patient group	HER2-positive patients with locally advanced breast cancers
Treatment arms	Sequential chemotherapy with taxane / trastuzumab x 4 cycles followed by EC (epirubicin, cyclophosphamide) x 4 cycles.
Sample size	40
Centre	KCL

Schedule of the clinical trials

All appropriate procedures to run clinical trials according to ICH GCP, EC Clinical Trial Directive and national legislation are already in place in the different clinical centres and have been inspected and audited successfully on many occasions including ethical review, GCP compliance, tissue storage and use.

CT1 will run from M 18 to M42, considering M1 as the first month of the project as soon as the contract is signed by the EC.

CT2 will run from M18 to M42, considering M1 as the first month of the project as soon as the contract is signed by the EC.

B.4.2. Use of human samples and Data protection

Human samples sources:

Tissue Human samples to be used are both formalin-fixed paraffin-embedded and frozen breast cancer tissues (derived from both the KCL breast tissue bank and prospective clinical studies in this proposal; as well as from collaborators such as those collected in the Neo-ALTTO study.

UCL

Research conducted using human biological materials will only occur after the approval of the relevant local and/or national ethics committees, according to national and EU legislation.

KCL

Research conducted using human biological materials will only occur after the approval of the relevant local and/or national ethics committees, according to national and EU legislation.

Informed consent

Patients and donors donating **samples** for use in IMAGINT will not be exposed to treatment or research without their permission having been obtained in accordance with the regulations of the country in which their sample is obtained, which is generally free and informed consent. Should the IMAGINT patient physical state prevent the patient from signing, another method (i.e. finger printing of informed consent form) may be used if this is in line with local ethical regulations. Should the patient mental state prevent him/her from making an informed decision, he/she will not be requested to participate in the study unless a guardian is legally authorised to sign the informed consent form by local ethical regulations. The form will clearly explain that the patient is free to withdraw his/her participation with no negative consequences.

Data protection

All human samples to be processed will be pseudo-anonymised. All data obtained using human samples will follow the Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data.

UCL
Data protection is in accordance to the local ethic committee, and personal information relating to the subject will be stored anonymously.

KCL
Data protection is in accordance with Research Ethics Committee requirements and KCL policy. Media containing personal identifiers will be stored securely and accessed only by authorised study members and regulatory officers.

B.4.3. Use and Protection of Animals

General remarks

The pre-clinical research described in the project (WP4) involves the use of common strains of laboratory mice to enable pre-clinical tests leading to therapies. No animal models other than mice will be used within IMAGINT.
All animal procedures will be approved by the local ethical committees and will adhere to the national and international laws and provisions regarding the protection of animals. In particular, all animal experiments will be performed by authorised personnel under the rules of each given country according to EC Directive 86/609. Approvals regarding existing animal studies have been obtained by all partners from their local authorities. The animal studies will be carried out under strict containment, in authorised animal facilities that meet legal requirements, under veterinarian control, and by qualified personnel to minimise any possible discomfort to the animals. The animals will be provided with food and beverage *ad libitum* and will be placed in proper cages with adequate bedding. All partners performing work, who envisage the use of animals, hold animal licenses and adhere to the national regulations on animal experiments.
Where possible, *in vitro* cell cultures and non-invasive imaging protocols will be used to minimize the need for procedures on living animals. When necessary, mice will be humanely sacrificed after being put to sleep by general anaesthesia according to general animal protection rules, to provide tissues or cells for in vitro experiments. Whenever possible, and in accordance with the Amsterdam protocol on animal protection and welfare, animal experiments conducted during the IMAGINT project will be replaced with alternatives. Due to the particular characteristics of some human diseases, that are topic of the IMAGINT research, the use in parallel of *in vitro* methods will allow reducing the number of experimental animals involved, but will not completely replace it. In the animal studies the negative effects should be balanced against the potential gains of knowledge necessary for the human studies. The strength of transgenic systems to gain definitive knowledge has an added ethical value and will reduce the need of mice in further studies.
The WP4 and WP5 involving the use of animal models are written according to the European Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member Sates regarding the protection of animal used for experimental and other scientific purposes. In addition, the project has taken / will take all national regulations for the use of animals in research into account and will seek approval through local Ethics Review Committees. Notably, all experiments with vertebrate animals are being performed at UU/GEHC in accordance to the Animal Welfare Act, the Public Health Service Policy on Human Care and Use of Laboratory Animals by Awardee Institutions, the US Government Principles for the Utilization and Care of Vertebrate Animals using in Testing, Research and Training, and the NIH Guide for the Care and Use of Laboratory Animals.
All partners have considered using non-living alternatives to their animal models, however the nature of the studies necessitates that an entire organism be used. The principles of the “3Rs” (Reduction, Refinement and Replacement) have been applied.

Ethical sensitivity and the 3Rs

The challenge for the research community is to develop alternatives to animal-based tests. IMAGINT is committed to better organising the research and development and to promoting and applying the “3Rs” (reduction, refinement and replacement) concept in order to find alternatives to animal experimentation.

Animal Positron Emission Tomography (PET) enables translation from pre-clinical environment to clinical practice. This provides options for non-invasive in vivo imaging and quantification of a target expression. In vivo animal studies allow repeated investigations to be performed on the same animal. This is particularly valuable because it results in an increase in the statistical quality of the data (subjects can be used as their own control) and reduces the number of animals required for a given study.
However, although advanced techniques such as animal PET offer a considerable reduction in the number of animals required for a study, there are a number of factors, e.g. time aspects, economical concern, and ethical issues that need to be considered prior to planning of animal experiments. It is therefore desirable to introduce less resource-demanding yet still reliable preclinical methods for obtaining valuable data allowing a more efficient planning of studies in animal models and human trials. In preclinical cancer research, the Multicellular Tumour Spheroid (MTS) model has gained an important role as an intermediary system between cells growing as monolayer and solid tumours in experimental animals or patients. MTS are biologically and physiologically more similar to in vivo grown tumours than 2D-cultured cells and are less laborious to use than animal models. The model provides the possibility of minimizing animal experiments and helping to set up in vivo studies more efficiently.

More precisely, IMAGINT will take initiatives to:

- **Reduce animal experimentation:**

UU/GEHC uses a number of mice as low as possible for each experiment, provided however that a sufficient statistical power is obtained in the experiment.
Furthermore, UU/GEHC have developed experimental plans in close collaboration with biostatisticians, and in most cases experiments will be performed and repeated once to assure reproducibility, with a group size of 3 animals. In experiments requiring the sacrifice of animals, protocols have been developed that analyze analysis of all relevant organs and tissues by a specially assembled team of researchers in the laboratory. The number of animals employed will not exceed that required for reaching statistical significance.

