SEVENTH FRAMEWORK PROGRAMME

THEME [HEALTH.2010.2.1.2-1]
[Tackling Human Diseases through Systems Biology Approaches. FP7-HEALTH-2010-two-stage]

Grant agreement for: Collaborative project*

Annex I - "Description of Work"

Project acronym: ASSET

Project full title: " ASSET: Analysing and Striking the Sensitivities of Embryonal Tumours "

Grant agreement no: 259348

Date of preparation of Annex I (latest version): 2010-09-09

Date of last change: 2010-09-08

Date of approval of Annex I by Commission:

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Part A

Workplan Tables

A1:
Project summary

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per project

General information			
Project title [3]	ASSET: Analysing and Striking the Sensitivities of Embryonal Tumours		
Starting date [4]	01/11/2010		
Duration in months [5]	60		
Call (part) identifier [6]	FP7-HEALTH-2010-two-stage		
Activity code(s) most relevant to your topic [7]	HEALTH.2010.2.1.2-1: Tackling Human Diseases through Systems Biology Approaches. FP7-HEALTH-2010-two-stage		
Free keywords [8]		Embryonal tumours; modelling; transcriptomics; proteomics; high content perturbation studies	

Abstract [9]

Cancer is hallmarked by multiple genetic aberrations that lead to a functional derangement of cellular signalling networks. Embryonal tumours (ETs) comprising neuroblastoma, medulloblastoma and Ewing sarcoma, occur early in life, and thus may reveal pathogenetically relevant lesions clearer than adulthood tumours which carry passenger mutations accumulated during life. ASSET will exploit this fact by focussing on unravelling the signalling networks and their alterations in ETs. The basic hypothesis is that ETs share common pathogenetic principles that can be captured and made accessible to rational analysis by combining high-throughput and high content analysis of the genome, transcriptome and proteome with mathematical modelling. ASSET builds on a previous FP6 consortium, the European Embryonal Tumour Pipeline (EEPT), which generated high-throughput genomic and transcriptomic data on ETs. ASSET is the next logical step to add crucial functional information that will allow us to generate (i) defined in vitro and in vivo tumour systems; (ii) combined analysis of genomic mutations, transcriptome, miRNA expression and dynamic proteome changes; (iii) systematic perturbations; (iv) mathematical modelling to elucidate pathogenetic networks and their emergent behaviour; (v) the virtuous cycle of model validation in relevant biological model systems and clinical samples towards a major goal. This goal is to identify mechanistically understood network vulnerabilities that can be exploited for new approaches to the diagnosis and treatment of major paediatric tumours. Elucidating such core mechanisms will (i) improve understanding of and therapeutic options for these devastating childhood malignancies and (ii) enable a rational approach to deal with the complexity of the pathogenesis of adulthood cancers.

A2:
List of Beneficiaries

Project Number [1]	259348	Project Acronym [2]	ASSET

List of Beneficiaries

No	Name	Short name	Country	Project entry month[10]	Project exit month
1	UNIVERSITY COLLEGE DUBLIN, NATIONAL UNIVERSITY OF IRELAND, DUBLIN	NUID-UCD	Ireland	1	60
2	ST. ANNA KINDERKREBSFORSCHUNG	CCRI	Austria	1	60
3	CEMM - FORSCHUNGSZENTRUM FUER MOLEKULARE MEDIZIN GMBH	CEMM	Austria	1	60
4	INSTITUT CURIE	CURIE	France	1	60
5	DEUTSCHES KREBSFORSCHUNGSZENTRUM	DKFZ	Germany	1	60
6	VALTION TEKNILLINEN TUTKIMUSKESKUS	VTT	Finland	1	60
7	UNIVERSITAETSKLINIKUM ESSEN	UKE	Germany	1	60
8	UNIVERSITEIT GENT	UGENT	Belgium	1	60
9	UNIVERSITY COLLEGE LONDON	UCL	United Kingdom	1	60
10	UNIVERSITAET BERN	UBERN	Switzerland	1	60
11	WEIZMANN INSTITUTE OF SCIENCE	Weizmann	Israel	1	60
12	BAYER SCHWEIZ AG	ZEPTO	Switzerland	1	60
13	Københavns Universitet	UCPH	Denmark	1	60
14	FUNDACION CENTRO NACIONAL DE INVESTIGACIONES ONCOLOGICAS CARLOS III	CNIO	Spain	1	60

A3:
Budget Breakdown

Project Number [1]	259348	Project Acronym [2]	ASSET

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	NUID-UCD	75.0	T	1,865,702.40	0.00	274,096.00	50,800.00	2,190,598.40	0.00	1,724,172.80
2	CCRI	75.0	T	1,020,256.00	0.00	22,000.00	35,200.00	1,077,456.00	0.00	822,392.00
3	CEMM	75.0	A	1,053,091.00	0.00	11,000.00	15,000.00	1,079,091.00	0.00	815,818.25
4	CURIE	75.0	T	1,086,400.00	0.00	14,000.00	32,000.00	1,132,400.00	0.00	860,800.00
5	DKFZ	75.0	A	2,379,785.00	0.00	10,000.00	21,000.00	2,410,785.00	0.00	1,815,838.75
6	VTT	75.0	A	850,544.00	0.00	4,000.00	19,000.00	873,544.00	0.00	660,908.00
7	UKE	75.0	T	1,031,360.00	0.00	10,800.00	16,000.00	1,058,160.00	0.00	800,320.00
8	UGENT	75.0	T	1,032,000.00	0.00	22,000.00	32,000.00	1,086,000.00	0.00	828,000.00
9	UCL	75.0	T	591,414.40	0.00	24,000.00	27,200.00	642,614.40	0.00	494,760.80
10	UBERN	75.0	T	934,400.00	0.00	14,000.00	16,000.00	964,400.00	0.00	730,800.00
11	Weizmann	75.0	T	638,016.00	0.00	5,200.00	3,200.00	646,416.00	0.00	486,912.00
12	ZEPTO	50.0	A	467,000.00	0.00	8,000.00	43,000.00	518,000.00	0.00	284,500.00
13	UCPH	75.0	T	1,586,068.80	0.00	23,600.00	41,008.00	1,650,676.80	0.00	1,254,159.60
14	CNIO	75.0	A	533,832.00	0.00	7,400.00	12,000.00	553,232.00	0.00	419,774.00
Total				15,069,869.60	0.00	450,096.00	363,408.00	15,883,373.60	0.00	11,999,156.20

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

259348

Project title

ASSET—ASSET: Analysing and Striking the Sensitivities of Embryonal Tumours

Call (part) identifier

FP7-HEALTH-2010-two-stage

Funding scheme

Collaborative project

WT1
List of work packages

Project Number [1]	259348	Project Acronym [2]	ASSET

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	Reconstruction of Gene Regulatory Networks (GRN)	RTD	9	152.50	1	48
WP 2	Targeted and global high content network perturbation screens w/siRNAs and drugs for network reco	RTD	6	228.50	1	60
WP 3	microRNA expression in ETs: rRegulation of by ET oncogenes, identification of targets & effects	RTD	6	173.50	1	60
WP 4	ET transcription factor protein networks	RTD	1	112.25	1	60
WP 5	Mapping cytosolic ET signaling protein networks by quantitative proteomics	RTD	13	131.75	1	48
WP 6	Computational-kinetic models of critical Myc-dependent regulatory networks that drive ETs	RTD	1	100.75	1	60
WP 7	Network fragility analysis of apoptosis and proliferation decisions	RTD	5	168.50	1	60
WP 8	Probing network fragilities with kinase inhibitors	RTD	3	125.50	1	60
WP 9	Validation of mathematical models in biological models and clinical samples	RTD	2	184.50	13	60
WP 10	Data management	RTD	13	75.00	1	60
WP 11	Systems level knowledge integration and exome sequencing	RTD	14	49.20	19	60
WP 12	Project Management	MGT	1	19.00	1	60
WP 13	Training	OTHER	1	18.25	1	60
			Total	1,539.20		

WT2:
List of Deliverables

Project Number [1]	259348	Project Acronym [2]	ASSET

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	Formal Methods & Algorithms	1	9	48.00	R	PU	30
D1.2	Formal methodology for reconstructing GRNs	1	5	37.50	R	PU	48
D1.3	GRNs and motif structures for ET TFs	1	9	67.00	R	PU	48
D2.1	Compounds inhibiting ET Cell Growth	2	6	46.00	R	PU	60
D2.2	List of kinases whose inhibition by siRNA molecules sensitized ET cells to selected drugs	2	6	76.00	R	PU	48
D2.3	Genes whose function is critical for ET cell survival	2	11	106.50	R	PU	60
D3.1	miRNAs regulating ET cell viability	3	6	86.00	R	PU	24
D3.2	miRNAs regulated by the key oncogenes in ET cells	3	4	87.50	R	PU	48
D4.1	MS interaction maps of selected ET transcription factors	4	1	60.25	R	PU	48
D4.2	Models of GRNs enriched by interaction proteomics	4	5	52.00	R	PU	60
D5.1	quantitative phosphoproteome analyses of the activated ALK- and	5	13	75.00	R	PU	24

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]
	TrkA-expressing NB cell lines						
D5.2	SRM-based assays for the routine quantitative measurement of selected protein species	5	1	30.00	R	PU	36
D5.3	Parallel relative quantitation of ET protein network components by reverse protein arrays	5	12	26.75	R	PU	48
D6.1	Core model of Myc protein regulation by TrkA in NB cell lines	6	1	49.75	R	PU	24
D6.2	Predicted fragility points in the Myc network	6	5	51.00	R	PU	60
D7.1	A comprehensive core model of the CDK-Rb-E2F-Skp2 and p53-Mdm2/ MDMX modules in NB	7	5	92.00	R	PU	48
D7.2	Extension of the NB core model to EWS and MB	7	4	76.50	R	PU	60
D8.1	Identification of tumour-type specific synergistic drug combinations	8	3	49.50	R	PU	36
D8.2	Identification of drug-dependent changes in phosphotyrosine signatures of synergistic drug combinati	8	13	76.00	R	PU	60

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]
D9.1	Initial validation results for model improvement from data re-analysis	9	5	38.00	R	PU	48
D9.2	Functional validation of in silico predicted fragility nodes in ET cell lines	9	2	70.00	R	PU	60
D9.3	Correlation of drug sensitivities, GRNs and gene mutations	9	10	76.50	R	PU	60
D10.1	A description of sources of pathways, protein-protein complexes and mutation-phenotype data	10	14	24.00	R	CO	24
D10.2	A description of the project catalogue of analysis workflows and their web service components	10	13	51.00	R	CO	60
D11.1	A description of a data integration pipeline facilitating query and linking to disease complexes and	11	13	25.00	R	PU	36
D11.2	A description of a specific genetic alterations and the corresponding influenced pathways/ network co	11	14	24.20	R	PU	60
D12.1	Project Websites On-line	12	1	8.00	O	PU	6

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]
D12.2	Review Meetings	12	1	7.00	R	RE	30
D12.3	Final report	12	1	4.00	R	PU	60
D13.1	Establishment of efficient training structures	13	1	16.25	O	PU	12
D13.2	Organisation of the Biosystems workshops 1	13	1	2.00	O	PU	60
			Total	1,539.20			

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP1	Type of activity [54]	RTD
Work package title	Reconstruction of Gene Regulatory Networks (GRN)		
Start month	1		
End month	48		
Lead beneficiary number [55]	9		

Objectives

In this WP we will systematically reconstruct plausible Gene Regulatory Networks (GRN) (i) involving the transcription factor EWS-FLI1 implicated in ESFTs and (ii) mediating transcription of N-Myc and c-Myc targets specific to NB and MB by assessing posterior probability distribution over network structures and structural motifs based on prior available evidence. These inferred structures shall form the basis for subsequent mechanistic modelling and associated dynamic simulations. In the inference process, we will utilise time-resolved mRNA and miRNA data as well as ChIP-chip/seq data in a manner that evaluates and maximises the information content regarding network structures. All uncertainty in predicted structures will be characterised so that this can be systematically propagated during the overall modelling process and analysed in subsequent workpackages and projects.

Description of work and role of partners

This WP will focus on reconstructing GRNs of ESFT, NB and MB. In particular, the basis for the inference of a distribution of structures will be pre-existing experimental data, which will be complemented by the new data generated in this WP, and a mapped network of molecular interactions downstream of EWS-FLI1, N-Myc and c-Myc regulating apoptosis and proliferation based on inducible cell culture model systems (see also the SITCON project web-page http://bioinfo-out.curie.fr/projects/sitcon/; and the E.E.T.-pipeline web-page http://www.eet-pipeline.eu/).

Task 1.1. Generation of time resolved mRNA and miRNA data and ChIP-chip/seq data.
In addition to the unique data currently available from the E.E.T.-Pipeline that will be employed in developing the EWS-FLI1, N-Myc, and c-Myc network structures, further experimental work will be conducted to complement the existing time-course data of the core cell models. Time-resolved mRNA and miRNA expression data exist for the ESFT core cell model (conditional EWS-FLI1 knockdown in a p53 mutant) and will be generated for a p53 wildtype ESFT cell line. Time-course data also exist for 7 time points between 2 and 14 days after EWS-FLI1 reactivation in the same p53 mutant cell line and will be produced for the p53 wildtype model. Expression data also exist from ESFT in vitro cultures1 and xenografts2, as well as time-resolved mRNA and miRNA expression data for conditional c-Myc knock-down in the ESFT core cell model and wildtype p53 ESFT cell lines. ChIP-chip data exist for N-myc and c-myc target genes for all tumour entities, and two independent sets of ChIP-seq for EWS-FLI1 will also be provided. Existing time-course transcriptomic data for the core NB and MB cell models before and after expression of N-Myc and c-Myc, respectively, will be supplemented with new experimental data to be used to derive GRN driven by the dysregulation of Myc TFs. For MB, time-resolved mRNA and miRNA expression data for conditional c-Myc over-expression will be generated in the UW-228 cell line. Thus, this WP will have a unique and information-rich data resource with which to infer the pathway motif and network structures.
Task 1.2. Inference of network structures of EWS-FLI1 and N-Myc/c-Myc (dys)regulated GRNs.
o define a prior distribution on plausible structures, we will adopt (i) a statistical clustering methodology and linear regression analysis to map statistically significant correlations of gene expression in gene array data and ChIP-chip/seq data from task 1.1, and (ii) in collaboration with WP10, a literature-curated network of downstream effects of EWS-FLI1, N-Myc and c-Myc to help develop a prior distribution of network connectivities, which then will be employed in network posterior inference. We have successfully used this approach to infer biochemical network structures4,5. The likelihood of the data – individual and integrated – given the structures, will then be

used along with the prior to define a Markov-chain which will enable exploration of the posterior over network structures. A distribution over structures can be obtained from this, which will characterise the levels of evidential certainty-related micro-structures6. These become important when studying the fragility of pathways, developing detailed mechanistic models and suggesting subsequent experimental protocols in SP4. A main effort in this WP will be devoted to the design of Markov-chain sampling methods and associated algorithms sufficiently efficient to explore the manifold of network structures in a timely manner. In addition we will also use dynamic Bayesian modelling methodologies (e.g. state-space models with regime switching) to infer posterior intervals for time points of gene regulation ("switching points") and their probable relations, thus infering dynamic regulatory maps7,8. This may reveal additional regulators and suggest links between gene regulatory and signalling networks. This information will directly feed into the mechanistic modelling WPs. One main objective of this task is to discriminate between immediate targets of EWS-FLI1 and Myc TFs and genes that are indirectly regulated through other TFs or miRNAs. The latter studies will be performed in collaboration with WPs 6 & 7 for consistency with mechanistic models of GRN submodules. High performance computing (HPC) capability at UCL will be exploited in this WP.
Task 1.3. Refinement of inferred GRNs by integration of promoter occupancy data.
Within this WP, our existing ChIP-chip and ChIP-seq data on occupancy of TFBS will be provided for TFs of the Myc family members and EWS-FLI13. We will use these data to identify co-occurring TFBS in genes with coherent dynamical profiles detected by clustering techniques. TFBS will be identified by matching of probability weight matrices from the TRANSFAC database to promoter regions of relevant genes. Moreover, we will identify potential employment of TFs at different time points in the integrative analysis of the TFBS occurrences and the dynamic gene expression data measured after over-expression or inhibition of Myc family members or EWS-FLI1. We will use transcriptomic data, data on gene knock-down of Myc targets in NB (this WP & WP7) and prediction of relevant TFs to refine the networks inferred in this WP. Results will be consolidated with WP7 to infer direct regulation of NB cell cycle via N-Myc, c-Myc, MDM2/MDMX and CDK4 entry points. Results from WP9 on functional characterisation of candidate Myc targets will be used to further refine GRNs.

Output of the WP
This WP will use advanced statistical modelling and inference methods (within the Bayesian framework) to deliver global GRNs aberrant in ETs. The results will inform and guide the design of further wet experimentation to test the inferred network structures, as well as help to elaborate important subnetworks for mechanistic, dynamic modelling.

Role of Participants
FS will generate miRNA profiles for time-resolved experiments. HK (CCRI) and OD (CURIE) will provide gene expression and ChIP-seq data for EWS-FLI1 and generate de-novo time-resolved mRNA and miRNA expression data in two conditional EWS-FLI1 shRNA engineered ESFT cell lines (mutant and wildtype p53) to fill existing gaps. HK (CCRI) will provide c-MYC ChIP-chip data in ESFT. AA (UBERN) will conduct time-resolved experiments in MB. FW (DKFZ) will provide ChIP-chip data for all ET entities, carry out time-resolved experiments in NB and provide mRNA profiling for time-resolved experiments. MG (UCL), AZ (Curie) and SBu (DKFZ) will perform the statistical modelling and inference.

References
1 Kauer, M., Ban, J., Kofler, R., Walker, B., Davis, S., Meltzer, P. & Kovar, H. A molecular function map of Ewing's sarcoma. PLoS ONE 4, e5415 (2009).
2 Tirode, F., Laud-Duval, K., Prieur, A., Delorme, B., Charbord, P. & Delattre, O. Mesenchymal stem cell features of Ewing tumors. Cancer Cell 11, 421-429 (2007).
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Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
2	CCRI	18.50
4	CURIE	14.00
5	DKFZ	46.00
8	UGENT	6.00
9	UCL	48.00
10	UBERN	20.00
	Total	152.50

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	Formal Methods & Algorithms	9	48.00	R	PU	30
D1.2	Formal methodology for reconstructing GRNs	5	37.50	R	PU	48
D1.3	GRNs and motif structures for ET TFs	9	67.00	R	PU	48
		Total	152.50			

Description of deliverables

D1.1) Formal Methods & Algorithms: Formal methods & algorithms to produce distributions over GRN & motif structures from integrated heterogeneous data. [month 30]

D1.2) Formal methodology for reconstructing GRNs: Formal methodology for reconstructing GRNs from dynamic gene expression data and TFBS data systematic shRNA knock-downs and Chip-chip/seq data [month 48]

D1.3) GRNs and motif structures for ET TFs: GRNs and motif structures for ET TFs [month 48]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS1	Generation,normalisation and preparation of existing and new data and assessment of data information	4	18	
MS2	Reconstruction of prior GRN and motif structures	9	30	

WT3:
Work package description

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS3	Production of posterior GRN and motif structures	9	36	
MS4	GRN and motif structures for EWS-FLI1	2	36	
MS5	GRN and motif structures for N-Myc and c-Myc	5	48	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP2	Type of activity [54]	RTD
Work package title	Targeted and global high content network perturbation screens w/siRNAs and drugs for network reco		
Start month	1		
End month	60		
Lead beneficiary number [55]	6		

Objectives

Targeted and global high content network perturbation screens with siRNAs and drugs for network reconstruction
This WP will employ initial models of ET GRNs produced in WP1 to generate high-content perturbation data that will be used to (i) test GRN structures predicted by the models; (ii) enrich and refine GRN structures by functional data, thereby enabling the iterative cycle of modelling informing experimental work and experimental work informing the modelling; (iii) functionally link GRN structures to biological outcomes such as cell viability. In addition, we will perform (iv) hypothesis driven and mechanistically oriented drug discovery work by unbiased compound screens as well as (v) predicted synthetic effects between siRNA – siRNA and siRNA – drug combinations.

Description of work and role of partners

We will deploy our high content screening platforms to systematically interrogate and refine initial ET network models. Once predictive network models have been identified, we will design a comprehensive siRNA library targeting the nodes predicted to be functionally important, and screen ET cell lines for validation of the biological consequences (i.e. proliferation, apoptosis). As appropriate, targeted studies for selected perturbations using a range of specific output parameters (DNA damage, differentiation, cell cycle defects) and live-cell continuous imaging will also be included.. High-throughput compound screens will be used to explore, and informed by modelling, constrain the search space for new rational drug combinations. Using our high-throughput cell based platform, we will screen compound libraries that include most currently used drugs and phosphatase and kinase inhibitors in genetically engineered isogenic ET cell line pairs to identify growth inhibitory compounds and synthetic lethalities. Results from synthetic lethal screens will help to elucidate cross-talk between pathway sub-circuits or disease complexes. The screens will also include drugs combined with siRNAs introduced by reverse transfection microarrays as well as combinatorial siRNA screens targeting selected kinases in order to test and validate multiple parameters of models and streamline the discovery of drug combinations.

Task 2.1.Functional role of EWS-FLI1 downstream genes to refine the ESFT network model.
ESFT cell models, where the EWS-FLI1 oncogene can be conditionally knocked down, have been used in the E.E.T.-Pipeline project to generate time-resolved mRNA and miRNA expression profiles as well as ChIP-chip/seq data. In WP1 we use these results for Bayesian inference and qualitative modelling approaches to reconstruct the GRNs through which the EWS-FLI1 oncogene regulates cell proliferation and apoptosis. Here, we will use a targeted siRNA-based gene knockdown approach in order to examine the functional role of the genes predicted to be regulated by EWS-FLI1 on cell viability and proliferation. Phenotypic screening and further mathematical modelling will be performed to refine the model in more detail with focus on how EWS-FLI1 regulates cell viability.

Task 2.2. Identification of synthetic lethal genes with amplified or deregulated N-Myc in NB cells.
The ChIP-chip/ seq and gene expression data from NB cell lines and tumours with amplified N-Myc produced by the E.E.T.-Pipeline project are used by WP1 to reconstruct a GRN controlled by the N-Myc transcription factor in NB cells. To identify crucial genes for NB cell survival, siRNA screens will be performed in NB cells using proliferation and apoptosis as readouts. As in task 2.1. these data will be used to refine the GRN models with the

aim is to predict genes that are synthetically lethal with N-Myc. These predictions will be tested by a subsequent siRNA screen.

Task 2.3. Identification of biological consequences of global ET GRN perturbations in cultured ET cells to refine the GRN models.
While the first two tasks use siRNA screens to examine two GRNs known to be critical in ET in a targeted hypothesis driven manner, this task will perform a network wide siRNA screen to probe the GRN structures predicted in WP1 in an unbiased way. Custom tailored siRNA libraries targeting the nodes of the signalling networks inferred in WP1 will be designed and utilised in an ultra-high density cell microarray screening system. This technique enables >200x screening throughput compared to 384-well-based assays with a corresponding reduction in reagent consumption. siRNAs, mixed with transfection agents, are printed as a microarray and cells are then grown directly on the array, where they become transfected with the siRNAs. After incubation for 2-3 days and fixation, the arrays can be stained with appropriate antibodies to study changes in cell proliferation, cell cycle, apoptosis, DNA damage and cell differentiation. The screens will be performed in two selected cell lines per tumour type (NB, MB and ESFT), and will be conducted in at least two biological replicates. The role of node genes will be compared between the NB, MB and ESFT cell models to identify commonalities as well as differences between the ET cell models. These data will provide a stringent test for the validity of the GRN models as well as important contributions towards the refinement of the models.

Task 2.4. Application of cell based HTS to identify growth inhibitory compounds and synthetic lethalities in genetically engineered isogenic ET cell line pairs. The high-throughput cell-based compound screening will be done in a 384-well plate format using a robotic screening system at the VTT Medical Biotechnology. The instrumentation allows for over 40,000 cell biological experiments to be carried out at a time. The screens will be performed in isogenic ET cell pairs (NB, MB and ESFT) with cell proliferation as the primary endpoint. The following ET models will be utilized in the HT screens:
• NB: N-Myc-inducible NB (SY5Y) cells.
• MB: c-Myc inducible MB (UW-228) cells.
• ESFT: conditional EWS-FLI1 knockdown ESFT (A673) cells.
• Mesenchymal stem cells, which are an additional excellent control for ESFT1

The compound libraries that will be utilized in the screens contain approximately 3000 drug-like molecules including most of the drugs currently used in the clinic, about 1500 natural products, as well as an additional set of more selective drugs, including e.g. phosphatase and kinase inhibitors. The data will be processed with bioinformatic methods to identify growth inhibitory compounds and differences in vulnerabilities between the isogenic cell line pairs. Selected compounds will be taken through a second step biological validation procedure using a wider range of assays including measurements of cell proliferation, apoptosis, and invasive behaviour in semisolid matrices.

Task 2.5. Combination of data from chemical biology with signalling network model to refine critical pathways for ET proliferation. Proteomic and chemical perturbation data from the siRNA interference screens will be integrated and enriched with available in-house cancer-specific protein-protein interaction data. From the constructed biological networks, we will be able to identify disease complexes and study the drug effect on them. Predictions based on structural similarities and available experimental data for the biological activity of small compounds on proteins in the disease complexes will be integrated in the networks, and will be used to find connections between small molecules sharing a mechanism of action. The systems biology analysis of the data from synthetic lethal screens will help elucidate the cross-talk between pathway subcircuits, disease complexes and drug mode of action. The biological networks that emerge from this study will provide a powerful discovery platform to understand the rewiring of pathways and their functional connections to disease and drug action.

Task 2.6. Identification of siRNAs sensitizing NB and MB cells to selected drugs. Kinome-wide siRNA screens will be performed in NB and MB cell lines in combination with drugs currently used in the clinic. The differences in drug responses between NB and MB will be analysed. Similar screens have been performed with cisplatin treatment and the results indicate that down-regulation of a few kinases sensitized NB and MB cells to cisplatin2. These findings have also been validated with pharmacological inhibitors in a broader panel of ET cell lines and potential clinical significance supported by expression analysis in primary tumours from ET patients.

Task 2.7. Use of siRNA screens to ascertain the identity of the kinases which mediate synergistic response induced by kinase inhibitor combinations. Combinatorial screens using siRNAs targeting selected kinases will be performed in ET cells. The siRNAs will be chosen based on the results of combinatorial screens with 10 kinase inhibitors performed in WP8. The aim is to deconvolute the kinase targets that mediate the synergistic

effects. This is important as even the clinically used kinase inhibitors block the function of multiple kinases. The elucidation of the critical kinase targets responsible for the synergistic effects will inform the mechanistic pathway models, the validation studies, and enable advanced mechanistic interpretations of the results of the chemical proteomics screens.

Output of WP
The results from targeted perturbation screens will test the mathematical models and generate feedback data for their refinement. Using cell viability as an endpoint will provide links between network models and biological effects, and hence enhance the value of the models. This structured approach will help us in understanding the functional role of the nodes in the ET signalling networks and identify vulnerabilities, and also provide the tools (siRNAs, drugs, drug combinations) to exploit these vulnerabilities. Thus, the results also will feed into the biological validation. Since combinatorial screens with siRNAs will be performed with 1) the clinically used drugs in ET tumours and 2) siRNAs targeting clinically approved kinase inhibitors showing synergistic effect in ET cells, the results are likely to provide information that can be taken forward in pre-clinical models.

Role of participants

OD and AZ (CURIE) will perform the work on the functional role of EWS-FLI1 downstream genes to refine the ESFT network and mathematical modelling of the results. FW (DKFZ) will perform the dentification of synthetic lethal genes with amplified or deregulated N-Myc in NB cells. OK and KI (VTT) will perform the ET network model node perturbation screens in cultured ET cells and the HT compound screens. OK and KI (VTT), AA (UBERN), YY (WEIZMANN) will perform the identification of siRNAs sensitizing NB and MB cells to selected drugs. OK and KI (VTT), YY (WEIZMANN), GSF (CEMM) will perform combinatorial siRNA screens against selected kinases to understand synergistic response of kinase inhibitors. SBr (UCPH) will analyse pathway cross-talk. YY (WEIZMANN) will perform cell culture experiments using readouts of cell proliferation, apoptosis and migration through extracellular matrix barriers.

References
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2. Tirode, F., Laud-Duval, K., Prieur, A., Delorme, B., Charbord, P. & Delattre, O. Mesenchymal stem cell features of Ewing tumors. Cancer Cell 11, 421-429 (2007).

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
3	CEMM	5.00
4	CURIE	38.00
5	DKFZ	18.00
6	VTT	39.00
10	UBERN	16.00
11	Weizmann	106.50
13	UCPH	6.00
	Total	228.50

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]
D2.1	Compounds inhibiting ET Cell Growth	6	46.00	R	PU	60
D2.2	List of kinases whose inhibition by siRNA molecules sensitized ET cells to selected drugs	6	76.00	R	PU	48
D2.3	Genes whose function is critical for ET cell survival	11	106.50	R	PU	60
		Total	228.50			

Description of deliverables

D2.1) Compounds inhibiting ET Cell Growth: List of compounds inhibiting ET cell growth [month 60]

D2.2) List of kinases whose inhibition by siRNA molecules sensitized ET cells to selected drugs: List of kinases whose inhibition by siRNA molecules sensitized ET cells to selected drugs [month 48]

D2.3) Genes whose function is critical for ET cell survival: List of genes originating from ET network whose function is critical for ET cell survival [month 60]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS6	List of selective growth inhibitory compounds between isogenic ET cell lines	6	48	
MS7	Identification of EWS-FLI1 and Myc downstream genes regulating ET cell survival	4	48	
MS8	A map of drug/siRNA interactions and refined network models explaining synergistic drug/siRNA effect	6	60	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP3	Type of activity [54]	RTD
Work package title	microRNA expression in ETs: rRegulation of by ET oncogenes, identification of targets & effects		
Start month	1		
End month	60		
Lead beneficiary number [55]	6		

Objectives

MicroRNA expression in ETs: rRegulation of by ET oncogenes, identification of targets & effects on proteomes. MicroRNAs (miRNAs) are central connections between the genome, transcriptome and proteome. Their crucial roles in cancer are increasingly recognised. We will assess aberrations of miRNA expression in ETs and their effects on the transcriptome and proteome to include miRNA effects into our signalling network models. Global and focussed approaches are combined: (i) high-throughput cell-based miRNA transfection screens with a comprehensive library of >800 pre-mirs will generate data concerning ET cell proliferation; (ii) analysis of the effects of selected miRNAs regulated by EWS-FLI1, N-Myc/c-Myc (for instance miR-17-92), ALK and TrkA on the transcriptome and proteomes of ET cells; and (iii) generation of time-resolved miRNA expression data after EWS-FLI1, N-Myc and c-Myc perturbation.

Description of work and role of partners

Description of work
This WP will focus on miRNA regulation and targets in order to cover an important but largely unexplored area that connects the genome with the proteome. It will provide a link between the GRN and the proteomic studies, and is designed from two conceptually complementary angles: (i) an unbiased screen to discover new regulatory miRNAs; and (ii) a hypothesis driven focussed approach to study the function of miRNAs likely to be involved in the pathogenesis of ETs. Both approaches are based on miRNA expression data in ETs and informed by our reconstruction of ET signalling networks, in this case including both GRNs and protein networks.

Task 3.1. Profiling miRNA expression in ETs. Large data sets consisting of miRNA expression profiles in clinical NB, MB and ESFT samples, and cell lines (from E.E.T.-Pipeline and OD) will be provided and expanded to use as a base to analyse the in vivo relevance of functional miRNA results collected in this workpackage. For instance, an NB dataset for at least 100 cases assessed for 450 miRNAs exists, which we plan to expand to apprx. 650 miRNAs combined with sequencing of miRNAs from fetal neuroblasts.

Task 3.2. Global approaches to identify critical miRNAs for ET cell viability using high-throughput screening (HTS). VTT Medical Biotechnology is equipped to conduct HTS in a 384 well plate format using a robotic HTS screening system. The pre-mir screens will be performed in the selected cell lines and conditions per tumour type (NB, MB and ESFT) described in WP2 as the core cell models, and conducted in at least two biological replicates. The primary screening method is CellTiterGlo cell proliferation assay (Promega Inc., Madison WI, USA). Data are processed using bioinformatic methods, and miRNA hits are defined. It is expected that several of the miRNAs identified in the HTS may have been previously associated with ETs (e.g. miR-17-92), and therefore highlight critical roles of the signalling network models studied in detail in ASSET. In addition, the global screen planned here is expected to lead to the identification of several novel pre-mirs regulating ET cell viability.

Task 3.3. Targeted approaches to identify targets of miR-17-92 (Myc) targets, and TrkA- and ALK-regulated miRNAs in NB by transcriptomics and proteomics. To understand the complex network regulated by N-Myc in NB, the effects of modulating the miR-17-92 cluster (valid target of Myc oncogenes) on the proteomes of NB cells will be analysed. We can make use of existing and well-characterised NB cells with an inducible miR-17-92 construct to identify the direct targets of this cluster using the ribonucleoprotein immunoprecipitation–microarray (RIP-ChIP) method9 or other methods under development based on high-throughput sequencing. The effects

of mutated and overexpressed ALK as well as TrkA overexpression on the miRNAome will be studied using an automated stem-loop RT-qPCR platform enabling simultaneous analysis of 650 mature miRNAs10,11. In parallel, the analyses of the transcriptome using RNA sequencing (Genome Analyser II, Illumina) and proteome will be performed to facilitate miRNA target gene detection. Proteome analysis will be done by MS-based protein expression profiling using SILAC labelling to identify and quantify changes in protein expression caused by miRNAs12.

Task 3.4. Characterization of the functional interrelation between TrkA and N-Myc in regulating NB cell fate. We already have analysed the miRNA expression patterns associated with N-Myc as well as TrkA in cultured NB cells. Also a set of primary NBs, which were selected by high and low TrkA expression, have been profiled. A comparison of miRNAs associated with TrkA in vivo to those associated with an in vitro model (SH-SY5Y with ectopic expression of TrkA), revealed an overlapping list of miRNAs. Deep sequencing has been performed from ten primary NB tumours to reveal the exact quantification of mature miRNAs and extensive variation in the expression of isomiRs and miRNAs. However, for a robust statistical analysis of miRNA variation, the profiling of small RNA transcriptomes of ca. 100 NBs is required and will be performed. Here, a qPCR based approach will be used to generate data for modelling the interplay between N-Myc and TrkA in modulating miRNA expression. . These data will also be utilised to assess the interplay between the good prognostic marker, TrkA, and the bad prognostic marker, N-Myc, on the miRNome, and will feed into modelling efforts in WPs 6 & 7 to explore driving effectors and the balance between proliferation and apoptosis in NB cells.

Task 3.5. Analysis of time-resolved EWS-FLI1 dependent miRNA expression profiles in order to study molecular and phenotypic effects of selected miRNAs in ESFT cell lines. miRNA expression profiles have been collected before/after stable (3 wildtype p53 and 2 mutant ESFT cell lines) and conditional (1 mutant p53 cell line) EWS-FLI1 knockdown, and for a series of primary ESFTs and mesenchymal stem cells (MSC) as the most relevant control tissue1,13. Here, the time-resolved miRNA profiling will be refined and extended using the stem-loop RQ-PCR platform in mutant and wildtype p53 ESFT cell lines and compared to an extended panel of primary ESFT and MSC to establish the EWS-FLI1 miRNA signature of ESFT. We then will focus on miRNAs which either directly or indirectly regulated by EWS-FLI1 based on time course experiments and on already available ChIP-SEQ data. Informed by the global HTS to identify functional miRNA regulating ET cell viability selected miRNAs for conditional pre-miRNA overexpression experiments will be further studied in ESFT cell line models. Both miRNAs activated and repressed by EWS-FLI1 will be considered.

Task 3.6. Modelling to identify commonly influenced miRNAs in ETs and separate miRNA profiles for NB, MB and ESFT. One of the most frequently used unsupervised frameworks to exploit large and complex signal datasets are linear models. They are successfully applied to reduce the multidimensional complexity of gene expression microarray data to a smaller set of latent variables. In these models, the measured expression level is considered as a weighted sum of mixed signals, possibly related to distinct biological influences. The analysis of gene expression by a linear model can help to discriminate the different original biological signals. One approach to address this problem is to assume statistical independence of the original signals and to decompose the expression dataset by independent components. In Independent Component Analysis (ICA) the miRNA expression values in the sample space have been decomposed in the space of the independent components, so that groups of commonly influenced miRNAs can be defined. Once robust components have been identified, this analysis reveals those individual miRNAs that have the strongest contribution to each component. ICA has been used to analyse a large dataset containing the expression profiles of 650 miRNAs for 40 ESFT patients. The same approach will be used here for miRNAs in NB and MB to extract a miRNA-based characterisation of the different tumours.

Output of WP
Here the previous information on miRNA expression on ET tumours and cell lines will be complemented by defining the functionally important miRNAs regulating ET cell survival. In addition, miRNA regulation by key oncogenes in ET tumours will be studied and modelling used to identify commonalities and differences on miRNA expression profiles between NB, MB and ESFT. Since miRNAs are candidate therapeutic targets, identification of miRNAs overexpressed in ET patient samples, regulated by key oncogenes and having a critical role for ET cell survival may help in defining the most suited miRNAs for antagomir based approaches for the treatment of ET tumours.

Role of participants
OK and KI (VTT) will perform the HT miRNA screens. FS (UGENT) will define Myc targets and identify ALK regulated miRNAS in NB. AE (UKE) will study the interplay of TrkA and N-Myc in NB. HK (CCRI) will focus on the molecular effects of EWS-FLI1 and MYC dependent miRNA modulation on ESFT transcriptome, OD

(CURIE) on the phenotypic analysis of ESFT cell lines upon modulation of the selected miRNAs. AA (UBERN) will perform validation experiments with functionally important miRNAs in MB cells. AZ (CURIE) will define commonly influenced as well as ET tumour type specific miRNAs. WK (UCD) will perform the quantitative proteomics experiments.

References
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5 Kauer, M., Ban, J., Kofler, R., Walker, B., Davis, S., Meltzer, P. & Kovar, H. A molecular function map of Ewing's sarcoma. PLoS One 4, e5415 (2009).
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Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	NUID-UCD	36.00
2	CCRI	34.00
4	CURIE	20.00
6	VTT	16.00
7	UKE	42.00
8	UGENT	12.00
10	UBERN	13.50
	Total	173.50

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]
D3.1	miRNAs regulating ET cell viability	6	86.00	R	PU	24
D3.2	miRNAs regulated by the key oncogenes in ET cells	4	87.50	R	PU	48
		Total	173.50			

Description of deliverables

D3.1) miRNAs regulating ET cell viability: List of miRNAs regulating ET cell viability [month 24]

D3.2) miRNAs regulated by the key oncogenes in ET cells: Lists of miRNAs regulated by the key oncogenes in ET cells [month 48]

WT3:
Work package description

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead beneficiary number	Delivery date from Annex I [60]	Comments
MS9	Data integration in order to mine a large dataset of miRNA profiles from ETs.	4	30	
MS10	A combined biological and mathematical model of miRNA function in ET cells	2	60	
MS11	Changes of proteome expression profile induced by Myc and TrkA regulated miRNAs	7	60	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP4	Type of activity [54]	RTD
Work package title	ET transcription factor protein networks		
Start month	1		
End month	60		
Lead beneficiary number [55]	1		

Objectives

ET transcription factor protein networks:
This WP will characterise TF networks on the functional protein level. Towards this we will map the dynamic protein-protein interactions of TFs altered in ETs, such as N-Myc, c-Myc, EWS-FLI1, p53 and Rb using quantitative proteomics. These data will be used to enrich mathematical models of GRNs by dynamic and mechanistic data

Description of work and role of partners

Description of work
The approach pursues is based on the observation that the proteins responsible for transcription assemble in multiprotein complexes and that the function of these complexes is determined by the composition of proteins. Therefore, we will map the protein interactions occurring in different TF complexes and their dynamic changes in response to perturbations using quantitative proteomics. We have previously shown that assessing dynamic changes in ERK-interacting proteins in response to differentiation and mitogenic factors is a powerful tool to elucidate signalling pathways that specifically regulate cell fate decisions14.

Task 4.1. Dynamic analysis of protein-protein interactions in ET TF complexes. This will analyse the dynamic changes in the composition of ET TF complexes to derive comparative interaction maps of the core ET TFs. The consortium has antibodies proven suitable for the immunoprecipitation (IP) of endogenous N-Myc, c-Myc and EWS-FLI1 proteins. Thus, most of the work can be carried out using endogenous protein IPs. OtherWeizmanne, we will use Flag-tagged exogenously expressed proteins, which in our hands give comparable results to endogenous proteins, when mildly overexpressed3. We have standard protocols for optimising IP conditions for individual antibodies to yield the best possible MS results. TF complexes will be isolated from SILAC-labeled cells using fast immunoprecipitation, which we have developed to preserve transient protein interactions4, and analysed by Orbitrap MS. Based on the results of the E.E.T.-Pipeline we already know a core TF network that is important in ETs, comprising N-Myc, c-MYC, EWS-FLI1, p53 and Rb. Thus, dynamic proteomic pathway mapping will focus on these factors and expanded as required dependent on and informed by the results of our GRN maps.

