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E.E.T.-Pipeline

European Embryonal Tumor Pipeline

Thematic Priority »Life sciences, genomics and biotechnology for health«
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Publishable Final Activity Report

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Administrative Coordinator:
Dr. Kathy Astrahantseff

Coordinator Organisation: University Hospital Essen

Final Activity Report
Section 1 - Project Execution

www.eet-pipeline.eu/

1. Background

Second to accidents, cancer is still the leading cause of death for children in Europe. Approximately 30% of childhood malignancies are embryonal tumours (ET), often demonstrating resistance to conventional treatment approaches and being associated with lower survival rates compared to other childhood cancers. Thus, novel diagnostic and therapeutic options are urgently needed in particular for this group of tumours to improve survival rates and quality of life of paediatric cancer patients in Europe. We therefore focus our combined research efforts on ET. ET are dysontogenetic tumours whose pathological features resemble those of the developing organ or tissue of origin and include the entities neuroblastoma (NB), nephroblastoma/Wilms' Tumour (WT), medulloblastoma (MB), retinoblastoma (RB), the Ewing sarcoma family of tumours (ESFT) and rhabdoid tumours (RT). Their early manifestation in infants and young children suggests that only a limited number of genetic changes lead to the transformed phenotype, making ET an ideal model for the post-genomic investigation of cancer-related expression changes. Due to the high genetic complexity of solid tumours in adults, it is difficult to identify suitable drug targets. The genetic low complexity of ET tumours provides a more suitable system to identify targets, which may also be important for adult tumours, and therefore, have a more general impact. Treatment of embryonal tumours (ET) is a challenge for the paediatric oncologist. Innovative translational research is required to exploit available genomic data and implement state-of-the-art technologies to overcome the deficits of current diagnostic and treatment strategies.

2. General Project Objectives

We set up a consortium of leading European institutions and SMEs with extensive clinical and technological expertise to establish a unique pipeline for the comprehensive development and validation of novel diagnostic tools in addition to efficient preclinical drug development for ET. The E.E.T.-Pipeline provided a comprehensive, multi-team approach for improving ET diagnostics and treatment by the integration, assessment and validation of information generated by basic research utilising high-throughput technologies. In this integrated post-genomic research effort, we established dual pipelines concentrating on (1) state-of-the-art diagnostics and (2) innovative drug development and preclinical testing to channel these efforts. Efficient use and distribution of resources as well as optimised data analysis and fast gain of molecular knowledge was ensured by linking both pipelines to a central bioinformatics platform. Meta-analysis integrating newly obtained and existing data for the ET entities derived from different genomic and proteomic platforms derived further gain of knowledge from the data. The driving force of the project was a set of 10 additional workpackages (WPs), each specifically addressing the integration of a key technological platform in the ET diagnostics and drug development pipelines and making all platforms available for use by all consortium partners.

Our holistic approach incorporated the following work:

1) Validation of a **chip-based diagnostic platform** including analysis of genes previously shown by the consortium to be affected in ET
2) Generation of ET-specific data on a **novel array-based platform** for the development of **diagnostics** at the microRNA level
3) Extension of an existing **database** designed to warehouse complete clinical and experimental data for neuroblastoma to include all ET entities
4) Functional characterisation of the most **promising molecular targets** previously identified by the partners as a foundation for entry into a drug development pipeline
5) Integration of existing disease-specific **mouse models** to evaluate new treatment modalities *in vivo*
6) Application of novel bioinformatic solutions for the **meta-analysis** of data generated on different platforms for different ET entities
7) **Dissemination** of the novel tools to researchers and clinical study centers in Europe via the continuation of the E.E.T.-Pipeline as a consortium and its participation in the newly established ENCCA network for pediatric oncology.

Our coordinated effort was designed to achieve a critical mass facilitating the necessary integration of research capacities for translating ET "omics" data into significant medical progress. Involvement of clinical study centers was sought to ensure a direct link to the bedside, for improving child health and quality of life, and will now be extended within the ENCCA network.