We will reduce animal experimentation by screening of the tracer candidates in Multicellular Tumour Spheroid model. Applying the MTS model, we have the possibility to examine the cellular pharmacokinetics (cellular tracer uptake and retention), specificity and affinity of the tracer candidates. The model provides the possibility of minimizing animal experiments and helping to set up in vivo studies more efficiently. The evaluations in MTS is a suitable starting point for the translational activities which should be followed by rationally designed studies in xenografts, based on the MTS results and finally planned inclusion of PET in clinical trials (105).

- **Refine animal experimentation** by using best practices which alleviate or minimise potential pain, suffering and distress and enhance animal well being.

UU/GEHC constantly review their models, and at present no alternative for the state-of-the-art animal models can be used. **Mice** will be maintained in groups wherever possible and will be inspected daily by research and/ or Biological Services Unit staff. The Project License that provides legal authority for this work contains a series of guidelines that will be used to assess non-specific or unexpected adverse effects in animals undergoing regulated procedures in this project. Animals showing two or more of the limiting clinical signs in the category equivalent to the protocol severity limit will be monitored closely and veterinary advice will be sought. In the event that any animal shows more than three limiting clinical signs in the category equivalent to the protocol severity limit, it will be killed. The use of imaging to monitor tumour status will allows the incorporation of humane endpoints into tumour challenge studies. Pilot studies will be performed to validate imaging tool as a reliable indicator of tumour status in animals. Once established, this will allow the culling of mice with circulating tumour cells even at a point when they remain clinically well.
Mice that are subject to the experiments described in the proposal are examined daily for the appearance of distress or injury. Animals that appear to be in distress or whose tumours might be causing distress due to size or position are euthanized using CO_2. Anaesthetic drugs are used to sedate or put mice to sleep before procedures involving bleeding. Animals required restraint for any reason are also anesthetized to put them to sleep. Pain and distress will be relieved by anaesthesia under guidelines approved by the Institutional Animal Care and Use Committee (IACUC). Animals requiring euthanasia are sacrificed using CO_2 by the animal facility staff upon notification by the investigators or upon discovery of problems by the animal facility staff. The method of euthanasia is consistent with the recommendations of the panel on euthanasia of the American

Veterinary Medical Association. Animals will receive varying doses of radioactive substances. Due to the sensitivity of the method the doses are so low that no toxic effects arise (microdosing).
Replace animal experimentation, when possible, with methods that do not require experimentation or other scientific procedures on animals.
Actually, there is no alternative to *in vivo* testing for biodistribution studies and for developing clinical imaging tool.
Experiments using animal models will only be performed when this is strictly necessary, in case in vitro studies will not suffice to translate laboratory findings into treatment modalities in humans, or in case diagnostic procedures can not be analysed in patients (UCL, KCL, UU).

Level of suffering of animals

Potential discomfort ('suffering') must be evaluated at the level of each individual animal. Animal discomfort must clearly be minimised for ethical and experimental reasons. Severe acute or chronic mild discomfort might compromise the outcome and goals of long-term studies. Animals will be observed daily for signs of injuries, illness or abnormal behaviour by trained personnel. Any such cases must be reported to the attending veterinarian and treated as required. The animals will be acclimatised to the experimental conditions and care will be taken to exclude pain, fear and illness. Both short and long-term stress and pain will be avoided by using refined experimental conditions including appropriate anaesthesia and analgesia, state-of-the-art surgical facilities and techniques (including sterile conditions); highly experienced personnel performing these procedures. Optimal housing conditions (supervised by trained veterinarians) will be sought and provided, habituation of the animals to the procedures involved (including training/gentling prior to the experimental tests and manipulations), regular control of body weight and competent pre- and post-operative care, are all strategies that aim at minimising animal suffering. Animal experiments will be approved and supervised by the respective authorities (*e.g.* Government).

Justification of species chosen

Research in animals will be conducted according to the EU directive 86/609/EC, regarding the protection of animals used for experimental and other scientific purposes. The research involving animals will only be performed after having obtained approval by the local/national ethical committee, as appropriate.
The choice of the species to be used in animal experiments is based on scientific relevance and ethical considerations. **Mice are widely recognized as one of the best and most practical models for tumour and imaging tool development**. In addition, more reagents are available for studies in mice than for studies in any other preclinical model. Mice with the following background will be used

- In oncology research, xenograft in immunodeficient mice is the most common and relevant model that can represent and can be extrapolated to the human data. SKOV-3 xenograft mice model is an excellent choice for in vivo investigation of HER2 expression in the human tumour (171)).

Animal use by individual partners

UU/GEHC (WP4)

The animals will be obtained from research institutions or from authorized vendors, and will be housed in approved animal facilities which are part of the University or Institute. The animal will be followed for endpoints, and euthanized at the earliest possible time.

Twelve mice (BALB/c nu/nu) bearing SKOV-3 xenografts are prepared for in vivo tracer validation.

The animals will be divided in four groups (each group including 3 animals):
Anesthetised animals are injected with high SRA ^{68}Ga-DARPin and subjected to 2h PET scan for following the kinetic behaviour of the tracer. The same experiment with same animals will be repeated following day after with low SRA ^{68}Ga-DARPin.
In order to follow the time course of binding with high signal to noise as a function of concentration, anesthetised animals are subjected to dual PET/SPECT/CT study with the PET tracer ^{68}Ga and the SPECT tracer ^{67}Ga.
In order to investigate the eventually anaesthesia effect on tracer clearance, non-anesthetised animals are injected with ^{68}Ga-DARPin, subjected to PET scan after 1h and euthanatized with CO_2 for organ distribution study.
In order to investigate the sensitivity of the tracer, animals are treated with a $HSP90_{inh}$ followed by repetitive PET scans in different time points after treatment.

The studies are followed by quantitative analysis of the tracer retention in the tumour and other vital organs following the comparison of the results of experiments with different specific radioactivity.

The Multicellular Tumour Spheroid model
Applying the MTS model, we have the possibility to examine the cellular pharmacokinetics (cellular tracer uptake and retention), specificity and affinity of the tracer candidates. The model provides the possibility of minimizing animal experiments and helping to set up in vivo studies more efficiently. The evaluations in MTS is a suitable starting point for the translational activities which should be followed by rationally designed studies in xenografts, based on the MTS results and finally planned inclusion of PET in clinical trials (105).

Characterisation of the ^{68}Ga-DARPins binding to HER2 is evaluated using ovarian carcinoma SKOV-3 and breast carcinoma BT474 cell lines. The cell lines are selected because they have a high level of HER2 expression (97,172).

In vitro specificity test
MTSs are pre-incubated with increasing concentrations of 3 different well-characterised HER2 blockers, incubated with 67Ga-DARPins for 1h, washed three times and the radioactivity is measured to calculate specific receptor-bound activity.

In vitro sensitivity test
MTSs are treated with an increasing concentration of 2 different well-characterised $Hsp90_{inh}$, incubated with ^{67}Ga-DARPins for 1h, washed three times and the radioactivity is measured to calculate the sensitivity of the tracer to treatment response.