We will use cell model systems where TF activity can be regulated by conditional expression systems or where isogenic pairs plus/minus oncogene are available. We have NB cell lines available where N-Myc is tetracycline-regulatable5, and isogenic cell lines that overexpress the TrkA receptor. The former cell line expresses high levels of c-Myc, which is downregulated when N-Myc is induced. Thus, a c-Myc vs. N-Myc interactome comparison will be done. As NB cells with normal N-Myc often express high levels of c-Myc we hypothesise that interaction partners critical for transformation should be common to both N-Myc and c-Myc. In addition, as the combination of non-amplified MYCN and TrkA overexpression in primary tumours confers good prognosis, we will examine the N-Myc interactome in presence and absence of TrkA overexpression. A comparison of these conditions should enable us to filter out the interaction partners important for transformation and prognosis. As N-Myc also regulates p53 and Mdm2 expression5, we will also “walk along this TF pathway” to extend the analysis to p53 interaction partners and eventually Rb interaction partners in order to map connections between the p53 / Rb tumour suppressor axis. We will map the EWS-FLI1 interactome in ESFT. The aim is to interaction map the core TF network in ETs.

Task 4.2. Validation of results by perturbation studies with siRNA and drugs. We will use siRNA knockdown of interacting proteins predicted to regulate the TFs or mediate their effects in order to functionally validate the MS studies. The data will also be evaluated against biochemical and/or biological parameters of the specific cell models and predicted effects from the mathematical models. In addition, we will perform time-resolved perturbation studies using the kinase inhibitors, which we characterise in this project. This will allow the correlation of specific kinase activity with signalling events (phosphorylation), multiprotein complex formation, promoter assembly and transcription.

Task 4.3. Generation of dynamic protein-DNA and protein-protein interaction maps for modelling. We will use data-driven modelling methods, such as clustering and linear regression analysis, to elaborate the changes which most significantly correlate with the oncogenic action of TFs as measured in above cell models. The resulting data will be used to refine models of inference- and/or rule-based GRNs and also support mechanistic dynamic models generated in other WPs.

Output of the WP
This WP will deliver an integrated view of TF complexes and their roles in ET networks both on biochemical and modelling levels by providing data on the dynamic interactions between proteins present in the TF complexes and their alterations induced by ET oncogenes. These results will feed into the modelling as well as validation WPs as they provide direct information how signalling networks interact with TF networks to regulate gene expression.

Role of participants
BK (UCD), MG (UCL), AV (CNIO) and TH (DKFZ) will support integration of the data with inferred GRN structures and dynamic mechanistic models, and incorporate the dynamic interaction data into the models, and use models to make testable predictions. WK (UCD) will perform the dynamic and quantitative analysis of TF interactions to be used for modelling. HK (CCRI), AE (UKE), FW (DKFZ) will contribute the cellular systems, expertise in analysing them and assistance with validation of MS hits and validation of results predicted by the models. HK (CCRI) and FS (UGENT) will investigate promoter occupancy by identified TF complex components in ESFT by ChIP-PCR and reporter gene assays.

References
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Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	NUID-UCD	45.75
2	CCRI	13.00
5	DKFZ	19.00
7	UKE	6.00
8	UGENT	11.50
9	UCL	5.00
14	CNIO	12.00
	Total	112.25

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]
D4.1	MS interaction maps of selected ET transcription factors	1	60.25	R	PU	48
D4.2	Models of GRNs enriched by interaction proteomics	5	52.00	R	PU	60
		Total	112.25			

Description of deliverables

D4.1) MS interaction maps of selected ET transcription factors: MS interaction maps of selected ET transcription factors [month 48]

D4.2) Models of GRNs enriched by interaction proteomics: Models of GRNs enriched by interaction proteomics [month 60]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS12	Biochemical interaction maps of TFs altered in ETs	1	36	
MS13	Validated interaction maps of selected ET TFs	8	42	
MS14	Validated GRN models incorporating TF interaction data	5	60	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP5	Type of activity [54]	RTD
Work package title	Mapping cytosolic ET signaling protein networks by quantitative proteomics		
Start month	1		
End month	48		
Lead beneficiary number [55]	13		

Objectives

Mapping cytosolic ET signaling protein networks by quantitative proteomics:
Protein functions, hence, network connectivities are critically regulated by phosphorylation. Subsets of NBs feature aberrant receptor tyrosine kinase (RTK) signalling by TrkA and ALK. Our recent unpublished data indicate that ALK signalling is also relevant to ESFT. To identify the regulatory circuits downstream of ALK and TrkA we will use quantitative proteomics to map (i) tyrosine phosphorylation sites on TrkA and ALK, (ii) proteins which bind them and (iii) global phosphoproteome changes downstream of these RTKs. ALK expression is a better prognostic predictor than ALK mutational status in NB. We have NB cell models with regulatable ALK or TrkA expression, which will be used for these studies to deliver rich dynamic data for the mechanistic models. In order to further parameterise our kinetic models we will quantitate protein network components and their phosphorylation states using (i) Zeptosens' reverse protein arrays for highly parallel relative quantitation; (ii) MS-based proteomics (AQUA) for absolute quantitation, and (iii) Selected Reaction Monitoring (SRM) for the development of routine assays permitting parallel monitoring of 50-100 protein species and their PTMs over many timepoints.

Description of work and role of partners

We will apply our SILAC based phosphoproteomics technology1,2 that enables the routine identification and quantification of more than 10,000 phosphorylation sites to map the ALK and TrkA associated and global phosphoproteomes of the regulatory circuits downstream of ALK and TrkA. Further we will determine the absolute concentrations of critical network proteins for the parameterisation of kinetic mechanistic models. These data will complement the protein interaction and protein profiling studies, and will also contribute to dynamic modelling approaches and target validation efforts.

Task 5.1. Global profiling of protein expression of the core ET cell lines by quantitative mass spectrometry.
We will establish stable isotope labelling via amino acids in cell culture (SILAC) of the different ET cell lines. The proteome of induced and untreated SILAC lysates from the core ET cell models for MB, NB and ESFT will be analysed by high-resolution MS. Whole-cell lysates will be mixed and digested with trypsin. The resulting peptides separated off-line by iso-electric focusing into 12 fractions before online nanoflow RP-HPLC-MS/MS analysis. This standardised and largely automated workflow uses the latest generation of state-of-the-art high-resolution MS instrumentation, i.e. the LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific) coupled to an online nano HPLC system (Proxeon Biosystems) available at UCPH. From replicated experiments, we expect to be able to quantify more than six thousand proteins in each of the cell lines.

Task 5.2. Quantifying the phosphoproteome of different NB cell lines with induced TrkA and ALK expression/activation levels. For in-depth coverage of the phosphoproteome, we will fractionate peptides from tryptic digests of whole-cell lysates by strong-cationic exchange chromatography (SCX), and enrich phosphopeptides to high purity using TiO2 and antibody-based methods. Each phosphopeptide fraction will be analysed and quantified by high-resolution MS as we have described1,2.

Task 5.3. Identification of phospho-tyrosine dependent protein-protein interactions for ALK and TrkA using antibody-based affinity-purification coupled to high-resolution MS. The proteomic approach will be similar to our previous analysis of the EGF receptor3. SILAC-labeled NB cells expressing ALK or TrkA proteins will be used to affinity purify the tyrosine kinase complexes using anti-phosphotyrosine antibody-conjugated beads and micro-columns, which will be directly analysed by LC-MS. This will allow us to map the proteins involved

in the phosphotyrosine-dependent signalling complexes and quantify the tyrosine phosphorylation sites on ALK and TrkA using the MaxQuant software suite. As biochemical validation we will perform confirmatory co-immunoprecipitation and western blotting analyses of selected proteins and phosphoproteins.

Task 5.4. Absolute quantification of selected phosphorylation sites and proteins using AQUA. Peptides and corresponding phosphopeptides harbouring regulated phosphorylation sites identified above will be synthesised using our automated tabletop peptide synthesiser in stable isotope-labelled (13C and 15N) versions. These so-named AQUA peptides will be added (spiked) to whole-cell lysates of different cell lines. Comparison between the AQUA peptides of known concentration and the corresponding endogenous peptides permits the accurate and reliable absolute quantification of endogenous peptides by MS4.

Task 5.5. Routine MS-based measurements of selected proteins and PTMs. Based on the above effort, we also plan to establish selected reaction-monitoring (SRM) MS methods to routinely quantify a selected smaller number (50-100) of proteins and protein phosphorylations5. With the above data available we can establish SRM transitions for a smaller number of proteins that are of interest to assay routinely and over many more timepoints or perturbation conditions. This will yield high quality data for dynamic modelling, while being much more economical than western blotting. It also will complement the array work described below. Either technology has limitations and bottlenecks, i.e. the quality of antibodies in the array work, protein detection and availability of suitable SRM transitions in the MS work. Combining both technologies will extend our capabilities to cover a wide range of quantitative measurements on a routine fashion.

Task 5.6. Investigation of the dynamic behaviour of protein signalling networks using reverse protein microarrays. Reverse arrays based on planar wave guide nano-structured chips6 will be used to complement the MS-based methods. These arrays have the exceptional benefit to provide quantitative information on expression and activation changes of typically 100 to 200 signalling pathway proteins from different signalling cascades in the same cellular sample containing as little as 105 to 106 cells without prior labelling. Recognition of proteins is based on well established immunodetection, however, the array format allows generation of the data equivalent to 10,000 western blots within a week. Reverse arrays yield high quality quantitative and time-resolved data that capture the dynamics of protein expression changes and posttranslational modifications for multiple pathways at affordable cost. With a high quality antibody the limit of quantification is 2,000 proteins per spot. They reliably identify protein expression and posttranslational activation changes >25% between samples. Absolute quantification can be achieved by generating a calibration curve from corresponding recombinant proteins spotted on the array. Thus, tasks 5.5 and 5.6 will give us a excellent capacity to produce dynamic protein data for mechanistic kinetic modeling of ET protein signaling networks.

Output of WP
The output of this WP will be an integrated view of aberrant tyrosine kinase signalling and the PTMs involved in the regulation of ET networks both on the biochemical and modelling levels. These datasets will feed into the construction of predictive kinetic mathematical models. Results also will be used in the biological validation WPs in SP4.

Role of participants
This WP draws on consortium resources in quantitative phosphoproteomics and the core cell models for ET. JO (UCPH) will perform the dynamic phosphorylation site mapping, receptor kinase interaction screens and the AQUA-based optimisation and quantification. AE (UKE) will contribute ET cell models including the SY5Y-TR-TrkA cell model and assist in cell culture of the core ET cell lines in JO's lab by providing materials and staff in short-term exchanges. ME (Zeptosens) will provide expertise on protein arrays and analyse all core cell lines using this technology. WK (UCD) will develop the SRM based techniques.

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Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	NUID-UCD	30.00
7	UKE	18.00
12	ZEPTO	23.75
13	UCPH	60.00
	Total	131.75

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]
D5.1	quantitative phosphoproteome analyses of the activated ALK- and TrkA-expressing NB cell lines	13	75.00	R	PU	24
D5.2	SRM-based assays for the routine quantitative measurement of selected protein species	1	30.00	R	PU	36
D5.3	Parallel relative quantitation of ET protein network components by reverse protein arrays	12	26.75	R	PU	48
		Total	131.75			

Description of deliverables

D5.1) quantitative phosphoproteome analyses of the activated ALK- and TrkA-expressing NB cell lines: Large-scale quantitative phosphoproteome analyses of the activated ALK- and TrkA-expressing NB cell lines [month 24]

D5.2) SRM-based assays for the routine quantitative measurement of selected protein species: SRM-based assays for the routine quantitative measurement of selected protein species [month 36]

D5.3) Parallel relative quantitation of ET protein network components by reverse protein arrays: Parallel relative quantitation of ET protein network components by reverse protein arrays [month 48]

WT3:
Work package description

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead beneficiary number	Delivery date from Annex I [60]	Comments
MS15	Identification of differentially regulated phosphorylation sites between NB cell lines	13	30	
MS16	Quantitative dynamic Interactome of TrkA and ALK receptor tyrosine kinases	1	36	
MS17	Dataset of all absolution quantitation of all selected ALK and TrkA network components	1	48	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP6	Type of activity [54]	RTD
Work package title	Computational-kinetic models of critical Myc-dependent regulatory networks that drive ETs		
Start month	1		
End month	60		
Lead beneficiary number [55]	1		

Objectives

Computational-kinetic models of critical Myc-dependent regulatory networks that drive ETs:
To make critical network parts accessible to mechanistic simulations and predictions, we will develop predictive ODE models from the global network models developed in SP1 based on perturbation analyses and populated by data on protein-protein and protein-DNA interactions obtained in SP2. Stochastic features will be added using advanced statistical approaches and ensemble modelling which considers the distribution of output functions of an “ensemble” of models rather than single deterministic models. The aim is to develop experimentally validated mathematical models of the core regulatory networks, deregulation of which drives Myc-dependent ETs

Description of work and role of partners

Previous genome-wide screens by consortium members have shown that diverse molecular aberrations in ETs converge on the interacting Trk signalling, CDK-Rb-E2F-Skp2 and p53-Mdm2 modules of cell cycle and apoptosis regulation. A critical connecting hub in this network is deregulated N-Myc and c-Myc expression. Myc target genes include p53, E2F transcription factors and cyclins that regulate CDK2. Myc protein expression in ETs can be deregulated due to gene amplification or functionally through signalling by the Akt and Ras/ERK pathways downstream of the TrkA receptor. Phosphorylation of Myc by ERK, GSK3 and CDK2 regulate Myc protein stability. The mechanism of Myc deregulation is relevant for disease progression as NBs with MYCN amplification have poor prognosis, while tumours with high TrkA and high (but not gene amplified) N-Myc expression have good prognosis. This points to important and disease relevant roles (i) for Myc gene dosage; (ii) for the signalling networks that connect TrkA and N-Myc; and (iii) the Myc transcriptional network that programs the effectors of the different biological responses. Emerging evidence suggests that these networks are also deregulated in ESFT and MB. Therefore, we will (i) develop models of N-Myc regulation by TrkA and upstream signalling pathways, and (ii) in close collaboration with WP7, expand those models into the transcriptional effector networks and analyse network fragilities.

Task 6.1. Modelling of the effects of TrkA on N-Myc and c-Myc expression and protein stability. This will involve measurements of activities of TrkA and downstream pathways1,2, in particular Ras/ERK and Akt/GSK3, which are known to regulate Myc protein stability by direct phosphorylation3. These measurements will be taken in part from the quantitative phospho-proteomics experiments conducted in WP5, and especially for the iterative phase of model refinement, supplemented by additional wet-lab experiments to obtain detailed kinetic data on selected network components. These measurements will be correlated with myc gene promoter activity and Myc protein stability to develop a core kinetic model considering regulation of N-Myc gene activity and protein stability by TrkA. This core model will be used to test how functionally induced elevation of N-Myc and c-Myc expression or activity compare with genetically induced (by gene amplification) Myc deregulation.

Task 6.2. Modelling of the N-Myc and c-Myc interaction network. Based on the Myc protein-protein and protein-DNA interaction data from WPs4& 5 and in close collaboration with SP1, we will generate a kinetic Myc interaction network4 in order to examine dynamic properties5 arising as a consequence of Myc deregulation and Myc dosage effects. Differentiation, apoptosis and proliferation will be assayed as biological read-outs in Myc-regulatable NB cells. Additional kinetic data on key components (as emerging from mathematical modelling) will need to be generated for the model refinement using the MS and array technologies from WP5. We will expand the Myc core networks by including transcriptional Myc effectors such as p53, E2F and cyclins. Models

for these signalling modules will be developed in WP7. Both WPs will integrate their respective models at the level of input-output maps to expand the networks and be able to perform more extensive simulations and fragility analysis.

Task 6.3. Fragility analysis. This will be done both on the level of the core networks and the extended networks in close collaboration with WPs7&9. The experimental work will be based on the model predictions, and also include testing of kinase inhibitors and siRNAs characterised in other WPs2& 8. We will focus on compounds and combination of compounds that can block proliferation or trigger apoptosis or differentiation.

Output of WP
This WP will integrate input from global network models, biochemical experimentation from previous WPs and apply dynamic kinetic modelling methods in order to elaborate models of network submodules providing mechanistic insights. These results will feed back into the experimental WPs as well as into the validation WPs by providing concrete testable predictions of a quantitative rather than qualitative nature. For instance, these models will be able to simulate the effects of partial inhibition of network components, a scenario that is highly relevant to predict the effects of drugs or siRNA interference.

Role of participants
BK (UCD) will develop and refine ODE models in close collaboration with TH and SBu (DKFZ), the latter will also contribute to the parameter estimation and application of stochastic methods to dynamic models to account for the inherent stochastic nature of biochemical processes in collaboration with MG (UCL). WK (UCD) and FW (DKFZ) will perform the biochemical and molecular biology experiments necessary for model development and refinement as well as cell culture experiments investigating the myc proteins and validation. AE (UKE) will perform cell culture experiments investigating TrkA.

References
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Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	NUID-UCD	47.75
5	DKFZ	30.00
7	UKE	5.00
9	UCL	18.00
	Total	100.75

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]
D6.1	Core model of Myc protein regulation by TrkA in NB cell lines	1	49.75	R	PU	24
D6.2	Predicted fragility points in the Myc network	5	51.00	R	PU	60
		Total	100.75			

Description of deliverables

D6.1) Core model of Myc protein regulation by TrkA in NB cell lines: Core model of Myc protein regulation by TrkA in NB cell lines [month 24]

D6.2) Predicted fragility points in the Myc network: Predicted fragility points in the Myc network [month 60]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS18	Kinetic core model of Myc network	1	36	
MS19	Fragility analysis of the Myc network	5	48	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP7	Type of activity [54]	RTD
Work package title	Network fragility analysis of apoptosis and proliferation decisions		
Start month	1		
End month	60		
Lead beneficiary number [55]	5		

Objectives

Network fragility analysis of apoptosis and proliferation decisions:
Here, we will develop mathematical models of the network that determines the balance between decisions for proliferation or apoptosis in ETs, centering around the CDK-Rb-E2F-Skp2 and p53-Mdm2/MDMX modules. Our initial focus will be on N-Myc-driven NB, where in collaboration with WP6 we aim to rationalise on a mechanistic level how the different degrees of N-Myc overexpression or amplification observed in NBs give rise to the various clinically recognised stages of the tumour. NBs range from moderately hyperproliferative through hyperproliferative yet apoptosis-sensitive (both of which respond to current therapies) to therapy-resistant hyperproliferative and apoptosis-insensitive stages. This model will be extended to dissect the regulatory effects of molecular aberrations in ESFT and MB. New molecular players and regulatory links identified in our functional genomic and proteomic screens will be incorporated into the models as required. We will systematically characterise in silico fragile nodes of the network that can be targeted to (i) sensitise tumour cells toward apoptosis or/and (ii) inhibit unchecked cell cycle progression. The results of this workpackage and WP6 will inform the biological validation SP4 using cell lines and mouse models.

Description of work and role of partners

Previous genome-wide screens by members of the consortium have shown that diverse molecular aberrations in ETs converge on the interacting CDK-Rb-E2F-Skp2 and p53-Mdm2/MDMX modules of cell cycle and apoptosis regulation1-3. The severity of a tumour (and its prognosis under chemotherapy) correlate both with the proliferative potential of the cells and their sensitivity to apoptosis4,5. Depending on the molecular aberrations present, these two properties combine in complex patterns including (1) slowly proliferating cells with low apoptotic sensitivity, (2) rapidly proliferating cells with high apoptotic sensitivity and (3) rapidly proliferating cells robust against apoptosis and therapy resistant. To dissect the complex regulatory networks governing decisions for cell proliferation and apoptosis, we will integrate mechanistically-based mathematical modelling of coupled gene-regulatory and signalling networks6,7 with quantitative biochemical experiments on prototypical ET cell lines. We will begin by constructing models based on existing functional genomics data for N-Myc-driven NB1, and parameterise the models using time-resolved data from the E.E.T.-Pipeline and WPs 4 & 5. Then we will analyse model behaviour using techniques from nonlinear dynamics and numerical simulations, and conduct systematic sensitivity analyses. In other ETs, such as ESFT and MB, different molecular aberrations appear to have similar effects on the core regulatory networks as seen in NB. We will, therefore, extend the model to these tumour entities. The present WP is linked tightly with WP6 investigating how deregulated signal transduction feeds into the core modules studied here, and the models of the two domains will be interfaced. The results of functional genomics and bioinformatics (SPs1&5) approaches on new molecular players and interactions will be incorporated into the mathematical models. This work will lead to predictions on how pharmacological or genetic intervention could halt deregulated cell proliferation, sensitise cells toward apoptosis and/or induce differentiation.

Task 7.1. Modelling of the CDK-Rb-E2F-Skp2 and p53-Mdm2/MDMX modules in NB. We will develop models based on chemical kinetic equations for critical players of these modules, incorporating gene expression, mRNA and protein turnover, as well as key PTMs (especially phosphorylation). Both modules will be coupled to investigate cross-regulation of cell proliferation, as well as cell-cycle arrest and apoptosis. The models will be parameterised using time-resolved data on mRNA, phosphorylation data and protein abundance generated as described above. The model will be characterised in terms of emergent behaviour and sensitivity using

numerical simulations and bifurcation analyses. This analysis will focus on the network mechanisms that give rise to switches or gradual shifts in proliferative potential and apoptosis sensitivity.

Task 7.2. Connection with signal transduction. In collaboration with WPs4-6, we will interface the model with models of signal transduction modules to investigate how molecular mechanisms other than MYCN amplification deregulate the cell cycle and apoptosis in NB. This includes TrkA signalling to N-Myc, which is a key pathway in NBs without MYCN amplification.

Task 7.3. Adaptation of the model to ESFT and MB. We will adapt the model to simulate key pathogenic mechanisms in ESFT (i.e. induction of c-Myc, SKP2, CDK2, MDMX and repression of p53 by EWS-FLI1 in tumours with and without p53 mutations) and MB (i.e. induction of N-Myc by sonic hedgehog or c-Myc by the Wnt pathway). This will allow us to test whether the cell cycle machinery in these ETs is deregulated by the same principles found in NB, or whether there are important differences. This work will be informed by existing data from partners HK, OD, AA and the results of WP4 characterising the protein complexes formed at cMyc and nMyc promoters.

Task 7.4. Predictive fragility analysis of single and combined nodes. We will conduct systematic sensitivity analyses to identify parameters and (binary) parameter combinations that exert strong control on the molecular cell-fate switches. Salient predictions will be tested in perturbation experiments on tumour cell lines as necessary for the iterative refinement of models in close collaboration with SP4. It is important to note that some experimental validation is required as direct feedback during the modelling process in order to produce a high quality model that makes useful predictions and subsequently can be applied to more challenging biological questions. We will monitor cell cycle progression, response to DNA damage and apoptosis, together with the kinetics of key molecular players (phosphorylations, protein levels, activation/deactivation) .

Output of WP
Experimentally-validated models for the dynamic functioning of the CDK-Rb-E2F-Skp2 and p53-Mdm2 in ETs, and prediction of new intervention approaches for validation in SP4.

Role of participants
DKFZ: FW will conduct the biochemical and molecular biology experiments on NB cell lines for model parameterisation, testing and refinement. TH will develop the kinetic models. UCD (BK) will participate in model building, sensitivity and bifurcation analyses. CURIE: (AZ) will work on the definition of the CDK-Rb-E2F-Skp2 and p53-Mdm2 modules and their analysis based on the previous experience of modelling cell cycle and cell fate decisions8 and adaptation of the modelling to ESFT.CURIE (OD) will provide experimental testing and model validation for ESFT based on inducible model systems. CCRI (HK) will perform genetic perturbation experiments of players identified in CDK-Rb-E2F-Skp2 and p53-MDM2/MDMX networks in ESFT cell lines. UKE (AE) will perform experimental work on NB in relation to TrkA for model refinement.

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WT3:
Work package description

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	NUID-UCD	23.00
2	CCRI	12.00
4	CURIE	22.00
5	DKFZ	106.00
7	UKE	5.50
	Total	168.50

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]
D7.1	A comprehensive core model of the CDK-Rb-E2F-Skp2 and p53-Mdm2/MDMX modules in NB	5	92.00	R	PU	48
D7.2	Extension of the NB core model to EWS and MB	4	76.50	R	PU	60
		Total	168.50			

Description of deliverables

D7.1) A comprehensive core model of the CDK-Rb-E2F-Skp2 and p53-Mdm2/MDMX modules in NB: A comprehensive core model of the CDK-Rb-E2F-Skp2 and p53-Mdm2/MDMX modules in NB [month 48]

D7.2) Extension of the NB core model to EWS and MB: Extension of the NB core model to EWS and MB [month 60]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS20	Validated kinetic model p53-Mdm2/MDMX module in NB cell lines	5	18	
MS21	Validated kinetic model CDK-Rb-E2F-Skp2 module in NB cell lines	5	24	
MS22	Integrated model of the core network governing proliferation versus apoptosis decisions in NB	4	36	
MS23	In silico quantification of effect of individual network nodes and pairs of nodes on proliferative a	1	48	

WT3:
Work package description

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS24	In silico adaptation of the model to ESFT and MB	4	60	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP8	Type of activity [54]	RTD
Work package title	Probing network fragilities with kinase inhibitors		
Start month	1		
End month	60		
Lead beneficiary number [55]	3		

Objectives

Probing network fragilities with kinase inhibitors:
Kinases are crucial in signal transduction. Ten selected kinase inhibitors that were shown to be safe in adult humans, and that cumulatively cover the majority of the druggable kinome will be characterised by combinatorial perturbation experiments in different ET cell lines. They include approved drugs: nilotinib, dasatinib, erlotinib, lapatinib, sunitinib, sorafenib; and drugs in clinical development: bosutinib, bafetinib, danusertib, midostaurin. We will use this panel (i) to identify synergistic drug combinations1; and (ii) to perform proteomic analyses to identify direct and distal protein targets of the most promising drug combinations. These datasets will be used to (i) correlate targets and biological effects using transcriptomics, phospho- and chemical proteomic approaches; (ii) identify vulnerable nodes via mechanistic modelling in order to predict effective inhibitors and inhibitor combination.

Description of work and role of partners

We have developed a robotics-assisted combinatorial drug screening platform supported by CEMM's bioinformatics facility, which enables a systematic investigation and mathematical evaluation of synergistic drug effects of all combinations of the described kinase inhibitors in ET cell line panels. The selection of ten inhibitors streamlines the screening logistics, but we will regularly review and possibly expand this panel depending on our results and interesting new drugs becoming available, e.g. pazopanib and ALK inhibitors. In order to define the biological target space of the drugs we will (i) use immobilised drugs as affinity probes for the identification of protein targets by MS; and (ii) investigate drug induced global protein phosphorylation signatures in order to characterise distal drug actions. We have already successfully applied these technologies to the characterisation of target specificity and mechanism-of-action of several clinical kinase inhibitors in haematological malignancies (e.g. Chronic Myeloid Leukemia, Fig. 1)1-3 and lung cancer4, and we also have excellent experience in investigating global protein phosphorylation signatures by MS5.

Task 8.1. Establishing the screening cell line panel and screening conditions. Establishment of the screening cell line panel (4 NB conditions: SH-SY5Y inducible for TrkA or N-Myc; 2 MB conditions: UW-228 with c-Myc on/off; 2 ESFT conditions: A673 with EWS-FLI1 on/off) with matching mRNA and miRNA expression and DNA copy number profiles from the consortium's resources (E.E.T.-PIPELINE data and AA). Screening conditions will be defined, comprising logistic planning and Standard Operating Procedures for the cell culture, screen conduction and data collection.

Task 8.2. Binary combinatorial drug screen. We will first determine the individual drug effects on each cell line for all 10 compounds in proliferation assays using Cell Titer Glo (Promega) as the readout2. The respective EC50 values will serve as starting points for the binary combinatorial screens. Starting with the most potent compound for each cell line, we will screen the remaining panel of compounds for synergistic antiproliferative effects through pairWeizmanne combinations. The resulting hree-dimensional response surfaces (Fig. 1) and isobolograms contain valuable information that can be used to validate vulnerabilities predicted by the models. Further analysis with the Loewe, Bliss and HSA models will identify the most promising synergistic drug interactions3. Priority will be given to those combinations that are selective for a particular ET type while assuring that all three different ETs are covered.
Task 8.3. Synthesis and validation of coupleable drug analogues. Four (nilotinib, dasatinib, bosutinib, bafetinib) out of the 10 selected drugs are already available in the Superti-Furga lab as analogues suitable for

immobilisation. These compounds are fully validated against a large number of kinase and non-kinase targets. For the remaining drugs, we will design coupleable analogues based on kinase co-crystal and structure-activity relationship data from the literature. Compound syntheses will either be performed in-house or outsourced to commercial providers. Subsequently, these drug analogues will be tested for in vitro kinase inhibition in comparison with the parent compounds against their cognate targets as well as in pilot drug pull-down experiments.

Task 8.4. Generation of drug target profiles. Using the validated immobilised kinase inhibitor analogues we will perform MS-supported drug affinity chromatography experiments as described in previous publications from our laboratory (Fig.2)4,5. We will focus on those drugs that showed synergistic effects in the combinatorial screen (task 8.2). Control experiments for target deconvolution will use unrelated drugs (e.g. ampicillin), other kinase inhibitors from our drug panel which have no significant effect on the cell line of interest, or competition with high concentrations of the parent drug (GSF).

Task 8.5. Characterisation of drug-dependent phosphoproteome signatures. In parallel to the described chemical proteomics approach, we will investigate the kinase inhibitor-dependent global phosphoproteome changes for the selected drug pairs using our established phosphopreoteomics MS platform6. The drug profile and phosphoproteome signatures will be further used in SP4 to control validation experiments and in SP5 to generate dynamic non-linear mathematical models.

Output of the WP
This WP will deliver drugs and drug combinations that will enable the matching of drug sensitivities to specific genetic and functional aberrations in ET cells. It also will deliver objective information on the radius of efficacy of the eleven kinase inhibitors by assessing their protein targeting and global effects on network functions. The WP will produce valuable tools for the validation WPs and for testing network fragilities predicted by the mathematical models.

Role of Participants
GSF (CEMM) will perform combinatorial drug screens and the chemical proteomics experiments. AA (UBERN) will characterise the cell line panel. JO (UCPH) will perform the global phosphoproteomics. SBr (UCPH) and AV (CNIO) will perform the computational analysis and modelling.

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3. Rix, U. & Superti-Furga G. Target profiling of small molecules by chemical proteomics, Nat Chem Biol 5 616-624 (2009).
4. Rix, U. et al. Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and dasatinib reveal novel kinase and nonkinase targets. Blood 110 4055-4063 (2007).
5. Remsing Rix, L.L. et al. Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. Leukemia 23 477-485 (2009).
6. Olsen, J.V. et al. Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. Sci Signal 3 ra3 (2010).

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
3	CEMM	80.50
10	UBERN	12.00
13	UCPH	18.00
14	CNIO	15.00
	Total	125.50

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]
D8.1	Identification of tumour-type specific synergistic drug combinations	3	49.50	R	PU	36
D8.2	Identification of drug-dependent changes in phosphotyrosine signatures of synergistic drug combinati	13	76.00	R	PU	60
		Total	125.50			

Description of deliverables

D8.1) Identification of tumour-type specific synergistic drug combinations: Identification of tumour-type specific synergistic drug combinations [month 36]

D8.2) Identification of drug-dependent changes in phosphotyrosine signatures of synergistic drug combinati: Identification of drug-dependent changes in phosphotyrosine signatures of synergistic drug combinations [month 60]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS25	Dose response curves of 11 selected kinase inhibitors in a well characterised cell line panel	3	18	
MS26	Synergistic pairwise drug combinations based on the most potent single drug	10	24	
MS27	Panel of validated drug analogues suitable for immobilisation	3	24	
MS28	Target profiles for synergistic drugs	3	54	
MS29	Global and quantitative drug-induced changes of the phosphoproteome for synergistic combinations	13	60	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP9	Type of activity [54]	RTD
Work package title	Validation of mathematical models in biological models and clinical samples		
Start month	13		
End month	60		
Lead beneficiary number [55]	2		

Objectives

Validation of mathematical models in biological models and clinical samples:
The focus of this WP is on validating network nodes predicted to be effective drug targets in ET cell lines engineered to permit tunable modulation of the involved genes (e.g. inducible regulation via tetracycline, shRNA expression, etc.), mouse xenograft models and human clinical samples. This multi-scaled approach across biological models of different complexities ensures that the transition from molecular mechanisms to clinical phenotypes remains firmly footed in tractable entities. We will use a combination of drug treatment and/or shRNA knockdown in cell lines and xenograft tumours in nude mice. Additionally, predictions can be assessed in the extensive, existing data from primary embryonal tumours (ET), which is available to the ASSET consortium and was generated by the E.E.T.-Pipeline. The primary objective is to validate model predictions within the background of the ET tumour cell and tumour within the context of the organism in order to rationally predict and identify effective novel drug combinations that will improve treatment options for paediatric solid tumours.

Description of work and role of partners

Here we will test the predictions made by refined and proven mathematical models including a second step of biological validation that will examine the model outside the particular context in which the model was developed. We also will use xenograft tumour models in nude mice to assess the efficacy of drugs or shRNA knockdown of predicted targets. Knockdown / inhibition of the targets will be verified in the tumour xenografts using immunohistochemistry, real-time RT-PCR or other assays available in the consortium.The ASSET consortium has access to the largest ET sample collection in Europe, and has generated extensive data for these tumour samples on the genomic, epigenomic, transcriptomic and protein levels. This will enable us to test salient predictions of the models generated above in the existing data, and assess predicted protein expression in primary tumour tissue arrays. Thus, we can correlate gene mutations or overexpression with functional changes in global and local network behaviour.

Task 9.1. Validation of predicted fragility nodes in cellular models for ET. Based on the predictions of the mathematical models and results of the perturbation screens described above we will select at least 2 different shared and 2 different ET entity-specific fragility nodes for further interrogation. To prioritise fragility nodes we will consider (i) sensitivity analysis; (ii) data from the combinatorial screens; and (iii) the drugability of candidate targets with a priority on kinase inhibitors. Validation of fragility nodes will be performed in 3 parallel approaches. First, the core ET cell lines, which harbour inducible systems for the key oncogenes and/or kinases, will be used to target predicted sensitivity nodes by transient siRNA expression and drugs if amenable. The molecular (mRNA and miRNA expression) and phenotypic (cell growth/apoptosis) consequences will be assayed and used as input for further refinement of the mathematical models. This work will be done in close collaboration with the respective WPs. Second, we will test for consistency of these fragility nodes by interrogation in additional ET cell lines. Stable ET cell lines will be generated with inducible modulation of the identified fragility nodes using derivatives of a single-vector lentiviral inducible RNAi system1. The functional consequences of perturbing the expression of (aberrant) genes central to ET biology (EWS-FLI1 in ESFT, N-Myc/Myc in MB and NB, TrkA and ALK in NB) and of up-/downstream fragility nodes will be tested in respect to proliferation and apoptosis. Third, we will establish an extended panel of up to 5 genetically well characterised cell lines per tumour entity (i.e. >15 cell lines in total) representing the scope of the genetic background of the tumour entity (i.e. ESFT cell lines with both wildtype and mutant p53, NB cell line with and without MYCN amplification, MB with and without amplified

c-Myc, etc.). The extended cell line panel will be used to test the predicted efficacy of single drugs and drug combinations against the three ET entities in vitro.

Task 9.2. Transcriptomic expression profiles for selected combinations of kinase inhibitors in the ETcell line panel. The binary combinations of kinase inhibitors identified as having synergistic anti-proliferative effects in ET cell lines will be further characterised in terms of their impact on global gene expression using cDNA microarrays. The experiment will include 4 NB conditions: SH-SY5Y inducible for TrkA or N-Myc with either gene on/off; 2 MB conditions: UW-228 with c-Myc on/off; and 2 ESFT conditions: A673 with EWS-FLI1 on/off .

Task 9.3. Testing of top drug combinations for each ET entitiy in xenograft mouse models. Based on the mathematical model predictions and cell line validation the top one or two candidate drug/siRNA treatment combinations will be tested in nude mouse xenografts of the appropriate tumour entity according to standard protocols. Two cell lines will be used per tumour entity, for instance, MYCN-amplified IMR-32 and single copy SH-SY5Y for NB, c-myc-amplified D-341 and single copy DAOY for MB and ESFT cell lines with (A673) and without (TC252) p53 mutations. Treatment will be initiated after tumours have reached a volume of 0.25cm3. 10 mice will be treated with each inhibitor separately as well as the combinations. Control groups will be treated only with carrier. This scheme requires 240 mice per drug combination when tested against all 3 ET entities with 2 cell lines each.Tumour growth and survival will be used as endpoints, and analysed using t-tests and Kaplan-Meier survival analysis.

Task 9.4. Re-analysis of existing high-throughput data sets from primary human NB, MB and ESFT for the validation of model predictions. We will re-analyse primary tumour data with a view to test the predictions of mathematical models for targeting the tumour via drugs and/or shRNAs. Where appropriate, protein expression will be assessed in primary ET tumour tissue arrays. Predicted models will be evaluated in all available expression data from ETs by calculating a pathway activity score (i.e. the summed ranks of the expression levels of all genes that are part of the model) and considering databases such as the Connectivity Map. In this way, we can confirm whether the models that were predicted on the cell line models, can be extrapolated to the tumour samples or certain subgroups of tumours. In the expression data reanalysis of primary tumour materials, we will pay particular attention to the choice of the relevant reference tissue. By comparing genomic and transcriptomic (mRNA and miRNA) data of the tumours with a low versus high pathway activity score, one should get a broader view of the deregulated genes and pathways (and in this way also new therapeutic targets), using differentially expression analysis followed by initial pathway analysis with the Ingenuity software.

Task 9.5. Immunohistochemical validation of selected proteins on existing tissue microarrays. For prioritisation of the drug or siRNA combinations to be tested it will be important to assess the expression of the prospective target in primary tumour material. The consortium has tissue microarrays of primary tumour samples available that were generated within the E.E.T.-Pipeline project. These will be used for the immunohistochemical analysis of selected proteins.

Output of WP
This WP will provide a stringent functional validation of mathematical model predictions within cellular and mouse preclinical models and tumour samples. The results will be provided to the heads of the respective European clinical trials (SIOPEN for NB, HIT for MB and EURO-EWING for ESFT) as a biology-based input for prioritisation of drugs to be tested in early clinical trials. This ensures a direct link of data and knowledge gained in ASSET to the bedside of the patient. There is high chance that this approach will change clinical practice as currently no algorithm exists to select molecular targeted drugs available on the market to be tested in early clinical trials. Thus, the output may contribute to the urgently needed shift from current drug selection based merely on the “gut feeling” of the treating physician towards future biology-guided drug selection.

Role of participants
HK (CCRI) and OD (CURIE) will perform all genetic targeting experiments (transient and inducible) of the selected sensitivity nodes in the ESFT cell line models (first validation phase) and additional ESFT cell lines (second validation phase) as described. They will also share the work on the analysis of engineered cell lines. HK (CCRI) will primarily focus on analysis of mRNA and miR expression, and OD (CURIE) on phenotypic consequences (apoptosis, proliferation, differentiation) using a high cell content screening platform. AE (UKE) will perform and analyse perturbation experiments of the selected sensitivity nodes in TrkA-, FS (UGENT) in ALK-, and FW (DKFZ) in Myc-engineered NB cell lines. AA (UBERN) will perform genetic targeting of the selected sensitivity nodes in Myc-engineered MB cell lines and will be responsible for the assembly and characterisation of the extended ET cell line panel and the in vitro drug combination testing with selected combinations of kinase inhibitors. He will be assisted by FW (DKFZ) and FS (UGENT) in the genetic

characterisation (including mRNA and miRNA expression. UKE (AE) will conduct xenograft experiments for NB and MB including xenograft tumour analysis, where appropriate, and contribute to immunohistochemical evaluation on TMAs. CURIE (OD) conduct xenograft experiments for ESFT including xenograft tumour analysis, where appropriate. UGENT (FS) will contribute to immunohistochemical evaluation on TMAs and data re-analysis. CCRI (HK) will contribute to data re-analysis DKFZ (FW, TH), and UBERN (AA) will contribute cell lines and models for NB and ESFT and contribute to data re-analysis, where appropriate.

References
1 Wiederschain, D. et al. Single-vector inducible lentiviral RNAi system for oncology target validation. Cell cycle (Georgetown, Tex 8, 498-504 (2009).