3. Contractors involved and co-ordinator contact details

The E.E.T.-Pipeline is composed of 11 Partners from 8 different European Countries:

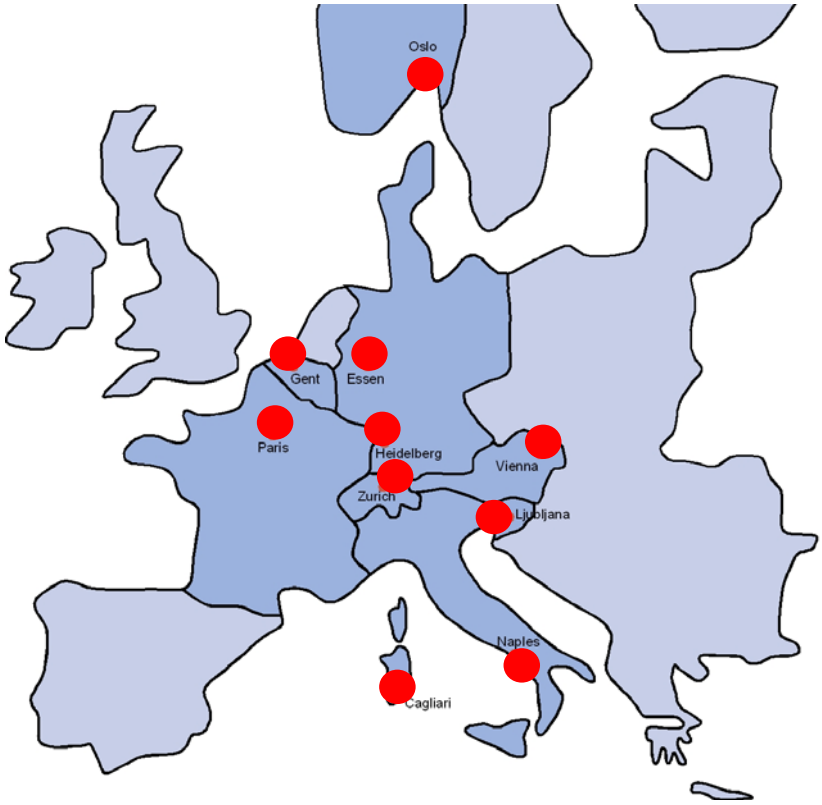

Fig.1: E.E.T.-Pipeline Partner Institutions

Academic Coordinator: Prof. Angelika Eggert

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Other **Partners** (in brackets, the acronym of the Institution):

- Prof. Manfred Schwab, DKFZ Heidelberg (DKFZ-Tumour Genetics), Heidelberg, Germany;
- Prof. Roland Eils, DKFZ Heidelberg (DKFZ-Theoretical Bioinformatics), Heidelberg, Germany;
- Prof. Saso Dzeroski, Jozef Stefan Institute (JSI), Ljubljana, Slovenia;
- Prof. Michael Grotzer, University Children's Hospital Zurich (UZH), Zurich, Switzerland;
- Prof. Heinrich Kovar, Children's Cancer Research Institute / St. Anna Kinderspital (CCRI), Vienna, Austria;
- Prof. Olivier Delattre, Institut Curie (CURIE), Paris, France;
- Prof. Massimo Zollo, CEINGE Biotecnologie Avanzate, (CEINGE), Naples, Italy;
- Prof. Frank Speleman, University of Ghent, (UGENT), Ghent, Belgium;
- Dr. Herald Reiersen (AFFITECH), Oslo, Norway;
- Dr. Alessandro Bulfone (BIOFLAG), Cagliari, Italy.

4. Work performed, main achievements and end results

A. Development, validation and use of new high-throughput platforms to support target gene discovery in pathways important for ET biology and improve the information base connecting transcriptome and proteome in ETs.