In vitro study concerning cellular retention of the tracer
For characterisation of time and concentration dependence of tracer uptake MTS are incubated with different concentration of ^{67}Ga-DARPins for a time range of 1 to 5 hours.
For dissociation characteristic of the tracer, MTS incubated for 1 hour with the tracer are washed with tracer-free medium for a time range of 0.5 to 5 hours before measurements.

UCL (WP5)
All experiments will be performed in compliance with the Animals (Scientific Procedures) Act 1986 and the UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia. It is anticipated that four different experiments, using nu/nu mice bearing human HER2+ve and HER2-ve xenografts, will be required in order to obtain a good indication of the pharmacokinetics and tumour uptake of the radiolabelled GMP cys-tagged antihER2 DARPin. 48 animals are required in total. In general, 4 animals per group will be used for these biodistribution experiments as agreed by the statistician at UCL when preparing the Oncology Department Home Office Project Licence PPL70/6481, expiry date 2011, in which the 3Rs were fully addressed and adopted. Mice will be humanely killed by cervical dislocation on completion of experiments.

B.4.4. Safety provision – Use of GMOs

GMOs will be used in IMAGINT by UCL, UZH, and M-GMP.

The IMAGINT project entails the use of Genetically Modified microorganisms, which will be kept and manipulated according to national and European directives (90/219/EEC; 93/88/EEC; 98/81/EC). In the experiments involving the use of vectors, safety regarding the protection of the workforce and the environment by contamination and/or spreading of vectors may be questioned. This matter will be dealt with appropriately, following existing national laws and guidelines, and in compliance with the EC Directive on the Protection of Workers from Risks Related to Biological agents at Work (90/679/EEC).
The *Escherichia coli* strains XL1-Blue, TOP10 and DH5α are routinely used in laboratories and are non-pathogenic to humans. Standard precautions of sterility and containment of bacteria will be followed including personnel protection equipments (lab coats, gloves, disinfectants).

*Regarding the use of genetically modified organisms at **UCL***, for the use of all GMOs (*E. coli* strains XL1-Blue, TOP10 and DH5α and vectors), a full risk assessment has been conducted (March 2008) and has been approved

by the UCL Genetic Modification Safety Committee. All activities are low risk level 1 and are performed in a level 2 environment.
A full copy of the risk assessment can be forwarded on request.
In brief, the following control measures are in use:
- Good laboratory practice is enforced at all times.
- Departmental safety inspections are undertaken monthly.
- All staff members have received training in the handling of genetically modified organisms. New members of staff undergo induction and must attend Occupational Health.
- Standard operating procedures are in place to discourage the use of sharps and to reduce the production of aerosols.
- Tissue culture work is performed in a designated room in a class 2 microbiological safety cabinet (subjected to 6 monthly testing). All materials that have been in contact with genetically modified material will be decontaminated. Solid waste will be autoclaved prior to incineration and liquid waste will be decontaminated by overnight incubation with freshly prepared 1% Virkon. Autoclaves are subject to annual validation (temperature mapping and load validation).
- There is a standard operating procedure for fumigation of the containment facility.
- There is an accident plan for GM work.

Regarding the use of genetically modified organisms at the Department of Biochemistry at ***UZH***, a full risk assessment/safety concept for work with GMOs has been conducted on 19 September, 2008 and has been approved by the Swiss Federal Government represented by the Office for Waste, Water, Energy and Air (AWEL), Zurich, Switzerland and the Federal Office for the Environment (FOEN) at Bern, Switzerland on 28 October, 2008 by a previous on-site inspection.
A full copy of the safety concept and the inspection report can be forwarded on request.

The specific project on 'Protein Engineering and Biophysical Characterization' which includes the expression of recombinant protein expression in bacteria, yeasts or eukaryotic cell culture is registered with the number A000546 at the Swiss Federal Office for the Environment FOEN at Bern since 30 August, 2000, with a recent update on 28 April, 2009 as a biosafety level 1 project.

Biosafety issues are dealt with by an experienced senior scientist who represents the biosafety officer of the institute. He is in close and direct contact to the safety officials of the University of Zurich and has personal contacts to the Swiss state authorities.

In brief, the following control measures are in use:
- Good laboratory practice is enforced at all times.
- Departmental safety inspections are undertaken.
- All institute members have participated in a lecture series about general, chemical, bio and radioactive safety. These lectures are given on a regular basis by the respective department's safety officers.
- All new members get a specific and individual thorough introduction into lab life, touching all facets of practical safety in the respective lab.
- All young students are supervised by an experienced PhD student or Post Doctoral Researcher during all theoretical and practical aspects of lab activities in everyday life.

Regarding the use of genetically modified organisms at ***M-GMP: a***s for the contained use of genetically modified organisms Medipolis GMP follows the regulations described in the Gene Technology Act (377/1995). The Gene Technology Act has been set to fulfil the requirements defined in EU directives 90/219/EEC and 2001/18/EC.

Every operator using GMOs has certain duties laid down by the Act. The operator must make a risk assessment of each GMO and show care when using them. The operator has a duty to obtain information on the properties of the GMOs and to keep a record of the contained use. Also, the operator has a duty to submit relevant notifications to the Board for Gene Technology and to update the documents as specified by the Act.

The Board for Gene Technology is constituted by the Gene Technology Act (No.377/1995). In addition to being a national authority in Finland, the Board functions as a competent authority towards the European Community.

It processes notifications concerning the use and release of genetically modified organisms as defined in directives 90/219/EEC and 2001/18/EC, and responds to them within its authority to make legally binding decisions.

Besides following the national laws and EU directives concerning the use genetically modified organisms, Medipolis GMP is also following GMP principles to fulfil the requirements concerning pharmaceutical manufacture in order to guarantee adequate safety of the manufactured products and to prevent cross contamination between products. These principles have been described in internal standard operating procedures covering, but not limited to, adequate microbiological purity testing of used organisms and change over cleaning procedures between products and different organisms. These principles have been considered to be adequate as indicated by the manufacturing license granted by the Finnish Medicinal Agency FIMEA.

The minimum requirement for any incoming microbial organism to be used in clean room facility is to guarantee its microbiological purity (e.g. in case of and E.coli absence of bacteriophage and other microbial contaminants). The extent of any other characterization testing performed depends on the development phase/ use of the product.

B.4.5. Safety provision – Use of human samples

The standard laboratory safety techniques are used for dealing with human material. Even though these samples may not be high risk the same precautions are used.

B.4.6. Dual use

All the technologies and results in the project will not and have no potential to be used for any kind of military action.

B.4.7. National and European legislation and codes of conduct

The beneficiaries ensure that appropriate health and safety procedures conforming to relevant local/national/European guidelines/legislations are followed for staff involved in the project.