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
2	CCRI	35.00
4	CURIE	17.50
5	DKFZ	43.00
7	UKE	42.00
8	UGENT	5.00
10	UBERN	42.00
	Total	184.50

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]
D9.1	Initial validation results for model improvement from data re-analysis	5	38.00	R	PU	48
D9.2	Functional validation of in silico predicted fragility nodes in ET cell lines	2	70.00	R	PU	60
D9.3	Correlation of drug sensitivities, GRNs and gene mutations	10	76.50	R	PU	60
		Total	184.50			

Description of deliverables

D9.1) Initial validation results for model improvement from data re-analysis: Initial validation results provided for model improvement from re-analysis of existing data and/or selected target molecule assessment in primary tumour samples on an individual basis (Mo 48) [month 48]

D9.2) Functional validation of in silico predicted fragility nodes in ET cell lines: Functional validation of in silico predicted fragility nodes in ET cell lines [month 60]

D9.3) Correlation of drug sensitivities, GRNs and gene mutations: Correlation of drug sensitivities, GRNs and gene mutations [month 60]

WT3:
Work package description

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead beneficiary number	Delivery date from Annex I [60]	Comments
MS30	Transcriptomic profiles of expanded cell line panel with dose-response curves for validating selecte	10	60	
MS31	ET cell lines with inducible tuning of entity-specific and shared fragility nodes	2	48	
MS32	Results from data re-analysis from the initial model predictions	5	48	
MS33	Molecular and phenotypic characterisation of fragility node perturbation in ET cell lines	2	54	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP10	Type of activity [54]	RTD
Work package title	Data management		
Start month	1		
End month	60		
Lead beneficiary number [55]	13		

Objectives

Data management:
This work package will provide a complete framework for all experimental data and models generated in ASSET. It will establish an interrogable project database for holding shared experimental data, and a repository for mathematical models and data integration pipelines that also exploit publicly available data. It will construct a data warehouse to support and enable combination of project proprietary data with publicly available data sources. The use of semantic technologies, where appropriate, will allow integrating and interrogating data at many levels. Data frameworks will be built with future maintenance in mind, with provisions to integrate with alternative systems, for example through the use of web services. This database will be made accessible to the scientific community at the end of the project, and represents the lasting legacy of ASSET to the scientific community.

Description of work and role of partners

Data, their integration, and their use to design and validate quantitative models are a key binding theme in this project. Technically, WP10 builds on many important advances for data integration. These include a richer and better understood set of computational tools around information structures (ontologies, controlled vocabularies and disease classification schemes), formal web services (and semantic technologies) and syntactic representations of information (XML). WP10 will also use clinical descriptions of cancer phenotypes as they can be extracted from electronic patient records to improve the search for new cancer-related genes and the protein complexes and pathways they relate to. The effort will build extensively on already existing tools and database structures, and will avoid any unnecessary duplication of effort. The work also includes a repository for all the modelling efforts, providing overview, interfaces and integration. This repository will be linked where relevant to Reactome, CellML, BioModels, DOQCS, ModelDB and similar community-wide efforts. Specifically, data management will include (i) state-of-the-art: current consortium data; (ii) data newly generated in ASSET; (iii) relevant publicly available data or links to it; (iv) models generated in ASSET with links to underlying data; and (v) primary data analysis results from ASSET. The database will also have an external interface, which allows other researchers to access the data as soon as IP is protected and results have been published.

Task 10.1. Construction of a state-of-the-art and data warehouse for ASSET. An analysis of data already generated by the consortium will include working with existing databases and data formats, understanding their semantic relationships and assessing gaps within the data as well as gaps in data connectivity and usability by the consortium for systems biology mining efforts. Similarly relevant publicly available data will be assessed. A datawarehouse will be constructed to host new data generated by the consortium as well as relevant publicly available data. Attention will be paid to facilitating queries across data types and linking to disease complexes and pathway reactions. Use of semantic web concepts will be evaluated if appropriate to enhance query possibilities. The developed database will be a shared resource of the ASSET consortium.

Task 10.2: Data mining of publicly available mutation-phenotype data, protein-protein complexes and pathways and incorporation of these data within the relational database framework. The task will include building a database of childhood cancer pathway knowledge available from publicly accessible databases with an emphasis on pathways active in the tumour types in the study. The task will involve additional annotation of selected pathway circuits and protein-protein complexes key to this project. Using clinical phenotypes based on public databases such as OMIM and electronic patient records when relevant, the WP bridges the molecular

level and the macroscopic description of cancer phenotypes in novel ways, building on recent advances in text mining and data integration1.

Task 10.3: Develop web-services and data visualisation. The aim is to make the database informative, user-friendly and appealing to a wide range of users from bioinformaticians, modellers, mathematicians and biologists. In-house development in this area will include pathway visualisation using, for instance, Cytoscape which is an open-source development framework from the Systems Biology Institute, Seattle. Various data types can be visualised within this framework as 'layers'. Further visualisation and data interaction will be developed through a suitable experienced sub-contractor. Models produced in ASSET can be connected, on a meta-level, with the use of semantic technologies. Using semantic tools, we plan to allow the researcher to study biological processes by zooming between different models at different resolutions. Web services are the current standard methodology for the integration of bioinformatics methods. The open distribution and usage of web services and the use of standard repositories guaranties the survival of the useful methods developed by the project beyond its existence. ASSET data will be exposed via suitable interfaces and web-services for the community once publications and IPR protection of relevant content has been secured.

Task 10.4: Linking the model repository for ASSET to community-wide efforts (e.g. Reactome, CellML, BioModels, DOQCS, ModelDB, etc.). This will support the modelling work within the consortium, but also facilitate dissemination to the scientific community, interactions with other modellers and collaborations beyond the project. Again, this will be a lasting ASSET legacy to the scientific community

Output of WP
This WP will summarise the project data, and provide a resource for the analysis and dissemination of the data and models produced in this project

Role of participants
All partners will provide data and descriptions of data from their work packages for inclusion in the data warehouse. UCPH (SBr) will carry out all work related in constructing the data warehouse as well as subcontracting of visualisation and custom curation. CNIO (AV) will collaborate for collecting and curating mutation-phenotype data and develop web-services. DFKZ (SBu) will assist with model collection, storage and dissemination.

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	NUID-UCD	0.50
2	CCRI	0.50
3	CEMM	0.50
4	CURIE	1.50
5	DKFZ	5.00
6	VTT	0.50
7	UKE	0.50
8	UGENT	0.50
9	UCL	0.50
10	UBERN	0.50
11	Weizmann	0.50
13	UCPH	22.00
14	CNIO	42.00
	Total	75.00

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]
D10.1	A description of sources of pathways, protein-protein complexes and mutation-phenotype data	14	24.00	R	CO	24
D10.2	A description of the project catalogue of analysis workflows and their web service components	13	51.00	R	CO	60
		Total	75.00			

Description of deliverables

D10.1) A description of sources of pathways, protein-protein complexes and mutation-phenotype data: A description of sources of pathways, protein-protein complexes and mutation-phenotype data. [month 24]

D10.2) A description of the project catalogue of analysis workflows and their web service components: A description of the project data warehouse, its relational principles, data formats, web services components and links to external sources [month 60]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS34	An analysis of state-of-the-art data and databases	13	18	
MS35	Assessment of public data that should be included with recommendations for appropriate linking	14	24	
MS36	Datawarehouse design	13	30	
MS37	A complete description of a models repository and its external links	14	54	
MS38	A webservice-based resource for the analysis of genetic and functional aberrations in ETs	13	60	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP11	Type of activity [54]	RTD
Work package title	Systems level knowledge integration and exome sequencing		
Start month	19		
End month	60		
Lead beneficiary number [55]	14		

Objectives

Systems level knowledge integration and exome sequencing:

This work package will provide a systems level approach to integrating various data from the WP10 data warehouse. Emphasis will be on mechanistic interpretations based on pathways and protein-protein interaction complexes linking the generated data with clinical phenotypes or endpoints. This workpackage aims to combine qualitative and quantitative modelling, where qualitative data integration schemes will lead to models that can be used to select and design quantitative simulation efforts targeting key pathways and temporal cancer-linked mechanisms. WP11 will, thus, address the issue of integrating data and models at two levels. Components can be linked using semantic annotation to allow viewing and component analysis in different modelling contexts. This allows, for instance, the integration of data across regulatory and signalling networks. Secondly, expanding the semantic annotations into rule-based frameworks will permit zooming in and out of models from coarse- to fine-grained resolution. A systems level analysis of data and association with phenotypes will go beyond current studies where single entity results or perturbations show limited reproducibility due to the multiple routes of signalling in a cell leading to similar end effects.

Description of work and role of partners

Task 11.1. Construction of cancer-specific protein-protein interaction networks (rewired cancer interactomes) linked to expert annotation of selected disease-related protein complexes. Integration of high-content screening data, protein interaction data, phosphoproteomics and expression profiling data and kinase inhibitor data (all of which are generated in the current project) through a pathway-centred approach will be very valuable to understand perturbation in cellular networks, as well as in putting together mechanistic understandings of drug action which would facilitate discovery of drug combinations. The approach of using orthogonal data, such as protein-protein complexes, inhibitor data and pathway subcircuits, overcomes the dilemma that dissimilar pairs of perturbations can lead to the same phenotype. Uncovering such a mechanistic understanding will provide a systems level signature that is more replicable across studies instead of a single entity association. Moreover, integration of the high-content screening data as well as kinase inhibitor data through a pathways-based approach will be very valuable in understanding perturbations in cellular networks, as well as for summarising mechanistic understandings of drug action.

Task 11.2. Interpretation and mapping variation data. This WP will produce interpretation of variation data progressively from the molecular / genetic levels (genes and proteins) to the network level, including standard and cancer-specific pathways (taken from the database developed in WP10), including predictions of protein interaction sites, promoter sites and changes in corresponding expression levels, microRNA binding sites, splicing sites and others. The resulting information will be projected onto network topologies.

Task 11.3. Exome and targeted exon sequencing of ETs. In order to have a standardised reference for data comparison and integration, this workpackage will use next-generation sequencing technology to fully sequence the exome of core cell models used in ASSET. The core panel comprises SH-SY5Y (NB) with tet-regulatable TrkA and N-Myc; UW-228 (MB) with tet-regulatable c-Myc; and A673 (ESFT) with tet-regulatable EWS-FLI1. In addition, for the ALK work, we will use SK-N-AS (NB) with regulated expression of the ALK F1174L mutant, because this accessory cell line is necessary to provide information about the role of ALK as input for evaluation in the validation WPs and the choice of inhibitor combinations chosen for NB. We will sequence the complete exomes of the core cell lines in order to survey their exact mutational status. Based on the results, we will define

a set of exons for targeted exon sequencing of at least 50 ET samples using state-of-the-art multiplexed targeted sequencing.

Task 11.4. Comparative analysis of ET and adult cancer genetic alterations as an approach to delineate driver from passenger mutations. Information gathered by ASSET on genetic alterations in ETs and cell lines and their potential consequences will be compared with mutations (CN, mutations) in other cancer types at the levels of genes, pathways and networks with the intention of extending the understanding of the specific character of ET mutations, and contributing to the elucidation of issues related to the distribution of driver mutations in adult tumours and the accumulation of passenger mutations during tumour development.

Output of WP
This WP will summarise the project data, and provide a resource for the analysis and dissemination of the data and models produced in this project. New hypotheses will be generated from the systems biology level knowledge integration that will be used to propose specific validation experiments.

Role of participants
SBr (UCPH) will establish context specific protein-protein interaction networks and pathway subcircuits with custom curation. Integrative analysis and evidence layers relating variations to phenotypes will be constructed by SBr and AV. MG (UCL) will provide mathematical modelling to infer novel connections and interactions in signalling networks. SBu (DKFZ) will construct models for integrating regulatory and signalling networks as well as a framework for inference based analysis. The output of integrative data analysis will suggest mechanistic hypotheses, provide markers and suggestions for further validation as well as suggestions for individualised intervention. AZ (CURIE) will participate in the integrative data analysis and mathematical modelling and will provide complementary computational tools such as BiNoM Cytoscape plug-in (2).

References
1. Lage, K. et al. A human phenome-interactome network of protein complexes implicated in genetic disorders. Nat. Biotechnol 25, 309-16 (2007).
2. Zinovyev, A., Viara, E., Calzone, L. & Barillot, E. BiNoM: a Cytoscape plugin for manipulating and analyzing biological networks. Bioinformatics 24, 876-877 (2008).
3. Baudot, A., Real, F. X., Izarzugaza, J. M. & Valencia, A. From cancer genomes to cancer models: bridging the gaps. EMBO Rep 10, 359-366 (2009).

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	NUID-UCD	2.00
4	CURIE	5.00
5	DKFZ	6.00
13	UCPH	15.00
14	CNIO	21.20
	Total	49.20

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]
D11.1	A description of a data integration pipeline facilitating query and linking to disease complexes and	13	25.00	R	PU	36

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]
D11.2	A description of a specific genetic alterations and the corresponding influenced pathways/network co	14	24.20	R	PU	60
		Total	49.20			

Description of deliverables

D11.1) A description of a data integration pipeline facilitating query and linking to disease complexes and: A description of a data integration pipeline facilitating query and linking to disease complexes and pathway reactions [month 36]

D11.2) A description of a specific genetic alterations and the corresponding influenced pathways/network co: A description of a specific genetic alterations and the corresponding influenced pathways/network components of ETs in comparison to other tumour types [month 60]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS39	Construction of cancer-specific protein-protein complexes	14	24	
MS40	Construction of data integration pipeline	13	30	
MS41	Description of integrative models	4	48	
MS42	Description of generated hypotheses with their validation status	13	60	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP12	Type of activity [54]	MGT
Work package title	Project Management		
Start month	1		
End month	60		
Lead beneficiary number [55]	1		

Objectives

Project Management:
To ensure an efficient project coordination and to achieve the project objectives and goals, the management is divided into the following sections:
1. Project start
2. Day-to-day management
3. Project monitoring: technical and financial reporting
4. Dissemination
5. Other management-related issues: coordination of and decision-making on topics regulated by Annex II and by the consortium agreement
6. Project end and planning of follow-up

The management organization is described in detail in Part 2.1.

Description of work and role of partners

Task 12.1. Project start
The following management activities will be undertaken at project start:
1. The consortium agreement will be generated based on the DESCA model agreement for large collaboration projects. UCD will collect individual additions/amendment recommendations from the participants' representatives and their corresponding legal/patent offices.
2. The scientific advisory board will be set up
3. The Industrial Liaison and Exploitation board will be set up. This activity will be supported by the SBI business development manager as in-kind contribution from UCD.
4. Templates for reporting (incl. deliverables, milestone, finances, dissemination) will be generated and provided to the members of the PIs
5. The network of the project (related projects and scientific groups, members of the industry, clinicians, funding agencies, etc.) will be defined based on input from all participant groups. The list will be used for dissemination, networking activities and identification of synergistic collaborations.
6. The specifications of the project website will be defined. To optimise functionality and design we will subcontract a professional web designer according to the national regulations and to the organisational guidelines at UCD.
7. The kick-off event will be organised and hosted by UCD. Within its frame, the following meetings will take place for the first time: the general assembly meeting, the scientific meeting, and the scientific advisory board meeting. Further, a scientific workshop with invited international speakers will be organised to celebrate the project launch and publicise the project.
8. The kick-off meeting will be communicated to the public media, members of the paediatric cancer research and systems biology communities by means of a coordinated press release involving at least the organisations of the project management team (UCD, CCRI, UCL, CEMM, UKE).

Task 12.2 Ongoing management
UCD is responsible for the ongoing scientific, financial and administrative management. In order to ensure an efficient and active management of the financial, administrative and dissemination tasks, the Project Coordinator (PC) will be supported by the SBI grants manager (GM), Lauren Montague, and part-time by the SBI education

& outreach officer, Philip Smyth (the latter is an in-kind contribution of UCD). The subproject leaders (SPLs) will assist with scientific management and implementation of the scientific plans.

The project management team (PMT) consisting of the PC, GM and the SPLs will have the following tasks:

1. Managing all aspects of the reporting, including the delivery and follow-up of administrative and financial documents
2. Creating common working and reporting tools
3. Being a permanent contact point for all partners regarding their participation in the project, and responding to any relevant requests
4. Liaising with the EC on behalf of the consortium
5. Implementing any changes in the scientific direction or organisation of the project, e.g. changes to the consortium agreement, changes of participant status.
6. Following and updating the project indicators (Gantt chart, deliverables, milestones).The aim is to ensure that the technical objectives are followed, but also the project is completed within the approved budget.
7. Ensuring the flow of communication within the consortium. This includes keeping the project webpage updated; organising a quarterly project newsletter; notifying the consortium of due dates; making the consortium aware of relevant meetings and conferences. This and the following subtasks will closely work with Task 18.4 “internal dissemination”.
8. Organising the researchers’ exchange programme between participants
9. Organising the annual project meetings and assist with organising interim meetings as required

Task 12.3. Project monitoring
The main tool of monitoring project progress towards targets and expenditure will be periodical written reports.

Frequency of reporting
In order to ensure that the project management is kept up to date with the current state and progress SPLs will collect short biannual reports from the WP leaders and provide them to the PC. Full reports to the Commission in the format set by the Commission will be provided according to the frequency set up in the grant agreement.

Financial management and reporting
The PC will take the necessary measures to ensure the appropriate use of the FP7 grant between the participants by providing a time schedule for transferring of funds allocated within the consortium. The work will be done in collaboration, via agreed procedures in the consortium agreement. “Easy-to-use” templates for time and cost reporting will be implemented.

The consortium will follow the FP7 requirements for cost controlling,, including:
1. Monitoring cost performance to detect deviations from plan (regular follow-up by the PC),
2. Ensuring that all appropriate changes are recorded accurately in the cost baseline;
3. Preventing incorrect, inappropriate or unauthorised changes to the contract);

The PC will notify partners of due dates for financial reporting, provide support for completion of financial reports (and certificates on financial statements if relevant) and will collect documents for submission to the relevant EU Commission body.

The PC will be a day-to-day contact and serve as a central resource for the whole consortium to provide answers to queries such as costs eligibility, financial reporting and the official process for fund transfer. It will ensure that EC rules are respected for cost reporting on the basis of the information provided by the partners.

Technical reporting
An efficient reporting (complete and on time) will be ensured by
1. Using common templates as specified by the EU Commission;
2. notifying due dates and deadline reminders;
3. assisting partners to respect indications and guidelines assigned by FP7;
4. collecting and consolidating the reports (WP leaders → SP leaders or COU leaders → UCD),
5. representing the central contact point for checking that the data provided are in line with the FP7 rules and requirements.
.
The PC, with the support of the project management team, will consolidate the progress, deliverables and milestone reports to be submitted to the Commission. In addition, SP and WP leaders will be requested to

update the Coordinator every six months with a short written report. It is their responsibility to track any deviation and delay, and propose appropriate solutions. It will ensure that the PC is kept well informed about the progress of the project by all partners.

Task 12.4. Dissemination
While a main part of dissemination will occur at the level of individual research groups through publications in scientific journals and seminars and lectures at conferences, this task will deal with all dissemination activities that are project-wide. SBI has a dedicated education and outreach officer, Philip Smyth, who will be appointed to assist with ASSET outreach activities on a part-time basis (as in-kind contribution from UCD). In addition, SBI's business and development officer (official search in progress), will support the industrial liaison and dissemination of results to the biotech and pharmaceutical Industry (also on a part-time basis and as in-kind contribution from UCD).

The goals of dissemination in ASSET are (i) to communicate ASSET's mission and achievements to the scientific community (primarily scientists devoted to cancer research and systems biology) the industry and further network members (regulatory authorities, clinicians, etc.); and (ii) to position ASSET as a poster child for the successful integrative approach of experimental and systems research.

Specifically, project-wide, external dissemination tasks will include:
Establishment of a public interactive website. The PMT will provide regular updates and site maintenance.
Collection of publication references and publications on the public website. Whenever publishing rights allow, placement of open access publications on the website.
Organisation of press packages at timepoints in accordance with the achievement of selected milestones
Creation of a project identity and brand name, including logo, tag line and brochure order to promote the project and facilitate dissemination.
Liaison with industry and targeted showcasing of project results to industry to raise awareness, initiate collaborations and enable European industries to take instant advantage of ASSET's findings.
Organisation of three international systems biology workshops in conjunction with annual project meetings

Specifically, project-wide, internal dissemination tasks will include:
Organisation of the researchers' exchange programme between participant groups.
Stimulation of internal communication by arranging teleconferences and Skype-meetings
Quarterly teleconference meetings of the PMT
Issuing a quarterly ASSET Newsletter informing about current activities
Establish a dedicated backstage virtual space website that will facilitate the sharing of documents, discussions about results and plans, and organisation of ad hoc meetings.
Organisation of the annual meetings
Help with organising local meetings and meetings of SP or smaller working groups.
Assistance with exploitation and IPR protection if needed through SBI's business development manager.

Task 12.5. Other management related issues
The PC, in collaboration with the General Assembly and Scientific Advisory Board will coordinate further topics that will be addressed in the course of the project e.g. strategy towards related projects and potential synergies, development of further opportunities from results, especially at the end of the project, gender equity, coordinated approach to regulatory authorities if relevant, and matters that will be regulated by the consortium agreement i.e. IPR, exploitation, liabilities, changes of the consortium composition, amendments of the project i.e. financial allocation or content.

Task 12.6. Project end and planning of follow-up
At the project end the management team will ensure the proper wind-down of the project including:
Preparation of all final reports to the Commission
Organisation of the final technical meeting and the general assembly meeting
where final results and the project execution in total will be reflected
further collaborations and new joint initiatives will be discussed
Ensuring that the project data will be made publically available, in particular the mathematical models, the data warehouse and the associated data mining and semantic knowledge integration tools.

WT3:
Work package description

In kind contributions to this work package include: CCRI (HK) CEMM (GSF) UKE (AE) and UCL (MG)

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	NUID-UCD	19.00
	Total	19.00

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]
D12.1	Project Websites On-line	1	8.00	O	PU	6
D12.2	Review Meetings	1	7.00	R	RE	30
D12.3	Final report	1	4.00	R	PU	60
		Total	19.00			

Description of deliverables

D12.1) Project Websites On-line: Public and restricted websites online [month 6]

D12.2) Review Meetings: Review meetings organised and held [month 30]

D12.3) Final report: Final report delivered [month 60]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS43	Consortium agreement signed	1	3	
MS44	Appointment of the Boards	1	6	Appointment of the Industrial Liasion and Exploitation board
MS45	Establishment of project website	1	6	
MS46	Organization of Annual Project meetings	1	2	meetings to be held months 2, 12, 24, 36, 48 and 60
MS47	Summary on follow-up plans	1	60	

WT3:
Work package description

Project Number [1]	259348	Project Acronym [2]	ASSET

One form per Work Package

Work package number [53]	WP13	Type of activity [54]	OTHER
Work package title	Training		
Start month	1		
End month	60		
Lead beneficiary number [55]	1		

Objectives

Training:
Systems biology is an inherently highly interdisciplinary field. One of the biggest obstacles in the field are the barriers between the theoretical and experimental branches, which include differences in the scientific culture, language, thinking and hence can compromise successful collaborations. On the other hand, this difficult interface can also produce the creative friction and enlightening sparks that drive innovative science. Therefore, ASSET will introduce training programme to break down these barriers and encourage the innovative aspects. The training programme aims to facilitate the communication and active engagement between the wet and dry disciplines. Therefore, while it mainly targets PhD students and postdoctoral scientists, it also will engage PIs.

Description of work and role of partners

ASSET will offer a dynamic scientific environment for students and scientists at all stages of their career, both in a broad context as well as with a focus on project specific issues. The training programme is an integral part of this offering. It will include:

Task 13.1. A monthly lecture series will be used to highlight state of the art discoveries and the big questions in the fields of ET-research and systems biology. These lectures will be given by PIs and invited eminent scientists. They will be broadcast to project partners via videolinks and presentations will be posted in the restricted area of the project website.

Task 13.2. On demand sessions. In addition to the regular programme we will introduce on demand sessions where topics are suggested by researchers as need and interest arises. These can be question and answers sessions, or lectures on specific requested topics. Video conferencing will be used and the sessions will be made available in the project restricted area of the webpage.

Task 13.3. In a Quarterly round-table discussions will affress project-related scientific topics. These can include presentation of research highlights and achieved milestones, but also discussion of scientific challenges and problems. Again we will use videolinks for ease of participation.

Task 13.4. Must haves. PIs and senior scientists of ASSET will contribute to the education and knowledge of students and postdocs by sending a must have list of references to the outreach officer on a quarterly basis. He will then compile the list of references and the corresponding abstracts and distribute the must-haves through the newsletters. Each series will be made available at the project website.

Task 13.5. Biosystems workshops. ASSET will organize three in–depth workshops held in conjunction with project meetings in the spring of year 1 (opening of the new UCD-SBI building), at mid-term and at the end of the project targeting PhD students and young postdocs. Selected topics could be:
“(i) “Mathematical modelling in cancer biology” hosted by DKFZ in conjunction with the International Conference on Systems Biology (ICSB 2011) in Heidelberg, Germany
(ii) “Modelling and –omics technologies” or “ETs-biology and clinical treatment “ hosted by CCRI
(iii) “From systems biology towards systems medicine” hosted by UCD.

Lectures will be delivered by senior scientists and by invited guest speakers. Interactions with other scientific research institutions will provide opportunities to extend the students’ scope beyond the research topics investigated in ASSET. In order to provide junior workshop attendants the opportunity to present their work (i.e.

posters or oral presentations) within an international field, the final workshop will be organized as a satellite event to an international symposium within the field.

Task 13.6. Circuit training. Based on individual requirements and aspirations, PhD students and postdocs will be given the opportunity to visit collaborating participant groups. In particular, we will encourage “crossover” visits, where a dry partner visits a wet group and vice versa. These circuit training will facilitate communication, widen the horizon of the participating researchers and help build team spirit.

Task 13.7. Basics of knowledge and technology transfer. These training sessions will give students and postdocs insight into the basic aspects of knowledge and technology transfer, and different models of partnerships between academia and industry. This field is becoming ever more important but is not well covered by accompanying training opportunities. We expect that this training will encourage young scientists to become proactive in this area and stimulate entrepreneurial thinking. These courses will be given by UCD and CEMM via videolink. If the demand is very high, we will consider organising a life workshop in year 4.

Task 13.8 Biosystems knowledge transfer to experimentalists. Experimental research groups will have the opportunity to obtain training in state-of-the art systems biology algorithms and to build/increment their expertise in the field.

Person-Months per Participant

Participant number [10]	Participant short name [11]	Person-months per participant
1	NUID-UCD	3.00
2	CCRI	1.00
3	CEMM	1.00
4	CURIE	2.00
5	DKFZ	3.00
6	VTT	1.00
7	UKE	1.00
8	UGENT	1.00
9	UCL	1.00
10	UBERN	1.00
11	Weizmann	1.00
12	ZEPTO	0.25
13	UCPH	1.00
14	CNIO	1.00
	Total	18.25

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]
D13.1	Establishment of efficient training structures	1	16.25	O	PU	12

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]
D13.2	Organisation of the Biosystems workshops 1	1	2.00	O	PU	60
		Total	18.25			

Description of deliverables

D13.1) Establishment of efficient training structures: Establishment of efficient training structures [month 12]

D13.2) Organisation of the Biosystems workshops 1: Organisation of the Biosystems workshops [month 60]

Schedule of relevant Milestones

Milestone number [59]	Milestone name	Lead benefi-ciary number	Delivery date from Annex I [60]	Comments
MS48	Establishment of videolinked lecture series	1	6	
MS49	Establishment of videolinked round table and on-demand sessions	1	12	
MS50	Establishment of the circuit training	1	12	
MS51	Establishment of the knowledge and technology transfer courses	1	24	

WT4:
List of Milestones

Project Number [1]	259348	Project Acronym [2]	ASSET

List and Schedule of Milestones

Milestone number [59]	Milestone name	WP number [53]	Lead beneficiary number	Delivery date from Annex I [60]	Comments
MS1	Generation,normalisation and preparation of existing and new data and assessment of data information	WP1	4	18	
MS2	Reconstruction of prior GRN and motif structures	WP1	9	30	
MS3	Production of posterior GRN and motif structures	WP1	9	36	
MS4	GRN and motif structures for EWS-FLI1	WP1	2	36	
MS5	GRN and motif structures for N-Myc and c-Myc	WP1	5	48	
MS6	List of selective growth inhibitory compounds between isogenic ET cell lines	WP2	6	48	
MS7	Identification of EWS-FLI1 and Myc downstream genes regulating ET cell survival	WP2	4	48	
MS8	A map of drug/siRNA interactions and refined network models explaining synergistic drug/siRNA effect	WP2	6	60	
MS9	Data integration in order to mine a large dataset of miRNA profiles from ETs.	WP3	4	30	
MS10	A combined biological and mathematical model of miRNA function in ET cells	WP3	2	60	
MS11	Changes of proteome expression profile induced	WP3	7	60	

WT4:
List of Milestones

Milestone number [59]	Milestone name	WP number [53]	Lead beneficiary number	Delivery date from Annex I [60]	Comments
	by Myc and TrkA regulated miRNAs				
MS12	Biochemical interaction maps of TFs altered in ETs	WP4	1	36	
MS13	Validated interaction maps of selected ET TFs	WP4	8	42	
MS14	Validated GRN models incorporating TF interaction data	WP4	5	60	
MS15	Identification of differentially regulated phosphorylation sites between NB cell lines	WP5	13	30	
MS16	Quantitative dynamic Interactome of TrkA and ALK receptor tyrosine kinases	WP5	1	36	
MS17	Dataset of all absolution quantitation of all selected ALK and TrkA network components	WP5	1	48	
MS18	Kinetic core model of Myc network	WP6	1	36	
MS19	Fragility analysis of the Myc network	WP6	5	48	
MS20	Validated kinetic model p53-Mdm2/MDMX module in NB cell lines	WP7	5	18	
MS21	Validated kinetic model CDK-Rb-E2F-Skp2 module in NB cell lines	WP7	5	24	
MS22	Integrated model of the core network governing proliferation versus apoptosis decisions in NB	WP7	4	36	

WT4:
List of Milestones

Milestone number [59]	Milestone name	WP number [53]	Lead beneficiary number	Delivery date from Annex I [60]	Comments
MS23	In silico quantification of effect of individual network nodes and pairs of nodes on proliferative a	WP7	1	48	
MS24	In silico adaptation of the model to ESFT and MB	WP7	4	60	
MS25	Dose response curves of 11 selected kinase inhibitors in a well characterised cell line panel	WP8	3	18	
MS26	Synergistic pairwise drug combinations based on the most potent single drug	WP8	10	24	
MS27	Panel of validated drug analogues suitable for immobilisation	WP8	3	24	
MS28	Target profiles for synergistic drugs	WP8	3	54	
MS29	Global and quantitative drug-induced changes of the phosphoproteome for synergistic combinations	WP8	13	60	
MS30	Transcriptomic profiles of expanded cell line panel with dose-response curves for validating selecte	WP9	10	60	
MS31	ET cell lines with inducible tuning of entity-specific and shared fragility nodes	WP9	2	48	
MS32	Results from data re-analysis from the initial model predictions	WP9	5	48	
MS33	Molecular and phenotypic characterisation	WP9	2	54	

WT4:
List of Milestones

Milestone number [59]	Milestone name	WP number [53]	Lead beneficiary number	Delivery date from Annex I [60]	Comments
	of fragility node perturbation in ET cell lines				
MS34	An analysis of state-of-the-art data and databases	WP10	13	18	
MS35	Assessment of public data that should be included with recommendations for appropriate linking	WP10	14	24	
MS36	Datawarehouse design	WP10	13	30	
MS37	A complete description of a models repository and its external links	WP10	14	54	
MS38	A webservice-based resource for the analysis of genetic and functional aberrations in ETs	WP10	13	60	
MS39	Construction of cancer-specific protein-protein complexes	WP11	14	24	
MS40	Construction of data integration pipeline	WP11	13	30	
MS41	Description of integrative models	WP11	4	48	
MS42	Description of generated hypotheses with their validation status	WP11	13	60	
MS43	Consortium agreement signed	WP12	1	3	
MS44	Appointment of the Boards	WP12	1	6	Appointment of the Industrial Liasion and Exploitation board
MS45	Establishment of project website	WP12	1	6	
MS46	Organization of Annual Project meetings	WP12	1	2	meetings to be held months 2, 12, 24, 36, 48 and 60
MS47	Summary on follow-up plans	WP12	1	60	

WT4:
List of Milestones

Milestone number [59]	Milestone name	WP number [53]	Lead beneficiary number	Delivery date from Annex I [60]	Comments
MS48	Establishment of videolinked lecture series	WP13	1	6	
MS49	Establishment of videolinked round table and on-demand sessions	WP13	1	12	
MS50	Establishment of the circuit training	WP13	1	12	
MS51	Establishment of the knowledge and technology transfer courses	WP13	1	24	

WT5:
Tentative schedule of Project Reviews

Project Number [1]	259348	Project Acronym [2]	ASSET

Tentative schedule of Project Reviews

Review number [65]	Tentative timing	Planned venue of review	Comments, if any
RV 1	30	Dublin, Ireland	RV1 - Project Review, mid-term

WT6:
Project Effort by Beneficiary and Work Package

Project Number [1]	259348	Project Acronym [2]	ASSET

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	WP 9	WP 10	WP 11	WP 12	WP 13	Total per Beneficiary
1 - NUID-UCD	0.00	0.00	36.00	45.75	30.00	47.75	23.00	0.00	0.00	0.50	2.00	19.00	3.00	207.00
2 - CCRI	18.50	0.00	34.00	13.00	0.00	0.00	12.00	0.00	35.00	0.50	0.00	0.00	1.00	114.00
3 - CEMM	0.00	5.00	0.00	0.00	0.00	0.00	0.00	80.50	0.00	0.50	0.00	0.00	1.00	87.00
4 - CURIE	14.00	38.00	20.00	0.00	0.00	0.00	22.00	0.00	17.50	1.50	5.00	0.00	2.00	120.00
5 - DKFZ	46.00	18.00	0.00	19.00	0.00	30.00	106.00	0.00	43.00	5.00	6.00	0.00	3.00	276.00
6 - VTT	0.00	39.00	16.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	1.00	56.50
7 - UKE	0.00	0.00	42.00	6.00	18.00	5.00	5.50	0.00	42.00	0.50	0.00	0.00	1.00	120.00
8 - UGENT	6.00	0.00	12.00	11.50	0.00	0.00	0.00	0.00	5.00	0.50	0.00	0.00	1.00	36.00
9 - UCL	48.00	0.00	0.00	5.00	0.00	18.00	0.00	0.00	0.00	0.50	0.00	0.00	1.00	72.50
10 - UBERN	20.00	16.00	13.50	0.00	0.00	0.00	0.00	12.00	42.00	0.50	0.00	0.00	1.00	105.00
11 - Weizmann	0.00	106.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	1.00	108.00
12 - ZEPTO	0.00	0.00	0.00	0.00	23.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	24.00
13 - UCPH	0.00	6.00	0.00	0.00	60.00	0.00	0.00	18.00	0.00	22.00	15.00	0.00	1.00	122.00
14 - CNIO	0.00	0.00	0.00	12.00	0.00	0.00	0.00	15.00	0.00	42.00	21.20	0.00	1.00	91.20
Total	152.50	228.50	173.50	112.25	131.75	100.75	168.50	125.50	184.50	75.00	49.20	19.00	18.25	1,539.20

WT7:
Project Effort by Activity type per Beneficiary

Project Number [1]	259348	Project Acronym [2]	ASSET

Indicative efforts per Activity Type per Beneficiary

Activity type	Part. 1 NUID-UC	Part. 2 CCRI	Part. 3 CEMM	Part. 4 CURIE	Part. 5 DKFZ	Part. 6 VTT	Part. 7 UKE	Part. 8 UGENT	Part. 9 UCL	Part. 10 UBERN	Part. 11 Weizman	Part. 12 ZEPTO	Part. 13 UCPH	Part. 14 CNIO	Total
1. RTD/Innovation activities															
WP 1	0.00	18.50	0.00	14.00	46.00	0.00	0.00	6.00	48.00	20.00	0.00	0.00	0.00	0.00	152.50
WP 2	0.00	0.00	5.00	38.00	18.00	39.00	0.00	0.00	0.00	16.00	106.50	0.00	6.00	0.00	228.50
WP 3	36.00	34.00	0.00	20.00	0.00	16.00	42.00	12.00	0.00	13.50	0.00	0.00	0.00	0.00	173.50
WP 4	45.75	13.00	0.00	0.00	19.00	0.00	6.00	11.50	5.00	0.00	0.00	0.00	0.00	12.00	112.25
WP 5	30.00	0.00	0.00	0.00	0.00	0.00	18.00	0.00	0.00	0.00	0.00	23.75	60.00	0.00	131.75
WP 6	47.75	0.00	0.00	0.00	30.00	0.00	5.00	0.00	18.00	0.00	0.00	0.00	0.00	0.00	100.75
WP 7	23.00	12.00	0.00	22.00	106.00	0.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	168.50
WP 8	0.00	0.00	80.50	0.00	0.00	0.00	0.00	0.00	0.00	12.00	0.00	0.00	18.00	15.00	125.50
WP 9	0.00	35.00	0.00	17.50	43.00	0.00	42.00	5.00	0.00	42.00	0.00	0.00	0.00	0.00	184.50
WP 10	0.50	0.50	0.50	1.50	5.00	0.50	0.50	0.50	0.50	0.50	0.50	0.00	22.00	42.00	75.00
WP 11	2.00	0.00	0.00	5.00	6.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.00	21.20	49.20
Total Research	185.00	113.00	86.00	118.00	273.00	55.50	119.00	35.00	71.50	104.00	107.00	23.75	121.00	90.20	1,501.95
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	0.00	0.00	0.00	0.00
3. Consortium Management activities															
WP 12	19.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.00
Total Management	19.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.00

WT7:
Project Effort by Activity type per Beneficiary

4. Other activities															
WP 13	3.00	1.00	1.00	2.00	3.00	1.00	1.00	1.00	1.00	1.00	1.00	0.25	1.00	1.00	18.25
Total other	3.00	1.00	1.00	2.00	3.00	1.00	1.00	1.00	1.00	1.00	1.00	0.25	1.00	1.00	18.25

Total	207.00	114.00	87.00	120.00	276.00	56.50	120.00	36.00	72.50	105.00	108.00	24.00	122.00	91.20	1,539.20

WT8:
Project Effort and costs

Project Number [1]	259348	Project Acronym [2]	ASSET

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	NUID-UCD	207.00	1,092,819.00	12,000.00	268,805.00	816,974.40	2,190,598.40	0.00	1,724,172.80
2	CCRI	114.00	469,660.00	6,000.00	200,000.00	401,796.00	1,077,456.00	0.00	822,392.00
3	CEMM	87.00	325,213.00	6,000.00	169,000.00	578,878.00	1,079,091.00	0.00	815,818.25
4	CURIE	120.00	600,000.00	6,000.00	104,000.00	422,400.00	1,132,400.00	0.00	860,800.00
5	DKFZ	276.00	1,167,500.00	0.00	344,000.00	899,285.00	2,410,785.00	0.00	1,815,838.75
6	VTT	56.50	340,003.00	4,000.00	110,000.00	419,541.00	873,544.00	0.00	660,908.00
7	UKE	120.00	340,000.00	6,000.00	317,600.00	394,560.00	1,058,160.00	0.00	800,320.00
8	UGENT	36.00	246,000.00	6,000.00	429,000.00	405,000.00	1,086,000.00	0.00	828,000.00
9	UCL	72.50	343,634.00	8,000.00	53,000.00	237,980.40	642,614.40	0.00	494,760.80
10	UBERN	105.00	425,000.00	6,000.00	174,000.00	359,400.00	964,400.00	0.00	730,800.00
11	Weizmann	108.00	265,760.00	5,200.00	135,000.00	240,456.00	646,416.00	0.00	486,912.00
12	ZEPTO	24.00	334,000.00	7,000.00	144,000.00	33,000.00	518,000.00	0.00	284,500.00
13	UCPH	122.00	749,774.00	79,800.00	232,024.00	589,078.80	1,650,676.80	0.00	1,254,159.60
14	CNIO	91.20	394,860.00	1,400.00	65,000.00	91,972.00	553,232.00	0.00	419,774.00
	Total	1,539.20	7,094,223.00	153,400.00	2,745,429.00	5,890,321.60	15,883,373.60	0.00	11,999,156.20

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.

1. 3. PART B

Table of Contents

B1. Concept and objectives, progress beyond state-of-the-art, S/T methodology and work plan

B1.1 Concept and objectives

B1.1.1 Concept

What halts progress in cancer research? Tumourigenesis involves complex genetic alterations impeding the definition of clear mechanistic principles to develop targeted therapeutic strategies. Damage caused by multiple environmental and endogenous sources accumulates many decades before cancer onset in the adult. Thus, it is difficult to delineate clear pathogenetic models and mechanistic insights on a molecular level. However, such knowledge is critical to develop rational approaches for therapeutic interventions, in particular, to exploit the armoury of signal transduction inhibitors that is now becoming available. Malignant tumours occurring in children offer a unique chance to elaborate and investigate core mechanisms by minimising noise from passenger mutations accumulated during a long lifespan. ASSET applies a systems level approach to study embryonal tumours (ET), which are dysontogenetic tumours whose pathological features resemble those of the developing organ or tissue of origin and include the entities neuroblastoma (NB), medulloblastoma (MB) and Ewing sarcoma family tumours (ESFT). These tumours arise early in infants and young children. Therefore, they are less burdened by passenger mutations and may reveal the pathogenetically relevant driver mutations more clearly than adulthood tumours. This is the first hypothesis of ASSET.