ChIP-chip and ChIP-seq platforms for identifying N-Myc and c-Myc target genes (DKFZ-Tumour Genetics)

The consortium agreed to use the Agilent 44K gene expression platform as the core platform to extend gene expression profiling on the mRNA level for primary ETs, and to centrally establish the new ChIP-chip / ChIP-seq technology. This platform was introduced to support researchers on their way to identifying common molecular mechanisms deregulated in all ETs, such as deregulated N-MYC or c-MYC functions. The epigenetic marks allowed the distinction of actively transcribed (H3K4me3) and elongated (H3K36me3) or repressed (H3K27me3) genes. Understanding c-MYC and N-MYC regulation and function in initiation and progression of ETs was a common goal of three WPs. This is important for risk assessment, and can be combined with drug discovery research to help interpret preclinical testing of targeted agents, such as the Myc inhibitor assessed in one WP or existing kinase inhibitors. Profiles for N-Myc and c-myc target genes including epigenetic marks for transcriptional activity were generated for NB (30 total). N-Myc and c-myc target profiles (20 total) were generated for MB, and 3 target gene profiles were generated for ESFT. MYC family target gene discovery also addressed entity-specific questions in *in vitro* ET model systems.

Affymetrix Exon arrays (UKE)

Affymetrix exon arrays are designed to interrogate every known and predicted exon in the human genome. The combination of coverage and precision enables the analysis of regulation events on sub-gene level, extending beyond conventional expression profiling. This is important since a substantial proportion of protein-coding genes are predicted to be alternatively spliced, and many non-coding genes including miRNAs and NATs are known also to be of biological significance. We profiled a cohort of 101 primary NBs using Affymetrix exon arrays, emphasising two major points: (1) increasing the potential for meta-analyses of expression profiling data as exon arrays can also be considered as whole transcriptome arrays, when focussing on gene expression only and (2) identifying alternatively spliced genes in local vs. metastatic NB. Alternatively spliced exons were verified in NBs as proof of concept for the usefulness of exon arrays in the analysis of solid paediatric tumours.

B. Extension of molecular profiles generated for ET entities using validated platforms to support meta-analysis across platforms and entities

Customised Agilent 44K gene expression profiling (DKFZ-Tumour Genetics)

The consortium decision to maintain the Agilent 44K gene expression platform in place of designing a customized ET-chip for entrance into clinical use provided more information for meta-analysis (based on the larger number of genes assessed) at an overall lower cost. This decision was based on the agreement that the current knowledge base was not yet rich enough to choose a small number of highly applicable marker genes for a chip designed for future routine clinical use. The consortium now has a total of 367 expression profiles stored in the iCHIP database. These data fed into meta-analysis efforts.

Custom-made Agilent CGH chip (UGENT)

For arrayCGH profiling, a customised 44K Embryonal Tumour array CGH-chip (ET-aCGH chip) was constructed with enrichment for critical regions of loss/gain for NB (10 kb resolution), miRNAs/T-UCRs (5 oligos), cancer gene sensus genes (5 oligos) and genes known to be important in RT, ESFT, MB, RB and NB (5 oligos). A total of 246 samples were profiled on this platform, including 101 NB, 53 MB, 30 RB, 10 RT, 10 ESFT, 32 NB cell lines and 10 cell lines derived from other ET entities. All data were imported in an in-house developed database (http://medgen.ugent.be/arrayCGHbase) allowing easy visualisation of the genomic profiles and allowing unbiased recognition of regions with equal copy number changes using the CBS algorithm (circular binary segmentation). This database was specifically designed to deal with the distribution and visualisation of arrayCGH data. The overall quality of arrayCGH data was good, but could be improved for some cases through implementation of a wave correction algorithm developed in the arrayCGHbase analysis pipeline. These data fed into meta-analysis efforts.

Stem-loop megaplex RT-qPCR platform for genome-wide miRNA profiling (UGENT)

The consortium decided to implement a centralised stem-loop, megaplex reverse-transcription quantitative PCR (RT-qPCR) platform. This platform was extensively evaluated and optimised. A limited-cycle pre-amplification step was introduced to reduce the required input RNA to 20 ng per tumour sample. Expression profiling of 384 miRNAs was carried out for 75 primary untreated NBs and 61 MB in the initial pilot testing of the platform, and currently encompases miRNA profiles for 253 primary ETs (156 NB, 61 MB, 30 RB, 3 normal retina controls, 6 ESFT and 5 mesenchymal controls). Whole miRNAome profiling using the RT-qPCR platform was also performed on 24 different ET model systems (12 NB, 6 MB and 6 ESFT). These results fed into meta-analyses, and helped define the role of miRNAs in gene regulation in ETs.