National legislation

Use of human biological samples

Use of Human Biological Samples
Germany
Rechtslage Zentrale Ethik-Kommission §§8,9 StZG
Verwaltungsakt der zuständigen Ethik-Kommission i.S.d. §35 S.1 VwVfG
Ethik-Kommission §20 Abs. 7 MPG
Forschung mit nicht einwilligungfähigen Menschen (Art.17 Abs.2 der Konvention)
Gentests in Medizin, Arbeitsleben und Versicherungen – Bundestag 11 März 2003
Gesetz zur Regelung des Transfusionswesens (Transfusionsgesetz) Date: 1 July 1998 (published in the Official Journal Bundesgesetzblatt BGBl I, S. 1752)
Spain
Act 41/2002, of 14 November, a basic regulation of the autonomy of the patient, and of the rights and duties of providing information and clinical documentation
Royal Decree 411/1996, of 1 March which regulated the activities relating the use of human tissues
Royal Decree 2070/1999, of 30 December, on the activities of collection and clinical use of human organs
Act 42/88 of 28 December, on donation and use of human embryos and foetuses or of their cells, tissues or organs
Switzerland
Ordinance on Clinical Trials of Therapeutic Products (SR 812.214.2)

Use of Human Biological Samples
ICH-GCP guidelines on clinical trials
Sweden
Kommissionens direktiv 2006/17/EG av den 8 februari 2006 om genomförande av Europaparlamentets och rådets direktiv 2004/23/EG när det gäller vissa tekniska krav för donation, tillvaratagande och kontroll av mänskliga vävnader och celler, Kommissionens direktiv 2006/86/EG av den 24 oktober 2006 om tillämpning av Europaparlamentets och rådets direktiv 2004/23/EG med avseende på spårbarhetskrav, anmälan av allvarliga biverkningar och komplikationer samt vissa tekniska krav för kodning, bearbetning, konservering, förvaring och distribution av mänskliga vävnader och celler, Förordning om kvalitets- och säkerhetsnormer vid hantering av mänskliga vävnader och celler - SFS 2008:414 LVFS 2008:12 - Läkemedelsverkets föreskrifter om hantering av mänskliga vävnader och celler avsedda för läkemedelstillverkning Socialstyrelsens föreskrifter om donation och tillvaratagande av vävnader och celler - SOSFS 2008:22 SFS 2008:286 - Lag om kvalitets- och säkerhetsnormer vid hantering av mänskliga vävnader och celler SFS 2008:414 - Förordning om kvalitets- och säkerhetsnormer vid hantering av mänskliga vävnader och celler
United Kingdom
Human Tissue Act 2004 Human Tissue Authority Code of Practice 8: Import and export of human bodies, body parts and tissue

Confidentiality of personal data

Confidentiality of personal data
Germany
Durchführung von Klinischen Prüfungen mit Arzneimitteln (GCP – Verordnung – GCP – V ; 9 August 2004 BGBl. I, S. 2081)
12. Novelle des Arzneimittelgesetzes
Rechtslage Zentrale Ethik-Kommission §§8,9 StZG
Verwaltungsakt der zuständigen Ethik-Kommission i.S.d. §35 S.1 VwVfG
Ethik-Kommission §20 Abs. 7 MPG
Federal Data Protection Law de 1 de enero de 2003
Spain
“Ley Organica 15/1999, de 13 de Diciembre, on Personal Data Protection - the “LOPD”) and subsequent modifications (Ley 62/2003, y Recurso de Inconstitucionalidad 1463/2000)
Ley 41/2002, de 14 de noviembre (básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica) / a basic regulation of the autonomy of the patient, and of the rights and duties of providing information and clinical documentation
Ley 8/2001, de 13 de julio, de la Presidencia de la Comunidad de Madrid de Protección de Datos de Carácter Personal en la Comunidad de Madrid (autonómica)
Real Decreto 428/1993, de 26 de marzo, por el que se aprueba el Estatuto de la Agencia Española de Protección de Datos
Real Decreto 994/1999, de 11 de junio, por el que se aprueba el Reglamento de Medidas de Seguridad de los ficheros automatizados que contengan datos de carácter personal
Instrucción 1/1998, de 19 de enero, de la APD, relativa al ejercicio de los derechos de acceso, rectificación y cancelación
Federal Data Protection Law de 1 de enero de 2003
Switzerland
Datenschutzgesetz Artikel 6,7,8,11,16,24 and 36
United Kingdom
All data must be treated in accordance with the European directive 95/46 EC and the Data Protection Act (UK) 1998 ; Human Rights Act 1998 ; The Law of Confidentiality (common law duty of confidence) ; Special permission must be granted by the Department of Health’s; MRC operational and ethical guidelines for the use of human tissue and biological samples in research

Human studies and clinical trials, Good clinical Practice

Human studies & Clinical trials; Good Clinical Practice ***Information applying to Genetic Testing & Biobanking included***
Germany
Durchführung von Klinischen Prüfungen mit Arzneimitteln (GCP – Verordnung – GCP – V ; 9 August 2004 BGBl. I, S. 2081), die durch Artikel 4 der Verordnung vom 3 November 2006 (B6B1 I S. 2523) geändert worden ist.
12. Novelle des Arzneimittelgesetzes
Rechtslage Zentrale Ethik-Kommission §§8,9 StZG
Verwaltungsakt der zuständigen Ethik-Kommission i.S.d. §35 S.1 VwVfG
Ethik-Kommission §20 Abs. 7 MPG
Forschung mit nicht einwilligungfähigen Menschen (Art.17 Abs.2 der Konvention)
Gentests in Medizin, Arbeitsleben und Versicherungen – Bundestag 11 März 2003
Gesetz zur Regelung des Transfusionswesens (Transfusionsgesetz) Date: 1 July 1998 (published in the Official Journal Bundesgesetzblatt BGBl I, S. 1752)
Spain
Act 41/2002, of 14 November, a basic regulation of the autonomy of the patient, and of the rights and duties of providing information and clinical documentation
Royal Decree 411/1996, of 1 March which regulated the activities relating the use of human tissues
Royal Decree 2070/1999, of 30 December, on the activities of collection and clinical use of human organs
Act 42/88 of 28 December, on donation and use of human embryos and foetuses or of their cells, tissues or organs
Royal Decree 223/2004, of 6 February, which regulates the clinical essays with drugs
Switzerland
Ordinance on Clinical Trials of Therapeutic Products (SR 812.214.2)
ICH-GCP guidelines on clinical trials
United Kingdom
European Clinical Trials Directive (2001/20/EC) transposed into UK law as « The Medicines for Human Use (Clinical Trials) Regulation 2004, SI 2004/1031 & Amendment Regulations 2006, SI 2006/1928. Medicines for Human Use (Clinical Trials) Regulations 2004 Under this regulation, all clinical trials must receive approval from an ethics committee before the trial can start Research Governance Framework for Health and Social Care. Department of Health, 2nd edition 2005 Human Tissue Act 2004
Sweden
Europaparlamentets och rådets direktiv 2001/20/EG av den 4 april 2001 om tillnärmning av medlemsstaternas lagar och andra författningar rörande tillämpning av god klinisk sed vid kliniska prövningar av humanläkemedel Kommissionens direktiv 2005/28/EG om fastställande av principer och detaljerade riktlinjer för god klinisk sed i fråga om prövningsläkemedel för humant bruk samt av krav för att få tillstånd till tillverkning eller import av sådana produkter