ETs seem to share common aberrations in core signalling networks with "modulator" pathways determining disease-specific manifestations. This is suggested by previous work (Gustafson and Weiss ; Eggert, Ho et al. 2000; Kovar 2003; Woo, Lucarelli et al. 2004; Pession and Tonelli 2005; Ban, Bennani-Baiti et al. 2008; Westermann, Muth et al. 2008; Zwerner, Joo et al. 2008; Brodeur, Minturn et al. 2009; Grotzer, Castelletti et al. 2009; von Bueren, Shalaby et al. 2009) and also is a salient outcome of a recently finished FP6 project, the European Embryonal Tumour Pipeline (EETP; http://www.eet-pipeline.eu/). This is the second hypothesis ASSET builds on. Combining state-of-the-art genomics, proteomics and mathematical modelling, ASSET will analyse ETs with the aim to deconvolute the plethora of molecular pathogenetic cancer aetiologies to the common core principles. If this hypothesis is correct, it will make phenotypically diverse cancers accessible to therapeutic approaches by targeting the shared core networks. ASSET will test this hypothesis by (i) predictive models that identify these network vulnerabilities; and by (ii) rationally designed screens for inhibitors (drugs, siRNAs) that target these vulnerabilities. The resulting information on drugs/siRNAs and efficacious combinations in ETs will be the main practical output of ASSET. It should be noted that while ASSET focuses on examining this hypothesis in respect to ETs, a positive validation could also open completely new concepts for the treatment of adulthood cancers by shifting the focus from trying to address the diversity of cancers towards a focus on finding and exploiting the commonalities.

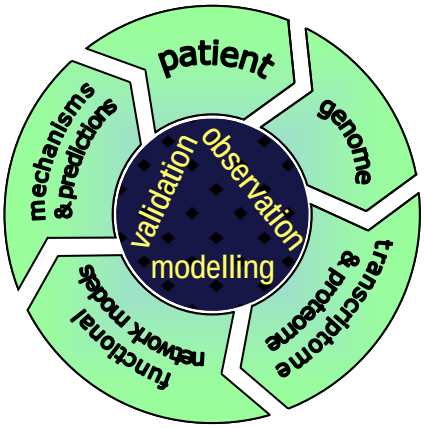
B1.1.2 Objectives

ASSET objectives arise from four central hypotheses motivating this project:

1. As ETs arise early in life, pathogenetic lesions that drive tumourigenesis are less confounded by passenger mutations and can be uncovered more easily than in adulthood tumours
2. Despite their clinical heterogeneity ETs share common functional aberrations, which may enable definition of a pathogenetic ET core signalling network
3. The deregulation of a core network is essential for ET pathogenesis, while the disease-specific features are manifestations of modifying factors
4. Mathematical models can be used to identify individual or combinatorially critical points of vulnerability in networks, which lend themselves as ideal targets for drugs

ASSET operates a systems biology-driven discovery and validation engine to achieve the following objectives:

- the combined analysis of genomic mutations, transcriptome, miRNA expression and dynamic proteome changes
- mathematical modelling to elucidate molecular pathogenetic networks and their emergent properties
- systematic perturbations to probe and refine these networks
- implementation of a virtuous cycle of model making and validation in relevant biological model systems (cell culture models and preclinical mouse models) and clinical samples



These research activities converge towards a common goal. ASSET's major goal is to identify mechanistically understood network vulnerabilities that can be exploited for new approaches to the diagnosis and treatment of major paediatric tumours.

Elucidating such core mechanisms will (i) improve the understanding of and therapeutic options for these devastating childhood malignancies, and (ii) inform a rational approach to deal with the complexity of the pathogenesis of adulthood cancers. Several single targeted drugs with promising clinical activity have already been approved for the treatment of advanced cancer types. However, most single agents fail to induce complete responses, and the treated patients often develop resistance during therapy. Here, we will go beyond these initial targeted approaches to identify intelligent and complementary combinations of targeted agents based on mechanistic insights into ET-specific signalling networks. This approach matches therapy to genetic and functional aberrations, and represents the personalised medicine needed to increase treatment responses and to overcome therapy resistance induced by single-agents.

As a result, we expect to obtain comprehensive insight into pathogenetic mechanisms of ETs based on validated computational models that are useful for (i) identifying fragile nodes where pharmacological interference will have maximal disease-specific effects while minimising side effects, (ii) improving therapeutic stratification of patients by molecular functional features and (iii) guiding the search for similar "core pathogenetic networks in adulthood cancers.

General Objectives

ASSET operates a systems biology driven discovery and validation engine that will enable

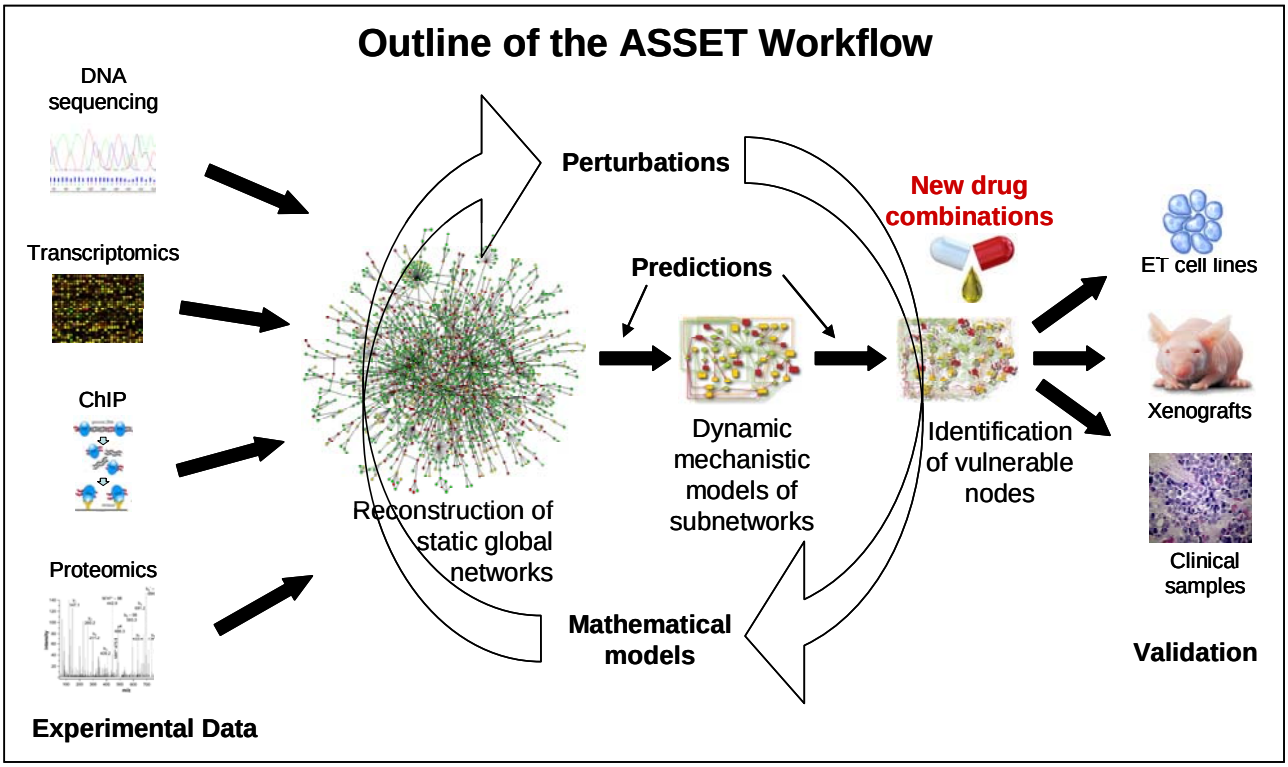
- The combined analysis of genomic mutations, transcriptome, miRNA expression and dynamic proteome changes
- Mathematical modelling to elucidate pathogenetic networks and their emergent properties
- Systematic perturbations to probe and refine these networks
- The virtuous cycle of model making and validation in relevant biological model systems (cell culture models and preclinical mouse models) and clinical samples

Specific Objectives

The general conceptual flow of the project is to start from the reconstruction of global, but static networks (GRN networks based on existing EETP data and protein signalling networks based on the quantitative proteomics experiments performed in ASSET), then use large-scale and targeted perturbation analysis to validate the network topologies, and in combination with model predictions, extract functionally important subnetworks that subsequently will be modelled using dynamic, kinetic methods. The latter models will allow us to analyse emergent network properties and simulate the behaviour of networks to perturbances. This analysis will be deployed to identify nodes that are individually, or in combination, vulnerable to interference by drugs or siRNAs. These predictions will be tested in ET cell models with conditional oncogene expression, preclinical xenograft

models and clinical specimens. The data throughout the project are collected in a data warehouse in a semantically linked form so that both experimental data and models are tractable and amenable to meta-analysis. As a result, we expect to achieve a rational prediction and validation of drug sensitivities in pathogenetic ET signalling networks.

This workflow is schematically represented in the overview scheme.



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

State-of-the-art

ETs are a group of childhood tumours with a heterogeneous clinical appearance and prognosis, but sharing the same morphology of so-called small blue round cell tumours and a similar biology. They provide unique features of tumour biology including a high incidence of spontaneous regression and differentiation. However, aggressive forms are highly lethal. They often respond well to initial therapy, but become resistant and progress. Although ETs are rare diseases, cancer is still a leading cause of death for children in developed countries. In this proposal, we will focus on three highly aggressive tumour entities with a particularly poor outcome in the disseminated stages of disease: neuroblastoma (NB), medulloblastoma (MB) and Ewing sarcoma family tumours (ESFT). We will briefly describe the disease entities and what is known about the molecular signalling pathways that are disturbed as a consequence of genetic aberrations.

Neuroblastoma

NB arises from the primitive precursor cells of the sympathetic nervous system, and most commonly affects the adrenal glands or paraspinal sympathetic ganglia. It is the most common childhood cancer in infancy accounting for 7–10% of all childhood cancers and 15% of all cancer deaths in children. The clinical presentation and prognosis is highly variable (Brodeur 2003; Schwab, Westermann et al. 2003). A remarkably large subset of tumours undergoes spontaneous regression or differentiation while others show relentless progression. Around half of all cases are currently classified as high-risk for disease relapse, with overall survival rates less than 40% despite intensive multimodal therapy. Accumulating evidence suggests that at least three biological subtypes of NB can be distinguished (Brodeur 2003; Maris, Hogarty et al. 2007). The first group, which is characterised by

elevated TrkA expression, low tumour stage, a hyperdiploid karyotype and lack of structural chromosomal changes, is concomitant with a favourable prognosis due to a high frequency of spontaneous regression or differentiation. A second group of intermediate prognosis includes patients with near-diploid or tetraploid tumours, low TrkA expression, and structural chromosomal anomalies, but lacking *MYCN* gene amplification. The last group exhibits highly malignant clinical behaviour, and is characterised by *MYCN* amplification, 1p deletion, and elevated TrkB expression.

It is particularly intriguing that TrkA overexpression is associated with favourable prognosis especially in combination with overexpression of N-Myc from a non-amplified gene locus, whereas *MYCN* gene amplification indicates a bad prognosis(Tang, Zhao et al. 2006; Westermann, Muth et al. 2008). N-Myc functionally inhibits Rb and p53 tumour suppressive functions(Hernando, Nahle et al. 2004; Westermann, Henrich et al. 2007; Van Maerken, Vandesompele et al. 2009), and leads to increased proliferation of NB cells. TrkA leads to differentiation, apoptosis and spontaneous regression of NB (Eggert, Ho et al. 2000; Eggert, Ikegaki et al. 2000; Schramm, Schulte et al. 2005; Brodeur, Minturn et al. 2009), and plays a central role in NB. Recently, overexpression or mutation of the ALK receptor tyrosine kinase (RTK) has been suggested to be a major player in the pathogenesis of both familial and sporadic NB (Chen, Takita et al. 2008; George, Sanda et al. 2008; Janoueix-Lerosey, Lequin et al. 2008; Mosse, Laudenslager et al. 2008). The availability of ALK inhibitors not only makes it a possible therapeutic target(Janoueix-Lerosey, Schleiermacher et al. 2010), but also has redirected major efforts of NB researchers towards understanding and targeting ALK signalling.

Medulloblastoma

MB is the most common malignant brain tumour in children and represents more than 20% of all paediatric brain tumours (Dhall 2009). It is a highly invasive tumour tending to disseminate throughout the brain. With aggressive multimodal therapy including surgery, radiotherapy and chemotherapy, overall 5-year survival rates of approximately 50% are achievable, and 80-90% of children without disseminated disease can be cured. However, most metastatic and recurrent MB tumours are resistant to current therapeutic approaches, including high-dose chemotherapy with autologous hematopoietic stem cell rescue (Zeltzer, Boyett et al. 1999). In addition, MB treatment often results in significant endocrine and intellectual impairments. Thus, new therapies are desperately needed. In order to identify new therapeutic targets, it is imperative to achieve a better understanding of the molecular basis of MB pathogenesis.

Recently, some crucial signalling pathways involved in the development and progression of MB were identified. The sonic hedgehog (Shh) and wingless/int (Wnt) signalling pathways(Fogarty, Kessler et al. 2005; Marino 2005) are implicated in MB development(Read, Hegedus et al. 2006), by inducing an upregulation of N-Myc and consequently an increase in the cell proliferation rate (Kenney, Cole et al. 2003; Hatton, Knoepfler et al. 2006). High levels of N-Myc, in turn, activate the cyclin D family (cyclin-dependent kinase activators) and repress the expression of some cyclin-dependent kinase inhibitors thereby inducing cell cycle progression (Oliver, Grasfeder et al. 2003). Increased expression and stability of N-Myc synergise with the Shh signalling pathway in the induction of MB(Browd, Kenney et al. 2006). In addition, cyclin D promotes the phosphorylation and functional inactivation of the Rb tumour suppressor protein, and although Rb mutations are rare in MB, knock-out mouse models suggest that Rb inactivation may play a major pathogenetic role (Shakhova, Leung et al. 2006).

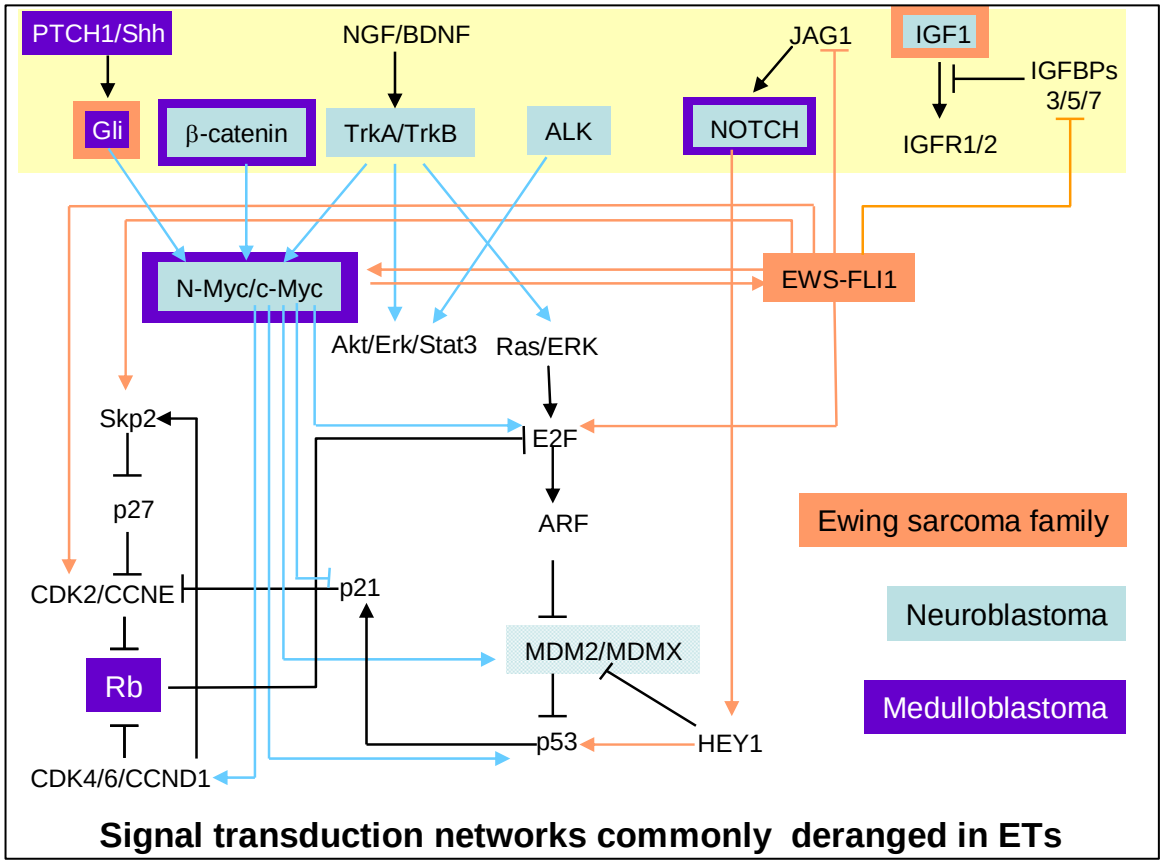
Although the involvement of N-Myc provided one of the first lines of evidence for the deregulation of signalling pathways in MB, c-Myc is also involved in MB (Herms, Neidt et al. 2000; Grotzer, Hogarty et al. 2001). Both *MYCN* and *CMYC* genes are amplified in 5 to 10% of MB patients, increasing to 17% in high-risk patients (Scheurlen, Schwabe et al. 1998; Eberhart, Kepner et al. 2002). Amplification of the *CMYC* gene and high levels of its gene product indicate poor prognosis (Herms, Neidt et al. 2000; Grotzer, Hogarty et al. 2001; Rutkowski, von Bueren et al. 2007). Another complementary histopathological prognostic factor is the large cell/anaplastic (LC/A) phenotype, indicative of a very aggressive clinical behaviour and poor outcome (Eberhart, Kratz et al. 2004; Stearns, Chaudhry et al. 2006). In contrast, low expression of c-Myc, alone or in combination with high TrkC mRNA levels, is a strong predictor of a favourable outcome in MB (Grotzer, Hogarty et al. 2001). Recent studies suggest that c-Myc may be a useful therapeutic target, as its downregulation by siRNA(Zhang, Li et al. 2006; von Bueren, Shalaby et al. 2009) or reduction of gene expression(Shalaby, von Bueren et al.) inhibited MB cell proliferation, but may also reduce apoptosis (von Bueren, Shalaby et al. 2009). Thus, it is likely that in order to exploit c-Myc as target, combination therapy will be necessary.

Ewing Sarcoma Family Tumours

ESFT are the second most frequent bone tumours in children and adolescents, comprising Ewing´s sarcoma and peripheral primitive neuroectodermal tumours along a gradient of neuroectodermal differentiation(Kovar 1998). ESFTs are highly aggressive tumours with a pronounced tendency to early metastatic spread, and a high propensity to recur following therapy. Currently, ESFTs are treated with a combination of surgery, radiation and chemotherapy. However, survival rates are poor, with 60% surviving at 5 years, and <25% surviving when metastases are present at diagnosis(Bernstein, Kovar et al. 2006). ESFT are hallmarked by chromosomal translocations, which lead to the expression of chimeric ETS transcription factors, EWS-FLI1 being the prototype and central to ESFT pathogenesis and progression(Bernstein, Kovar et al. 2006). While the histogenesis of ESFT is still not fully understood, evidence is accumulating that they derive from a pluripotent mesenchymal progenitor(Riggi, Cironi et al. 2005; Tirode, Laud-Duval et al. 2007; Kauer, Ban et al. 2009), and that most of the observed partial neural and endothelial differentiation in ESFTs is a consequence of the chimeric ETS transcription factor expression. EWS-FLI1 acts as a direct transcriptional activator of >300 genes(Hancock and Lessnick 2007; Kauer, Ban et al. 2009). An almost equal number of genes are repressed, however, mainly by indirect mechanisms(Kovar 2010). Recent ChIP-chip(Gangwal and Lessnick 2008) and ChIP-seq(Guillon, Tirode et al. 2009) studies revealed that EWS-FLI1 interacts with chromatin at two types of binding sites: distal enhancers enriched in GGAA microsatellites and proximal promoters harbouring canonical ETS binding motifs along with binding sites for other transcription factors(Kovar 2010). EWS-FLI1 primarily activates genes involved with cell cycle control and replication(Kauer, Ban et al. 2009), which show co-enrichment of binding sites for E2F and NFY transcription factors(Kauer, Ban et al. 2009). Among these are *CMYC(Dauphinot, De Oliveira et al. 2001), E2F1,3,5(Kauer, Ban et al. 2009)*, *CCND1(Dauphinot, De Oliveira et al. 2001)*, *CDK2* and *SKP2* (EETP data, unpublished). When introduced into NB cells, EWS-FLI1 switches *MYC* expression from *NMYC* to *CMYC*, further supporting its role in the regulation of *MYC* family genes(Rorie, Thomas et al. 2004). On the other hand, we found that the c-Myc protein feeds back on EWS-FLI1 expression suggesting a delicate balance between these oncoproteins (EETP data, unpublished). EWS-FLI1-suppressed genes mainly annotate to cell signalling and differentiation pathways(Kauer, Ban et al. 2009), including BMP/TGFβ, β-catenin/TCF and NOTCH signalling pathways.

Similar to NB and MB, *p53* tumour suppressor mutations or amplification of its negative regulator, *MDM2,* are rare in ESFT(Kovar, Auinger et al. 1993). However, EWS-FLI1-mediated suppression of NOTCH signalling results in reduction of basal p53 levels, functionally impairing p53 in ESFT(Ban, Bennani-Baiti et al. 2008). There is also evidence for functional inactivation of the pRb1 tumour suppressor(Hu, Zielinska-Kwiatkowska et al. 2008). While in about 20% of cases this may be due to homozygous loss of p16/p14ARF encoding the *INK4A* gene, which occurs as the most frequent molecularly defined secondary aberration in ESFT(Kovar 1998), the origin of pRb1 inactivation remains to be defined in the majority of the remaining cases. Other signalling pathways affected in ESFT include IGF1, required for EWS-FLI1-mediated transformation and holding promise as a target for treatment of resistant disease(Toretsky and Gorlick 2009), and Aurora kinases, whose expression is upregulated by EWS-FLI1(Wakahara, Ohno et al. 2008) and may also be promising drug targets(Maris, Morton et al. 2010) (and our unpublished results). The role and therapeutic value of ALK, which is also frequently expressed at high levels in ESFT(Dirks, Fahnrich et al. 2002) (and our unpublished results), remains to be established.

A summary of signalling pathway components found deregulated in ETs is shown in the figure.



These are the baseline data from where the project starts and against which the progress will be measured.

How ASSET will progress beyond the state-of-the-art and how progress will be measured

Despite prolific research activities that have identified diverse molecular players involved in tumourigenesis, the cancer field still suffers from a lack of mechanistic insights to inform strategies for disease interference or stratification based on confirmed molecular mechanisms of pathogenesis. Due to high-throughput sequencing and –omics technologies we are now in a situation where we can produce vast amounts of data and map genetic alterations with unprecedented speed and accuracy. The emerging picture from these studies has confirmed that cancer is a genetic disease, but has also revealed an enormous complexity of different mutations and genetic aberrations (Parmigiani, Boca et al. 2009; Sara, Kallioniemi et al. 2010). It becomes difficult to distinguish which mutations are driver mutations that are causally involved in pathogenesis, and which are passenger mutations that have accumulated coincidentally. Estimates show that <10% are driver mutations(Carter, Chen et al. 2009). As every cell division produces spontaneous mutations and cancer incidence increases with age, passenger mutations may accrue disproportionally in adulthood cancers(Stratton, Campbell et al. 2009). Therefore, cancers arising early in life, like ETs, are likely to be less contaminated by passenger mutations and present a clearer picture of the driver mutations. Recent high-throughput sequencing of cancer genomes has shown that medulloblastoma had 5-6 fold fewer mutations than adulthood tumours (Bert Vogelstein, John Hopkins University, Baltimore, Maryland, USA; personal communication). ASSET takes advantage of this evidence in order to deconvolute a complex disease phenotype.

ASSET builds on a wealth of high quality, high-throughput "omics" datasets generated in the EETP project, and also will benefit from access to the largest collection of clinical ET samples in Europe. Existing EETP data comprise >1000 molecularly characterised clinical samples, and mRNA and miRNA transcriptome profiles derived from ETs and cell lines. Importantly, ASSET will advance from an observatory domain into the functional, mechanistic domain informed and driven by mathematical models. This will be achieved by generating and

integrating quantitative, large-scale datasets to produce predictive models that will be rigorously validated in disease models and clinical samples.

In particular, we expect decisive progress beyond the state-of-the-art in the following areas described below.

How genetic mutations reprogram the dynamic properties of signalling networks

In keeping with the above, an extension of the mutation hypothesis is the view that cancer is a disease of signal transduction pathways that are derailed due to mutations(Hahn and Weinberg 2002). This functional view is probably more relevant in terms of therapy, as no therapeutic agent can fix multiple genetic lesions, but there is a much better chance to interfere with the pathological consequences arising from them. However, this adds further complexity. It has become clear now that signal transduction pathways are not individual pipelines, but form interconnected networks (Hahn and Weinberg 2002; Kolch 2005). In addition, the design of these signalling networks can give rise to complex dynamic behaviour and non-linear emergent properties(Kholodenko 2006). Importantly, these dynamic parameters can specify cell fate decisions, but are difficult to rationalise or understand without mathematical modelling (Santos, Verveer et al. 2007; von Kriegsheim, Baiocchi et al. 2009). Moreover, signalling networks interact in complex ways with gene-regulatory circuits to drive cell fate decisions, and mathematical modelling has again been instrumental in dissecting these networks(Schulz, Mariani et al. 2009). In particular, we lack insight into how genetic lesions found in cancer impinge on the dynamic properties of signalling networks determining cellular behaviour. This is one of the core gaps ASSET will address. The strategy to fill this gap is to develop computational models of these signalling networks, which are firmly footed in biomedical questions and tested against experimental realities. Again, the lesser genetic complexity of ETs will be a distinct advantage, as it reduces the complexity of possible inputs into signalling networks emanating from genetic lesions. Most importantly, the consortium comprises a unique set of complementary expertise to build these bridges. We perceive this as an organic process where models act to translate findings from basic, translational and clinical research into a common language, whose vocabulary is provided by experimental evidence and whose grammar is defined by mathematical rules.
Measureable outcomes: ASSET will deliver a computational / mathematical description and analysis how genetic mutations affect signalling networks.

The definition and functional characterisation of a pathogenetic ET core network

Despite this complexity and heterogeneity, recent work and the results of the EETP project have indicated the existence of a core network that is altered in all three ET entities(Gustafson and Weiss ; Eggert, Ho et al. 2000; Dauphinot, De Oliveira et al. 2001; Kovar 2003; Woo, Lucarelli et al. 2004; Li, Tanaka et al. 2005; Pession and Tonelli 2005; van Limpt, Chan et al. 2005; Ban, Bennani-Baiti et al. 2008; Hu, Zielinska-Kwiatkowska et al. 2008; Westermann, Muth et al. 2008; Brodeur, Minturn et al. 2009). This pathogenetic core network is centred around Myc, p53 and Rb and involves a functional inhibition of the tumour suppressor functions of p53 and Rb. However, the ET entities differ in how they derail this core network. For instance, ESFTs use EWS-FLI1 to regulate p53, while NB use MDM2/MDMX. This hypothesis refocuses the view from trying to describe the complexities of cancer to finding the commonalities. This quest is stimulated by observations that cancers share a few common biological traits that are fundamental to their pathogenesis and behaviour. They have been called the hallmarks of cancer and include growth, self renewal, differentiation, cell death, angiogenesis, invasion and metastasis(Hanahan and Weinberg 2000). But, can this apparent functional simplicity really be reconciled with the huge genetic complexity observed in cancer? Systems biology may provide an answer. Due to emergent properties encoded by the network structure or reaction mechanisms even simple biological networks can display a multitude of different behaviours (Kholodenko 2006; Ortega, Garces et al. 2006). On the other hand, complex systems may produce similar behaviour in response to different inputs (Kitano 2004; Orton, Sturm et al. 2008). This means that in order to deconvolute the complexity of cancer we need to understand the emergent properties of the deregulated networks. This represents the third hypothesis of ASSET, that deregulation of a core network is essential for ET pathogenesis, and that the disease-specific features are manifestations of modifying factors. Therefore, rather than trying to address all pathways altered in ETs we will concentrate on the core network. The modifying factors certainly comprise genetic mutations, but are also likely to include functional dynamic aberrations in signalling networks, that due to emergent network properties, can give rise to different biochemical and biological phenotypes. By speculation, this may contribute to the heterogeneity of disease manifestation in situations such as NB, where the relative level of N-Myc expression can profoundly affect prognosis. This hypothesis is only addressable by a modelling approach capturing these emergent network properties. Conceptually, this is an ideal scenario for the application of systems biology to a complex disease: The problem

is complex, but can be broken down into a logical framework by combining hypothesis-driven and data-based inductive thinking with model-based deductive reasoning. ASSET fully implements this concept. The result will be experimentally validated mathematical models that answer biomedical questions and provide mechanistic insight into pathogenesis.
Measureable outcomes: This research will yield an integrated model of ET relevant TF signaling that can be used for predictions and to simulate and analyse molecular behaviour.

Using mathematical models to identify vulnerable network nodes that can be used as therapeutic targets: towards a rational prediction of combination therapy
Another direct result of this concept is that such models can be applied to identify vulnerable network nodes to guide therapy choice. Currently, the treatment of ETs is based on the classic three pillars of cancer therapy, i.e. surgery, radiotherapy and cytotoxic chemotherapy. While childhood malignancies such as leukaemias and some tumours have good cure rates, the prognosis and treatment of MB, NB and ESFT remains poor. Advanced stages respond well to initial therapy, but still pose a formidable treatment problem. Relapse is common and usually therapy resistant, and 5-year survival can be as low as <10%. For instance, NB represents 8-10% of all paediatric malignancies, but accounts for 15% of cancer deaths in children. Thus, a huge unmet clinical need for new therapeutic approaches to these aggressive ETs remains. To improve current therapy, we must go beyond the cytotoxic approach and take advantage of new targeted approaches, such as those afforded by signal transduction inhibitors. Several multitargeted kinase inhibitors with promising clinical activity have already been approved for treatment of advanced cancer types. Nevertheless, all single agents rarely, if ever, induce complete responses, and at present, all patients develop resistance and progress during therapy. A critical need exists to develop biology-guided strategies that can increase the degree of antitumor effects. Combinations of targeted agents may overcome resistance developing after single-agent therapy, and could be incorporated either as part of initial therapy or later when disease resistance develops. Approaches aimed at combining these agents must be based on novel insights into tumour genetics and biology. Empirical approaches are slow and should be superseded by rationally developed advances based on mechanistic insights and combined intervention afforded by systems biology approaches. Thus, ASSET's fourth hypothesis is that mathematical models can be used to identify critical points of vulnerability in networks. These points of vulnerability are ideal drug targets. More importantly, modelling can identify combinatorial synergies difficult to uncover by experimental means only. Drug-based cancer therapies are usually combination therapies, thereby empirically validating the concept that more than one network node must be hit in order to achieve a therapeutic effect. ASSET will use the mathematical models to predict individual and synthetic network vulnerabilities. ASSET will also test these predictions using drugs and siRNAs, thereby making concrete suggestions for the rational application of chemotherapy to ET treatment.
Measureable outcomes: This will be the most important and application relevant output of ASSET as it will suggest concrete drugs and/or drug combinations that can change clinical practice in regard how to treat ETs.

Establishing a paradigm for systems biology driven by experimental data
Finally, ASSET will also break new ground in the field of systems biology. A decisive conceptual advance of our approach is the model-based optimisation of experimental design to guide the generation of information-rich data, and integration of different data features, such as perturbation data, protein interaction, modification and profiling data. This task will be accomplished through dovetailing mathematical modelling and targeted experimentation informed by the models and drawing on state-of-the-art technologies in modelling, genomics, proteomics, biochemistry and cell biology. The models will be continuously validated and improved by orthogonal types of perturbation analysis (regulatable gene expression, siRNA and drugs) and testing of salient predictions in clinical samples, e.g. profiling of gene and protein expression, post-translational modifications (PTMs), and correlations of molecular changes with clinical outcomes. We will derive mathematical models at two different granularities: (i) global network topology reconstructions that identify common molecular principles shared by ETs; and (ii) focussed mechanistic dynamic models of selected sub-processes that identify network vulnerabilities for therapeutic interference. As a result, we expect to obtain comprehensive insight into pathogenetic mechanisms of ETs based on validated computational models useful for (i) identifying fragile nodes where pharmacological interference will have maximal disease-specific effects while minimising side effects, (ii) improving therapeutic stratification of patients by molecular functional features and (iii) guiding the search for similar "core pathogenetic modules" in adulthood cancers. A unique strength of ASSET is the ability to rigorously validate the predictions of mathematical models by having access to and deploying biological ET models on all levels, including primary and established ET cell lines, xenograft mouse models, and the largest collections of patient materials in Europe.

Measureable outcomes: This output incorporates two elements. One is to establish a paradigm for how systems bioogy research can be done in order to deliver tangible outputs in forms of treatments, patents and publications. The second element is one that is much more difficult to measure but ultimately more important. It is the establishment of a modus how basic and clinical research can work together. While this may not have an output measurable by metrics it will introduce a change in research culture and collaborations between clinical and basic scientists that eventually will open up a free flowing communication between basic and clinical reseachers.

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1.3 S/T methodology and associated work plan

1.3.1.1 Overall strategy and general description of the workplan

As starting point ASSET will use a wealth of high quality, high-throughput genomic and transcriptomic data generated in the EETP. Importantly, ASSET advances from observation and classification into functional and mechanistic studies informed and driven by mathematical models generated from experimental data and rigorously validated in biological models comprising ET cell lines, xenografts and clinical samples.

Our general strategy is to (i) derive global network structures based on high-throughput data; (ii) identify functionally important subnetworks by combining existing knowledge and interrogation of the global networks by perturbation experiments using drugs and siRNA; (iii) establish detailed kinetic and mechanistic subnetwork models; (iv) analyse the subnetworks for emergent properties and vulnerable nodes, especially those which confer synthetic fragility; (v) test predictions in biological models (cell lines and xenografts) and clinical samples; and (vi) establish a datawarehouse and semantic tools that enable an advanced multiscale analysis of the combined data. The implementation of the work plan follows this logic.

Subproject 1 (SP1) will define the genetic aberrations in ETs and how they relate to clinical phenotypes and impinge on the proteome. To this end will derive global network topologies for gene regulatory networks (GRNs) by data-driven modelling and Bayesian inference mainly using mRNA, miRNA transcriptome profiles and ChIP-chip/seq data from EETP.

SP2 will focus on generating data that connect GRNs with cytosolic signalling networks by performing (i) quantitative proteomics experiments on transcription factor (TF) interactions with DNA and proteins; (ii) global phosphoproteomics of TF upstream pathways; (iii) chemical proteomics on a panel of 10 kinase inhibitors; and (iv) high-content screening of drugs and siRNAs as perturbations that inform modelling.

SP3 will develop the mechanistic kinetic models of critical subnetworks based on (i) quantitation of protein network components; (ii) known ET TF oncogene modules (Myc, EWS-FLI1) and as informed by the perturbation and proteomics experiments. These models will be used to identify emergent functional network properties and fragile nodes that can be targeted by drugs, siRNAs and combinations thereof.

SP4 will perform validation of the mathematical model predictions in biological models comprising (i) ET cell lines with tunable oncogene expression; (ii) preclinical ET xenograft models; and (iii) clinical specimens.

SP5 will establish a datawarehouse to (i) store and manage all ASSET data; (ii) link the data to enable zooming between different resolution models; (iii) enable advanced data analysis and interpretation on a semantic meta-level; and (iv) ensure that the data can be made available to the scientific community.

Standardisation of data generation. In order to guarantee consistency and comparability of the data generated in ASSET we will (i) use a defined panel of core cell lines representing the three ET entities: UW-228 (MB) with tet-regulatable c-Myc; A673 (ESFT) with tet-regulatable EWS-FLI1; SH-SY5Y (NB) with differentially regulatable TrkA and N-Myc; and for the ALK work, SK-N-AS (NB) with regulated expression of the ALK F1174L mutant. The core cell line panel will be exome sequenced and all partners will work with the same cells; (ii) establish standard operating procedures (SOPs) for handling the cell lines and experimental measurements; and (iii) establish a short-term exchange programme through which wet and dry researchers will visit partner labs to ensure that data generation and modelling work is pursued according to agreed standards.

Consolidation and integration of the modelling work. The modelling work in this project has to cope with a large amount of diverse data sets coming from genetics, transcriptomics, proteomics and high throughput perturbation experiments. While this richness of data is a key asset of the project, it is essential to assure a dovetailed integration between the different strands of work to generate the models that can competently address the questions we are asking. To this end we have developed the following strategy and workflow

1) Bioinformatic capture of the experimental data in the datawarehouse. This will be done through WP10 drawing on the expertise of partners 13 and 14.

2) Normalization and bioinformatic analysis of the data to prepare datasets suitable for modelling. Normalization will be done by the individual groups generating the data. For the bioinformatic analysis we importantly can draw on the expertise of partners 4, 13, and 14. The key advantage is that consortium members are aware of the purpose for which the data will be used and hence can decide which of the many bioinformatic analysis methods are most appropriate for generating the datasets suitable for the different kinds of modelling.
3) Coarse grained modelling of global networks will be established using data from the transcriptomics and proteomics experiments using data driven modelling and inference methods based on Bayesian statistical methods and logical rules by partners 5 and 9. This approach can deal with large amounts of experimental data to extract functional information and reveal functional connections of molecules within datasets. The risk is that due to the inevitable sparsity of the data points many of the inferred connection will be incompletely validated. As Bayesian inference assigns probabilities of (un)certainty, we at all times are aware of the exact errors and confidence of our network reconstructions. The uncertainities will be minimised by the information of the high throughput perturbation screens by Bayesian based procedures that we have descried previously (Xu TR, Vyshemirsky V, Gormand A, von Kriegsheim A, Girolami M, Baillie GS, Ketley D, Dunlop AJ, Milligan G, Houslay MD, Kolch W .Inferring signaling pathway topologies from multiple perturbation measurements of specific biochemical species. Sci Signal. 2010 Aug 10;3 (134):ra20. In addition, we will use the drug perturbation data and the data from the chemical proteomics screens performed in WPs 2,4,5 as they become available. Thus, the inferred networks will be continuosly refined during the project by the virtuous cycle of hypothesis making and experimental testing of predictions.
4) The coarse grained network models will be used to identify subnetworks, i.e. regions of the global networks, which are critical for the biological network functions, such as cell survival, cell transformation and drug responses. Our preliminary work has shown that it is possible to identify such subnetworks (Santra, T., Girolami, M., and Kolch, W. Evolution of biochemical networks: Engrained and plastic modules among different yeast species. Submitted).
5) These subnetworks will be further analysed by mechanistic dynamic modelling by partners 1 and 5. These models will allow us to generate fine grained models that give information on the dynamic responses to perturbations by ET oncogene expression and overexpression as well as drugs. In cases where we already have solid evidence that aberrations, such as c-Myc and N-Myc overexpression, play important roles in ET tumourigenesis we will generate dynamic models right away. The dynamic models will be used to (i) analyse the effects of ET oncogenes on network behaviour; (ii) the effect of drugs on the ET networks; and (iii) for the prediction of fragile nodes that should be targeted by drugs. These predictions are one of the major outcomes of the project. They will be tested experimentally in ET cell lines and animal xenograft models. The predicted alterations induced by ET oncogenes will be validated against an ET cell line panel and also in human clinical ET samples as appropriate.
6) The models and associated experimental data will be deposited in the datawarehouse. This will ensure that models can be easily exchanged and used by different consortium members. This repository will be made publicly available after publication and IP protection. We expect that this will be a lasting legacy of the project that will be very useful for the scientific community even after the project has ended.
7) The models and experimental data captured in the datawarehouse also will be used to develop higher level models that can integrate different types of models and experimental data in WP11. Emphasis will be on mechanistic interpretations based on pathways and protein-protein interaction complexes linking the generated data with clinical phenotypes or endpoints. We will address the issue of integrating data and models at two levels. First, components can be linked using semantic annotation to allow viewing and component analysis in different modelling contexts. This allows, for instance, the integration of data across regulatory and signalling networks. Second, expanding the semantic annotations into rule-based frameworks will permit zooming in and out of models from coarse- to fine-grained resolution. This systems level analysis of data and association with phenotypes will go beyond current studies and allow a truly systems wide view and analysis of the data.