ET-TMA (UKE)

Tissue microarrays (TMA) were developed for ET entities to enable the investigation of cell and tissue-localisation of target protein expression. This information is critical to detect target protein involvement in tumourigenetic processes regulated at the level of the expressed protein, such as relocalisation to another subcellular compartment or expression only in certain cell types of the tumour. TMAs also included appropriate normal tissue controls, and eased screening efforts for candidate target proteins in the consortium. Existing TMAs were extensively used by the consortium, and 13 studies for target proteins have been finalised. This platform has proved valuable to link molecular profiling and functional studies in models back to the tumour entity of interest as well as to rapidly assess if a marker for one ET entity is interesting for other entities.

C. Validation of the feasibility of molecular platforms for clinical application

MAQ (UGENT)

MAQ (multiplex amplicon quantification) is an alternative PCR-based method for the quantification of copy number changes, and consists of the simultaneous PCR amplification of several fluorescently labeled target and reference sequences.This methodology was fully tested, and found to be superior to another method that was tested, MLPA (multiplex ligation-dependent probe amplification). The NB MAQ assay was constructed in collaboration with the developers of the MAQ technology (www.multiplicon.com), and validated on a cohort of 48 NB samples, for which arrayCGH data were also available. The comparison of normalised peak areas between the test individual and control individuals results in a dosage quotient indicating the copy number of the target amplicon. In comparison to MLPA, MAQ required only a single PCR step without a challenging ligation step, and only 20-50 ng of DNA as input, in contrast to 100 ng for MLPA. MAQ was highly accurate in detecting *MYCN*-amplification, 1p-, 3p- and 11q-deletions and 17q-gain (regions commonly affected in NB) in this cohort. MAQ analysis costed only 66 € per sample in comparison to 88 € for MLPA and 205 € for the customised 44K arrayCGH. We demonstrated that the newly developed MAQ method can be used as a valuable diagnostic tool for reliable detection of copy number changes with prognostic relevance in NB. PCR-based techniques harbour advantages in comparison to arrayCGH that include reduced cost and sample handling while maintaining high performance. Moreover, the equipment needed (a thermal cycler and a capillary electrophoresis system) is present in the majority of molecular biology laboratories performing routine diagnostics. MAQ can measure the copy number status of up to 40 targets in one reaction, making it sufficient for many routine tests. MAQ is a feasible clinical option for assessing gene copy number to better support therapy choice on an individualised patient basis.

D. Application of novel bioinformatics solutions for the meta-analysis of ET data originating from all high-throughput platforms used here

The iCHIP database was extended to inlcude data produced on other ET entities **(DKFZ-Bioinformatics)**. Methods were developed for meta-analysis and integrative analysis of ET genomics data as well as for the preparation of data for use in these analyses. This included (1) developing methods for mapping of probes between different microarray platforms to enable meta-analysis (**DKFZ-Bioinformatics**), (2) developing algorithms to integrate information about the genome (from arrayCGH), mRNA and miRNA transcriptomes, transcription factor binding (from ChIP-chip) and the proteome (**JSI** and **DKFZ-Bioinformatics**) and (3) establishing methods for cross-entity analysis of the different tumour types examined (**JSI** and **DKFZ-Bioinformatics**). The objective of these methods was to help investigate common mechnisms in pathogenesis and progression of ET and to identify points for interference that could be validated and further investigated on the “wet bench”. One focus was identifying common mechanisms involving Myc transcription factors

in NB and MB. Another focus addressed common patterns of transcriptional signature activation, and genes and concepts important for NB progression. This work provided methodology and novel information supporting a basis for similarities of some core pathways in ET pathogenesis, which are activated or repressed by entity-specific mechanisms. Target molecules were identified from the combined data from molecular platforms assessing genomic and transcriptomic levels that may be points for therapeutic intervention.

E. Functional characterisation of previously identified target genes

Function characterisation was carried out for several targets of tumourigenic properties. Two WPs addressed cell cycle progression and mechanisms of cell death, one addressed the therapeutic possibilities for inhibition of promigratory / proinvasive effectors, one addressed the development of resistance and one addressed the role of miRNAs in regulating several processes associated with ET tumourigenesis.