Use of GMOs, Good Laboratorary Practice (GLP), Good Manufacturing Practice (GMP)

Use of GMOs, Good Laboratory Practice (GLP) / Good Manufacturing Practice (GMP)
Switzerland
Ordinance on the Contained Use of Organisms (SR 814.912), nov.1999 Ordinance on the Protection of Workers against Risks Originating from Intended or Unintended Use of Microorganisms (SR 832.321), nov.1999 January 1st 2002 new federal law on medicinal products and medical devices (Law on Therapeutic Products, SR 812.21) European Guide to Good Manufacturing Practice for gene therapy vectors production Ordinance Concerning the Control of Transplants (SR 818.111.3)
United Kingdom
MRC operational and ethical guidelines for the use of human tissue and biological samples in research Genetically Modified Organisms (Deliberate Release) Regulations 1992 SI 1992 No. 3280

Use of GMOs, Good Laboratory Practice (GLP) / Good Manufacturing Practice (GMP)
Genetically Modified Organisms (Contained Use) (Amendment) Regulations 2005 SI 2005 No. 24662000 Use of GMOs, Good Laboratory Practice (GLP) / Good Manufacturing Practice (GMP) – Rules & Guidance for Pharmaceutical Manufacturers & Distributors 2007 – the 'Orange Guide'. GLP: OECD Principles of GLP and Compliance Monitoring, issued as EU directives 99/11/EEC & 99/12/EEC and adopted in UK as SI 3106, 1999 as amended by SI 994, 2004.
Finland
Gene Technology Act (No.377/1995) Directives 90/219/EEC and 2001/18/EC Manufacturing license granted by the Finnish Medicinal Agency FIMEA

Protection of animals

Protection of animals
Germany
Law for Animal Protection (TierSchG,) §§4,6(Anzeige,Organe), §8 (Genehmigung), §11 (Haltung), VersuchtiermeldeVO, EU Richtlinien zur Haltung + Versorgung von Tieren
Switzerland
Small laboratory animals under national legislation (normal mice, MDX mice)
According to the Swiss Constitution; Article 120: Dignity of the creature and the restrictions on the use of non-human gene technology are regulated in the detailed law issued by the Swiss Ethics Committee on Non-Human Gene Technology
United Kingdom
SI 1998/1974 Animals (Scientic procedures) Act 1986 (Amendment) Regulations 1998.
United States
Code of Federal Regulations: 21CFR501, 511, 514, 515, 530, 558 (drugs for animals); 9CFR1, 9CFR2 (Animal Welfare Act)
Sweden
Law for Animal Protection (Djurskyddslag) 1988:534 , Centrala försöksdjursnämndens föreskrifter om den etiska prövningen av användningen av djur för vetenskapliga ändamål 1988:45 , Centrala försöksdjursnämndens kungörelse med föreskrifter och allmänna råd om utbildningskrav vid användning av djur för vetenskapliga ändamål m.m 1992:11 , Djurskyddsmyndighetens föreskrifter om statistikföring vid djurförsök 2004:13 , Djurskyddsmyndighetens föreskrifter och allmänna råd om djurförsök m.m. 2004:4 ,
All Partners
Animal ethical issues will be dealt with in compliance with the recently revised standards of Council of Europe Convention ETS123 on top of each country's national guidelines

Good Laboratory Practive (GLP), Good Manufacturing Practice (GMP)

Good Laboratory Practice (GLP) / Good Manufacturing Practice (GMP)
Germany
German Drug Law (AMG, 12. Novelle des Arzeimittelgesetzes)
Zentrale Kommission f. Biologische Sicherheit
Finland
Directives 90/219/EEC and 2001/18/EC
Manufacturing license granted by the Finnish Medicinal Agency FIMEA
Sweden
AFS 2005:01 - Mikrobiologiska arbetsmiljörisker - smitta, toxinpåverkan, överkänslighet , AFS 1990:11 - Arbete med försöksdjur
Switzerland

Good Laboratory Practice (GLP) / Good Manufacturing Practice (GMP)
Ordinance on the Contained Use of Organisms (SR 814.912), nov.1999
Ordinance on the Protection of Workers against Risks Originating from Intended or Unintended Use of Microorganisms (SR 832.321), nov.1999
January 1st 2002 new federal law on medicinal products and medical devices (Law on Therapeutic Products, SR 812.21)
European Guide to Good Manufacturing Practice for gene therapy vectors production
United Kingdom
Use of GMOs, Good Laboratory Practice (GLP) / Good Manufacturing Practice (GMP) – Rules & Guidance for Pharmaceutical Manufacturers & Distributors 2007 – the 'Orange Guide'. GLP: OECD Principles of GLP and Compliance Monitoring, issued as EU directives 99/11/EEC & 99/12/EEC and adopted in UK as SI 3106, 1999 as amended by SI 994, 2004.

Other

Recommendations and regulations regarding international transport of human biological samples
International
UNECE (United Nations Economic Commission for Europe) *UN Recommendations on the Transport of Dangerous Goods. Model Regulations.* http://www.unece.org/trans/danger/publi/unrec/rev13/13files_e.html
IATA (International Air Transport Association) *Dangerous Goods Regulations 2005.* http://www.iata.org/ps/publications/9065.htm
ICAO (International Civil Aviation Organization) ICAO (International Civil Aviation Organization) http://www.icao.int/icao/en/m_publications.html
International Air Shipping Rules
The European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR) (2007) (ECE/TRANS/185, Vol. I and II)

EU legislation

IMAGINT partners will conform to relevant EU legislation such as:

- The Charter of Fundamental Rights of the EU
- Directive 95/46/EC of 24 October 1995 on the protection of individuals with regards to processing of personal data and the movement of such data
- Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use
- Detailed guidance on the application format and documentation to be submitted in an application for an Ethics Committee opinion
- Detailed guidance for the request for authorisation of a clinical trial to the competent authorities
- (see next line)Directive 2003/63/EC of 25 June 2003 amending the GMP Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use
- Directive 2003/94/EC of 8 October 2003 laying down the principles & guidelines of GMP in respect of Medicinal Products for human use and investigational medicinal products for human use.
- Regulation (EC) No 1084/2003 of 3 June 2003 concerning the examination of variations to the terms of a marketing authorisation for medicinal products for human use and veterinary medicinal products granted by a competent authority of a Member State
- Regulation (EC) No 1085/2003 of 3 June 2003 concerning the examination of variations to the terms of a marketing authorisation for medicinal products for human use and veterinary medicinal products falling within the scope of Council Regulation (EEC) No 2309/93
- Directive 98/44/EC of the European Parliament and of the Council of 6 July 1998 on the legal protection of biotechnological inventions