SP1: The dysregulated ET genome – SP Leader (SPL): Mark Girolami

WP1. Reconstruction of Gene Regulatory Networks (GRN) driven by the ET transcription factor oncogenes, N-Myc, c-Myc and EWS-FLI1. Prominent aberrations in ETs are overexpression or gene amplification of N-Myc or c-Myc (NB, MB) and expression of the fusion protein EWS-FLI1 (ESFTs). We will use advanced Bayesian inference methods to reconstruct GRNs driven by these oncogenic transcription factors based on large-scale mRNA and miRNA expression profiles as well as ChIP-chip/seq data (from the EETP).

WP2. Targeted and global high content network perturbation screens with siRNAs and drugs for network reconstruction. Here, we will employ initial models of ET GRNs produced in WP1 to generate high-content perturbation data that will be used to (i) test GRN structures predicted by the models; (ii) enrich and refine GRN structures by functional data, thereby enabling the iterative cycle of modelling informing experimental work and experimental work informing the modelling; (iii) functionally link GRN structures to biological outcomes such as cell viability. In addition, we will perform (iv) hypothesis driven and mechanistically oriented drug discovery work by unbiased compound screens as well as (v) predicted synthetic effects between siRNA – siRNA and siRNA – drug combinations.

WP3. microRNA expression in ETs: regulation by ET oncogenes, identification of targets & effects on proteomes. MicroRNAs (miRNAs) are central connections between the genome, transcriptome and proteome and play crucial roles in cancer. We will assess aberrations of miRNA expression in ETs and their effects on the transcriptome and proteome. Finally we will integrate data in order to mine a large dataset of miRNA profiles from ETs. These experiments are designed to probe the links between protein signalling networks and GRNs. Linking the transcriptome to the proteome is a largely unexplored area of systems biology, and miRNA analysis is a logical connector.

SP2: Mapping ET signalling networks – SPL: Giulio Superti Furga

WP4. ET transcription factor protein networks. This WP will characterise ET transcription factor (TF) networks on the functional protein level. Towards this we will map the dynamic protein-protein interactions of TFs altered in ETs, such as N-Myc, c-Myc, EWS-FLI1, p53 and Rb using quantitative proteomics. These data will be used to enrich mathematical models of GRNs by dynamic and mechanistic data.

WP5. Mapping cytosolic ET signaling protein networks by quantitative proteomics. Protein functions, hence, network connectivities are critically regulated by phosphorylation. Here, we will map regulatory circuits downstream of peripheral ET proteins that are relevant to pathogenesis (TrkA and ALK tyrosine kinase receptors) using quantitative phosphoproteomics. This approach will come from the other side to close the gap between GRNs and protein networks. Mechanistic modelling needs quantitative data including absolute amounts of the protein species involved and their relative changes in response to perturbations of the network due to mutations or drug/siRNA interference. Here, we will generate these data using MS-based proteomics (AQUA) for absolute quantitation and Zeptosens' reverse protein arrays for highly parallel relative quantitation.

SP3: Dynamic mechanistic modelling of ET-driving protein and gene networks – SPL: Boris Kholodenko

WP6. Computational-kinetic models of critical Myc-dependent regulatory networks that drive ETs. This WP marks the transition from global models to mechanistic kinetic models of pathogenetically important subnetworks. We will develop predictive ODE models from the global network models, based on perturbation analyses and parameterised by data on protein-protein and protein-DNA interactions. Stochastic features will be added using advanced statistical approaches and ensemble modelling which considers the distribution of output functions of an "ensemble" of models rather than single deterministic models. The aim is to develop experimentally validated mathematical models of the core regulatory networks, whose deregulation drive Myc-dependent ET s.

WP7. Network fragility analysis of apoptosis and proliferation decisions. Here, we will expand on the theme above by developing mathematical models of the core network determining the balance between proliferation and apoptosis decisions in ETs, and which can identify fragile network nodes.

SP4: Validation of mathematical models in biological models – SPLs: Heinrich Kovar & Angelika Eggert

WP8. Probing network fragilities with kinase inhibitors. Kinases are crucial in signal transduction.Ten kinase inhibitors that are in or close to clinical use will be tested against ET cell lines individually and in combination. The single and combinatorial drug profiles will be used as perturbation inputs for mathematical dynamic pathway models. We further will use quantitative chemical proteomics with immobilised drugs as baits to identify direct and distal protein targets of the drug and drug combinations proven effective in objective 3.

WP9. Validation of mathematical models in biological models and clinical samples. The focus of this WP is on validating network nodes predicted to be effective drug targets in ET cell lines engineered to permit tunable modulation of the involved genes (e.g. inducible regulation via tetracycline, shRNA expression, etc.), mouse xenograft models and human clinical samples. This multi-scaled approach across biological models of different complexities ensures that the transition from molecular mechanisms to clinical phenotypes remains firmly footed in tractable entities. We will use a combination of drug treatment and/or shRNA knockdown in cell lines and xenograft tumours in nude mice. Additionally, predictions can be assessed in the extensive, existing data from primary embryonal tumours (ET), which is available to the ASSET consortium and was generated by the E.E.T.-Pipeline. The primary objective is to validate model predictions within the background of the ET tumour cell and tumour within the context of the organism in order to rationally predict and identify effective novel drug combinations that will improve treatment options for paediatric solid tumours.

SP5. Data management & knowledge integration – SPLs: Soren Brunak and Alfonso Valencia

WP10. Data management & Systems level knowledge integration. This work package will provide a complete framework for all experimental data and models generated in ASSET. It will establish an interrogable project database for holding shared experimental data, and a repository for mathematical models and data integration pipelines that also exploit publicly available data. It will construct a data warehouse to support and enable combination of project proprietary data with publicly available data sources. The use of semantic technologies, where appropriate, will allow integrating and interrogating data at many levels. Data frameworks will be built with future maintenance in mind, with provisions to integrate with alternative systems, for example through the use of web services. This database will be made accessible to the scientific community at the end of the project, and represents the lasting legacy of ASSET to the scientific community.

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WP11. Build a network/dynamic model as a reference framework to correlate genetic alterations with clinical cancer phenotypes. This work package will provide a systems level approach to integrating various data from the WP10 data warehouse. Emphasis will be on mechanistic interpretations based on pathways and protein-protein interaction complexes linking the generated data with clinical phenotypes or endpoints. This workpackage aims to combine qualitative and quantitative modelling, where qualitative data integration schemes will lead to models that can be used to select and design quantitative simulation efforts targeting key pathways and temporal cancer-linked mechanisms. WP11 will, thus, address the issue of integrating data and models at two levels. Components can be linked using semantic annotation to allow viewing and component analysis in different modelling contexts. This allows, for instance, the integration of data across regulatory and signalling networks. Secondly, expanding the semantic annotations into rule-based frameworks will permit zooming in and out of models from coarse- to fine-grained resolution. A systems level analysis of data and association with phenotypes will go beyond current studies where single entity results or perturbations show limited reproducibility due to the multiple routes of signalling in a cell leading to similar end effects.

SP6 Project Management & Training - SPLs: Walter Kolch & Lauren Montague

WP12. Project Management and Dissemination. UCD is responsible for the ongoing scientific, financial and administrative management. In order to ensure an efficient and active management of the financial, administrative and dissemination tasks, the Project Coordinator (PC) will be supported by the SBI grants manager (GM), Lauren Montague, and part-time by the SBI education & outreach officer, Philip Smyth (the latter is an in-kind contribution of UCD). The subproject leaders (SPLs) will assist with scientific management and implementation of the scientific plans. The project management team (PMT) consisting of the PC, GM and the SPLs will have the following tasks:

1. Project start: setting up the consortium agreement; setting up the boards; establish project website; organise kick-off meeting; generate and distribute reporting templates

2. *Day-to-day management:* Managing all aspects of the reporting, including the delivery and follow-up of administrative and financial documents; liaising with the EC on behalf of the consortium; implementing any changes in the scientific direction or organisation of the project, e.g. changes to the consortium agreement, changes of participant status; following and updating the project indicators (Gantt chart, deliverables, milestones); ensuring the flow of communication within the consortium; organising the researchers' exchange programme between participants; organising the annual project meetings and assist with organising interim meetings as required

3. Project monitoring: progress will be monitored formally through the technical and financial reporting activities, and informally by the day-to-day managementa activities described above.

4. Dissemination: While a main part of dissemination will occur at the level of individual research groups through publications in scientific journals and seminars and lectures at conferences, this task will deal with all dissemination activities that are project-wide. SBI has a dedicated education and outreach officer, Philip Smyth, who will be appointed to assist with ASSET outreach activities on a part-time basis (as in-kind contribution from UCD). In addition, SBI's business and development officer (official search in progress), will support the industrial liaison and dissemination of results to the biotech and pharmaceutical Industry (also on a part-time basis and as in-kind contribution from UCD).

Specifically, project-wide, *external dissemination* tasks will include:

Establishment of a public interactive website. The PMT will provide regular updates and site maintenance.

Collection of publication references and publications on the public website. Whenever publishing rights allow, placement of open access publications on the website. Organisation of press packages at timepoints in accordance with the achievement of selected milestones. Creation of a project identity and brand name, including logo, tag line and brochure in order to promote the project and facilitate dissemination. Liaison with industry and targeted showcasing of project results to industry to raise awareness, initiate collaborations and enable European industries to take instant advantage of ASSET's findings. Organisation of three international systems biology workshops in conjunction with annual project meetings

Specifically, project-wide, *internal dissemination* tasks will include:

Organisation of the researchers' exchange programme between participant groups. Stimulation of internal communication by arranging teleconferences and Skype-meetings. Quarterly teleconference meetings of the PMT Issuing a quarterly ASSET Newsletter informing about current activities. Establish a dedicated backstage virtual space website that will facilitate the sharing of documents, discussions about results and plans, and organisation of ad hoc meetings. Organisation of the annual meetings. Help with organising local meetings and meetings of SP or smaller working groups.Assistance with exploitation and IPR protection if needed through SBI's business development manager.

5. Other management-related issues: coordination of and decision-making on topics regulated by Annex II and the consortium agreement; development of further opportunities from results; strategy towards related projects.

6. Project end and planning of follow-up: At the project end the management team will ensure the proper wind-down of the project including the preparation of all final reports to the Commission; organisation of the final technical meeting and the general assembly meeting; ensuring that the project data will be made publically

available, in particular the mathematical models, the data warehouse and the associated data mining and semantic knowledge integration tools.

WP13. Training. Systems biology is an inherently highly interdisciplinary field. One of the biggest obstacles in the field are the barriers between the theoretical and experimental branches, which include differences in the scientific culture, language and thinking and hence can compromise successful collaborations. On the other hand, this difficult interface can also produce the creative friction and enlightening sparks that drive innovative science. Therefore, ASSET will introduce training programme to break down these barriers and encourage the innovative aspects. The training programme aims to facilitate the communication and active engagement between the wet and dry disciplines. Therefore, while it mainly targets PhD students and postdoctoral scientists, it also will engage PIs. It will include a strong internal dissemination programme with offerings of lectures, workshops, surgeries, and exchange of researchers between groups. Details are described in the WP description in part A.

1.3.1.2 Significant risks and associated contingency plans

General risks

We estimate the overall risk associated with the project as low.

A typical bottleneck in systems biology projects is that modelling requires a vast amount of data and wet experimentation has difficulties to keep data production aligned with the usually faster mathematical modelling work. This problem is vastly ameliorated by the availability of a large amount of data from the E.E.T.-Pipeline at project start. Thus, modelling work can start immediately. In addition, we have allocated ca. 2.5 times more manpower to wet experimentation than modelling. This ratio works well in our experience to make sure that wet experimentation keeps pace with the modelling, and that there is enough experimental capacity for testing model predictions.

Another general risk of large scale systems biology projects with several partners is that experimental data from different partner laboratories are not comparable and may lead to erroneous input into the modelling work. We have addressed this by the following measures.
We have defined a core ET cell line panel (described in the WP section), which will be distributed to all partner labs. These cell lines will be exome sequenced to ascertain their mutational status. They contain regulatable ET oncogenes so that each cell line can serve as its own control, i.e. experiments can be performed plus/minus oncogene expression.
We will establish standard operating procedures (SOPs) for handling the cells and performing experiments. Through the researchers' exchange programme we will assure that experimental procedures and data generation are synchronised between partner laboratories

Specific risks

We also have identified specific risks that are discussed below, and have designed contingency plans to overcome them.

1) The reconstruction of global GRN in SP1 bears the risk that the existing data from the E.E.T.-Pipeline may produce a number of meaningful network structures which may be difficult to rank in a robust manner, and therefore, make the selection of a small number of relevant sub-network structures challenging. However, the integration of orthogonal types of data, i.e. shRNA, mRNA, miRNA and ChIP-chip/seq data, provides a reduction in uncertainty, although it may not be common to all ETs. These risks will be assessed immediately by formally measuring the information content in the available data using established methods as available in the MG, SBu and TH groups. Although there may be multiple structures, possibly with varying levels of uncertainty in different tumour types, this can still be effectively managed within the Bayesian formalism. In particular, the ample production of perturbation data in the project will address and help resolve this issue. There also is a risk that the

inferred network structures may not provide a complete structure for extracting the mechanistic models. This risk will be mitigated by close collaboration with biologists, supported by the planned short-term exchanges between wet- and dry-lab groups, to constantly assess and experimentally validate the utility of the suggested network structures and define potential motifs, which may fill out the suggested structures for the models. In addition, we already have hypothesis driven, and experimentally well founded assumptions on the importance of some network modules, such as the Myc module.

2) The combinatorial drug (WP3) and drug/siRNA (WP9) screening efforts may not reveal any synergistic combinations. Based on our own preliminary results (Giulio Superti-Furga) this is highly unlikely, even with the limited set of 10 kinase inhibitors used in WP3. However, to eliminate this risk we also have included an unbiased large screen using the high-throughput capacities set up at VTT (Olli Kallioniemi).

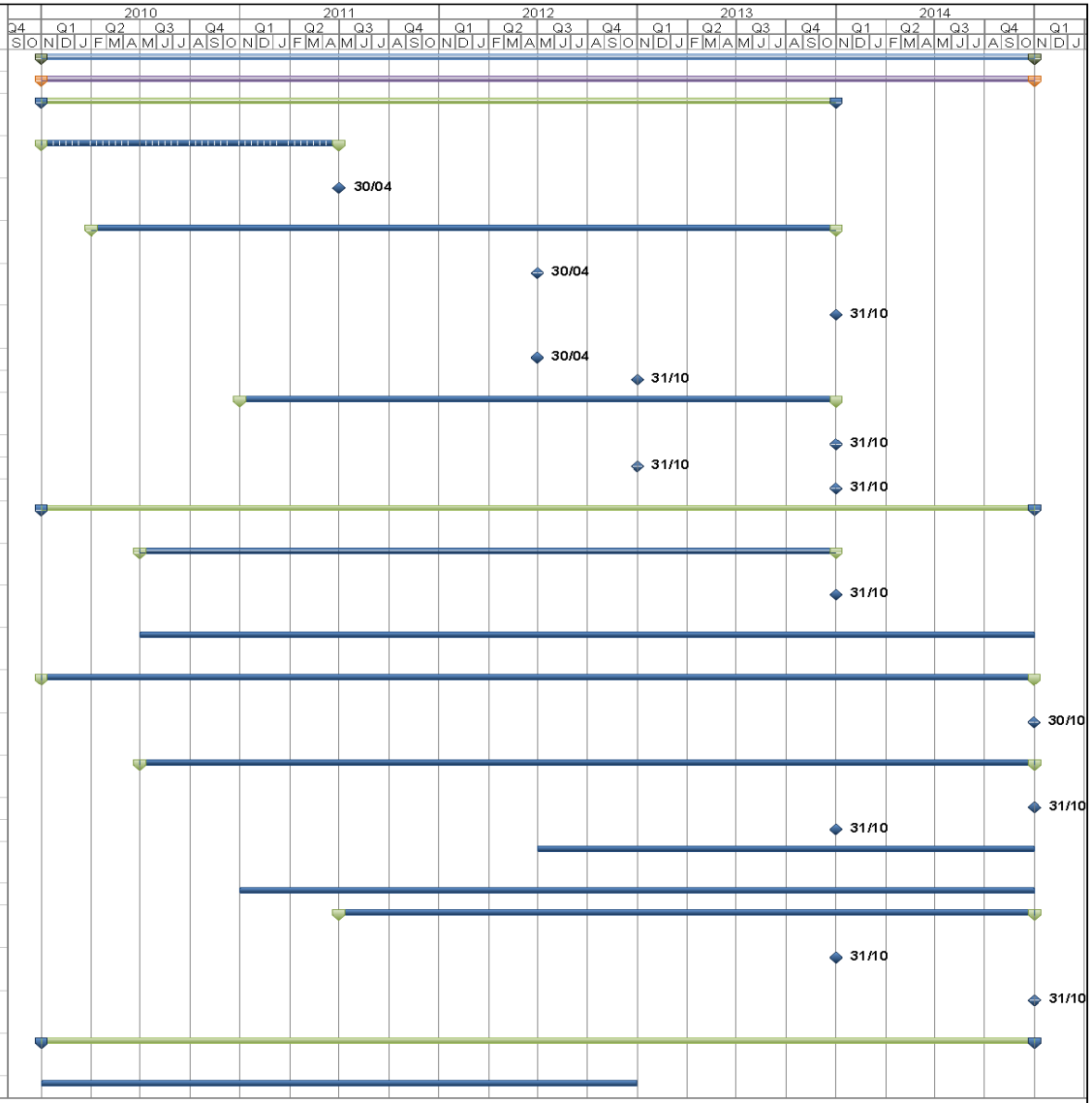
3) The quantitative proteomics technologies are all well established in the participants' laboratories (Jesper Olsen, Giulio Superti-Furga, Walter Kolch). There is a generic risk that no differences will be observed. While this in our longstanding experience is highly unlikely, we have alternative ET cell systems available in addition to the chosen core ET cell line panel.

4) For the generation of mechanistic dynamic models large amounts of quantitative, time-resolved data are required. Although we have planned for the production of these data, there is the possibility that the available experimental data may be insufficient for model refinement based on parameter identifiability. As contingency we will use bifurcation techniques and statistical approaches to characterise the dynamic behaviour of the models and fragility nodes.

5) The success of the datawarehousing in SP5 is contingent on partners depositing the data. Although we do not anticipate any compliance problems, this will be enforced by the coordinator if necessary. Warehousing all data to solve all questions is also a rather large and somewhat unrealistic undertaking. Another challenge is to setup a realistic and usable data integration framework that accomplishes specific and meaningful biological user queries, and does not try to "do it all". This risk will be mitigated by the fact that the CBS-DTU group (Soren Brunak) has extensive experience and a track record in software and database design. The group and the collaborators at CNIO (Alfonso Valencia) have also demonstrated successful and useful systems biology level data integration.

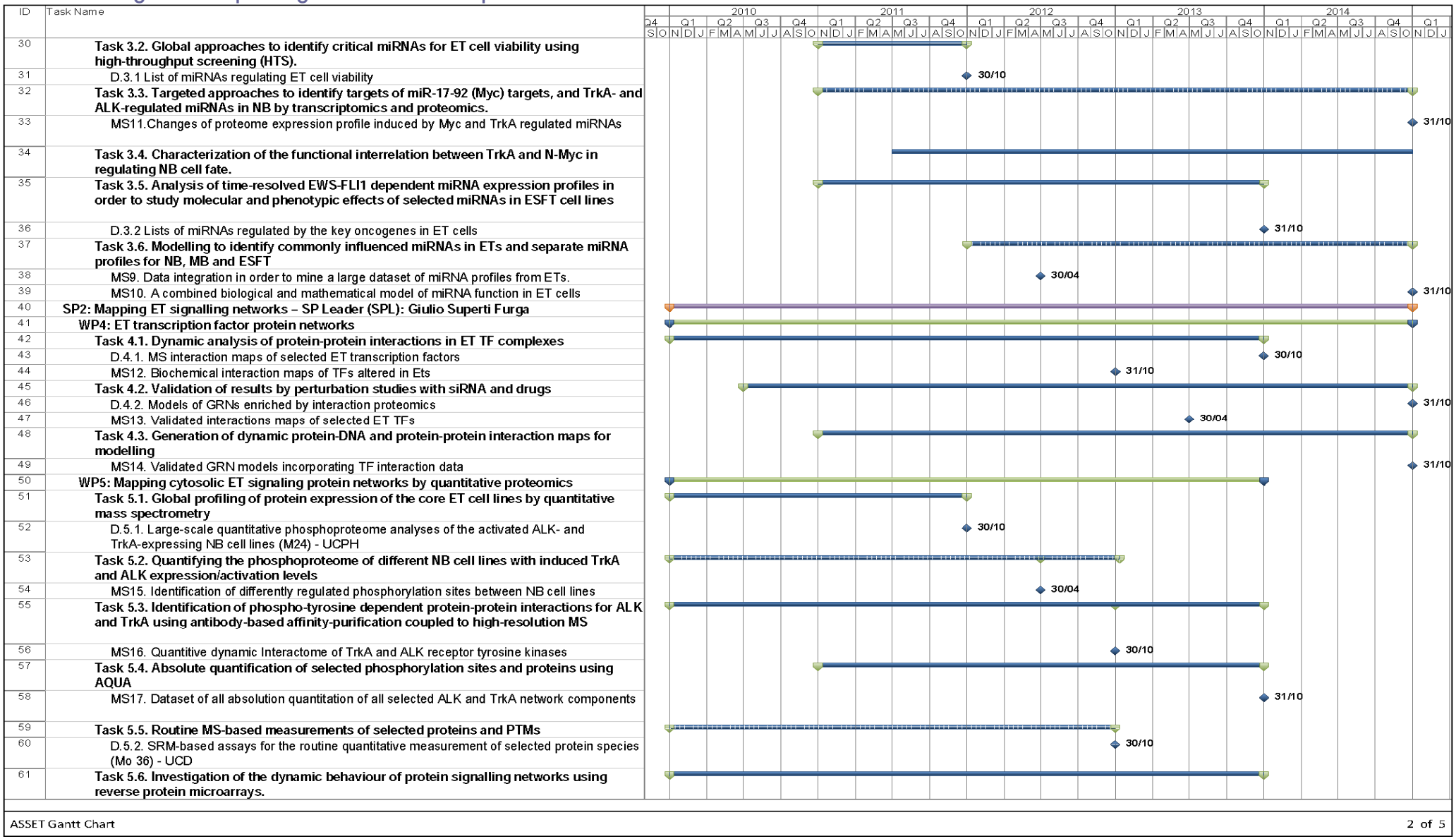
B 1.3.2 Timing of work packages and their components

ID	Task Name
0	**ASSET**
1	**SP1: The dysregulated ET genome – SP Leader (SPL): Mark Girolami**
2	**WP1. Reconstruction of Gene Regulatory Networks (GRN) driven by the ET transcription factor oncogenes, N-Myc, c-Myc and EWS-FLI1.**
3	**Task 1.1. Generation of time resolved mRNA and miRNA data and ChIP-chip/seq data**
4	MS1.Normalisation and preparation of existing and new data and assessment of data information
5	**Task 1.2. Inference of network structures of EWS-FLI1 and N-Myc/c-Myc (dys)regulated GRNs**
6	D.1.1 Formal methods & algorithms to produce distributions over GRN & motif structures from integrated heterogeneous data
7	D.1.2 Formal methodology for reconstructing GRNs from dynamic gene expression data and TFBS data systematic shRNA knock-downs and Chip-chip/seq dataw
8	MS2.Reconstruction of prior GRN and motif structures
9	MS3.Production of posterior GRN and motif structures
10	**Task 1.3. Refinement of inferred GRNs by integration of promoter occupancy data.**
11	D.1.3 GRNs and motif structures for ET TFs
12	MS4.GRN and motif structures for EWS-FLI
13	MS5.GRN and motif structures for N-Myc and c-Myc
14	**WP2: Targeted and global high content network perturbation screens with siRNAs and drugs for network reconstruction**
15	**Task 2.1 Functional role of EWS-FLI1 downstream genes to refine the ESFT network model**
16	MS7. Identification of EWS-FLI1 and Myc downstream genes regulating ET cell survival
17	**Task 2.2. Identification of synthetic lethal genes with amplified or deregulated N-Myc in NB cells.**
18	**Task 2.3. Identification of biological consequences of global ET GRN perturbations in cultured ET cells to refine the GRN models**
19	D.2.3. List of genes originating from ET network whose function is critical for ET cell survival
20	**Task 2.4. Application of cell based HTS to identify growth inhibitory compounds and synthetic lethalities in genetically engineered isogenic ET cell line pairs**
21	D.2.1. List of compounds inhibiting ET cell growth
22	MS6. List of selective growth inhibitory compounds between isogenic ET cell lines
23	**Task 2.5. Combination of data from chemical biology with signalling network model to refine critical pathways for ET proliferation**
24	**Task 2.6. Identification of siRNAs sensitizing NB and MB cells to selected drugs**
25	**Task 2.7. Use of siRNA screens to ascertain the identity of the kinases which mediate synergistic response induced by kinase inhibitor combinations**
26	D.2.2. List of kinases whose inhibition by siRNA molecules sensitized ET cells to selected drugs
27	MS8. A map of drug/siRNA interactions and refined network models explaining synergistic drug/siRNA effects
28	**WP3: microRNA expression in ETs: regulation by ET oncogenes, identification of targets & effects on proteomes**
29	**Task 3.1. Profiling miRNA expression in Ets**



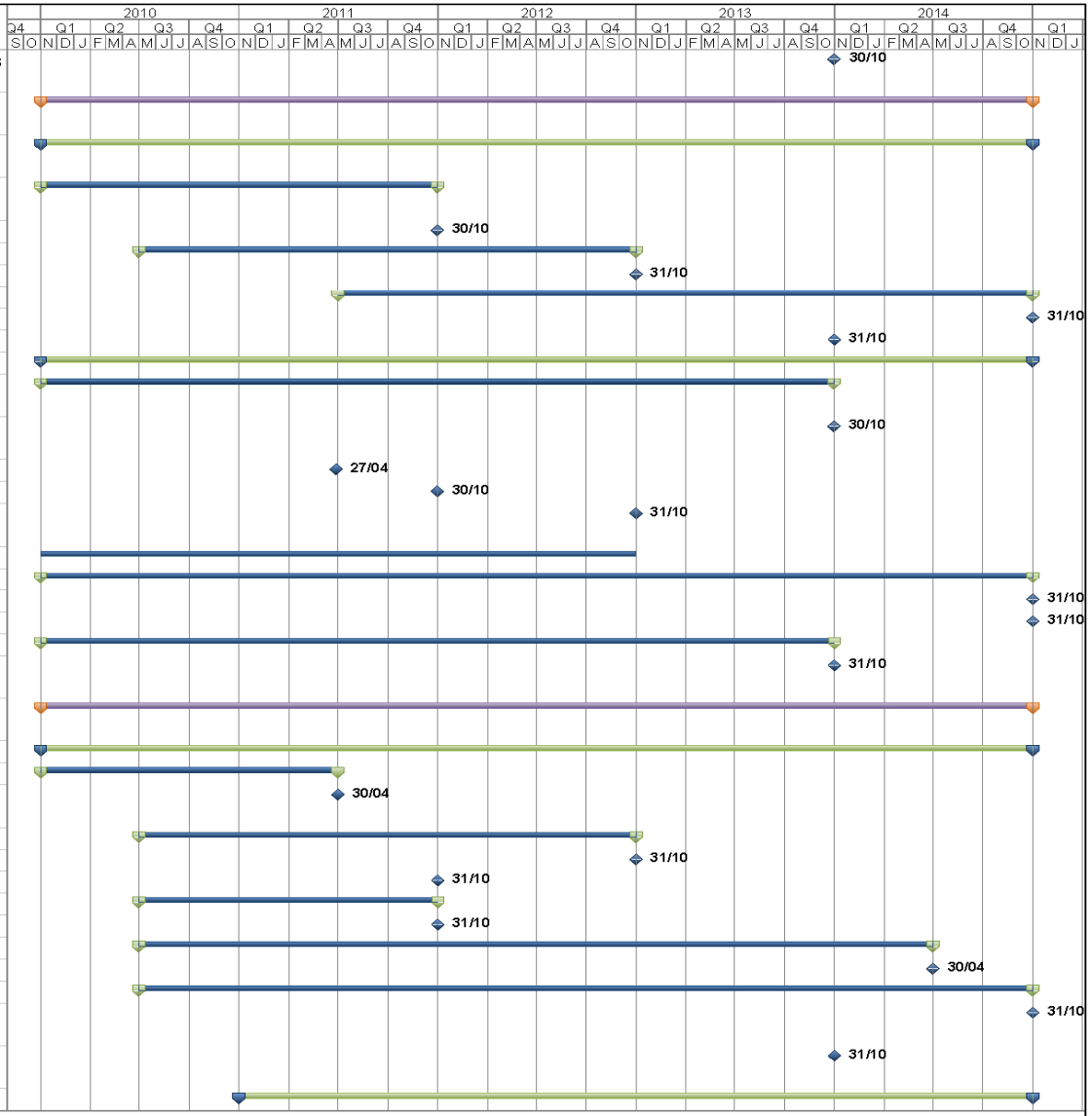
ASSET Gantt Chart 1 of 5

B 1.3.2 Timing of work packages and their components



B 1.3.2 Timing of work packages and their components

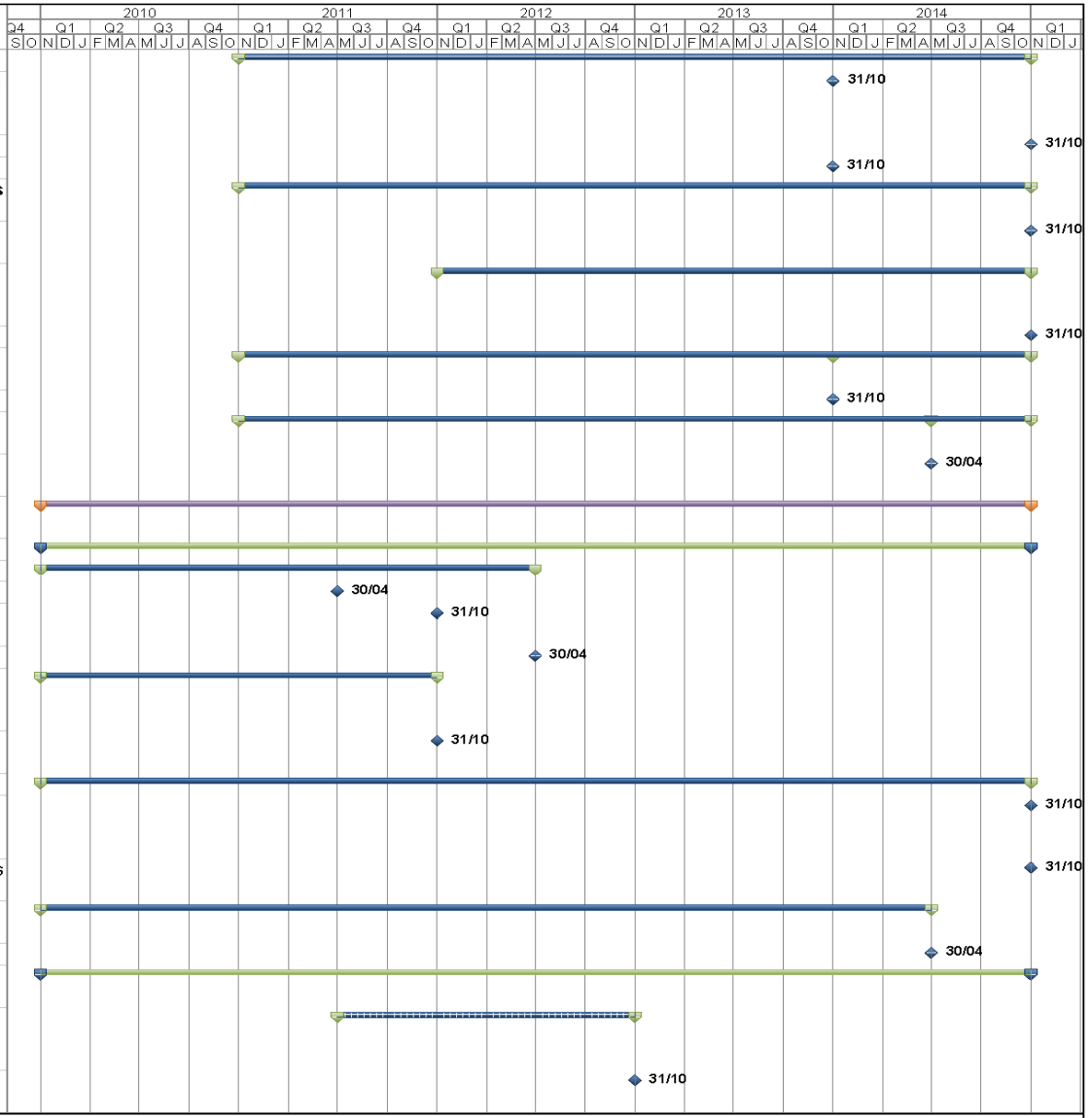
ID	Task Name
62	D.5.3. Parallel relative quantitation of ET protein network components by reverse protein arrays (Mo 48) - ZEPTO
63	**SP3: Dynamic mechanistic modelling of ET-driving protein and gene networks- SPL: Boris Kholodenko**
64	**WP6: Computational-kinetic models of critical Myc-dependent regulatory networks that drive Ets**
65	**Task 6.1. Modelling of the effects of TrkA on N-Myc and c-Myc expression and protein stability**
66	D.6.1. Core model of Myc protein regulation by TrkA in NB cell lines
67	**Task 6.2. Modelling of the N-Myc and c-Myc interaction network**
68	MS18. Kinetic core model p53-Mdm2/MDMX module in NB cell lines
69	**Task 6.3. Fragility analysis**
70	D.6.2. Predicted fragility points in the Myc network
71	MS19.Fragility analysis of the Myc network
72	**WP7:Network fragility analysis of apoptosis and proliferation decisions**
73	**Task 7.1. Modelling of the CDK-Rb-E2F-Skp2 and p53-Mdm2/MDMX modules in NB**
74	D.7.1. A comprehensive core model of the CDK-Rb-E2F-Skp2 and p53-Mdm2/MDMX modules in NB
75	MS20. Validated kinetic model p53-Mdm2/MDMX module in NB cell lines
76	MS21. Validated Kinetic model CDK-Rb-E2F-Skp module in NB cell lines
77	MS22. Integrated model of the core network governing proliferation versus apoptosis decisions in NB
78	**Task 7.2. Connection with signal transduction.**
79	**Task 7.3. Adaptation of the model to ESFT and MB**
80	D.7.2. Extension of the NB core model to EWS and MB
81	MS24. In silico adaptation of the model to ESFT and MB
82	**Task 7.4. Predictive fragility analysis of single and combined nodes.**
83	MS23. In silico quantification of effect of individual network nodes and pairs of nodes on proliferative and apoptotic potential
84	**SP4: Validation of mathematical models in biological models – SPLs: Heinrich Kovar & Angelika Eggert**
85	**WP8. Probing network fragilities with kinase inhibitors.**
86	**Task 8.1. Establishing the screening cell line panel and screening conditions**
87	MS25. Dose response curves of 11 selected kinase inhibitors in a well characterised cell line panel
88	**Task 8.2. Binary combinatorial drug screen**
89	D.8.1 Identification of tumour-type specific synergistic drug combinations
90	MS26. Synergistic pairwise drug combinations based on the most potent single drug
91	**Task 8.3. Synthesis and validation of coupleable drug analogues.**
92	MS27. Panel of validated drug analogures suitable for immobilisation
93	**Task 8.4. Generation of drug target profiles**
94	MS28. Target profiles for synergistic drugs
95	**Task 8.5. Characterisation of drug-dependent phosphoproteome signatures**
96	D.8.2.Identification of drug-dependent changes in phosphotyrosine signatures of synergistic drug combinations
97	MS29. Global and quantitive drug-induced changes of the phosphoproteome for synergistic combinations
98	**WP9. Validation of mathematical models in biological models and clinical samples**



ASSET Gantt Chart 3 of 5

B 1.3.2 Timing of work packages and their components

ID	Task Name
99	**Task 9.1. Validation of predicted fragility nodes in cellular models for ET.**
100	D.9.1. Initial validation results provided for model improvement from re-analysis of existing data and/or selected target molecule assessment in primary tumour samples on an individual basis
101	D.9.2. Functional validation of in silico predicted fragility nodes in ET cell
102	MS31. ET cell lines with inducible tuning of entity-specific and shared fragility nodes
103	**Task 9.2. Transcriptomic expression profiles for selected combinations of kinase inhibitors in the ETcell line panel**
104	MS30.Transcriptomic profiles of expanded cell line panel with dose-response curves for validating selected drug combinations found in WP8
105	**Task 9.3. Testing of selected drug combinations for each ET entity in xenograft mouse models. Task 9.3. Testing of top drug combinations for each ET entitiy in xenograft mouse models**
106	D. 9.3. Correlation of drug sensitivities, GRNs and gene mutations
107	**Task 9.4. Re-analysis of existing high-throughput data sets from primary human NB, MB and ESFT for the validation of model predictions**
108	MS32. Results from data re-analysis from the initial model predictions
109	**Task 9.5. Immunohistochemical validation of selected proteins on existing tissue microarrays**
110	MS33. Molecular and phenotypic characterisation of fragility node perturbation in ET cell lines
111	**SP5. Data management & knowledge integration – SPLs: Soren Brunak and Alfonso Valencia**
112	**WP10. Data management (UCPH, CNIO, & All Partners)**
113	**Task 10.1. Construction of a state-of-the-art and data warehouse for ASSET**
114	M34. An analysis of state-of-the-art data and databases
115	M35. Assessment of public data that should be included with recommendations for appropriate linking
116	M36. Datawarehouse design
117	**Task 10.2: Data mining of publicly available mutation-phenotype data, protein-protein complexes and pathways and incorporation of these data within the relational database framework**
118	D10.1. A description of sources of pathways, protein-protein complexes and mutation-phenotype data
119	**Task 10.3: Develop web-services and data visualisation**
120	D.10.2. A description of the project catalogue of analysis workflows and their web service components: A description of the project data warehouse, its relational principles, data formats, web services components and links to external sources
121	M38. A webservice-based resource for the analysis of genetic and functional aberrations in ETs
122	**Task 10.4: Linking the model repository for ASSET to community-wide efforts (e.g. Reactome, CellML, BioModels, DOQCS, ModelDB, etc.).**
123	M37. A complete description of a models repository and its external links
124	**WP11. Build a network/dynamic model as a reference framework to correlate genetic alterations with clinical cancer phenotypes.**
125	**Task 11.1. Construction of cancer-specific protein-protein interaction networks (rewired cancer interactomes) linked to expert annotation of selected disease-related protein complexes**
126	D.11.1. A description of a data integration pipeline facilitating query and linking to disease complexes and pathway reactions