(CCRI): Doxicycline-inducible systems were established in ESFT cell lines (A673, SK-N-MC), and complemented transient knockdown/expression systems in ESFT cell lines (TC252, WE68, VH64, STA-ET-1, STA-ET-7.2, SK-N-MC). These model systems allowed independent modulation of EWS-FLI1 and cMYC expression and the restoration of cMYC expression upon EWS-FLI1 knockdown, and were used to generate time-resolved gene and miRNA expression data to define the degree of overlap between genes regulated by EWS-FLI1 and MYC in ESFT. Candidate genes regulated by EWS-FLI1 and MYC were validated in primary tumour material, the *in vitro* model systems, under different growth conditions (normoxia vs. hypoxia; adherent vs. anchorage-independent) and distinguished directly from indirectly regulated genes via ChIP-chip (c-MYC) and ChIP-seq (EWS-FLI1).

(UZH): Complete expression profiling of MB-derived cell lines (DAOY) and subclones engineered to overexpress c-MYC were carried out. The DAOY M2 subclone was also profiled following siRNA-mediated downregulation of c-MYC. These data produced a group of 274 MYC-regulated genes, which were futher reduced to a gene ontology-based subset of cell cycle-related genes for validation as potential druggable targets.

(DKFZ-Tumour Genetics): A set of genes regulated by MYCN/c-MYC in NB-derived cell lines were identified, for which the expression significantly associated with poor patient outcome in data from primary NBs. The genes downstream of MYCN/c-MYC critical for malignant progression were assessed using functional *in vitro* assays, and the mechanisms of deregulated *SKP2* expression in NB cells downstream of MYCN were further characterised.

(CURIE): A diagnostic assay was developed for RT based on deletions of the *hSNF5/INI1* gene, and was tested on a large series of primary RTs. It proved useful in routine patient diagnosis. The functional consequences of *hSNF5/INI1* loss were investigated, specifically for genes that distinguish RT from other ETs.

(CEINGE): High expression levels of h-prune and nm23-H1/H2 have been associated with more agressive tumour phenotypes and worse prognosis in cancers other than ETs. Stable clones of NB- and MB-derived cell lines were created over-expressing h-prune or nm23-H1/H2 proteins, and their growth and metastatic capabilities were assessed. Both cell motility and proliferatation were enhanced in over-expressing clones. Nuclear staining for h-Prune and expression of nm23-H1 correlated with poor patient prognosis for MB and NB, respectively. Viral-mediated prune downregulation supressed proliferation and migration of NB and MB cells in vitro, and slowed the growth and caused differentiation of MB xenograft tumours in mice. Syomicin A was shown to have anticancer activity for NB and MB *in vitro*, but is not yet ready for delivery into animals since it is not water-soluble. These experiments provide proof-of-concept for targeting h-prune-nm23-H2 for NB and MB therapy.

(UKE): Interfering with Gal-1 function reduced invasiveness and migration of human SY5Y NB cells *in vitro*. These aggressive features were tightly linked to the expression and activation of the neurotrophin receptor, TrkB, which is associated with poor prognosis in NB. However, immunohistochemistry revealed no preferential expression of Gal-1 in aggressive primary NB. Three strategies were available to analyse the role of Galectin-1 *in vivo*: (1) interfering with Gal-1 by siRNA-treatment prior to grafting the cells, (2) knocking down Gal-1 via inducible shRNA and (3) pharmacological blocking of Gal-1/HRAS interaction using FTS (farnesyl-thiosalicylate). We primarily utilised the siRNA-based approach, since the inducible knockdown of Gal-1 via shRNA caused major technical problems, and FTS became commercially available too late in the project, allowing analysis in only one study cohort. We compared Gal-1 treatment to other innovative treatment options in NB, Temozolomide and Vorinostat (SAHA), which are currently tested in clinical trials for the treatment of solid cancers of childhood. Knockdown of Gal-1 reduced xenograft tumour burden and prolonged survival *in vivo*, validating it as a bona fide cancer target for ETs.