- Directive 86/609/EEC of 24 Nov. 1986 on the protection of animals used for experimental and other scientific purposes
- Protocol on Protection and welfare of animals (protocol to the Amsterdam Treaty)
- Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified micro-organisms.
- Directive 98/81/EC of 26 October 1998 amending Directive 90/219/EEC on the contained use of genetically modified micro-organisms
- Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.
- Regulation (EC) No 1946/2003 of the European Parliament and of the Council of 15 July 2003 on transboundary movements of genetically modified organisms (Text with EEA relevance)
- Regulation (EC) No 65/2004 of 14 January 2004 establishing a system for the development and assignment of unique identifiers for genetically modified organisms
- Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from the risks related to exposure to biological agents at work (7th individual directive within the meaning of Article 16(1) of Directive 89/391/EC)
- Directive 2004/23/EC of the European Parliament and of the Council on Setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells", code number 2002/0128 (COD), Strasbourg, 31 March 2004.
- Directive 89/391/EEC on the Management of Health and Safety at Work Regulations 1999 (SI 1999/3242)
- Directive 89/655/EEC on the Safe Use of Work Equipment, Provision and Use of Work Equipment Regulations 1998 (SI 1998/2306)
- Directive 90/269/EEC on Manual Handling Operations Regulations 1992 (SI 1992/2793)

International conventions and declarations

The IMAGINT members will respect the following international conventions and declarations:

- Helsinki Declaration: Clinical trials will be conducted in accordance with the World Medical Association Declaration of Helsinki

Convention of the Council of Europe on Human Rights and Biomedicine signed in Oviedo on 4 April 1997, and the Additional Protocol on the Prohibition of Cloning Human Beings signed in Paris on 12 January 1998
UN Convention on the Rights of the Child
Universal Declaration on the human genome and human rights adopted by UNESCO

Ethical Issues Table

The IMAGINT partners confirmed that the proposed research project does not involve:
Research activity aiming at human cloning for reproductive purposes.
Research activity intended to modify the genetic heritage of human beings which could make such changes heritable.
Research activities intended to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer.

B.4.8. Ethical Issues Table

Research on Human Embryo/Foetus	YES	PAGE
Does the proposed research involve human Embryos?	-	
Does the proposed research involve human Foetal Tissues/Cells?	-	
Does the proposed research involve human Embryonic Stem Cells (hESCs)?	-	
Does the proposed research on human Embryonic Stem Cells involve cells in culture?	-	
Does the proposed research on human Embryonic Stem Cells involve the derivation of cells from embryos?	-	
I CONFIRM THAT NONE OF ABOVE ISSUES APPLY TO MY PROPOSAL	**YES**	
Research on Humans	**YES**	**PAGE**
Does the proposed research involve children?	-	
Does the proposed research involve patients?	YES	WP5
Does the proposed research involve persons not able to give consent?	-	
Does the proposed research involve adult healthy volunteers?	YES	WP5
Does the proposed research involve Human genetic material?	YES	WP2-3; WP5
Does the proposed research involve Human biological samples?	YES	WP2-3; WP5
Does the proposed research involve Human data collection?	YES	WP5-6
I CONFIRM THAT NONE OF ABOVE ISSUES APPLY TO MY PROPOSAL	**NO**	
Privacy	**YES**	**PAGE**
Does the proposed research involve processing of genetic information or personal date (e.g. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	-	
Does the proposed research involve tracking the location or observation of people?	-	
I CONFIRM THAT NONE OF ABOVE ISSUES APPLY TO MY PROPOSAL	**YES**	
Research on Animals	**YES**	**PAGE**
Does the proposed research involve research on animals?	YES	
Are those animals transgenic small laboratory animals?	YES	WP2-3
Are those animals transgenic farm animals?	-	
Are those animals non-human primates?	-	
Are those animals cloned farm animals?	-	
I CONFIRM THAT NONE OF ABOVE ISSUES APPLY TO MY PROPOSAL	**NO**	
Research Involving Developing Countries	**YES**	**PAGE**
Does the proposed research involve the use of local resources (genetic, animals, plant, etc)?	-	
Is the proposed research benefit to local communities (e.g. capacity building, access to healthcare, education, etc)?	-	
I CONFIRM THAT NONE OF ABOVE ISSUES APPLY TO MY PROPOSAL	**YES**	
Dual Use	**YES**	**PAGE**
Research having direct military use	-	
Research having the potential for terrorist abuse	-	
I CONFIRM THAT NONE OF ABOVE ISSUES APPLY TO MY PROPOSAL	NO	

B.5. CONSIDERATION OF GENDER ASPECTS

B.5.1. Addressing the gender-based dimension in IMAGINT

The Helsinki Group on Women and Science, commissioned by the EC to investigate gender distribution in scientific careers, has clearly highlighted gender disparity in science. The "She Figures 2009" database reveals that women remain a minority in scientific research: on average, women account for 30% of all researchers in the EU-27 in 2006. However, positive trends have been observed: in between 2002 and 2007, the share of women among scientists and engineers has grown significantly (+6.2%) compared to +3.7% for men. Furthermore, the participation of women at PhD level has increased at a significantly higher rate (7.3% per year) compared with that of men (3.8% increase/year), whatever the disciplinary. While there is an encouraging trend, female researchers are substantially less numerous than their male counterparts; and although the proportion of female researchers varies considerably between countries, there is a clear scissor pattern male-female throughout the stages of a typical academic career. As a consequence, women are under represented on boards and at the head of high-education establishments.

In IMAGINT, women are well represented: the coordinator, in project management and in scientific activities within all WP and the different organisations (UCL -4/10; KCL – 5/12; UZH – 2/4; Firalis 1/3; INO – 1/2; C-GMP/TNL – 1/4; ACIES_P2R - 2/3) (17 out of 48, i.e. 35%).

The first WiS (Women in Science) data collection, carried out in 2007, emphasised a decrease in the proportion of female higher grade staff as age increases. This observation could point towards the existence of a generation effect. In IMAGINT, it is noteworthy that the percentage of **women scientists – 52% researchers** - reflects a fair participation of women and men. Women are present in all teams, from fundamental research to clinical teams, and from technician to experienced scientists. While no information is available as per their age, the female researchers are unambiguously experienced researchers and post Doc researchers.

B.5.2. Gender action plan

IMAGINT members are highly committed to **promoting equality in the form of equal employment opportunities**.