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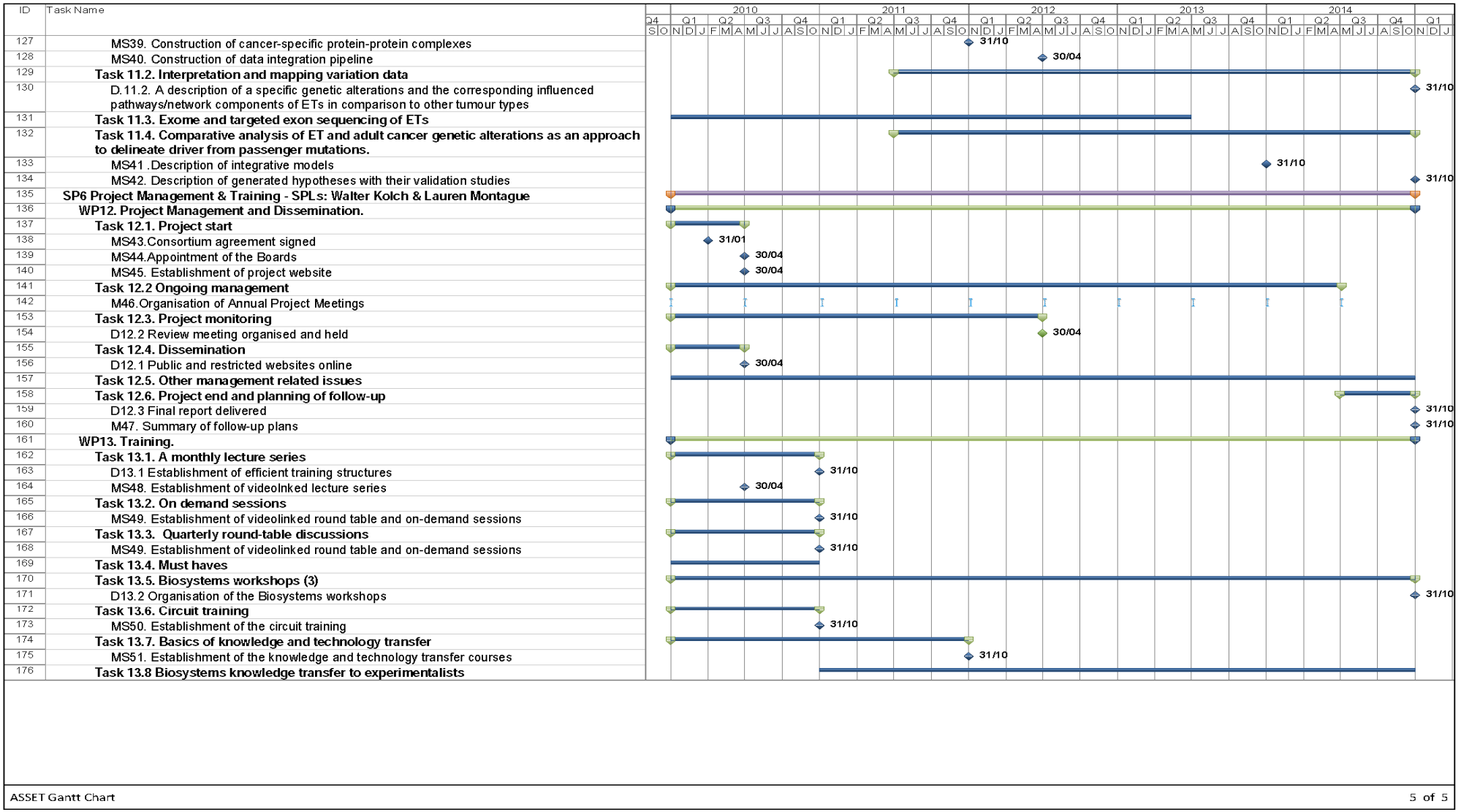
B 1.3.2 Timing of work packages and their components

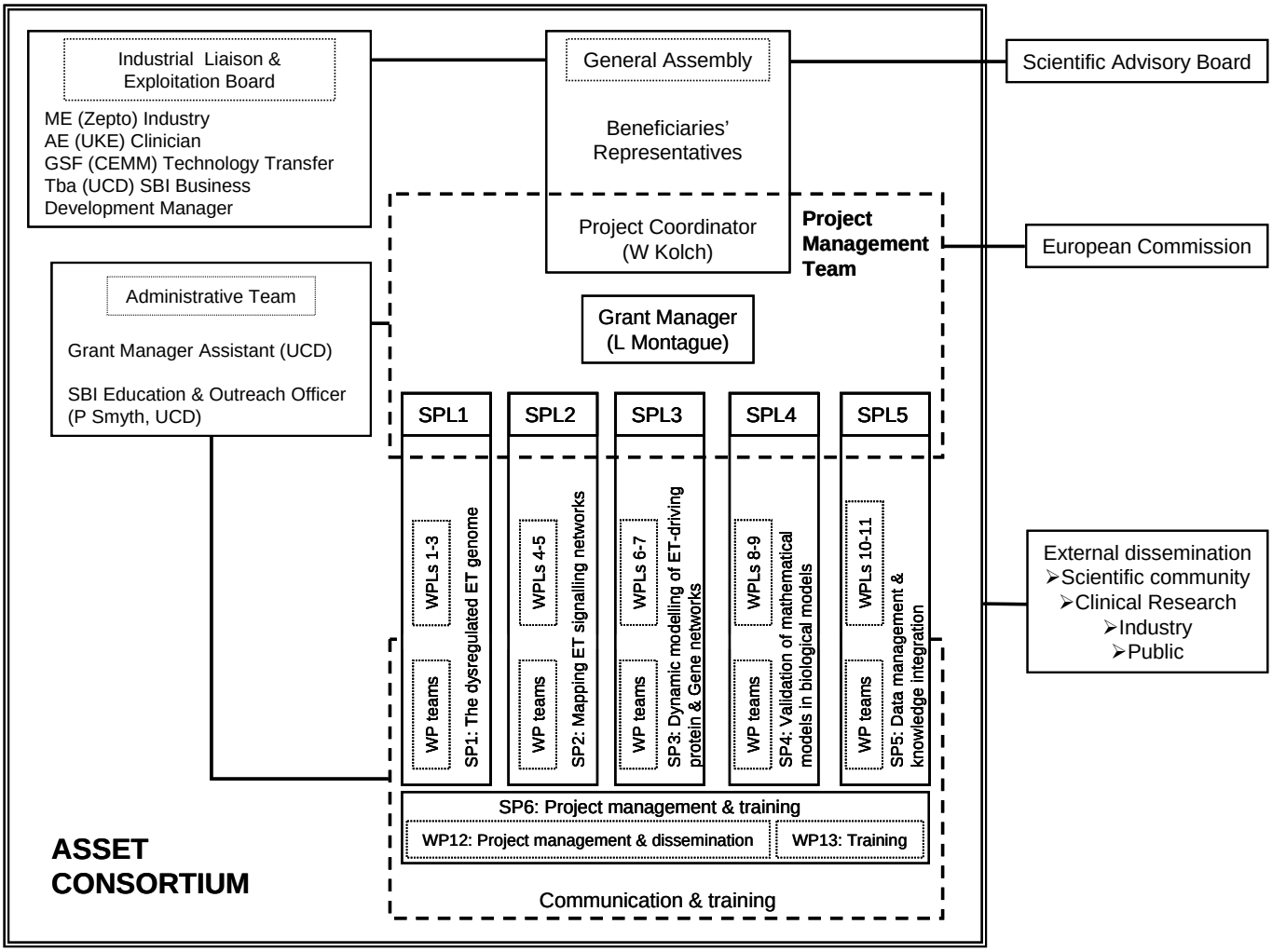


2. B2. Implementation

B 2.1 Management structure and procedures

ASSET will implement an organisational structure that is adequate to the complexity and aims of the project, yet simple and transparent enough to provide effective management and clear decision making mechanisms. The project coordinator, Walter Kolch, has longstanding and proven experience in the successful management of large interdisciplinary projects. For instance, before he moved to Ireland to head the Systems Biology Ireland Institute (SBI), he was the lead PI and coordinator of (i) the Interdisciplinary Research Collaboration in Proteomics Technologies RASOR (http://www.gla.ac.uk/rasor/), which is a ca. €18Mi project across four Scottish universities bringing together engineers, physicists, biologists, chemists and clinicians; and (ii) the Molecular Nose (http://www.molecularnose.org/), a ca. €6Mi project combining engineering, molecular biology and mathematical modelling.

The management structure is shown in the organisation diagram and described below:



a. The Project Coordinator (PC)

The project coordinator (PC), Walter Kolch, has the overall responsibility for the project. His role is to

1. chair the General Assembly (GA)
2. guide and as necessary adjust the scientific strategy of the project in consultation with the GA
3. chair the Project Management Team (PMT)
4. be the intermediate to the Commission
5. ensure that all EC guidelines and procedures applying to this project are followed

In order to allow the consortium t o concentrate fully on the research, most of the administrative and logistic work will be undertaken by the PC's office. For this the PC will be directly supported by the UCD/SBI grants manager, Lauren Montague, the UCD/SBI outreach and education officer, Philip Smyth, and the UCD/SBI business development manager, who is currently being recruited. While the grants manager time is charged to the management costs, the latter two functions are in kind contributions by UCD. This team will be responsible for the

1. Administrative project management
2. The project wide training activities
3. The project wide internal and external dissemination activities

b. The Project Management Team (PMT)

The Project management team (PMT) consists of the PC, the grant manager (GM) and the Sub-Project Leaders (SPL). Its role is the

1. Coordination of the scientific effort
2. Continuous development and implementation of the scientific strategy

c. The General Assembly (GA)

The governing and main decision making body of the consortium will be the general assembly (GA) which will consist of one representative of each research group, usually the principal investigator (PI) named as a Scientist in Charge of the work carried out in ASSET. Therefore, partners consisting of more than one research group will be represented by the corresponding number of delegates. Decisions will be made by majority vote, but if no majority vote can be achieved, the project coordinator will decide.

In general the GA will be in charge of the strategic decision making of the Consortium and will navigate the project according to the regulations settled in a consortium agreement. These comprise:

i) organisation/management: in general monitor the fulfilment of roles and responsibilities of each party from an administrative, legal, financial and technical point of view, communication procedures, methods and lines, decision making procedures, technical and operational monitoring, join of or withdrawal from the consortium, general changes to the consortium.
ii) Financial issues: reallocation of the budget if necessary according to the procedures specified in the consortium agreement in case of changes in the execution strategy.
iii) Confidentiality and publications: Information disclosure, confidentiality, publication and dissemination regulations
iv) Intellectual property rights, access rights and exploitation: Disclosure and protection of background for the project execution, regulation of transfer of background and foreground to third parties, property rights allocation of foreground, management of joint knowledge and of the protection strategy.
v) project execution: monitoring of project objectives, risk analysis and management of major issues, strategic-decision making and eventual re-orientation if relevant on the technical execution of the project

The above three groupings represent the executive and operational management of ASSET. They will be assisted by two advisory groups. These are

d. Scientific Advisory Board (SAB)

The role of the SAB will be to help optimising ASSET's output and impact by

1. Evaluating progress in comparison to the field in general
2. Evaluate the scientific strategy and its implementation, and suggest improvements
3. Providing constructive critique on the project in general
4. Recommending and facilitating new collaborations that will enhance output and impact

Therefore, the SAB will be populated with internationally recognised experts in the fields of ETs, signal transduction and systems biology. We aim to have a SAB whose expertise covers the whole scope of the project. As these requirements may change as the project progresses, we will ask SAB members to serve for a two year term, so that we can have the flexibility of turnover. For the initial SAB membership we plan to ask the following experts: Lee Helman, Head of the Molecular Oncology Section of the Pediatric Oncology branch the National Cancer Institute/NIH, USA, and an expert in childhood cancers. Huib Caron at the Amsterdam Medical Centre, Netherlands; he is also an expert in childhood cancers and new therapies. He coordinates the KidsCancerKinome project. Paul Meltzer, Head of Genetics Branch at the NCI/NIH, and expert in high-through-put genomics and bioinformatics. Aviv Regev, Broad Institute, MIT, Boston, MA, USA, who has both bioinformatics and

mathematical modeling expertise in complex mammalian systems. Michael Stumpf, Imperial College London, UK, expert in the statistical and mathematical analysis of molecular interaction networks and dynamical processes. Andrew Hopkins, College of Life Sciences Dundee, UK, an expert in "network pharmacology" and the discovery and rational design of multi-target drugs.

e. Industrial Liaison and Exploitation Committee (ILEC)

The Industrial Liaison and Exploitation Committee has the function

1. to screen the project output for results that could be useful for IP protection
2. liaise with industry to ensure the translation of project results into application and further development

The ILEC will be chaired by Markus Ehrat, our industrial partner in the project. Other members include Giulio Superti-Furga (academia & biotech expertise), Angelika Eggert (clinical expertise), Olli Kallioniemi (technology and technology exploitation expertise) and the SBI business development manager (whom we will be recruiting shortly).

2.1.2 Meetings

a. Annual General Meetings

On a yearly basis all project partners and the research staff involved in the project will meet and report on overall achievements of the previous year, discuss miscellaneous topics to be addressed and present plans for the next year. For cost effectiveness this meeting will also encompass the

1. *GA-Meetings.* The GA will be in continuous communication by email, telephone and the interactive project webpage. Formal GA-meetings will take place at least once a year. Here decisions concerning topics that will be regulated by the consortium agreement i.e. IPR, budget reallocation, changes to the consortium will be taken.
2. *SAB-Meetings.* In addition, we will make continuous use of the SAB by asking them to comment on the quarterly internal reports and specific issues as they arise.
3. *ILEC Meetings.*

b. Other Executive Meetings will be held as required. In particular, PMT meetings by teleconference will be scheduled quarterly coinciding with the quarterly internal reporting to discuss current issues.

2.1.3. Reporting

Biannual Internal reports

The WPLs will biannually compile a short written report on the work progress of their teams and provide them to the SPLs in order to anticipate and resolve any issue that may arise. The SPLs will compile the corresponding WP reports and provide them to SP participants and the PC.

Reports to the Commission

The periodicity of reports to the commission will be every 12 month. Reports and compilation of reports will be generated according to the work responsibilities (WPLs → SPLs → PC), and reports will be submitted by the PC.

2.1.4. Decision making mechanisms

The decision making mechanisms will be regulated by the consortium agreement.

The objective is to implement a management structure within the consortium which will give the possibility to take rapid and efficient decisions (avoid status-quo or blocking situation) whenever necessary and allow simultaneously to have each individual organization represented, in order to anticipate and avoid the occurrence of disagreements/conflicts.

The chosen structure will:

- Ensure the possibility to take strategic decisions and decisions regulated by the consortium agreement to all the members (GA). This is the reason why not only each beneficiary, but each research group will be represented in the GA.

-Allow a close collaboration between the strategic and operational level (implementation of the scientific activities) represented by the WPLs and the SPLs.
- Avoid slow and complex decision processes and limit the use of veto power.

In particular, specific decisions will be taken according to the following rules:
-Matters concerning topics ruled by the consortium agreement will be decided upon on by a 75% quorum of the General Assembly.
-All other matters require a simple majority.
-In the case of a split vote, the Project Coordinator will carry the deciding vote.

Addition of beneficiaries
Will be done if required for the progress and success of the project. New beneficiaries can be suggested by any existing beneficiary or by any of the boards, and presented to the GA for discussion. The decision about admission to the consortium will be made by the GA requiring simple majority vote. New beneficiaries will be integrated with the same rights, duties and benefitsas existing beneficiaries.

2.1.5. Consortium Agreement

A Consortium Agreement (CA) will be prepared on the basis of the DESCA model. It will be validated by the PMT with the advice of each corresponding legal offices and signed at the initiation phase of the project. The CA will state the objectives of the collaboration within parties and their corresponding expected deliveries to the project. Further, the project management structure and operational provisions for the execution of the project i.e roles and responsibilities, budget allocation and grant transfer process, access rights to pre-existing know-how (background) and knowledge arising from the project (foreground), IPR, exploitation, publication rights and processes, liabilities, knowledge disclosure, confidentiality, etc.

B2.2 Beneficiaries

1	UCD	The National University of Ireland, Dublin – University College Dublin (NUID UCD)

The National University of Ireland, Dublin - University College Dublin, (NUID UCD) is Ireland's largest University with over 28,000 students, and ranked by the 2009 Times Higher Education Supplement at number 89 worldwide. Systems Biology Ireland (SBI) is a newly founded Institute at UCD that uniquely combines wet (Kolch) and dry (Kholodenko) disciplines under one roof. This integration of computational scientists with lab-based scientists affords iterative project design and instant feedback of results that exceeds what can normally be accomplished by traditional collaborations. The Systems Biology group currently has 30 technical and non-technical employees, a number that is projected to double over the next five years. SBI is located in the multidisciplinary Conway Institute of Biomolecular and Biomedical Research at UCD, a state-of-the-art facility housing over 550 researchers working on molecular mechanisms underlying disease. SBI's research combines predictive mathematical modeling with experimental research probing signaling networks and their emergent properties. See both http://www.ucd.ie/sbi/ and http://www.ucd.ie/conway/ for further information.

Role within the project

The UCD Systems Biology Group, which combines both wet and dry lab facilities and has expertise in Biochemical Analysis and Dynamic Modeling of Signal Pathways, will take a lead scientific and programmatic role in the project.

UCD involves two research groups working together to achieve parallel and complementary results - The Kolch Group and The Kholodenko Group:

1. Walter Kolch is the Project Coordinator (PC) and responsible for the scientific and operational delivery of the project aims. He heads the Project Management team (PTM) and the Management & Training SP6. WK will perform MS-based proteomics experiments to track signal flux via changing protein interactions to map pathways altered in ETs, and the SRM experiments for absolute protein quantification.

2. Boris Kholodenko will lead SP3 and WP6. His group will develop experimentally validated mathematical models of the core regulatory networks. He is also a member of the PM team.

UCD will coordinate training and dissemination programs in which all participants will be involved , willl host the kick-off of the project which will take place within the frame of the new SBI building's inaguration and two in-depth workshops that will be held within the frame of the yearly technical meetings and of international scientific symposia. The UCD business development manager at UCD (the post is about to be advertised) will be member of ASSET's industrial liason and exploitation committee.

Previous R&D Experience relevant to the project

Professor Walter Kolch is the Director of SBI and the Conway Institute for Biomolecular and Biomedical Research at UCD and is an international expert in the analysis of signal transduction pathways using state of the art imaging, biochemical and proteomics methods. He was continuously involved in EU projects over the last decade as coordinator and participant, and has managed large interdisciplinary consortia, e.g. RASOR (until July 2009; http://www.gla.ac.uk/faculties/fbls/rasor/).

Professor Boris Kholodenko is the Deputy Director of SBI and has a long-standing track record and broad experience in systems biology. His work focuses on dynamic predictive mathematical modeling and computer simulation of biochemical networks that determine cellular responses to external cues, and thereby cell fate decisions.

Key people involved in the project

Walter Kolch -Project Coordinator (PC) of this project, leads SP6. He is Director of Systems Biology Ireland and the Conway Institute of Biomolecular and Biomedical Research. Previously, Senior Group Leader at the Beatson Institute for Cancer Research & Prof for Molecular Cell Biology, University of Glasgow, UK. Career includes work in drug discovery as Department Head in pharmaceutical industry (Parke Davies). >140 peer reviewed publications; >70 invited lectures since 2004. President of the British Society for Proteome Research, Fellow of the Royal Society of Edinburgh.

Boris Kholodenko – Leader of SP3 and WP12. Stokes Professor of Systems Biology and Deputy Director of

SBI. PhD 1976 in Biophysics from the Moscow Institute of Physics and Technology. Dr.Sci. 1989 in Biophysics from Moscow State University. Boardmember of the International Society for Systems Biology, Chairman International Consortium on Systems Biology of Receptor Tyrosine Kinase Networks. Advisory Editorial Board, Molecular Systems Biology EMBO, Editorial Board, IET Systems Biology (IET), Systems and Synthetic Biology, Springer. Over 190 peer reviewed publications.
Dr Alexander von Kriegsheim – Senior Postdoctoral Fellow. AvK is an expert in proteomics applied to the mapping of signalling networks, and also has participated in the FP6 Interaction Proteome grant. He will supervise the proteomic pathway mapping efforts at SBI and coordinate the wet lab activities with the modelling activities.

Lauren Montague is SBI's grant manager and has over 15 years of experience developing and supporting large-interdisciplinary research consortiums. She will be responsible for the overall administrative and financial management of the award and will serve as the liason to and resource for the partners on the logistical and reporting aspects of the project.

References related to the project

1. Kholodenko, B. N. (2006) Cell-signalling dynamics in time and space. Nat Rev Mol Cell Biol. 7, 165-176.
2. Borisov, N., Aksamitiene, E., Kiyatkin, A., Legewie, S., Berkhout, J., Maiwald, T., Kaimachnikov, N. P., Timmer, J., Hoek, J. B. & Kholodenko, B. N. (2009) Systems-level interactions between insulin-EGF networks amplify mitogenic signaling, Mol Syst Biol. 5, 256.
3. von Kriegsheim, A., Baiocchi, D., BirtWeizmanntle M., Sumpton, D., Bienvenut, W., Morrice, N., Yamada, K., Lamond, A., Kalna, G., Orton, R., Gilbert, D., and Kolch, W. Cell fate decisions specified by the dynamic ERK interactome. Nat.Cell Biol. 11: 1458-1464, 2009.
4. Preisinger C, von Kriegsheim A, Matallanas D, Kolch W. Proteomics and phosphoproteomics for the mapping of cellular signalling networks. Proteomics 21: 4402-15, 2008.
5. von Kriegsheim, A., Baiocchi, D., BirtWeizmanntle M., Sumpton, D., Bienvenut, W., Morrice, N., Yamada, K., Lamond, A., Kalna, G., Orton, R., Gilbert, D., and Kolch, W. Cell fate decisions specified by the dynamic ERK interactome. *Nat.Cell Biol.* 11: 1458-1464, 2009.
6 .Matallanas, D., Romano, D., Yee, K., Meissl, K., Kucerova, L., Piazzolla, D., Baccarini, M., Vass, J. K., Kolch, W., O'Neill, E. RASSF1A elicits apoptosis through an MST2 pathway directing proapoptotic transcription by the p73 tumor suppressor protein. *Mol.Cell.* 27: 962-975, 2007

Participation in relevant National or European research projects

UCD has a major contractual record in successive EU Framework Programmes, both as Coordinator (FP6 - 30) and Partner (FP6-76). Kholodenko and Kolch are currently involved in several EU projects, and Kolch has longstanding experience in EU programmes being continuously involved in EU grants since 2001 as both coordinator and participant, including RASOR (until July 2009; http://www.gla.ac.uk/faculties/fbls/rasor/) and FP6: GROWTHSTOP. Kholodenko & Kolch are leading the €15Mi Systems Biology CSET programme awarded by Science Foundation Ireland in 2009.

Personnel involved in the project

3 Post-Docs to be recruited (two for WK and one for BK), one or two are likely to be female.
Further women involved in this project include the SBI grants manager, Lauren Montague, who will be responsible for the management of this grant; a part time assistant (Magdalena Bryla) for administrative support, n.n. UCD Business Developer (to be recruited in short) and Kate Johnston, the SBI centre manager (the latter three in kind contributions of UCD). Additionally, SBI's Education and Outreach Officer, Philip Smyth, will also help with dissemination efforts including the generation of core Systems Biology training modules. This will also be an in-kind contribution from UCD.

2	CCRI	St Anna Kinderkrebsforschung e.V.

The Children's Cancer Research Institute (CCRI) is owned by St. Anna Kinderkrebsforschung, a private charity-based association. It is one of the leading paediatric basic and translational research institutions in Europe. CCRI hosts 10 research groups and is closely affiliated with the St. Anna Children's Hospital, the major paediatric cancer centre in Austria. HK is scientific director of the CCRI, head of the ASSET-contributing Molecular Biology department. CCRI is equipped with state-of-the art cell culture, molecular genetic and biochemical analysis facilities. HK's group has been focussing on the molecular biology of paediatric bone tumours for twenty years and has made significant contributions to the understanding of the molecular pathogenesis of ESFT.

Role within the project (If more than one department involved, describe all the teams)

1. Perform *in-vitro* experimental perturbations of fragility nodes in ESFT to i) generate (time-resolved) data for *in silico* GRN modelling, and ii) experimentally validate in silico models in ESFT. 2. Provide genetically engineered ESFT cell line models for conditional expression of key oncogenes and fragility nodes identified in ASSET, for high-throughput miRNA and drug screening, for time-resolved high-throughput mRNA and miRNA profiling, and for proteomics and transcription factor interaction studies. 3. Provide our EWS-FLI1 and MYC target gene database as a starting point 4. Our collection of ESFT cell lines and of functional tools for experimental work in the ESFT will be central to all WPs. 5. Host of one in-deep workshop that will be held within an international scientific symposium.

Previous R&D Experience relevant to the project

HK coordinated functional and genomics studies related the role of EWS-FLI1 and MYC in ESFT in FP6-PROTHETS and EETP. We own one of the largest collections of ESFT cell lines and have generated derivatives that will be used in ASSET. Ample access to primary tumor data due to international cooperations i.e. the European clinical ESFT studies (EICESS92, Euro-EWING99, EWING2008). All techniques required for ASSET i.e. the generation of inducible gene expression systems in ESFT cell lines, lentiviral gene transfer, RNAi by si/shRNA, chromatin immunoprecipitations, reporter gene, proliferation and apoptosis assays are well established. Established a database combining the largest collection of EWS-FLI1 knockdown data in ESFT cell lines worldwide under normoxic and hypoxic conditions, with MYC knockdown, and primary tumour data as compared to 89 normal tissues, and ChIP-seq for EWS-FLI1 and ChIP-chip for MYC. Identifed several physical and functional interaction partners for EWS-FLI1 and established a link between the chimeric EWS-FLI1 oncogene and the p53 tumor suppressor pathway, which became part of the core GRN underlying the ASSET project. Close collaborators to ASSET participants OD, AE, FS, AA in FP6-EET. Recently established comprehensive gene expression database for genes and miRNAs regulated by EWS-FLI1, (major ESFT oncogene), integrating results from genetic knockdown experiments in 7 ESFT cell lines: Expression data of ~60 primary tumors in relation to a) mesenchymal stem cells and EWS-FLI1 ChIP-seq data, b) differential gene and miRNA expression data for MYC knockdown and c) MYC ChIP-chip data. This database combined with data from OD (Curie) will form the starting point for ASSET´s analyses of ESFT.

Description of key people involved in the project

Prof. Heinrich Kovar (SP4 leader): Since 1988 Head of Dept. Mol. Biol., since 2001 Scientific Director of CCRI, Vienna. Chair of the Molecular Biology Committee of the European Ewing´s sarcoma clinical trials. Publications in peer reviewed journals: 69; grants: 17; awards: 4. Maximilian Kauer (Bioinformatician): Postdoc at Yale School of Medicine, Dept. Genetics, Dr. Kevin P. White's lab; since 2008: Bioinformatics core facility of CCRI. Publications in peer reviewed journals: 11; grants: 1; awards: 1

References related to the project

Ban J, Bennani-Baiti I, Kauer M, Schaefer K-L, Poremba C, Jug G, Schwentner R, Smrzka O, Muehlbacher K, Aryee D, Kovar H (2008) EWS-FLI1 suppresses NOTCH-activated p53 in Ewing´s sarcoma. Cancer Res. 68, 7100-9.

Bachmaier R, Aryee DN, Jug G, Kauer M, Kreppel M, Lee KA, Kovar H (2009) O-GlcNAcylation is involved in the transcriptional activity of EWS-FLI1 in Ewing's sarcoma. Oncogene 28, 1280-84.

Kauer M, Ban J, Kofler R, Walker B, Davis S, Meltzer P, Kovar H (2009) A molecular function map of Ewing's sarcoma. PLoS ONE 4:e5415

Participation in relevant National or European research projects

International: EU-FP6: PROTHETS, nr. 503036:WP5 leader (therapeutic value of EWS-FLI1 and therap. tools for clinical application); EU-FP6: EET-Pipeline, nr. 037260: WP7 leader (cell cycle); National: GEN-AU Ch.I.L.D., contract GZ200.071/2-VI/1/02: project coordinator.

Personnel involved in the project

3 postdocs: D. Aryee, M. Kauer, ND; in kind: 1 PhD; 2 technicians, CCRI's grant management office, and HK.

3	CEMM	CeMM – Forschungszentrum für Molekulare Medizin GmbH

At CeMM, the Research Center for Molecular Medicine of the Austrian Academy of Sciences the mode-of-action of pathologically-relevant proteins and novel or existing drugs is determined. Pathological processes are linked via an interdisciplinary systems-biology strategy and implemented into alternative and innovative diagnostic and therapeutic approaches. These systems-biology techniques encompass modern post-genomic methods including proteomics, chemical biology, biological-chip technologies and bioinformatics. The research interests of CeMM focus on cancer, inflammation and immune disorders.

Role within the project

We have developed a robotics-assisted combinatorial drug screening platform supported by CEMM's bioinformatics department, which will serve as a starting point for the investigation and the mathematical evaluation of synergistic drug effects of all combinations of the described kinase inhibitor and ET cell line panels. Through drug target identification by phospho- and a taylor-made chemical proteomics approach to identify the target network, characterisation of drug-dependent transcriptome changes and subsequent pathway analysis, CeMM will identify the specific ET cancer cell vulnerabilities that are targeted by these drugs.

Previous R&D Experience relevant to the project

(i) Superti-Furga and his team's expertise in proteomics is witnessed by several studies that contributed in a decisive way our current understanding of the organization of eukaryotic proteomes. The specific chemical proteomics expertise is summarized in a recent Nat Chem Biol review. (ii) Giulio Superti-Furga is coordinating the project PLACEBO (Platform in Austria for Chemical Biology) and has organized three conferences on chemical biology. The aim of PLACEBO is to generate novel bioactive molecules for two important biological pathways, identify the mechanism of action of four clinically relevant small molecule drugs and to establish access to chemical biology resources for researchers in Austria and beyond. The laboratory has derivatized several kinas einhibitors that can be immobilized without losing activity.

Key people involved in the project

Name and responsability, Experience and background, number of publications, patents, awards, role in the project.

Giulio Superti-Furga, Subproject Leader, recognized as one of the world's leading researchers in the area of molecular medicine and systems biology. Senior ERC awardee. His work explaining molecular protein networks is acknowledged as one of the groundbreaking and most-cited works of the post-genomic era. 94 publications, 10 patents.

Keiryn Bennett, Head of Mass Spectrometry, a world-renowned mass spectrometrist with 10 years of experience in protein identification, leading a dedicated mass spectrometry team with 5 state-of-the-art machines. 25 publications.

Uwe Rix, Chemical Proteomics, top chemist interested in elucidating the mechanism of action of research compounds and drugs at the molecular level, familiar with state of the art protein mass spectrometry technologies. 21 publications, 1 patent.

References related to the project

Lehár, J., Stockwell, B.R., Giaever, G. & Nislow, C. Combination chemical genetics. Nat Chem Biol 4, 674-81 (2008).

Rix, U. & Superti-Furga G. Target profiling of small molecules by chemical proteomics, Nat Chem Biol 5 616-624 (2009).

Bürckstümmer, T., et al. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. Nat Immunol. 10 266-72 (2009).

Participation in relevant National or European research projects

PLACEBO (description see above)

EU-Openscreen: EU-OPENSCREEN is part of the updated ESFRI Roadmap 2008 which lists important future research infrastructures in Europe. The aim is to satisfy the needs for new bioactive compounds in many fields of the Life Sciences (e.g. human and veterinary medicine, systems biology, biotechnology, agriculture and nutrition). CeMM is the official representative of Austria.

Presonnel involved in the project

Post-Docs: Uwe Rix (m), PhD: Georg Winter (m). In kind: PhD Keiryn Bennett (f), contribution of GSF.

4	CURIE	INSTITUT CURIE, Genetics and Biology of Cancers Unit

The Curie Institute, a private non-profit foundation established in 1921 and directed since the January 2002 by the Professor Claude Huriet, is dedicated to pursuing two connected goals in the fight against cancer: patient management and oncology research. In Paris and in Orsay, over 2,200 people currently work for the Curie Institute's hospital and research center. Curie Institute is one of the main cancer centers in France with 75,000 people visiting per year, including 7,500 new patients. The areas of expertise are breast cancer, sarcomas, paediatric tumors and intraocular malignancies. The genetic and biology of peadiatric tumour group is part of the INSERM U 830 unit. Its main focus is to characterize the genetic abnormalities found in peadiatric cancers, then to study their role in oncogenesis through the construction of appropriate in vitro and in vivo models.

A main focus of this group is Ewing's sarcoma. In 1992 this group identified the EWS gene fusion resulting from the the t(11;22) translocation. To further investigate EWS-FLI-1 properties, this group has been involved in collaboration with the bioinformatics INSERM U 900 unit through the SITCON (modeling signal transduction induced by a chimeric oncogene) and SYBEWING (systems biology of Ewing's tumor: high-throughput phenotyping and mathematical modeling) projects that were funded by ANR and INCA respectively.

Role within the project (If more than one department involved, describe all the teams)

We will contribute our knowledge of Ewing's sarcoma and of its mechanism of development and at the level of reagents (inducible cell lines, xenograft models, validated shRNA, tumour samples, etc). Our role within the project will include the following: (i) sequencing the A673 cell line; profiling of miRNA expression in time resolved EWS-FLI systems and in tumours; (iii) testing the model predicted fragility nodes and refine the ESFT network; (iv) xenograft models, in particular those in which EWS-FLI can be modulated in vivo.

Previous R&D Experience relevant to the project

The Institut Curie is a reference center for Ewing's sarcoma molecular diagnosis in France. It receives frozen material for most Ewing's sarcoma diagnosed in this country. Ewing's sarcoma also constitutes one of the main themes of the genetic and biology of pediatric tumour group for 20 years. High throughput and systems biology approaches have been developed in collaboration with the INSERM 900 Unit.

Key people involved in the project

Olivier DELATTRE is a medical oncologist specialised in peadiatrics. He got his PhD in 1991 and is now head of the INSERM 830 Unit at the Curie Institute in Paris. One of his main research areas is the study of adult and childhood sarcomas. Number of publication: superior to 200 – H factor: 50

Franck TIRODE is an INSERM scientist working on the molecular biology of Ewing's sarcoma with a strong background in transcription mechanisms. Number of publication: 15 – H factor: 9

References related to the project

- Delattre, O., Zucman, J., Plougastel, B., Desmaze, C., Melot, T., Peter, M., Kovar, H., Joubert, I., De Jong, P., Rouleau, G., Aurias, A. and Thomas, G. Gene fusion with an ETS domain caused by chromosome translocation in human tumours. Nature 359:162-165, 1992.
- Tirode, F., Laud-Duval, K., Prieur, A., Delorme, B., Charbord, P., Delattre, O. Mesenchymal stem cell features of Ewing tumors. Cancer Cell, 11: 421-429, 2007.
- Guillon, N., Tirode, F., Boeva, V., Zynovyev, A., Barillot, E, Delattre, O. The oncogenic EWS-FLI1 protein binds in vivo GGAA microsatellite sequences with potential transcriptional activation function. PLOS One, 4 : 1-8, 2009.

Participation in relevant National or European research projects

FP6 - project KCK (KidsCancerKinome) – contract n° 037390

FP6- EET Pipeline (European Embryonal Tumour Pipeline) -– contract n° 037260

SITCON project (modeling signal transduction induced by a chimeric oncogene) funded by ANR

SYBEWING project (systems biology of Ewing's tumor: high-throughput phenotyping and mathematical modeling) funded by INCA

Personnel involved in the project

1 PostDoc. Further involved in the project and in kind contributions: 4 PostDocs, participation of O Delattre and F Tirode.

4	CURIE	INSTITUT CURIE, Department Bioinformatics and Computational Systems Biology of Cancer

The Bioinformatics and Computational Systems Biology of Cancer research group is part of the U900 INSERM unit, which comprises three other computational research groups and a bioinformatics platform. Our expertise covers the mathematical modeling of biological networks (and in particular pathways involved in tumorigenesis and tumor progression) and the analysis and integration of all types of large-scale biology data (microarrays, deep sequencing, siRNA screenings, etc.). Our research group collaborates with the other institut partner (INSERM U830) through the SITCON (modeling signal transduction induced by a chimeric oncogene) and SYBEWING (systems biology of Ewing's tumor: high-throughput phenotyping and mathematical modeling) projects that were funded by ANR and INCA respectively.

Role within the project

We will contribute to the project at the level of computational and mathematical modeling of pathways involved in the Ewing's sarcoma, analysis of high-throughput siRNA-based screenings, finding fragility points in the core network of embryonic tumors and integrative analysis and mathematical modelling of microRNA role in embryonic tumors. We will also contribute experience in integrative data analysis, mathematical modelling of molecular mechanisms of cancer, working with systems biology standards and developed software for analysis of biological networks and analysis of high-throughput sequencing data and can organize teaching seminars to share this expertise with other members of the consortium.

Previous R&D Experience relevant to the project

CURIE's Bioinformatics and Systems Biology of Cancer group has large experience in computational biology of cancer projects, including data integration, data analysis, application and invention of new of machine learning techniques, systems biology standards and methods development and mathematical modelling of biological pathways involved in tumor progression. In particular, since 2005 we are in tight collaboration with the department of Olivier Delattre from Institut Curie for the systems biology of Ewing's sarcoma. This collaboration was supported by several national grants in France (ANR SITCON, INCA SYBEWING). In this collaborative work, we created computational model of the molecular interaction network deregulated in Ewing's sarcoma, analysed high-throughput data on gene and microRNA expression, next generation sequencing data produced for studying the Ewing's sarcoma. We could develop several tools for analysis and modelling of biological networks widely used by bioinformatics and systems biology community worldwide.

Key people involved in the project

Andrei Zinovyev, coordinator of the Computational Systems Biology of Cancer team at Institut Curie, has background in theoretical physics (M.Sc.), computer science and bioinformatics (Ph.D.), has 29 publications in peer-reviewed journals, 4 book chapters, one monograph. In ASSET he will be involved in the mathematical modeling of pathways and data analysis.

Emmanuel Barillot, director of the U900 research department INSERM – Mines ParisTech – Institut Curie "Bioinformatics and Computational Systems Biology of Cancer", director of the institut Curie Bioinformatics platform, director of Bioinformatics, Institut Curie, Paris, France. Emmanuel Barillot has background in Biophysics (M.Sc.) and Computational Human Genetics (Ph.D.), has 20 years of experience of managing scientific projects, more than 60 publications in peer-reviewed journals. In the ASSET project Emmanuel Barillot will provide general coordination and support to the project from the Bioinformatics platform of Institut Curie, will participate in the mathematical pathway modeling and finding fragility points.

References related to the project

Calzone L., Gelay A., Zinovyev A., Radvanyi F., Barillot E. A comprehensive modular map of molecular interactions in RB/E2F pathway. 2008. Molecular Systems Biology 4:174

Zinovyev A., Viara E., Calzone L., Barillot E. BiNoM: a Cytoscape plugin for using and analyzing biological networks. 2008. Bioinformatics 24(6):876-877

Guillon N, Tirode F, Boeva V, Zynovyev A, Barillot E, Delattre O. The oncogenic EWS-FLI1 protein binds in vivo GGAA microsatellite sequences with potential transcriptional activation function. PLoS One. 2009;4(3):e4932.

Participation in relevant National or European research projects

1)FP7 – APO-SYS , HEALTH-F4-2007-200767, mathematical modeling of apoptosis pathways.

2)SITCON project (modeling signal transduction induced by a chimeric oncogene) funded by ANR (Andrei Zinovyev is the coordinator of the project)

3)SYBEWING project (systems biology of Ewing's tumor: high-throughput phenotyping and mathematical modeling) funded by INCA (Andrei Zinovyev is the sub-coordinator of the systems biology part of the project)

Personnel involved in the project

1 PostDoc. In kind: 1 PostDoc and the participation of A Zinovyev and E Barillot. Women involved: 1

5	DKFZ	German Cancer Research Centre (Deutsches Krebsforschungszentrum, Dept Tumour Genetics and Theoretical Bioinformatics)

The Deutsches Krebsforschungszentrum (German Cancer Research Center, DKFZ) has a worldwide reputation of excellence in basic and translational cancer research as one of the leading research centers in this area. Founded in 1964, the DKFZ has today a staff of about 1020 scientists and 1180 employees providing technical and administrative support. In 2009, the total budget amounted to 155,6 million Euro funded by the Federal German Government and the State of Baden-Württemberg and third-party funding. The DKFZ is a member institution of the Helmholtz Association which is Germany's largest research organisation. Research at the DKFZ contributes to a better understanding of fundamental processes in cancer and develops innovative methods of cancer diagnostics and therapy. The research programme features seven main areas: Cell biology and cancer; Structural functional genomics; Cancer risk factors and prevention; Tumour immunology; Innovative cancer diagnosis, prevention and therapy; Infection and cancer; Translational cancer research.
The DKFZ offers outstanding facilities for innovative, multidisciplinary research with state-of-the art scientific equipment. In Central Core Facilities it provides: Genome and proteome analyses, Microscopy, Animal care as well as Computer services and documentation. In addition to the technical infrastructure, the DKFZ provides extensive administrative and technology transfer support for scientific research: The Unit "Administrative Project Management" assists scientists in all financial, administrative and contractual matters related to the management of third party funded projects. Locally, the DKFZ has long-standing ties with the University of Heidelberg and the European Molecular Biology Laboratory (EMBL), which provide the basis for intensive exchange and collaboration between scientists and facilities. Cooperations with numerous clinics and hospitals in the area provide opportunities to link basic and clinical research.

Role within the project (If more than one department involved, describe all the teams)

Department "Modelling of biological systems" - Thomas Höfer and his colleagues will develop mathematical models for the core unit regulating cell proliferation and apoptosis decisions in ETs and lead the corresponding WP7. This work will build on an already established close collaboration with the experimental group of Frank Westermann, involving regular joint meetings, analysis of data and planning of experiments. In collaborating with theoreticians Mark Girolami, Boris Kholodenko and others as well as cell biologists and clinicians, including Angelika Eggert, Heinrich Kovar, Walter Kolch and Giulio Superti-Furga, the work of the Höfer group will provide a hub for the tight integration of experimental work and bioinformatics with mechanistic mathematical modeling in ASSET.

The department of "Tumour Genetics" – Frank Westerman will provide all large-scale genomics and proteomics data for NB related work. We will also make available to the consortium our CGH-array and gene expression data of more than 300 primary NB tumors together with the clinical follow up data. In addition, will contribute with our expertise on MYC transcription factors in the development of cancer. Experienced in the explorative analysis of molecular genetics and further experimental data, S. Bulashevska applies state-of-the-art statistical and computational techniques to model and dissect complex biological processes; works in close collaboration with the groups in Systems Biology Centre Bioquant at the University of Heidelberg.