(UKE): One target with a possible role in resistance development was identified in a protein array screen for auto-antibodies in NB patient serum. This protein was expressed in several NB cell lines, and elevated expression in the nuclei and cytoplasm of a cisplatin-resistant cell line was observed in comparison to parental cells. Cisplatin treatment induced target protein expression and nuclear translocation in both cell lines. Knockdown increased sensitivity to cisplatin in both cell lines, as well as the number of γH2AX foci, indicating reduced repair capacity for double-strand breaks. Target protein expression was also associated with MYCN activity, and was shown to be a direct MYCN target. These results implicate this protein as a possible target for therapeutic intervention downstream of Myc signaling. Additionally, it could also play a similar role in ESFT and RB, since it was also identified as a direct c-Myc target in for ESFT and was also more strongly expressed in resistant RB cell lines.

An inducible model for the oncomir, miR17-92 cluster, was used to analyse target genes of this important miR in NB **(UKE** and **UGENT)**. Additionally, miR34a function was assessed in MB **(CEINGE)**. Both these miRNAs were identified as important for Ets, underlying that targeting miRNAs may have therapeutic possibilties for ET entities.

The E.E.T.-Pipeline project has produced a wealth of new knowledge about target genes functioning in important regulatory networks driving ET tumourigenesis, progression and cell survival of chemotherapy. Due to the interactive nature of the E.E.T.-Pipeline, it was possible to more quickly identify pathways and target molecules common to different entities and persue collaborative work to investigate them. Additionally, the pipelines defined in this project for tumour marker investigation functioned well to rapidly progress through *in vitro*, *in vivo* and patient tumour sample (TMA) analyses, to produce well validated targets for ETs.

F. Use of the drug testing platform for preclinical evaluation of a targeted novel drug (UZH)

As a proof-of-principle for the functionality and efficiency of the platform, we completely evaluated the efficacy of the MYC-inhibitor, NBT-272, *in vitro* using our ET cell line panel. Viability of some of the cell lines was inhibited. We showed this panel to be valuable as *in vitro* preclinical models for testing novel anticancer agents and better understanding their methods of action. Genomic and transcriptomic signatures of the panel of 11 ET-derived cell lines were also generated, and proved to be instrumental for a more comprehensive interpretation of the results relative to any drug screening and validation. This data also fed into meta-analysis, and was useful to answer pathway-specific questions. The mechanism of drug action was also addressed, in order to identify the molecular characteristics necessary for drug efficacy. NBT-272 treatment induced autophagy in ET cells. The lack of correlation between NBT-272 sensitivity and Myc protein expression did not support a direct effect on Myc exclusively at the protein level, but our results rather supported that the strong effect of NBT-272 on protein synthesis was the mechanism of efficacy. Stabilisation of c-Myc was

observed after inhibition of a proteasome-dependent protein degradation pathway, and co-treatement with both NBT-272 and a proteasome inhibitor rescued Myc degradation almost completely. ET cells were more sensitive to NBT-272 than small-molecule inhibitors of the Myc/Max interaction, supporting a broader spectrum of action and an indirect depletion of Myc protein via NBT-272. PI3K/Akt/mTOR and Mek/Erk pathways were active after NBT-272 treatment of ET-derived cells. The downstream targets of these pathways are direct effectors of apoptosis, cell cycle progression and autophagy, and can be reasonably addressed as key molecular determinants orchestrating the NBT-272-mediated cell responses. Even though the compound cannot be described as a direct kinase inhibitor, it targets the activation of the Akt/mTOR and Mek/Erk pathways, by inducing a reduction/depletion of the active (phosphorylated) form of some of their components. More significantly, both pathways are directly involved in regulation of protein synthesis. Indeed, NBT-272 treatment prevented a full activation of the cap-dependent protein translation machinery by interfering with the activation status of both the eukaryotic translation initiation factor 4E (eIF4E) and its binding protein, 4E-BP1. This testing pipeline not only effectively tested efficacy of novel compounds, but pointed out specificity and mechanism of drug action, supporting the definition of the molecular background necessary for drug efficacy against patient tumours.

G. Human phage display library screening for human antibody production for one ET target (AFFITECH)

As proof-of-principle for the feasibility of this approach to identify novel paediatric anti-tumour antibodies, a human phage display library was screened with a previously identified target relevant for ET tumour biology (c-met). Affitech has delivered a fully human c-met-specific antibody (IgG) that was selected using Affitech's naive scFv-phagemid library coupled to Affitech's preferred method of choice for lead-optimisation, using light-chain shuffling and robot-assisted AffiScreen^{TM}. The final candidate was demonstrated to bind the target on live cells, and is cross-reactive to murine c-met.