The highly educated and competent women of Europe and throughout the world are a crucial resource in the innovation-based economies of Europe. The relative lack of women in scientific sectors is unsatisfactory, particularly in top management positions associated with upper responsibility. We feel that the promotion of this project on diagnostics, monitoring tools of breast cancer will provide an important example of a well-balanced project in which women play a substantial role, in full partnership with their male colleagues. In order to ensure a fair participation rate of women during the project hiring process, IMAGINT will implement the following specific measures:

- During IMAGINT, recruiters will strive to employ an **equal number** of women and men among the research staff (incl. Post Docs) to with specific efforts made to consider gender issues in recruitment practices. The target rate of 50% of women employed for different positions will be monitored.
- Employers will also give equal consideration to women when **delegating** responsibilities of IMAGINT and when designating employees for **promotion**.
- A system for monitoring gender equality in mobility schemes will be established, such as equality of access and participation and subsequent impact on professional careers.
- A set of **gender indicators** will be produced in order to **measure progress** towards gender equality in the diagnostics. Age to level of **responsibility** relationship will also be carefully monitored.
- Special attention will be paid to working conditions (flexibility) to enable women to participate in research.

Under no circumstances, women contributing to the IMAGINT project will be discriminated against for gender reasons or as a result of pregnancy or childcare responsibilities.

B.6. Annexes

B.6.1. Bibliography

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B.6.2. Coordinator CV

CURRENT RESEARCH FUNDING

Type of grant	Awarding body	Date	Amount	Detail
Project Grant (Co-investigator)	Cancer Research UK	2008-2013	£8,680,000	KCL and UCL Comprehensive Cancer Imaging Centre C1519/A10331. T Ng (Principal Investigator and Coordinator),
Technician Post (PI)	Cogent Ltd	2010-2011	£70,075	A humanised ADEPT system using reverse polarity human RNAse
Technician Post (PI)	Breast Cancer Campaign	2009-2012	£194,749	Novel agents for imaging HER2 positive breast cancer
Project Grant (Co-investigator: Oncology Lead)	EPSRC	2009-2012	£1,561,415	Bio-functional Magnetic Nanoparticles: Novel High-Efficiency Targeting Agents for Localised Treatment of Metastatic Cancer.
Development award (Joint PI)	Cancer Research UK	2009 - 2010	£55,870	Development of scFv antibodies as therapeutics for in vivo targeting of prostate cancer
Equipment (PI)	UCL Cancer Institute Research Trust	2009 - 2010	£235,895	Upgrading GMP facility
PhD Studentship (PI)	Clement Wheeler-Bennett Memorial Trust	2009 - 2013	£107,013	Developing an optimal system for Antibody Directed Enzyme Prodrug Therapy (ADEPT) of cancer
Programme (Co-investigator)	Department of Health and CR-UK	2007-2012	£2,353,355	Experimental Cancer Medicine Centre, infrastructure for future research (PI = R. Begent)
Programme (PI for section awarded £1,219,714)	Cancer Research UK	2005-2010	£4,953,701	Systems for the targeted therapy of cancer (PI = R. Begent)

RECENT INVITED TALKS

●March 2010, Gordon Research Conference: Antibody Biology and Engineering (USA).
●December 2009, IBC San Diego Antibody Engineering Conference (USA).
● November 2009, Informa's 5th Annual Next Generation Protein Therapeutics (UK).
● October 2009, Pichia 2009 Protein Expression Conference (USA).
● June 2009, 26th International Conference on Monoclonal Antibodies and Cancer Stem Cells (Greece).
● June 2009, SMi Global Proteins Summit (UK).
●April 2009, Fabisch-Symposium: Targeted Tumor Therapies (Germany).
●March 2009, Keystone Symposium, Antibodies as Drugs (Canada).
●January 2009, Visiongain 2nd Annual Recombinant Antibodies Conference (UK).

RESEARCH PUBLICATIONS

Vigor KL, Kyrtatos PG, Minogue S, Al-Jamal KT, Kogelberg H, Tolner B, Kostarelos K, Begent RH, Pankhurst QA, Lythgoe MF, Chester KA. (2010) Nanoparticles functionalised with recombinant single chain Fv antibody fragments (scFv) for the magnetic resonance imaging of cancer cells. Biomaterials 31:1307-15

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B.6.3. Letter of intent – SAB members

Gregory Adams

LETTER OF INTENT (SAB)

For collaboration with IMAGINT project

"Tools for detecting disease-related proteins and their functional complexes in clinical tissues"

Name of the organisation: Fox Chase Cancer Center
Having its registered address at: 333 Cottman Ave, Philadelphia, PA 19111, USA

Laboratory or department involved in the activities of IMAGINT: Adams Lab (Antibody engineering and therapeutics)

The organisation Fox Chase Cancer Center represented by Gregory Adams, Ph.D. declares its interest in IMAGINT and is willing to collaborate with the IMAGINT consortium, if the project proposal is accepted for funding by the EC, Dr. Adams will join the Scientific Advisory Board (SAB). The SAB is composed of external experts recognised for their expertise in the field of the project. The role of the SAB will be to advise the Governing Board and the Executive Management Committee on the project research and/or strategic orientations.

The organisation will also undertake a protective approach in keeping all information confidential that pertains to the structure as well as the technical content of the proposal set-up. It will only disclose said information to third parties upon a coordinator's approval.

Drawn up in Philadelphia, PA.. on 09/02/2010
In three original copies

Organisation name: Fox Chase Cancer Center

Representative name: Gregory P. Adams, Ph.D.

Position: Co-Leader, Molecular and Translational Medicine Program

Signature and stamp:

José Baselga

VALL D'HEBRON
Institute of Oncology

LETTER OF INTENT (SAB)

For collaboration with IMAGINT project

"Tools for detecting disease-related proteins and their functional complexes in clinical tissues"

Name of the organisation: **Fundació Privada Institut d'Investigació Oncològica de Vall Hebron (VHIO)**

Having its registered address at:

Paseo Vall d´Hebron, 119-129
Barcelona 08035

Laboratory or department involved in the activities of IMAGINT:

Medical Oncology Service

The organisation **Fundació Privada Institut d'Investigació Oncològica de Vall Hebron (VHIO)** represented by **Dr. Jose Baselga** declares its interest in IMAGINT and is willing to collaborate with the IMAGINT consortium, if the project proposal is accepted for funding by the EC. **Dr. Jose Baselga** will join the Scientific Advisory Board (SAB). The SAB is composed of external experts recognised for their expertise in the field of the project. The role of the SAB will be to advise the Governing Board and the Executive Management Committee on the project research and/or strategic orientations.

The organisation will also undertake a protective approach in keeping all information confidential that pertains to the structure as well as the technical content of the proposal set-up. It will only disclose said information to third parties upon a coordinator's approval.