Department "Theoretical Bioinformatics"- Dr. Svetlana Bulashevska's group develops novel methods of bioinformatics and statistical analysis of experimental data with a goal of understanding cellular processes.

Previous R&D Experience relevant to the project

The Höfer group has had long-standing expertise in developing experimentally-based mathematical models of regulatory networks in eukaryotic cells. Höfer has been well-recognized for his research in biological pattern formation and cellular calcium signalling. His current work focuses on the regulation of cell proliferation and differentiation decisions in mammalian cells. Recent discoveries include a novel switch for T-cell proliferation mediated by a cytokine network and the definition of a core gene-regulatory network of T-cell differentiation, which were made possible through the tight iteration of theoretical and experimental work. The group is currently developing a multi-scale modeling approach that aims at a mechanistic understanding of elementary modules of gene regulation, dynamic protein complex formation and posttranslational modification and their integration into predictive models of interacting signal-transduction and gene-regulatory networks. These cell-biological problems are addressed with a common modeling and simulation methodology, drawing on biochemical systems theory, nonlinear dynamics, stochastic processes, reaction-

transport theory, and statistical concepts for parameter estimation and model identification. The group consists of experienced postdocs and PhD students from biophysics, biochemistry, physics and mathematics as well as technical support staff. It provides an interdisciplinary environment that fosters tight collaborations with experimentalists.

F. Westermann, group leader, is heading the neuroblastoma research group in the department and the reference lab of the German Neuroblastoma Trial. Our major research interests are basic and translational aspects of NB research. We are using structural and functional genomics approaches to define the molecular mechanisms of NB spontaneous regression and malignant progression. Major achievements over the past 5 years include the assembly of a diagnostic NB chip and introduction into the clinic. Furthermore, we have established the first model of spontaneous NB regression, which is based on a low-level MYCN protein function gain in NB cells. We could dissect distinct MYCN and c-MYC functions in NB tumorigenesis. Finally, we have identified CAMTA1 as a new tumor suppressor gene on chromosome 1p36. My group has been among the first to use chromatin immunoprecipitation (ChIP) in a genome-wide scale to analyze MYCN functions in tumor cells. Currently we are working on the integration of different high-throughput platforms (array-CGH, gene expression arrays, ChIP-chip, ChIP-seq, siRNA functional screens,) and bioinformatics to address relevant questions in NB research.

S. Bulashevska developed novel methods for inferring genetic regulatory networks and transcriptional programs in various diseases, modelled protein-protein interaction networks etc. She applies probabilistic Bayesian modelling and MCMC simulation techniques, especially required in the project.

Key people involved in the project

Prof. Dr. Thomas Höfer, biophysicist, 1996 DPhil University of Oxford, currently head of research group Modeling of Biological Systems at DKFZ, is an expert in the mathematical modeling of regulatory networks in mammalian cells, >50 publications, Boehringer Ingelheim Fellowship (1993-96), Jowett Senior Scholar at Balliol College Oxford (1994-96), Scientific expert in EU-FP7, coordinator of German research network on Epigenetic networks in gene silencing and cancer.
Dr. Elan Gin, postdoc, 2007 PhD in applied mathematics from University of Auckland, 6 publications, recipient of several prizes and distinctions, is an expert in model simulation, parameter estimation and statistical model discrimination in systems biology, previous experience includes extended stay in experimental laboratory.
Florian Lamprecht, PhD student, studied physics and biophysics at University of Heidelberg and EMBL, MSc with distinction, develops mathematical models and closely interacts with experimentalists.

F. Westermann, Group leader and head of the NB reference lab of the German Neuroblastoma trial, No. of publications: 42, Awards: DFG research fellowship, role in the project: defining the role of MYCN or c-MYC in ET development and progression.

S. Bulashevska, Postdoctoral scientist. No. of publications: 10

References related to the project

Schulz, E., Mariani, L., Radbruch, A., and Höfer, T. Sequential polarization and imprinting of T-helper type 1 differentiation by interferon-γ and interleukin-12. Immunity 30, 678-688 (2009).
Busse, D., de la Rosa, M., Hobiger, K., Thurley, K., Floßdorf, M., Scheffold, A. and Höfer, T. Competing feedback loops shape IL-2 signaling between helper and regulatory T cells in cellular microenvironments. Proc. Natl. Acad. Sci. USA, in press (2010).
Diernfellner, A., Querfurth, C., Salazar, C., Höfer, T., and Brunner, M. (2009) Phosphorylation modulates rapid nucleo-cytoplasmic shuttling and cytoplasmic accumulation of Neurospora clock protein FRQ on a circadian time scale. Genes Dev. 23, 2192-2200.

Schwab, M., F. Westermann, B. Hero, and F. Berthold, Neuroblastoma: biology and molecular and chromosomal pathology. Lancet Oncol, 2003. 4(8): p. 472-80.
Westermann, F., K.O. Henrich, J.S. Wei, W. Lutz, M. Fischer, R. Konig, R. Wiedemeyer, V. Ehemann, B. Brors, K. Ernestus, I. Leuschner, A. Benner, J. Khan, and M. Schwab, High Skp2 expression characterizes high-risk neuroblastomas independent of MYCN status. Clin Cancer Res, 2007. 13(16): p. 4695-703.
Westermann, F., D. Muth, A. Benner, T. Bauer, K.O. Henrich, A. Oberthuer, B. Brors, T. Beissbarth, J. Vandesompele, F. Pattyn, B. Hero, R. Konig, M. Fischer, and M. Schwab, Distinct transcriptional MYCN/c-MYC activities are associated with spontaneous regression or malignant progression in neuroblastomas. Genome Biol, 2008. 9(10): p. R150.

Bulashevska, S. Inferring genetic regulatory interactions with Bayesian logic-based model. In Handbook of Research on Computational Methodologies in Gene Regulatory Networks, Das, S., Caragea, D., Welch, S.M., Hsu, W. H. eds.

(2009).
Bulashevska, S., Adebiyi, E., Brors, B. & Eils, R. New insights into the genetic regulation of Plasmodium falciparum obtained by Bayesian modelling, Gene Regul Syst Bio. Nov 29; 1:137-49 (2007)
Bulashevska S, Eils R. Inferring genetic regulatory logic from expression data. Bioinformatics, Jun 1;21(11): 2706-13 (2005)

Participation in relevant National or European research projects

T Hofer - FP7, SYBILLA, 201106, Systems Biology of T-cell Activation in Health and Disease, WP leader mathematical modelling. Numerous national research grants from DFG and Federal Ministry for Education and Research (BMBF), including BMBF-funded research consortium on Epigenetic networks of gene silencing (EpiSys), coordinator

F. Westermann, as a principal investigator contributed to numerous national (NGFN2 and NGFNplus funded by the BMBF in Germany) and international (EU EET Pipeline, FP6, # 037260) collaborative research projects.
Principal investigator of the project DKFZ.V.10 in the network "Systems Biology of Signalling in Cancer (SBCancer)" of the Helmholtz Alliance on Systems Biology, an alliance intended to promote Systems Biology research in Germany.

The DKFZ has a strong record of participation in EU framework programmes (in the 6th framework programme participating in about 60 projects with funding for the DKFZ of 24 Mio. €; in the 7th framework programme participating in 16 projects with funding for the DKFZ of approx. 7 Mio. €). The Technology Transfer Department of the DKFZ assists scientists in applying for patents and cooperating with industry to develop commercially viable products (currently 227 patent families with 1011 national and foreign patents), realizing licensing agreements (currently 123 license agreements), and founding start-ups based on DKFZ intellectual property.

Personnel involved in the project

T Hofer: 1 PostDoc. In kind contribution: 1 PhD student; 1 Postdocs (Dr. Elan Gin.), T Höfer.

F. Westermann: 1 SenPostDoc, 1 PhD (Dr. Kai Henrich), 1 Technician (Steffen Bannert).
In kind contribution: 1 PhD student, F Westermann

S Bulashevska: 1 PostDoc. In kind contribution: S Bulashevska.

Total of women involved: so far 2.

6	VTT	VTT Technical Research Centre of Finland

The VTT Technical Research Centre of Finland is the biggest contract research organisation in Scandinavia, providing high-end technology solutions and innovation services through its international scientific and technology networks.VTT Medical Biotechnology, with 100 employees, has unique capabilities to perform high-throughput RNAi and compound screening. Prof.Kallioniemi's group has established a state-of-the-art cell-based High Throughput Screening (HTS) system, widely applicable to cancer research, functional target identification, and early-stage lead discovery. The instrumentation allows for over 40000 cell biological experiments to be carried out at a time. The extensive experimental infrastructure currently available at VTT enables three screening techniques: 384 well plate-based, lysate-array and cell-array screening.

Role within the project

Olli Kallioniemi and Kristiina Iljin are WP leaders in WPs 2 & 3. Work will be carried out in teams headed by senior scientists; Kristiina Iljin leads "Cancer Systems Biology" and Merja Perälä leads "Target discovery".

Previous R&D Experience relevant to the project

Olli Kallioniemi. Expert in developing and leading scientific projects on high-throughput genomics and transcriptomics analyses of cancer, bioinformatic modelling and the functional, high-throughput analysis with RNAi and drugs. Codeveloper of e.g.Comparative Genomic Hybridization (CGH, 1992), tissue microarrays (1998), NMD-microarrays (2002), cell-based RNAi microarrays (2003) and In Silico Transcriptomics (IST) database (2008). Data integration from these platforms has generated novel information on several critical genes in cancer i.e. prostate cancer and breast cancer. Dr. Kristiina Iljin (PhD) studies downstream effects of TMPRRS2-ERG gene fusion in prostate cancer and associated ERG positive prostate cancers to increased HDAC inhibitor sensitivity. Her results using high-throughput compound screening on prostate cells have led to identification of several cancer selective compounds that may have therapeutic relevance. She is also involved in several siRNA screening studies and gene expression studies aiming at identification of novel biomarkers and drug targets in cancer. Merja Perälä has played a major role in setting up the HTS system at VTT Medical Biotechnology. Her research includes applying HT compound, miRNA and siRNA screening.

Key people involved in the project

Olli Kallioniemi - 1995-2002 - Head of Translational Genomics Section at the Cancer Genetics Branch, National Human Genome Research Institute, at NIH, Maryland. Leading Professor and Director of the Inst. for Molecular Medicine Finland. Director of the Academy of Finland Centre of Excellence in Translational Genome-Scale Biology. Author of 226 publications and an editor/member of the editorial board of six journals. 16 issued patents. EACR young investigator award -1994, Anders Jahre Prize -1998, NIH Director's lecture - 2000, Medal of the Swedish Medical Society - 2003, EU Marie Curie Centre of Excellence Grant - 2004, National Academy of Sciences membership (Finland)- 2005, EMBO Membership- 2006, and Harold G. Pritzker Memorial Lecture-University of Toronto in 2006, AACR team science award in 2008 and the IFCC-Abbot Award for Molecular Diagnostics in 2008.Kristiina Iljin M.Sc (Genetics) in 1997 at University of Turku and PhD (Cancer Molecular Biology) in 2003 at University of Helsinki. Postdoctoral research - University of Zurich joined Prof. Kallioniemi´s group in 2005. Since 2009 she leads the Cancer Systems Biology team. Author in 18 publications, co-inventor in 1 patent application and an AACR AstraZeneca International Scholar-in-Training Award (2008). Merja Perälä, leader of Target Discovery team, PhD (1997) and Adjunct Professorship (2002) in Molecular Biology. Author in 29 publications and co-inventor in 1 issued patent and in 1 patent application, 20 years experience in academia and in Pharma R&D. Dr. Perälä joined VTT Medical Biotechnology in 2002. Her team has carried out multiple high-throughput cell based screening projects, pursuing the identification of drug targets and therapies for breast, prostate and brain cancers.

References related to the project

(1) Iljin K., Ketola K., Vainio P., Halonen P, Kohonen P, Fey V, Grafström R., Perälä M, and Kallioniemi O. High-throughput cell-based screening of 4910 known drugs and drug-like small molecules identifies disulfiram as an inhibitor of prostate cancer cell growth. Clin Cancer Res. 2009 Oct 1;15(19):6070-8 (2) Mousses S, Caplen NJ, Cornelison R, Weaver D, Basik M, Hautaniemi S, Elkahloun AG, Lotufo RA, Choudary A, Dougherty ER, Suh E, Kallioniemi O. RNAi microarray analysis in cultured mammalian cells. Genome Res. 2003 Oct;13 (10):2341-7 (3) Leivonen SK, Mäkelä R, Ostling P, Kohonen P, Haapa-Paananen S, Kleivi K, Enerly E, Aakula A, Hellström K, Sahlberg N, Kristensen VN, Børresen-Dale AL, Saviranta P, Perälä M, Kallioniemi O. Protein lysate microarray analysis to identify microRNAs regulating estrogen receptor signaling in breast cancer cell lines. Oncogene. 2009 Nov 5; 28(44):3926-36.

Participation in relevant National or European research projects

FP7: (1) METACancer, HEALTH-2007-2.4.1-2: Predictive metabolomics in breast cancer (2) Genica, HEALTH-2007-2.4.1-3: Genomic Instability in Cancer and Precancer (3) Prosper HEALTH-2007-2.4.1-1: Prostate cancer: profiling and evaluation of ncRNA. FP7, ApoSys Health-F4-2007-200767: Apoptosis systems biology applied to cancer and AIDS.

Personnel involved in the project

Technician P Käpylä (40%); PostDocs V Fey (20%) and S Haap-Paananen (40%) Sen.reseach scientist M Perälä (15%) and K Iljin; Professor O Kallionemi (5%). Women involved: 4.

7	UKE	Universitaetsklinikum Essen

The UKE is a state-of-the art University Hospital hosting the WTZ, Germany's largest Comprehensive Cancer Center and includes a teaching hospital and medical school treating 20,000 inpatients and > 70,000 outpatients each year. The Dept. of Paediatric Oncology, directed by Prof. Angelika Eggert, is one of the largest Paediatric Oncology Centers in Germany with approx. 120 newly diagnosed paediatric cancer patients per year. Major research focus is the field of embryonal paediatric tumours including neuroblastoma (NB), retinoblastoma (RB) and medulloblastoma (MB). Molecular biology and genetics research is focussed on genomic, transcriptomic, proteomic and epigenomic profiling of paediatric tumours and cell culture models. An additional major interest is xenograft and transgenic mouse models of embryonal tumors. Long-standing interest in the role of the Trk kinases, the receptors for neurotrophin signalling, in neuroblastoma. Recently, the ALK kinase has been identified as important for NB, and has become a focus of NB research in the AE group. Kinases represent druggable targets for disease intervention, and are therefore an important focus of the AE group translational research efforts.

Role within the project (If more than one department involved, describe all the teams)

Our experience in the field of embryonal tumours in combination with a high degree of networking competence and continued coordination of the E.E.T.-Pipeline consortium will be a necessary asset to this project. The well developed and long-standing interests in Trk receptor signalling, neuroblastoma biology and kinases as druggable targets for the development of therapies is well fitted to the ASSET project. Technological expertise for the ASSET consortium i: ET cell culture models with numerous biological read-outs, construction of tissue microarrays and their use for IHC, high-throughput mRNA and miRNA profiling and the respective data analyses, next generation sequencing, xenograft and transgenic mouse models. AE will be member of ASSET's industrial liason and exploitation committee and provide input as experimentalist and clinician of the pediatric cancer field.

Previous R&D Experience relevant to the project

Extensive experience in molecular biology and genetics of embryonal tumors which is integrated to clinical expertise at the bedside. Support in the evaluation of the clinical relevance of the data produced. Clinical research networks to which they belong provide a direct link to the relevant clinical trial centers. The wealth of data derived from the E.E.T.-Pipeline project are available to the ASSET consortium, in an effort to extend the identification of novel targeted therapies for children with embryonal tumours using a systems approach. This input is integral to the ASSET project.

Key people involved in the project

Prof. Angelika Eggert, MD, Dept. head / group leader, Professor of Paediatric Oncology, Director, Dept. of Paediatrics III and Director, WTZ (Comprehensive Cancer Centre) Coordinator: EU FP6th (STREP E.E.T.-Pipeline) and the German NGFN (three funding periods since 2002); 65 publications, 7 scientific awards in the field of paediatric oncology. Dr. Johannes H. Schulte; MD: group leader, nominated for Junior Professorship, several years research experience in the molecular and cellular biology of embryonal tumours, especially neuroblastoma; PD Dr. Alexander Schramm, PhD : Eggert laboratory director; several years research experience in the molecular and cellular biology of embryonal tumours, especially neuroblastoma; teaching docent for the University Hospital Essen, 35 publications; 2 scientific awards in the field of paediatric oncology

References related to the project

Schulte JH, Schramm A, Klein-Hitpass L, Klenk M, Wessels H, Schweigerer L, Brodeur, G.M., Havers W, Eggert A. Microarray analysis reveals differential gene expression patterns and regulation of single target genes contributing to the opposing phenotype of TrkA- and TrkB-expressing neuroblastomas. Oncogene 2005;24:165-177.

Schramm A, Vandesompele J, Schulte JH, Dreesmann S, Kaderali L, Brors B, Eils R, Speleman F, Eggert A. Translating expression profiling into a clinically feasible test to predict neuroblastoma outcome. Clinical Cancer Res. 2007;13:1459-65.

Schulte JH, Horn S, Samans B, Heukamp L, Eilers UC, Otto T, Krause M, Astrahantseff K, Buettner R, Schramm A, Christiansen H, Eilers M, Eggert A, Berwanger B. N-Myc regulates oncogenic microRNAs in neuroblastoma. Int. J. Cancer 2008;122:699-704.

Participation in relevant National or European research projects

Coordinator AE (research) KA (admin): EU FP6 STREP, the E.E.T.Pipeline (Co.# 037260). The Neuroblastoma Network and the Extended Neuroblastoma Genome Interaction Network (ENGINE) projects financed within the German National Genome Research Project (NGFN2) and the follow-up, NGFNplus programs (still active) by the German Ministry of Research and Education (BMBF). AE (UKE) coordinated both networks with Manfred Schwab (DKFZ, Heidelberg).

Personnel involved in the project

2PhD students, 1 technician. In kind: A. Eggert's contribution.

8	UGENT	Universiteit Gent, Functional Oncogenomics Research Group, Center for Medical Genetics (CMGG)

CMGG is based at the Ghent University Hospital. Research activities of the CMGG are based within the Medical Research Building at the Hospital site. The Functional Oncogenomics Research Group is part of this research team and focuses primarily on functional genomics of childhood cancers. See also at http://medgen.ugent.be/CMGG/home.php

Role within the project

The past years our team has performed extensive high throughput genomic screening and set up functional analyses in the study of neuroblastoma. This has yielded a unique dataset concerning DNA copy number alterations, mRNA and miRNA expression. Our group has extensive expertise in high throughput qPCR and is part of the NXTGent deep sequencing consortium. We also have access to and will develop cell line model systems and mouse models. Our group has a bioinformatics core group involved in aspects related to high throughput genomics data analyses, gene signature analyses, deep sequencing etc.

Previous R&D Experience relevant to the project

Our group has participated in the discovery of the role of the ALK gene in neuroblastoma, and is the first and only group who has profiled normal fetal neuroblasts, which are considered to be the normal counterpart of neuroblastoma cells. We have optimised a qPCR based platform for high throughput screening of the expression of all miRNAs using limited amount of input material and produced unique large datasets in neuroblastoma cell lines and tumors. Using these datasets, we are studying the consequences of altered miRNA expression in neuroblastoma in great detail. These datasets and technical expertise will be of crucial importance for validation of the data gained from model systems. Our group also has access to model systems, in particular for ALK overexpression and knock down.

Key people involved in the project

Prof. Frank Speleman, principle investigator, Supervisor of the Diagnostic and Research Laboratory at CMGG since 1986. Biologist, Geneticist. 236 A1 publications. Patents: “Identification of neuroblastoma tumor suppressor genes” (ep.00870219.3-PCT/EP01/11199). Published patent application WO 02/26815. Inventors: F. Van Roy, K. Staes, K. Vandepoele, N. Van Roy, F. Speleman. Awards: Roland De Ruyck Cancer Award 2007, prognostic m(i)RNA patent !!!

Prof. Ir. Joke Vandesompele, principle investigator, Bio-engineer, biostatistician. Co-supervisor of the Functional Oncogenomics Research Group, Supervisor of the Bio-informatics Unit at CMGG, CEO Biogazelle,104 A1 publications.

References related to the project

Antitumor activity of the selective MDM2 antagonist nutlin-3 against chemoresistant neuroblastoma with wild-type p53.
Van Maerken T, Ferdinande L, Taildeman J, Lambertz I, Yigit N, Vercruysse L, Rihani A, Michaelis M, Cinatl J Jr, Cuvelier CA, Marine JC, De Paepe A, Bracke M, Speleman F, Vandesompele J. J Natl Cancer Inst. 2009 Nov 18; 101(22):1562-74. Epub 2009 Nov 10.

Identification of ALK as a major familial neuroblastoma predisposition gene. Mossé YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, Laquaglia MJ, Sennett R, Lynch JE, Perri P, Laureys G, Speleman F, Kim C, Hou C, Hakonarson H, Torkamani A, Schork NJ, Brodeur GM, Tonini GP, Rappaport E, Devoto M, Maris JM. Nature. 2008 Oct 16; 455(7215):930-5. Epub 2008 Aug 24

High-throughput stem-loop RT-qPCR miRNA expression profiling using minute amounts of input RNA.
Mestdagh P, Feys T, Bernard N, Guenther S, Chen C, Speleman F, Vandesompele J. Nucleic Acids Res. 2008 Dec; 36(21):e143. Epub 2008 Oct 21

Participation in relevant National or European research projects

EU projects: FP6 EET Pipeline, nr 037260, FP6 Marie Curie Conferences on Molecular Cytogenetics and array CGH, coordinator, 2002-2005.

Current national research projects: Foundation Against Cancer, 2008-2010, Identification of MYCN target genes, Flemish Fund for Scientific Research (FWO) grant, 2009-2012, the role of miRNAs in regulation of p53 in neuroblastoma, (FWO) grant, 2008-2011, study of the role of altered miRNAs in neuroblastoma development, Federal Government of Health (FOD), 2009-2011, the role of ALK in neuroblastoma development, FOD, 2009-2011, application of next generation sequencing for mutation detection in cancer. GOA, Concerted Ghent University Grant, 2010-2014, the role of miRNAs in the development of pediatric cancer. Flemish league against cancer (VLK), 2010-2012, study of epigenetic alterations in cancer, VLK, 2009-2011, miRNAs as prognostic markers in neuroblastoma. Fund Fournier Majoie, 2009-2011, validation of mRNA:miRNA prognostic classifier for neuroblastoma.

Personnel involved in the project

1 PostDoc (n.n). In kind: 4 PhD (2 Women, 1 to be hired) , three professors (1 women)

9	UCL	University College London

University College London is ranked fourth in the world and second in the UK in the 2009 QS World Rankings, with Competitively won research grants and contracts accounting for a third of UCL's income. UCL's defined research strategy includes building on this expertise to tackle four initial Grand Challenges by actively facilitating cross-disciplinary interaction, within and beyond UCL, and applying collective strengths, insights and creativity to overcome problems of global significance. The first of these is the Grand Challenge of Global Health. The Department of Statistical Science at UCL was the first statistics department in the world, and now specializes in, among other things, theoretical research on epidemic models and genetics, leading to applications in the life sciences and insight on biological mechanisms.

Role within the project

The main contribution to this project will be the statistical inference methodologies required in the model systems development cycle. The role of statistical inference in mathematical modelling of biological systems is important and this is increasingly being seen as such. The particular issues of network inference and uncertainty characterisation requires advanced computational statistics methods such as Markov Chain Monte Carlo for which Girolami has an internationally leading profile.

Previous R&D Experience relevant to the project

Girolami is a world leader in the development and deployment of advanced statistical methodology theory and practice .Girolami is one of a small handful of Computing Scientists and Statisticians that has contributed at the interface of the Life Sciences and Mathematical modelling – an example is advocacy of the Bayesian framework as a codification of the Scientific Method in Systems Biology – the recent Science Signalling paper (below) was instigated by him and the novel MCMC methods were developed by him to ensure precise estimates of Bayes factors in assessing evidential support for biochemical models.

Description of key people involved in the project

Mark Girolami is leading SP.1.Number of publications greater than 100, with an H-Index of 22, author on 3 patents, awarded the SPIE Pioneer Award in 2009 for his contributions to Signal Processing for Brain Imaging.

References related to the project

1. Riemannian Manifold Hamiltonian and Langevin Monte Carlo Method. The Royal Statistical Society, 2010.
2. Vyshemirsky, V. & Girolami, M. A. Bayesian ranking of biochemical system models. Bioinformatics 24, 833-839 (2008).
3. Xu, T. R., Vyshemirsky, V., Gormand, A., von Kriegsheim, A., Girolami, M., Baillie, G. S., Ketley, D., Milligan, G., Dunlop, A. J., Houslay, M. D. & Kolch, W. Inferring Signaling Pathway Topologies from Multiple Perturbation Measurements of Specific Biochemical Species. Science Signaling in press (2010).

Participation in relevant National or European research projects

Currently PI on 1 BBSRC (Biological and Biotechnological Science Research council of the UK) grant and 4 EPSRC (Engineering and Physical Sciences research Council of the UK) grants.

Personnel involved in the project

1 PostDoc, 1 technician and M Girolami's contribution. In kind: 1 SenPostDoc and 1 administrator. Women so far: 1.

10	UBERN	University of Bern

Dr. Alexandre Arcaro's group recently moved from the University Children's Hospital Zurich to the Department of Clinical Research (DCR) of the University of Bern. At Bern, the group will continue its research activities in the field of paediatric oncology research. The University of Bern is a highly regarded interdisciplinary research organization. The Department of Clinical Research (http://www.dkf.unibe.ch/31/homepage.html) is an Institution of the Medical Faculty housing over 40 research groups with access to state-of-the-art facilities, including animal facilities, DNA microarrays, flow cytometry, magnetic resonance, microscopy imaging center, mass spectrometry and proteomics.
Dr. Arcaro's group has set up several research projects that will contribute to the present proposal: (1) it has set up panels of embryonal tumor (ET) cell lines (ESFT, MB, NB) and performed testing of various kinase inhibitors as cytostatic and cytotoxic agents (2) it has performed kinome-wide siRNA screens in MB and NB cell lines to identify modulators of ET sensitivity to chemotherapeutic drugs (3) it has, within the framework of the previous FP6 project (EET-Pipeline) set up cellular MB models with constitutive or inducible expression of c-Myc, which have been profiled by DNA microarrays. The group also collaborates with the group of Michael A. Grotzer (University Children's Hospital Zurich) who has a long track record in clinical research in childhood embryonal tumours with a focus on medulloblastoma.

Role within the project

Dr. Arcaro's group will contribute the following: generation of data from expression profiling in MB cells with inducible c-Myc expression; assessing functional parameters of drug sensitivities; validation of miRNAs in MB; they will also contribute to the high content network perturbation screens and high throughput screens for synthetic phenotypes. In particular, the group will perform validation experiments in panels of ET cell lines using drug combinations and contribute to the validation in preclinical models and clinical samples.

Previous R&D Experience relevant to the project

Dr. Arcaro has been working on aspects of kinase signalling pathways in cancer, inflammation and T cell activation for 19 years. His PhD work focused on the elucidation of kinase signalling pathways involved in the regulation of cell migration. His postdoctoral work at the Ludwig Institute for Cancer Research resulted in the cloning and characterization of phosphoinositide 3-kinase (PI3K) isoforms, which are enzymes activated by receptor tyrosine kinases (RTKs), such as the epidermal growth factor receptor (EGFR), with a crucial role in tumour growth and survival. At Imperial College London, he characterized some important new molecular targets for lung cancer and developed targeted therapies for the disease. This research initiated 2 new clinical trials for lung cancer in the UK. He then headed a research group at the University Children's Hospital Zurich with main research topics in RTK signalling and the development of targeted therapies for childhood cancers, such as medulloblastoma, neuroblastoma and leukaemia.
Currently, Alexandre Arcaro is a research director in Paediatric Oncology at the Department of Clinical Research (DCR) of the University of Bern and the Inselspital (Bern University Hospital). He has been collaborating with Michael A. Grotzer (University Children's Hospital Zurich) who has a long track record in clinical research in childhood embryonal tumours with a focus on medulloblastoma, for over 6 years. Each collaborates with Partners 4, 5 (FW) and 8.

Key people involved in the project

PD Dr. Alexandre Arcaro obtained his PhD from the University of Fribourg. He did 5 years of postdoctoral work at the Ludwig Institute for Cancer Research (UCL and Lausanne Branches). He then worked as a Group Leader at Imperial College London and at the University Children's Hospital Zurich. He is research director in Paediatric Oncology at the DCR of the University of Bern. He has published over 40 articles and obtained over 2.0 million Euros in grants/awards.

References related to the project

(1) Guerreiro, A.S., Fattet, S., Fischer, B., Shalaby, T., Jackson, S.P., Schoenwaelder, S.M., Grotzer, M.A., Delattre, O., and Arcaro, A. (2008). Targeting the PI3K p110 isoform inhibits medulloblastoma proliferation, chemoresistance and migration. Clinical Cancer Research, 14, 6761-6769 (2) Boller, D., Schramm, A., Doepfner, KT., Shalaby, T., von Bueren, A.O., Eggert, A., Grotzer, M.A., and Arcaro, A. (2008). Targeting the phosphoinositide 3-kinase isoform p110 impairs growth and survival in neuroblastoma cells. Clinical Cancer Research, 14, 1172-1181 (3)Guerreiro, A.S., Boller, D., Shalaby, T., Grotzer, M.A., and Arcaro, A. (2006). Protein kinase B modulates the sensitivity of human neuroblastoma cells to insulin-like growth factor receptor inhibition. Int. J. Cancer, 119, 2527-2538

Participation in relevant National or European research projects

EU FP6 project (European Embryonal Tumor Pipeline) at the University of Zurich with Michael A. Grotzer.

Personnel involved in the project

2 PhD students, 1 postdoctoral scientist. In kind: 1 technician, AA. Women involved: 3

11	WEIZMANN	Weizmann Institute of Science

The Weizmann Institute of Science in Rehovot, Israel, is one of the world's top-ranking multidisciplinary research institutions. The Department of Biological Regulation tackles the regulation of processes responsible for the concerted action of cells, tissues, and organs. Our studies include the identification of signaling molecules such as hormones and growth factors, their specific receptors, their target cells, and the mechanisms involved in the transduction of their action. Since de-regulation of such processes are a cause for many human diseases (heart failure, stroke, cancer and abnormal growth, infertility, lack of nerve regeneration after injury, etc.), we make an effort to use our results for the development of tools for early diagnosis and for the design of new drugs for pharmacological intervention.

Role within the project

Our long experience is growth factor signalling, especially through receptor tyrosine kinases, will be employed. Furhter, proteomic and transcriptomic approaches on ETs. In addition, cellular models of disease, namely genetically modified cancer cells grown in 2D and 3D formats, including co-cultures and extracellular matrix components.

Previous R&D Experience relevant to the project

Our experience in the field of tumour progression and especially the role for growth factors will be disposed. We routinely carry out experiments in vitro and in animals, genetically manipulate cultured cells and perform large scale screens of mRNAs, microRNAs, mass-spectrometry and siRNA libraries. These methodologies will be made available when carrying out tasks for the consortium.

Description of key people involved in the project

Prof. Yosef Yarden pioneered the field of growth factor receptors and their roles in cancer. He cloned the first receptors and several new growth factors, defined the family of Receptor Tyrosine Kinases (RTKs), and discovered that RTKs are activated through dimerization, and inactivated upon ubiquitinylation. Studying HER2, Yarden first recognized the role of signaling networks in cancer. He has vast experience in conducting scientific projects at National and Multi International levels and has published more than 160 publications in this field. Prof. Yosef Yarden serves as Dean of the Institute's Feinberg Graduate School for the last 6 years.

References (publications), and patents related to the project

Avraham, R., Sas-Chen, A., Manor, O., Steinfeld, I., Shalgi, R., Tarcic, G., Bossel, N., Zeisel, A., Amit, I., Zwang, Y., et al. (2010). EGF decreases the abundance of microRNAs that restrain oncogenic transcription factors. Sci Signal 3, ra43.

Pines, G., Kostler, W.J., and Yarden, Y. (2010). Oncogenic mutant forms of EGFR: lessons in signal transduction and targets for cancer therapy. FEBS Lett 584, 2699-2706.

Seger, R., Rodeck, U., and Yarden, Y. (2008). Receptor tyrosine kinases: the emerging tip of systems control. IET Syst Biol 2, 1-4.

Participation in relevant National or European research projects

SIMAP; FP6 Specific Targeted Project, EU-FP6-IST-027265; Funding period: 2006-2008. TRANSFOG; EU - FP6 - LSHC-CT-2004; Funding period: 2004-2008.ErbB IN BREAST TUMOR; FP5-QLG1-CT-2000; Funding period: 2000-2004.

PhD, Post-Doctorates & number of women involved in the project

1PhD funded by ASSET; in kind contributions: 1PhD, 1 PostDoc and Y Yarden’s participation

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12	ZEPTO	Zeptosens - Division of Bayer (Schweiz)

Zeptosens is a division of Bayer (Schweiz) AG focussed on the development, production and commercialization of bioanalytical systems and research services. Zeptosens has a comprehensive array platform on the market comprising spotters, arrays, the array reader, the image analysis software as well as the necessary reagents for spotting and assay performance. The system is based on proprietary planar waveguide technology to achieve outstanding detection sensitivity. Zeptosens has a wide expertise in development of immunoassays in the capture as well as reverse array format.

Role within the project

The Zeptosens team has the experience to screen large numbers of tissue or cultivated cell samples for changes on a large number of signalling proteins. Currently 240 antibodies are validated for reverse array applications. The set is constantly expanded and the technology allows us to implement all the antibodies already used in western blots provided they are highly selective. A particular benefit of reverse arrays is their long shelf life of over one year, which allows us to investigate the sample months after the initial tests incorporating findings obtained in parallel by other methods, e.g. to test for changes in pathways initially not considered.

Previous R&D Experience relevant to the project

The investigation of changes in signaling pathways in diseased tissues as well as determining the efficacy of kinase inhibitors in selected pathways is one of the core competencies of Zeptosens. The amount of prestigious sample material is extremely small for reverse arrays with only 400 pL sample volume per spot.
Zeptosens provides protein-profiling studies to academic as well as industrial customers and since 2004 has focused on reverse arrays for protein profiling. 7 of the 10 largest pharmaceutical companies either use our protein profiling services or have our protein-profiling platform installed in their laboratories. The applications areas have been expanded from biomarker finding to monitoring of changes in signaling pathways in diseased tissues and determination of kinase inhibitors EC50 values to target deconvolution. Zeptosens provides state of the art profiling services as well as an array platform optimized for reverse protein arrays for quantitative detection of signaling changes.

Key people involved in the project

Zetposens currently has 3 laboratory teams providing R&D services on reverse and forward arrays.
Dr. Markus Ehrat will take over the project management role at Zeptosens. He received his Ph.D. at the ETH in Zürich, Postdoc at Northeastern University in Boston before joining Ciba-Geigy, later Novartis. Was head of the department of bioanalytical research before founding Zeptosens AG of which he was CEO. After the acquisition by Bayer he became the managing director. M. Ehrat is author and coauthor of numerous papers and patents in the field of advanced bioanalytical methods.

References related to the project

J.vanOostrum, H. Voshol, EUROPEAN PHARMACEUTICAL REVIEW IISSUE 2 I2008
Jan van Oostrum1, Claudio Calonder1, Daniel Rechsteiner1, Markus Ehrat1,
Juergen Mestan2, Doriano Fabbro2 and Hans Voshol3, Proteomics Clin. Appl. 2008, 2,412-422

Participation in relevant National or European research projects

Zeptosens has participated in three EU projects: AUTOROME, SEMOFS, SABIO.
The role of Zeptosens in AUTOROME was the development and commercialization of an array for the diagnosis of rare autoimmune diseases. The array was planned to include the new markers detected by the consortium members.
Both SEMOFS and SABIO were projects for research on novel optical detection methods for diagnostic tests based on immunoassays. The role of Zeptosens was the development of DNA and antibody based assays on these novel detection platforms. In addition Zeptosens was given the task to contribute information on the market situation and had an important role in all intellectual property aspects, including the search on potentially blocking patents.

Personnel involved in the project

1 SenPostDoc will be hired and funded by ASSET. In kind: M Ehrat's contribution.

13	UCPH	Department of Proteomics, University of Copenhagen, UCPH

The research teams at the department for proteomics at UCPH are headed by some of the best researches in the field of proteomics. We have developed many of the mass spectrometric and proteomics methods in routine use today worldwide. We have developed a generic phosphoproteomics technology that allows us to identify and quantify phosphorylation sites in an unbiased fashion and on a global scale.

Role within the project (If more than one department involved, describe all the teams)

We will contribute state-of-the-art mass spectrometry-based quantitative proteomics and phosphoproteomics analysis of the core ET cell lines. In addition, we will provide time-resolved kinetic phosphorylation site data as well as absolute quantitation of key regulatory molecules in the different cell lines.

Previous R&D Experience relevant to the project

We are one of the main partners in this ASSET consortium who will be able to provide a strong emphasis on quantitative proteomics in combination with high-performance mass spectrometry. We are one of the few laboratories world-wide that have a proven record in large-scale analysis of phosphorylation dynamics using quantitative mass spectrometry. Our experience, technology, and know –how is essential for the consortium to be successful.

Key people involved in the project

Jesper V. Olsen is since 2009 Research Professor and Group Leader in the Department for Proteomics at UCPH. Jesper Olsen's lab focuses on development of quantitative mass spectrometric tools and technologies for systems-wide analysis of proteins and their modifications, and they apply these to study disease-signalling networks. He is (co-) author of more than 60 scientific publications. Dr. Olsen received the Max Planck Institute's Junior Research Award in 2007 and the Human Proteome Organization (HUPO) Young Investigator Award in 2008.

Alicia Lundby is since 2009 a post-doctoral fellow in the Department for Proteomics at UCPH. Her main interest is identification and quantitation of post-translational modifications such as phosphorylations on membrane proteins. She is (co-) author of 10 scientific publications and has been awarded the Young Investigator Award from University of Copenhagen in 2005 and the Talent Award by the Lundbeck Foundation in 2007.

Chiara Francavilla is since 2010 a post-doctoral fellow in the Department for Proteomics at UCPH. Her main research interests are unbiased identification and quantitation of phosphorylation events that regulates signal transduction pathways and receptor tyrosine kinase endocytosis. She is (co-) author of 5 scientific publications and won an EMBO long-term fellowship as well as a Marie Curie Post-doctoral grant in 2010.

References related to the project

Global, in vivo, and site-specific phosphorylation dynamics in signaling networks.
Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M. Cell. 2006

Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast.
de Godoy LM, Olsen JV, Cox J, Nielsen ML, Hubner NC, Fröhlich F, Walther TC, Mann M. Nature. 2008

Lysine acetylation targets protein complexes and co-regulates major cellular functions.
Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV, Mann M. Science. 2009

Participation in relevant National or European research projects

FP7 SyBoSS (Project number: 242129) "Systems Biology of Stem Cells and Reprogramming"

Personnel involved in the project

1 PostDoc 100% and 1 PostDoc 20% will be funded by ASSET.

Dr. Alicia Lundby, Postdoc (f), Dr. Chiara Francavilla, Postdoc (f), Stephany Mundk, PhD student (f), Dr. Omid Hekmat, Postdoc (m) and Jesper Olsen will be involved in the project and funded by own resources..

13	UCPH	Dept of Disease Systems Biology, University of Copenhagen, Denmark (UCPH-CBS)

The Dept. of Disease Systems Biology at the Center for Protein Research (CPR) at works as multi-disciplinary basic research groups within bioinformatics and systems biology with particular focus on large data sets of relevance to human health. The center, started in 2008, comprises three research departments (Proteomics, Disease Systems Biology, and Disease Biology), in addition to a major effort on protein production in a facility which aims for characterization of all human proteins. The center has a highly multidisciplinary profile (molecular biologists, biochemists, medical doctors, physicists and computer scientists) with a ratio highly biased towards bio versus nonbio backgrounds. The three departments and the protein technology facility are highly collaboratory with many cross-center projects. The center has been formed by a grant from the Novo Nordisk Foundation, which together with contributions from the university exceeds 100 MEuro.

Role within the project

CPR-UCPH will use its experience of large scale data management, systems biology data integration as well as genomics experience in the area of DNA sequencing. This will be used for constructing the datawarehouse for the consortium (WP10) and building computational pipelines for data integration (WP11) and managed curation. The piloting of multiplexed targeted sequencing is unique and will be valuable to ASSET for cost effective exon sequencing in embryonic tumours.