H. Virtual drug screening (BIOFLAG)

BIOFLAG further developed two bioinformatics platforms for the identification of specific anti-sense transcripts and new surface protein markers, and initiated studies on a well-defined Affymetrix tumour expression dataset for MB and data from public genomic databases and expression data from the large NB cohort profiled on exon arrays, which yielded more usable information for AS-FLAG discovery. Polyclonal antibodies against selected targets were optimised for use on ET TMAs, and target protein expression was assessed.

5. Impact

Due to the relatively low patient numbers in paediatric oncology, treatment of patients in nationwide or mostly Europe-wide clinical trials is mandatory and has been well established for decades. The realisation of the E.E.T.-Pipeline, efficiently bridged state-of-the-art post-genomic research at the bench and the bedside at the European level. The continuing existence of the E.E.T.-Pipeline consortium of researchers, and its involvement in the ENCCA network of excellence for paediatric oncology provides a strengthened link towards rapid translation of “omics”-derived results into clinical application.

As cancer is a major threat to the health of the approximately 160 million children < 15 years of age in Europe, and ET account for approximately 30% of paediatric cancers, there is a considerable market for diagnostic and therapeutic tools developed in the E.E.T.-Pipeline. Combined research on all ET tumour entities is a novel way of increasing the potential market for drugs and diagnostic tools for ET. Drugs effective against the group of ET entities may also be applicable to other morphologically related adult cancers, including melanomas and lung cancers, further increasing the potential markets.

Section 2 - Dissemination and Use

Section 1 – Exploitable knowledge and its use

NA

Section 2 – Dissemination of knowledge

Overview table

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
BioPartnering North America (BPN), Vancouver, Canada 4-Feb-2007	Exhibition at Conference	*Industry* - Biology and biotechnology. - Medicine and human health	North America	thousands	BIOFLAG
98th Annual American Association for Cancer Research (AACR) Meeting, 13-18.04.2007	poster presentation	research international	all	Thousands	UKE, UGENT, DKFZ-Theoretical Bioinformatics
98th Annual AACR Meeting, 13-18.04.2007	poster presentation	research international	all	Thousands	CCRI
BIO 2007 conference, Boston, USA 6-May-2007	Exhibition at Conference	*Industry* - Biology and biotechnology. - Medicine and human health	North America	thousands	BIOFLAG
Bioforum Partnering Event, Milan 25-Sep-2007	Exhibition at Conference	*Industry* - Biology and biotechnology. - Medicine and human health	Europe	thousands	BIOFLAG
ECML/PKDD 2007 Conference, Warsaw, Poland, 07.09.2007	Conference and Publication	research international	all	100	JSI
TGIN Meeting, Washington D.C., 01.10.2007	oral presentation	research international	all	50	DKFZ-Tumour Genetics
Workshop on Computational Approaches in microRNA research, 2 Oct 2007	Conference	research international	European	100	DKFZ-Theoretical Bioinformatics
25.10.2007	Invited talk	Graduate Students and Faculty of Faculty of Electrical Engineering and Information Technologies, University Ss. Cyril and Methodius (Skopje, Macedonia)	Macedonia	50	JSI
39th International Society for Paediatric Oncology (SIOP) meeting, Mumbai, India, 1-3.11.2007	oral presentation	research international	all	800	UKE
39th annual SIOP meeting, Mumbai, India, 1-3.11.2007	conference	research international	all	800	CCRI
Workshop "From Bioinformatics to Medical Systems Biology", 12-13 Nov 2007	Conference	research international	European	120	DKFZ-Tumour Genetikcs, DKFZ-Theoretical Bioinformatics
"Schweizer Forschertag Pädiatrie" meeting, UKBB Bern, Switzerland, 13.11.2007	Oral presentation	Swiss research	European	>100	UZH
Cell Signaling and novel cancer therapeutics meeting, BACR, London, UK, 29-30.11.2007	Poster presentation	research international	all	>100	UZH
BRECOSM-METABRE EU-mestastasis research society meeting, Rome, 5-7.12.2007	Oral presentation	research European	Europe	100	CEINGE