Drawn up in **Barcelona** on **09/02/2010**
In three original copies

Organisation name: **Fundació Privada Institut d'Investigació Oncològica de Vall Hebron (VHIO)**

Representative name: **Dr. Jose Baselga**

Position: **Director**

Signature and stamp:

VALL D'HEBRON
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B.6.4. Letter of intent – ILC members

GEHC

LETTER OF INTENT (ILC)

For collaboration with the IMAGINT project

"Tools for detecting disease-related proteins and their functional complexes in clinical tissues"

Date: February 1, 2010

Name of the organisation: GE Medical Systems Israel Ltd.
Having its registered address at: 4 Hayozma Street
P.O. Box 170
Tirat Carmel, 30200
Israel

Laboratory or department involved in the activities of IMAG: GE Medical Systems Israel Ltd

The organisation GE Medical Systems Israel Ltd. represented by Aharon Peretz declares its interest in the IMAGINT and is willing to collaborate with the IMAGINT consortium, if the project proposal is accepted for funding by the European Commission. GE Medical Systems Israel Ltd. will participate in the consortium's Industrial Liaison Council meetings in order to evaluate the scientific and the technical output with regards to their commercial potential.

The organisation will also undertake a protective approach in keeping all information confidential that pertains to the structure as well as the technical content of the proposal set-up. It may only disclose said information to third party upon a coordinator's approval.

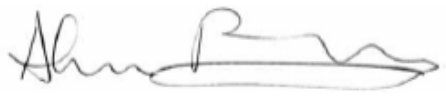
Drawn up in Haifa on February 1, 2010
In three original copies

Organisation name: GE Medical Systems Israel Ltd

Representative name: Aharon Peretz

Position:
Manager, Nuclear Medicine Research and Advanced Applications
GE Healthcare

Signature and stamp:



GE Healthcare

2010-02-02/ BN

Letter of Intent

For collaboration with the IMAGINT project

"Tools for detecting disease-related proteins and their functional complexes in clinical tissues"

GE Healthcare Europe would like to express our strong support for the project:

"Tools for detecting disease-related proteins and their functional complexes in clinical tissues", the IMAGINT project,

led by Professor KERRY CHESTER at UCL cancer Institute, University College London.

Specific interest from GE Healthcare:

^{68}Ga is one of the isotopes that GE Healthcare is focusing efforts on to bring to the marketplace with automated synthesis technology and labelling schemes. Here the objective has been to use the existing synthesis platform FastLab and incorporate the ^{68}Ga isotope produced via a generator into this platform. Ongoing research will reweal if the FastLab Gallea will give us high enough specific activity for some demanding applications.

In parallel we will develop a new, more compact synthesis platform that will focus on high specific activity applications. The IMAGINT proposal is attractive as it will push the need for high specific activity. The new ^{68}Ga- platform could therefore fit very well into the IMAGINT project. GE Healthcare also strongly support the SPECT/ CT application by using ^{67}Ga labelling. The comparative study of SPECT and PET will be done with in our triple modality preclinical imaging equipment, Triumph. These studies can be done in Uppsala, Sweden.

GE Healthcare

About GE Healthcare:
GE Healthcare is a $18 billion unit of General Electric Company (NYSE: GE). It is the world's leader in transformational medical technologies for patient care. Its expertise in medical imaging and information technologies, medical diagnostics, patient monitoring systems, drug discovery, and biopharmaceutical manufacturing technologies is helping clinicians around the world develop new ways to predict, diagnose, inform and treat disease. GE Healthcare's broad range of products and services enable healthcare providers to better diagnose and treat cancer, heart disease, neurological diseases, and other conditions earlier. The GE vision for the future is to enable a new "early health" model of care focused on earlier diagnosis, pre-symptomatic disease detection and disease prevention. Biomedical imaging systems include X-Ray, CT, MRI, Ultrasound, Nuclear Medicine and PET/CT imaging systems.

Previous Relevant Experience:
GE is the world's leader in transformational medical technologies for patient care. We are an important partner in many research programs in Europe. GE Healthcare has its Headquarters in Pollards Wood near London. GE Healthcare have manufacturing, engineering and research facilities in 11 European countries.

Organization number in Sweden: 556051-6725

GE Healthcare has approximately 43 000 employees World Wide. and Approximately 2000 of these are based in Sweden. Our Life Science Headquarter is in Uppsala and we are also manufacturing all cyclotrons for short lived isotope production including chemistry systems in Uppsala.

GE Healthcare

Dr. Bengt Nielsen is Chief Executive of GE Healthcare International Research Division. He studied at University of Linkoeping in Sweden where he received his Dr. Degree in radiation physics and medical science in 1985. He worked at the Department of Radiation Physics at the same university. From University of Linkoping he became the Technical Manager in General Electric CGR AB, Managing Director/Zone Manager in GE Medical Systems Sweden, MR Marketing Manager for Europe, MR Development Manager and Cardio- & Neurovascular MR Program Manager in GE Medical Systems Europe and Regional Sales Manager for Nordic Region in GE Medical Systems Nordic (Sweden, Denmark, Norway, Finland, Iceland and Estonia).

Since 2005 Bengt is the General Manager for the Academic Program in GE Healthcare Europe. He has 34 publications in peer-reviewed papers and has experience from work within expert groups in the EU FP-7 program. Bengt is also participating in several EU FP 7 projects and other national funding programs.

The commitment from GE Healthcare is to participate actively in the work of IMAGINT. Currently GE Healthcare as a Company has a strong program supporting discovery research widely with technical solutions and existing products but also the innovative approaches paving the way to future healthcare.

This letter is provided as evidence of our support for the IMAGINT project application and shall not constitute any legally binding agreement or commitment on behalf of GE Healthcare.

Yours sincerely,

Bengt Nielsen, Ph.D
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Bayer Schering Pharma AG

LETTER OF INTENT (ILC)
For collaboration with the IMAGINT project "Tools for detecting disease-related proteins and their functional complexes in clinical tissues"

Name of the organisation: **Bayer Schering Pharma AG**
Having its registered address at: **Bayer Schering Pharma AG Müllerstr. 178 13353 Berlin**

Laboratory or department involved in the activities of IMAGINT:
GDD-GTR-TD-Global Biomarker Research

The organisation **Bayer Schering Pharma AG** represented by **Dr. Axel KRETSCHMER** declares its interest in the IMAGINT and is willing to collaborate with the IMAGINT consortium, if the project proposal is accepted for funding by the European Commission. **Dr. Axel KRETSCHMER** will participate in the consortium's Industrial Liaison Council meetings in order to evaluate the scientific and the technical output with regards to their commercial potential.
The organisation will also undertake a protective approach in keeping all information confidential that pertains to the structure as well as the technical content of the proposal set-up. It may only disclose said information to third party upon a coordinator's approval.

Drawn up in Wuppertal on 10 / 02 / 2010

In three original copies
Organisation name : **Bayer Schering Pharma AG**
Representative name : **Dr. Axel KRETSCHMER**

Dr. A. Kretschmer

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