Previous R&D Experience relevant to the project

Long experience in working with Systems biology and genomics applied to various disease areas. This has aided several genome-wide association studies in making complex based ranking instead of at the individual SNP level. Computational pipelines integrating diverse data sets have produced protein function predictors, temporal regulation in cell cycle predictors as well as phenotype associations of protein-protein interaction. The development of large genotype-phenotype catalogues lead to being involved in the first ancient genome sequence that is recently published in Nature (Feb, 2010).

Description of key people involved in the project

Søren Brunak (SBr), Professor, Dr. Phil., Ph.D., center director for CBS-UCPH. Over 150 papers with peer-review, 4 books, 3 proceedings and edited books. Twenty papers with over 100 citations. Member of various scientific advisory boards including chairman on European Bioinformatics Institute, UK. Main advisor on 24 Master theses, 29 PhD theses (+ 15 on-going). Various awards including Villum Kann Rasmussen Prize (2006) for Research within the Natural and Technical Sciences and Grundfos-Prize for medical research (2009). Leads CBS-UCPH's involvement in ASSET.Ramneek Gupta (RGu), Associate Professor, Ph.D., Leads the Functional Human Variation group. 15 published peer-reviewed articles (h-factor 9, average citation 83). Ph.D. in 2001 in bioinformatics, group leader for Bioinformatics Development at Eli Lilly and Company, Singapore for five years. Experience in integrating genomic data associated with solid tumours; and is currently co-grant recipient of childhood asthma and childhood ALL leukemia projects. RGu is currently supervising 2 Masters, 4 PhD students and 2 post-docs. Systems Biology integration of genotype-phenotype data, exon sequencing and coordination of CBS-UCPH activities on ASSET.Irene Kouskoumvekaki (IK), Associate Professor, Ph.D., coordinator for Computational Chemical Biology group. 15 articles in international peer-reviewed journals, 5 of which in the area of chemoinformatics and data analysis with application in drug research. Young Researchers Award (2007) from the Danish Research Council. IK will supervise the hired postdoc in the area of systems biology data mining combining synthetic lethalities, kinase inhibitor data with genomics.

References (publications), and patents related to the project (max.3 references)

1. Lage, K. et al. A human phenome-interactome network of protein complexes implicated in genetic disorders. Nat. Biotechnol 25, 309-16 (2007).
2. Lage, K. et al. A large-scale analysis of tissue-specific pathology and gene expression of human disease genes and complexes. Proc. Natl. Acad. Sci. U.S.A 105, 20870-20875 (2008).
3. Bergholdt, R. et al. Integrative analysis for finding genes and networks involved in diabetes and other complex diseases. Genome Biol 8, R253 (2007).

Participation in relevant National or European research projects

* EU: DEER project nr. 212844, grant participant. * Villum Foundation Denmark funded the creation of Center for Disease Systems Biology to study Systems Biology and Gene-Environment interactions in Male Infertility, ca. 3.3M EUR, SBr is grant coordinator. * RGu is co-grant recipient of ca. 0.5M EUR to study impact of genetic polymorphisms on chemotherapeutic response in childhood ALL leukemia, funded by Danish Cancer Foundation and Danish Childhood Cancer Foundation.

PhD, Post-Doctorates & number of women involved in the project

1 PostDoc. In kind: I Kouskoumvekaki, R Gupta (SenPostDocs) and S Brunak. Women 2.

14	CNIO	Centro Nacional de Investigaciones Oncológicas Carlos III

The Spanish National Cancer Research Centre (CNIO) is one of the world's leading institutions in cancer research. Its activity includes both basic and applied research in an integrated fashion, thus supporting the interaction of basic research programmes with those of molecular diagnostics and drug discovery. The CNIO group and the Structural Biology and Biocomputing Programme are interested in the analysis of the function and structure of proteins related to cancer through the integration of genomic data, statistical analysis of genomic data, function prediction methods and information extracted from papers (text mining). The group has experience in the collaboration with consortia such as the scale-up ENCODE project, the ICGC cancer genome effort (including the Spanish CLL subproject of ICGC) the main bioinformatics European Networks (Biosapiens, EMBRACE and ENFIN).

Role within the project

We will: 1) Contribute to the Integration of data generated by the various workpackages 2) Enhance the functional information available by integrating DB and other sources of information including textual sources 3) Develop methods for the analysis of genomic information at the network level and 4) Collaborate with the project partners to the analysis of complex data in the context of the information available for other cancer genome projects.

Previous R&D Experience relevant to the project

Our group has recently contributed to the creation of new methods in various areas of Bioinformatics, including function annotation, information extraction and network analysis, which are directly related to this project. On the technological side, the group has created bioinformatics resources that are in use in a number of large-scale genome projects and /or available on the web for the annotation of genomes and the semantic enrichment of functional annotations. This expertise has been put at the work for the analysis of high-throughput genomic results, including genome variation studies and the prediction of the consequences of genetic variation in protein families. This work is developed in the context of the National Human Genome Research Institute (NHGRI, USA) ENCODE scale-up project, and the BioSapiens and ENFIN European Networks of Excellence. One methodology that has produced particularly relevant results for 2009 relates to information extraction in biological text developed around the BIOCREATIVE (Critical Assessment of Information Extraction systems in Biology) challenge.

Key people involved in the project

Alfonso Valencia is a biologist with formal training in population genetics and biophysics with a PhD (1988) from the U. Autónoma Madrid. He was Postdoctoral Fellow at the laboratory of C. Sander at the European Molecular Biology Laboratory (EMBL), Heidelberg. In 1994 Alfonso Valencia set up the Protein Design Group at the Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC) in Madrid where he was appointed as Research Professor in 2005. Member of EMBO, founder and former Vice President of the International Society for Computational Biology where he has been Chair of the Systems Biology since 2003. He serves on the Scientific Advisory Board of the SWeizmanns Institute for Bioinformatics, Biozentrum Basel, Curie Institute Paris, Interpro database, Spanish grant evaluation agency (ANEP), among others. Director of the Spanish National Bioinformatics Institute (INB), a platform of Genoma España. He is executive editor of journal Bioinformatics. - Daniel Rico, PhD, 3y postdoc training in Molecular Biology and Bioinformatics. Works in Cancer Genome analysis in special in CNV analysis. - Anäis Buadot, PhD 3 years postdoc experience, works in network analysis, including protein interaction networks and cancer –related gene funciton analysis.

References related to the project

Hoffmann R, Valencia A. A gene network for navigating the literature. Nat Genet. 2004 Jul;36(7):664.
Baudot A, Gómez-López G, Valencia A.Translational disease interpretation with molecular networks. Genome Biol 2009 10, 221-
Tress ML, other authors and Valencia A. The implications of alternative splicing in the ENCODE protein complement. Proc Natl Acad Sci U S A. 2007 Mar 27; 104(13):5495-500.
Juan D, Pazos F, Valencia A. High-confidence prediction of global interactomes based on genome-wide coevolutionary networks. Proc Natl Acad Sci U S A. 2008 Jan 22; 105(3):934-9.
Rausell, A, Juan D, Pazos F, Valencia A. Protein interactions and ligand binding: from protein subfamilies to functional specificity. Proc Natl Acad Sci U S A. 2010 in the press

Participation in relevant National or European research projects

CNIO had 96 national projects and 39 European projects (6th and 7th FP) running in 2009 (9 IP, 6 STREP,4 NoE, 4 CA, 1 Research Infrastructures, 6 Marie Curie, 5 ERC advanced grants, 3 ERC starting grants, 1 IMI JU. CNIO also had 6 international awards (Association for International Cancer Research (AICR) and US National Institutes of Health (NIH)).One NIH project (GENECODE part of ENCODE), a NSF grant (Biocreative), an IMI project (etox), three IEU projects (EMBRACE and ENFIN and Microme).

Personnel involved in the project

The Group: 5 postdocs, 7 students and 9 bionformaticians. 6 are non-spanish. 3 are female scientists.24% SenPostDocs M Tress and Daniel Rico; 5% Alfonso Valencia, 1PostDoc (n.n.) will be funded from ASSET.

B 2.3 Consortium as a whole

The logic behind the consortium setup

This project emerged from the FP6 EETP project. Thus, it builds on a solid stock of common interests and expertise, which now is extended to the logical next level by adding world leading capacity in high-throughput data generation and mathematical and computational modelling. The modelling will be on different scales and levels, but always validated and informed by biological experimentation. The formation of the ASSET consortium and the design of the proposed project were driven by the clear recognition of the huge potential impact for cancer research and the feasibility to achieve it. The logical flow of the project is from genetic aberrations to transcriptomics and proteomic changes that collectively cause decisive functional alterations in signalling networks. Our ability to capture and interpret these processes through mathematical models is the key to identifying new therapies. We have paid particular attention to match the need for new experimental data for advanced modelling with the capabilities to generate these data. The consortium composition was driven by this requirement, and will deliver to it. We unite basic researchers, translational and clinical researchers who can bridge the gap between modelling and clinically relevant applications.

Roles of partners

ASSET has ambitious goals. Therefore, we have very carefully designed the consortium membership to generate the required synergies between experimental and computational modelling work that will achieve these goals. The concept of the project acknowledges that no single technology or modelling approach is adequate to tackle a complex disease on a systems level. Thus, we need to combine high throughput data generation directed by hypothesis driven research with modelling of data on different scales and by different methods. We also need to keep the compass focussed on achievable and concrete outputs that ultimately can change clinical practice. This is only achievable by recruiting expertise spanning the whole spectrum from basic research to translational research and clinical research. Therefore, ASSET has assembled a consortium that can cover this scope in depth. Detailed partner descriptions are presented in the previous section, but are briefly summarised here to highlight the synergistic capabilities of the consortium to deliver against ASSET's goals.

Expertise in the analysis of genetic aberrations and large scale transcriptomics, miRNA expression and ChIP-chip/seq data are contributed by HK, OD, FW, FS, and AE. The concomitant bioinformatic analysis is provided by AZ, JV, AV and SBr. The reconstruction of global GRNs based on these data is provided by MG and SBu. This expertise will also be used to reconstruct signalling networks based on results from high-throughput data screening data provided by OK, protein array data contributed by ME and quantitative proteomics data generated by JO, GSF and WK. Combining hypothesis driven experimentation and perturbation by drugs and siRNA these large, but static network models will be explored to define subnetworks that are important for pathogenesis and are likely to contain relevant drug targets. These subnetworks will be dynamically modelled in order to simulate the effects of drugs and drug combinations. Dynamic modelling expertise is provided by BK and TH. Expertise in chemical proteomics and combinatorial drug screening is provided by GSF and AA. Expertise in the analysis of mammalian signal transduction networks is provided by WK, YY, BK and GSF. In order to parameterise the dynamic models targeted biochemical assays to monitor protein functions is required and contributed by YY, WK and GSF. For testing the model predictions we will use cell lines with conditional ET oncogene expression, xenografts and also clinical samples. Disease relevant validation models are contributed by FS, OD, FW, AE, AA, and HK. Clinical samples and clinical expertise is provided by AE, OD, FS, FW and HK. In order to enable in depth analysis all the data and models will be centrally stored in an interrogatable and semantically linked form. Expertise in datawarehousing and semantic computational methods is provided by SBr and AV. Thus, the consortium spans the wide range of expertise that can cover the project's scope from elucidation of basic molecular mechanisms to validation and translational aspects.

The consortium has one industrial partner, Zeptosens, who will provide array based profiling of protein expression and modifications. Zeptosens has a unique planar waveguide technology that vastly improves the signal to noise ratio, while the array format minimises sample consumption. Thus, this technology is ideally suited to examine

large numbers of samples or conditions. Both are needed in ASSET, and therefore Zeptosens is a logical addition to the consortium.

In order to ensure the exploitation of the results we have established the Industrial Liaison and Exploitation Committee with the remits to (i) monitor project results for IP protection and potential exploitation; and (ii) proactively establish relationships with industry.

Managing to deliver

The management structure is detailed in 2.1, but the general philosophy behind it is to have as many of the administrative management tasks as possible done by the PC's office in order to allow participants to concentrate on the research and outputs. From previous experience we are aware that for successful interdisciplinary collaborations we have to overcome the communication barriers between the different fields. Therefore, we have emphasised internal communication within the project and introduced mechanisms to promote it, such as different types of videolink meetings (round tables, seminars, on-demand sessions), workshops and a researcher exchange programme. In addition, the project management team (Project Coordinator, Subproject Leaders and Grants Manager) has vast experience in interdisciplinary collaborations including between wet and dry sciences as well as between basic, translational and clinical research.

The Project Coordinator, Walter Kolch, is Director of SBI, the newly founded Systems Biology Ireland Institute of UCD, which combines modelling and wet research under one roof. WK is trained as an MD, has worked in both pharmaceutical industry and academia, with a track record in cancer research, signal transduction, proteomics and network modelling. WK has coordinated major interdisciplinary projects on national and transnational levels.
The Grants Manager, *Lauren Montague* has >15 years experience in managing large interdisciplinary projects and the timely delivery of budget and timeline metrics.
The leader of SP1, *Mark Girolami*, is a renowned expert in machine learning and Bayesian inference, who has had longstanding collaborations with biologists and biomedical researchers working on the analysis of gene array data, mass spectra, biomarkers, neurocognition, and modelling of signalling networks.
Giulio Superti-Furga, who heads SP2, has an outstanding track record in academia as well as in Biotech industry. Working mainly in the field of signal transduction, proteomics, protein interactions and signal transduction pharmaceuticals, he is a co-founder of Cellzome, a successful drug discovery company, and is now Director of the Centre for Molecular Medicine where he closely collaborates with clinicians.
Boris Kholodenko, the leader of SP3, pioneered the dynamic mathematical modelling of signalling pathways and has made many seminal contributions to the field of systems biology. He also was amongst the first to have both dry and wet researchers in his group recognising the importance of close interactions between wet and dry disciplines.
The leaders of SP4, Angelika Eggert and Heinrich Kovar are renowned experts in ETs, combining vast experience in clinical and translational research research, respectively.
Soren Brunak and Alfonso Valencia, the leaders of SP5 have an eminent reputation for their contributions to bioinformatics in a particular cancer genetics, and also have an established track record in working with biologists and clinicians. Thus, the scientific integration and cross-fertilisation will rest in experienced hands

Training

We are convinced that a key parameter for systems biology to succeed is to equip researchers and postdocs with the abilities needed to work in an interdisciplinary environment. Therefore, we have integrated training activities into the project plan. An exchange programme will provide PhDs and postdocs with opportunities to visit partner facilities in order to experience the interface of knowledge transfer between experimentalists and systemists, enabling both sides to understand reliability and background of experimental data and the transfer of in vivo and in vitro results into the mathematical lab setting. Exchange of scientists will be encouraged in order to tighten the ASSET network and support career development, particularly of young scientists. Three workshops for students and postdocs are planned in order to address topics emphasising the need for interdisciplinary communication in systems biology. Prospective topics are (i) "Mathematical modelling in cancer biology"; (ii) "Modelling and –omics technologies"; (iii) "From systems biology towards systems medicine". The first workshop will be hosted by DKFZ

in conjunction with the International Conference on Systems Biology (ICSB 2011) in Heidelberg, Germany. We plan to also hold the other workshops as satellite events to major conferences, as this will raise ASSET's international profile and give ASSET researchers to interact with eminent scientists in the field.

SBI will co-ordinate the training programme. SBI is currently developing a PhD programme in systems biology, which includes taught modules on different aspects of systems biology and will make these available to the ASSET consortium. Partner DKFZ has close institutional links to the BioQuant Centre Heidelberg, a dedicated research centre for quantitative and systems biology that also runs regular international training courses (e.g., http://www.dkfz.de/en/sbcancer/modeling-school2009.html) as well as the MSc program in Molecular Cell Biology/Systems Biology. DKFZ will organize the international ASSET training workshop on Mathematical modelling in cancer biology in conjunction with ICSB 2011.

Subcontracting

Routine auditing work to certify periodic financial statements, necessary for the implementation of this EC project, have been budgeted under management costs for each beneficiary. Entities chosen to conduct EU project audits for participants have been awarded these contracts according to established principles of best value for money and transparency and typically have on-going relationships with the partners that predate the establishment of this project. Additional routine tasks to be subcontracted include those by the coordinator (UCD) for the wireframes necessary for the implementation of the internal and external project webpage and identity branding design work. UCD has a central Procurement Office that coordinates and establishes competitive bidding practices at the University, resulting in a series of sanctioned preferred vendors for different commodities (such as web design) to ensure that value for money is achieved in all purchasing activity. Other tasks to be subcontracted include the hiring of a patent attorney by ZEPTO, in accordance to their internal procedures and respecting the subcontracting provisions of the ECGA, as needed for IPR issues that may arise during the course of the project. UCPH has budgeted money for subcontracting tasks necessary to carry the objectives of WP 10 in establishing the interrogative project database and associated webservices. These include three possible areas of subcontracting: 1) Custom Curation (data-mining) to extend the knowledge base on relevant cancer pathways by mining literature with special emphasis on key pathway hubs of interest for the ASSET. The information is collected by human literature mining, and once collected, the information will be available within the consortium with freedom to publish 2) Data Visualisation, where development often requires specialised talent, both in design and implementation. The idea here is to layer data collected in the consortium onto pathway maps in a manner that facilitates interpretation and communication of results. Custom development is anticipated around pathways and targets of interest in the project. 3) Targeted Sequencing. UCPH has in house experience and infrastructure for multiplexed targeted sequencing library development. Once libraries have been prepared, the sequencing may be carried out in-house, with an academic partner or through a service provider. Since this field is rapidly evolving, we have budgeted subcontracting of these tasks to allow for the possibility using a service provider. This will provide flexibility to optimise sequencing of the most samples on a given budget. UCPH will undertake the subcontracting of these three tasks in accordance with the procurement policies established by their national authorities, and in accordance with ECGA principles of best value for money (best price-quality ratio) under procedures that assure transparency and equal treatment during the bidding process. Details of the cost estimation for these tasks are summarized below.

RTD	69.000,00	UCPH: custom data curation (10,000), data visualisation development (30,000) and targeted sequencing (29,000).
IPR	4.000,00	ZEPTO: patent attorney
Dissemination	6.000,00	UCD: partial coverage of public, restricted and public website and graphics for corporate identity
Management	84.400,00	ALL: Financial audits
Total	165.400,00	

Associated Third Parties

INSERM, the French Institute for Health & Medical Research, is a third party affilitated with Institute Curie and provides in kind support resources for them.

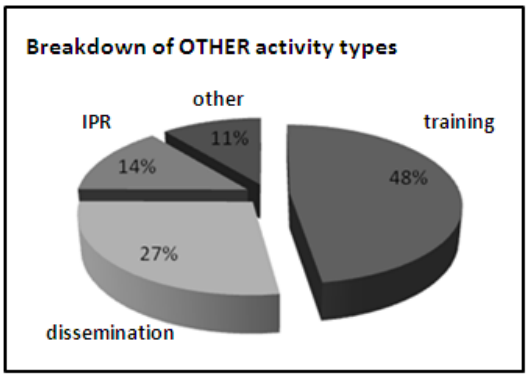
B 2.4 Resources to be committed

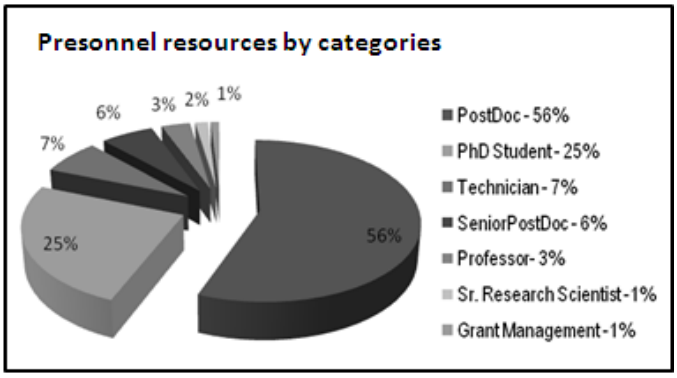
Allocation of costs within activity types

ASSET has estimated an overall budget of TEUR 15.873 which corresponds to TEUR 11.992 requested grant. The distribution of costs among activity types is displayed below. It can be seen that budget requested will be primarily invested in RTD (95%), whereas 3% and 2% respectively will supplement the total costs for management and other activities (training, dissemination, IPR), for which own funding has been mobilized i.e. an administrative assistant, the business development manager and the education & outreach officer at UCD/SBI and the Scientific Advisory board, who won't charge fees to the project.

	RTD	Management	Other	Total
costs [TEUR]	15.000	450	363	15.883
% costs	95%	3%	2%	100%
grant [TEUR]	11.185	450	363	11.992
% grant	93	4	3	100%

OTHER activities: We have included a reserve for IP-protection measures (TEUR 42), a budget for dissemination including an interactive website (TEUR 96,4) and for training (TEUR 188,6) i.e. summer and individual training measures (workshops and scientific symposia). The workplan complements the evidence on the consortium's serious effort to provide enough resources and expertise for project management, training, dissemination and IPR/Exploitation.





Human resources: ASSET unifies 20 research groups from 14 research/academic institutions and one industrial partner that work either in the high-end wet experimentation or in the computational field. The necessity of highly qualified experimentalists for the *in vitro* and *in vivo* models validation and generation of high through put data and of bioinformaticians for modeling, transcriptomics and proteomics experimentation is reflected on that the main performers will be predominantly postdocs For the five-year project, the total effort for the project is 1539.20 PMs. Since modelling is usually faster than data generation, we have allocated ca. 2.5 times the manpower to wet experimentation than to modelling. In our experience this distribution works well to balance data generation and modelling. It has to be accounted that this only represents a part of the actual human resources devoted to the project. Since this is the case for most of the principal investigators that are involved in the Project Management Team (PTM), the Industrial Liaison & Exploitation Committee (ILEC) and the extensive training programme, Management and Other costs requested from the Commission make only a small amount of the total project volume (3% and 2%). Important to note is that the members of the Scientific Advisory Board (SAB) will not charge honoraries to the project.

Breakdown of RTD costs: Out of TEUR 15.000 for RTD costs, TEUR 6.900(46%) are personnel costs. The rest will supplement materials/consumables (16%) and overheads (38%). Resources have been allocated according to tasks acknowledging that consumables and material costs of high-end wet experimentation are much higher than for computation. For instance, wet experimentation carries consumable costs of 15-25k per person per annum

especially when employing state of the art –omics technologies. In contrast, consumable costs for computational modelling are small and mainly comprise purchase of software. High costs are also incurred by the proteomics and transcriptomics experiments, especially when applied to clinical samples. We always will analyse enough numbers of samples to achieve statistical power and produce meaningful data. Although expensive, we consider this essential for the success of the project. The validation phase also will make extensive use of clinical sample analyses and xenograft mouse models to test new drugs and drug combinations. Again this is expensive, but necessary for deriving robust results that are transferable to clinical applications. Some partners (UCD, DKFZ, UGENT, CURIE) involve more than one independent research group, and hence have a larger share of the budget.

Overview on major cost items

Systemists	
UCPH	The multiplex exon libraries (18k EUR) and corresponding access charges (40k EUR) (UCPH).
CEMM	Drug pulldown experiments (CEMM),
All	Purchase of software and licenses, hardware, access to data infrastructures in general
Experimentalists	
CCRI, UBERN	Extensive cell culture work, transfections, reporter gene assays and mRNA/miRNA profiling
DKFZ	gene expression microarrays, QPCR, ChIP-chip/seq, flow cytometry
VTT	high-throughput miRNA, siRNA and compound screens for cell proliferation and apoptosis studies; siRNAs for kinases, kinase inhibitors for combinatorial siRNA-siRNA and siRNA-drug screens
UKE	siRNA knock-downs and 200-250 mice (~20.000€) +substances to be tested in vivo (~10.000€) , deep sequencing exome (100.000€)
UGENT	miRNA profiling and gene expression profiling, RIP-CHIP, Q-PCR, mutation analysis, kits for functional assays (growth, apoptosis), perMIRs, antagomirs

Since the EU contribution does not cover all RTD costs, all partners have dedicated additional resources of their own to ASSET.

Overview on kind contributions:

All	Scientific resources: 13 Professors, 10 senior postdocs, 9 postdocs, 10 PhD students and three technicians will be fully/partially involved in the project. Administrative resources: grant management assistant, grants office (all that have one)
UCD	Proteomics facility; part time grant manager (=ASSET's project manager), a part time outreach manager, part-time CSET manager and a business development manager
CCRI	Biochemical (protein, RNA) analysis facilities Computer hard- and software EWS-FLI1 ChIP-seq (done in collaboration with NCI)
DKFZ	Maintenance of infrastructure
UCPH	Maintenance of a fully operational High Perfomance Computing installation partially funded by the Danish Center for Scientific Computing; additional costs are offset by access charges such as those paid through this grant.
UKE	Laboratory infrastructure, equipment and basic costs
UBERN	Access to genomics labs and use of equipment, use of departmental facilities (FACS, etc.), standard chemicals, plasticware.

B3. Impact

B 3.1 Strategic impact

Cancer is still a major threat to the health of the approximately 160 million children < 15 years of age in Europe, leading to the death of approximately 2300 paediatric patients in Europe each year. According to calculations of years of life lost to disease in comparison to research funding, childhood tumours have one of the lowest levels of funding and highest numbers of years of life lost. Therefore, consortia like ASSET must fill the gap and develop the urgently needed state-of-the-art diagnostic and therapeutic platforms. As ET account for approximately 30% of paediatric cancers, ASSET will have a major impact on solving societal problems.

ASSET is ideally poised to address the impact remits as specified in the call as follows:

- *These projects are expected to deliver robust models for human diseases that will open new avenues for therapeutic interventions.*

The major outcomes of ASSET are computational models that (i) explain functional pathogenetic mechanisms underlying ETs, and (ii) can be used to predict vulnerabilities in ET networks that can be exploited for therapy. This latter point will be taken to the proof-of-principle stage via our siRNA and drug screening and combinatorial target prediction WPs, and the validation work in SP4. The mathematical models, which ASSET will produce, will be extensively validated on two levels: First, through the iterative cycle of prediction and experimentation during the construction of the model, which will be done at the WP level and which we have stringently built into all modelling work. The second level of validation is to use these pre-validated models to make predictions about pathogenetic mechanisms and possible points of interference. These predictions will be tested in SP4 in different cellular model systems, pre-clinical xenograft models and clinical samples. Thus, ASSET will deliver validated, robust models for ETs, and the results will open new avenues for therapeutic interventions. As a part of our screening efforts focuses on combinations of new drugs, which are or are about to be introduced into clinical use, our results may have a direct and fast impact on changing clinical practice. In this respect it is valuable that AE is a clinician heading a Paediatrics Clinic, and that several participant research groups (HK, AA, GSF) are located within hospitals.

- *The projects will apply systems biology approaches to investigate diseases as complex systems.*

ASSET takes full account of ETs as complex systems as evident by its multi-pronged and multi-level approach both on the experimental as well as on the modelling side. Global approaches usually lack predictive detail, whereas mechanistic approaches do not scale to global levels. ASSET overcomes these limitations by using global network reconstruction and analysis to identify disease relevant submodules that will be modelled in detail by mechanistic, dynamic methods. In addition, ASSET will link the different models using semantic tools in order to allow (i) “zooming” through models of different resolution and dynamics; and (ii) integrate the knowledge arising from the analysis of different models in WPs 16 & 17. ASSET also makes use of all modern –omics technologies to study genes, transcripts, regulatory RNAs and proteins, and will use these data for modelling of networks.

B 3.2 Plan for the use and dissemination of foreground

Dissemination

Dissemination is a hugely important aspect of any modern research project, but in particular for interdisciplinary projects where additional means of dissemination must be employed to whittle down the barriers between different disciplines. Successful dissemination should take into account (i) the purpose of the communication and (ii) the interest and level of the audience; and choose the best means of communication accordingly. The goals of dissemination of ASSET are (i) the communication of ASSET's mission and achievements to the scientific community (primarily scientists devoted to cancer research and systems biology) the industry and further network members (regulatory authorities, clinicians, etc.); and (ii) to position ASSET as a poster child for the successful integrative approach of experimental and systems research. Therefore, our dissemination strategy addresses four types of dissemination:

1. Dissemination within the project

The internal dissemination is very important to surmount interdisciplinary barriers, generate cohesion and team spirit. SBI has a dedicated education and outreach officer, Philip Smyth, who will assist with the dissemination activities on a part time basis (as in kind contribution from UCD). While email and telephone will be the major daily communication means between remote sites, we will implement the following measures to develop a strong communication network within ASSET:

Establishment of a researchers' exchange programme between participant groups. This is in particular for cross-over visits between disciplines, e.g. modellers visiting a proteomics lab and vice versa.

Establish a dedicated backstage virtual space on the project website that will facilitate the sharing of documents, discussions about results and plans, and organisation of ad hoc meeting.

Establishment of a monthly lecture series that highlights the big questions in the fields of ET-research and systems biology, and discusses new discoveries in the field in general. These lectures will be given by PIs and invited eminent scientists. They will be broadcast to project partners via videolinks and presentations will be posted in the restricted area of the project website.

Quarterly round tables to discuss current issues in the project. These can include presentation of progress and research highlights, but also discussion of scientific challenges and problems. Again we will use videolinks for ease of participation.

On demand sessions. In addition to the regular programme we will introduce on demand sessions where topics are suggested by researchers as need and interest arises. These can be question and answers sessions, or lectures on specific requested topics. Video conferencing will be used and the sessions will be made available in the project restricted area of the webpage.

Issue of a 3-monthly ASSET Newsletter informing about current activities

Annual project meetings where all partners meet and report on their results. These meetings will feature poster sessions, discussion rounds and team building exercises.

2. Dissemination to scientific peers

This is the classic form of dissemination of scientific results and will use all the well established mechanisms:

Publications in peer reviewed journals

Presentations at scientific conferences and workshops

Seminars and lectures

Project web page: the ASSET project webpage will contain a section for the scientific community describing

ASSET's concept and aims

Progress (unless confidential)

Scientific presentations and talks

Publications

Web based data sharing tools, such as repositories for high-throughput data, models etc. We will make contents of the project datawarehouse and analysis tools publicly accessible after IP has been protected or results published.
Biosystems workshops. ASSET will organize three in–depth workshops. Selected topics could be: "(i) "Mathematical modelling in cancer biology", (ii) "Modelling and –omics technologies", and (iii) "From systems biology towards systems medicine". Lectures will be delivered by senior scientists and by invited guest speakers. The first workshop will be organized as a satellite event to an international symposium within the field ICSB 2011 in Heidelberg, Germany, and we also will try to organise the other workshop in conjunction with international conferences, e.g. FOSBE or Dagstuhl.

3. Dissemination to industry
ASSET will pursue a proactive strategy to engage industry. SBI has currently six industrial collaborators (including both international corporations and biotech companies) and in our experience a proactive trust building approach is the most effective way to engage industry. This activity will be done mainly through the Industrial Liaison and Exploitation Committee (ILEC) with facilitation by the SBI Business Development Manager (BDM), but also by individual partners as appropriate. It involves
Showcasing ASSET and its results to industry through the ILEC and BDM
Inviting industrial representatives to the Annual Meetings and workshops

4. Dissemination to the wider public
This will be aimed at an interested lay audience. These activities will comprise:
Creation of a project identity and brand name, including logo, tag line and brochure order to promote the project and facilitate dissemination.
Establishment of a public interactive website, where the project, its aims and results are explained in laymen's terms
Press releases covering major achievements or publications

Exploitation and IP management

The intellectual property monitoring and patent survey is an indispensable prerequisite to ensure that the research and developments are driven properly. However, in order to assure that IP is captured and protected we have established the Industry Liasion and Exploitation Committee (ILEC). ILEC will, based on their access to ASSETs output through their ongoing communication with the general assembly and the project management team, support the continuous identification of exploitation opportunities by monitoring state of the art proceedings/literature and industrial developments. ILEC will advise the inventors and the general assembly on the qualification of tangible and intangible results that should be either kept confidential, legally protected, disseminated or transferred to third parties. ILEC will monitor the quarterly reports of ASSET and will receive drafts of papers before they are submitted for publication in order to identify results which warrant IP protection or have potential commercial value.

Intellectual Property agreements will be put in place and signed by all partners as a part of the Consortium Agreement and at project start-up the consortium participants will attempt to identify, evaluate and protect potential sources of Foreground Intellectual Property (IP) (ie Knowledge). This will be fully developed within the Consortium Agreement which will comply with the principles, rules and guidelines outlined by the European Commission. All agreements will also include that information brought to the project Background Information and Background Rights, ie pre-existing Know-How will remain the property of the Partner introducing the information into the project. Foreground IP (Knowledge) will be reviewed quarterly by ILEC. In case of joint inventions ownership rights will be fairly distributed between the inventors as specified in the consortium agreement. We expect IP mainly to arise from high-throughput perturbation data, predictive models and their preclinical validation. All partners have their own Technology Transfer Offices, who will protect and exploit IP. ILEC and the SBI BDM will assist as and if required.

Project results (such as gene array, large scale proteomics data), which cannot be IP protected, may still be very useful to research organisations and industry for exploitation in a pre-competitive context. These results will be disseminated to the scientific community and industry as described above. The datawarehouse and the associated analysis tools developed in WP16 & 17 will hold all the project data, results and models. We will make it accessible to the community as soon as IP is protected and results are published. Thus, it will be the lasting legacy of the project and a major vehicle for dissemination and exploitation of the data and tools generated in ASSET.

Contributions to standards

Standardisation of data generation. In order to guarantee consistency and comparability of the data generated in ASSET we will (i) use a defined panel of core cell lines representing the three ET entities: UW-228 (MB) with tet-regulatable c-Myc; A673 (ESFT) with tet-regulatable EWS-FLI1; SH-SY5Y (NB) with differentially regulatable TrkA and N-Myc; and for the ALK work, SK-N-AS (NB) with regulated expression of the ALK F1174L mutant. The core cell line panel will be exome sequenced and all partners will work with the same cells; (ii) establish standard operating procedures (SOPs) for handling the cell lines and experimental measurements; and (iii) establish a short-term exchange programme through which wet and dry researchers will visit partner labs to ensure that data generation and modelling work is pursued according to agreed standards.

B4. Ethical issues

The ultimate aim of ASSET is the identification of promising targeted combination therapies for childhood ET. The use of human biological samples (established tumor cell lines) and existing patient data as well as xenograft animal models in the validation phase of this project is an inescapable preclinical step to test the efficacy of combinations targeting nodes identified to be sensitive in the modeling approaches used in ASSET. Additionally, molecular biological and genetic information gathered from patient samples is essential as input for the systems biology approach proposed here. As the proposal is focused on childhood cancer, the human biological samples were also derived from pediatric patients unable to consent for themselves. Informed consent was given by the patient's parents, and is available for all patient materials and data.

Ethical approval for the use of patient material and data was obtained for all analyses performed within the framework of the E.E.T.-Pipeline and per the original protocol, is extendable for use in this, and other studies. As the countries represented in the E.E.T.-Pipeline consortium do not have uniform ethical regulations, appropriate local ethical approval according to national guidelines was obtained by the responsible principle investigators for the collection, documentation, storage and use of human biological material. Along the project duration ethical approval for the use of patient material and data from the E.E.T.-Pipeline project will be updated at the local ethics boards to include the new analyses planned here. Additional access to clinical data is enabled through the BFM-NB-2004 therapy optimization study for neuroblastoma patients, approved by Nr. 04-049 University of Cologne Ethics Committee. The EuroEwing99 treatment protocol covers Ewing patient data that will be studied in the frame of the ASSET program. Copies of approval documents have been made available to the European Commission.

All parts of the project will at all times conform to the CIOMS International Ethics Guidelines, the “Helsinki Declaration, Convention of the Council of Europe on Human Rights and Biomedicine”, the “UNESCO Declaration on Human Genome and Human Rights” the Recommendation of the Council of Europe on Research on Biological Materials of Human Origin, Rec (2006) 4, 15 March 2006, and national regulations concerning use of human biological materials in research. The patients, whose materials will be used in this project, are under the control and management of their local physicians and healthcare systems, for which the consortium has no responsibility. Neither the diagnosis nor the treatment of any patient will be altered in any way because of this project, and the project is not involved in patient care. However, this project has a high potential to provide extensive benefits to future patients and to the scientific and medical communities.

Human Biological Samples

Biological samples from paediatric ET patients to be used within this proposal only comprise samples of paraffin-embedded tumour tissue and established tumor cell lines. All samples have been collected as residues during routine diagnostic and therapeutic procedures. No additional procedure was performed only to obtain material for the projects in which the samples were collected to produce the molecular biological and genetic data used as input for this project or in tissue microarrays used for in validation workpackages of this project. There was no additional risk to the patient other than that usually associated with surgery as part of the patient's routine and treatment. Informed consent of the patients' parents exists for all cases, according to the guidelines of the German National Ethics Council, *BioBanks for Science*, D.3, p. 64, *Obligations concerning informed consent*. In the case of stored paraffin-embedded tissue (often > 10 years old), it is expected that retrospective consent will not be required, unless it is required by the country in which that tissue was taken (in Germany it has been recognised that retrospective consent is not usually required for paraffin-embedded material).

Protection of data

Data utilised from the E.E.T.-Pipeline project for input into ASSET conforms to the data protection legislation active in the ASSET partner's countries and to EU Directive on Data Protection passed in 1995. Data provided to the ASSET consortium is coded by a BioBank identification number for patient samples and patient data, and is provided in a strictly anonymised form, thus, is not attributable to any particular patient. Neither any of the results nor any of the samples will be identified with the patient's name. In addition, the patient's birth date will also not be identified in the documentation, so that it will not be possible to trace the samples back to any individual based on birth date. If any results of this study are published, only the anonymized code number will be used and the patient's identity will remain confidential. It is, therefore, impossible that an individual could be identified from the information reported from this project.

Summary of ASSET Relevant Ethical Consent and Protocol Documents

NB-2004 *Trial Protocol for Risk Adapted Children with Neuroblastoma*, University of Cologne, Germany, approved by 04-049 – University of Cologne Ethics Committee.
EURO-EWING99 *Protocol for the treatment of Ewing tumors: randomized trials comparing chemotherapy with consolidation, including a medico-economic evaluation*. Institut Curie 2007
EC/2007-137/Svdm dated 2007-06-20 and EC/2006-146/Svdm dated 2006-10-4 *The storage and use of biological material in pediatric patients,* UGENT
EC/148-2006/Ivdp dated 2006-10-05 *Use of tissue samples for scientific research in pediatric hematology-oncology patients,* UGENT
EC/208-2006/SVdm dated 2006-09-12 *Using tissue samples from neuroblastoma patients for research, INES amendment, HR-NB, LNESG-1, LNESG-2 and "stage 2 and 3 unresectable" protocols* (UGENT)

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 THE ABOVE ISSUES APPLY TO MY PROPOSAL	X	-

	Research on Humans	YES	Page
*	Does the proposed research involve children?		
*	Does the proposed research involve patients?		
*	Does the proposed research involve persons not able to give consent?		
*	Does the proposed research involve adult healthy volunteers?		
	Does the proposed research involve Human genetic material?	X	14-15
	Does the proposed research involve Human biological samples?	X	14-15
	Does the proposed research involve Human data collection?	X	14-15
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		-

	Privacy	YES	Page
	Does the proposed research involve processing of genetic information or personal data (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 THE ABOVE ISSUES APPLY TO MY PROPOSAL	X	-

	Research on Animals	YES	Page
	Does the proposed research involve research on animals?	X	14-15
	Are those animals transgenic small laboratory animals?		
	Are those animals transgenic farm animals?		
*	Are those animals non-human primates?		
	Are those animals cloned farm animals?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		-

	Research Involving Developing Countries	YES	Page
	Does the proposed research involve the use of local resources (genetic, animal, plant, etc)?		
	Is the proposed research of benefit to local communities (e.g. capacity building, access to healthcare, education, etc)?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	X	-

	Dual Use	YES	Page
	Research having direct military use		
	Research having the potential for terrorist abuse		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	X	-

B5. Gender aspects

Several documents released by the European Commission1 highlight the importance of taking into account the gender dimension for the execution of research programmes.
In ASSET we will ensure equal opportunities for hiring, in order to facilitate working positions for qualified female aspirants in competitive fields as are paediatric cancer research (rare disease, niche research area) and systems biology (raising research discipline).

Specifically, Principal Investigators (participant's representatives) of ASSET committed to pay particular attention in the sensitisation about the gender equality in research structures. They will make best efforts to support the recruitment of women in their research groups on the basis of their expertise and potential professional input to the group.

The topic of this project does not involve expected differences between genders so that both will be considered equally during the experimentation and both should benefit equally from the results.

ASSET proposes to promote gender equality in research in general, but importantly, in a field that due to the technical background tends to be dominated by male scientists, namely systems biology. Therefore this matter will be addressed in dissemination activities.
Flexible working hours will be considered as well as family-friendly policies. The rate of women employed at different positions will be monitored all along the project.

If ASSET succeeds in attracting a larger proportion of women students and experienced scientists to the project, we will have contributed to more qualified female personnel for work in the European medical, pharmaceutical and biotechnology fields and general more qualified women in the European society.