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Cancer Lett 2007 May 18;250(1):17-24. Epub 2006 Dec 4	publication	research international	all	Thousands	DKFZ-Tumour Genetics
BMC Cancer 2007 May 25;7:89	publication	research international	all	Thousands	DKFZ-Tumour Genetics, DKFZ-Theoretical Bioinformatics
Clin Cancer Res 2007 Aug 15;13(16):4695-703. Epub 2007 Jul 25.	publication	research international	all	Thousands	DKFZ-Tumour Genetics, DKFZ-Theoretical Bioinformatics
7th Annual McGill Workshop on Bioinformatics, 18-25 Jan 2008	Conference	research international	US / Canada	40	DKFZ-Theoretical Bioinformatics
13th Heidelberg Grand Rounds, 19.02.2008	Oral presentation	research European	Europe	>50	DKFZ-Tumour Genetics
Epigenetics Meeting, Heidelberg, 29.02.2008	Conference	research European	Europe	50	DKFZ-Tumour Genetics
52nd GTH Congress (Deutsche Gesellschaft für Thrombose- und Hämostaseforschung), 20-23 Feb 2008	Conference	research Germany	Germany	30	DKFZ-Theoretical Bioinformatics
29 Feb 2008	TV broadcast	Nano (TV Science Magazine): Report on neuroblastoma as a rare disease	Europe	approx. 200,000	DKFZ-Tumour Genetikcs, DKFZ-Theoretical Bioinformatics
NIH Bethesda Maryland-NCI-Clinical and Translational therapeutics program,27/02-02.03.2008	Oral presentation	Research international	USA	>100	CEINGE
Rockville Pike, Avalon pharmaceuticals-Clinical and Translational therapeutics program, 27.02-02.03.2008	Oral presentation	Research international	USA	50	CEINGE
13.03.2008	TV press release /NANO Sat3	General public / international	Europe	1 Mill	DKFZ-Tumour Genetikcs, DKFZ-Theoretical Bioinformatics
Analytica Conference, 1-4 Apr 2008	Conference	research international	all	80	DKFZ-Theoretical Bioinformatics
Beshg conference, Leuven, Belgium, 25. April 2008	poster presentation	research national	Belgium	100	UGENT
MC-Gard Meeting workshop, Braga, Portugal, 8-13 Sept 2008	poster presentation	research national	Belgium	100	UGENT
Rett Nearburg International Ewing's sarcoma Symposium IV, 9-11.04.2008	oral presentation	research international	all	~100	CCRI
BeSHG 2008: Meeting of the Belgian society for human genetics, Leuven, Belgium, 25.04.2008	Poster presentation	Research ()	All	>100	UGENT
BeSHG 2008: Meeting of the Belgian society for human genetics, Leuven, Belgium, 25.04.2008	Poster presentation	Research (BeSHG 2008: Belgian society of human genetics)	Belgium	>100	UGENT
08.05.2008	Invited talk	Graduate Students and Faculty of School of Electrical Engineering & Computer Science Oregon State University (Corvallis, OR, USA)	USA	50	JSI
Advances in Neuroblastoma Research (ANR) Conference, Chiba, Japan, 21-24.05.2008	oral presentation	research international	all	500	UKE
ANR Conference, Chiba, Japan, 21-24.05.2008	oral presentation	research international	all	500	CEINGE

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
ANR Conference, Chiba, Japan, 21-24.05.2008	oral presentation	research international	all	500	UGENT
ANR Conference, Chiba, Japan, 21-24.05.2008	oral presentation	research international	all	500	UGENT
ANR Conference, Chiba, Japan, 21-24.05.2008	oral presentation	research international	all	500	UGENT
ANR Conference, Chiba, Japan, 21-24.05.2008	oral presentation	research international	all	500	UGENT
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	all EET-Pipeline partners
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	UKE, UGENT
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	UKE
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	UKE, DKFZ-Theoretical Bioinformatics
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	UKE, DKFZ-Tumour Genetikcs, DKFZ-Theoretical Bioinformatics
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	UKE, CEINGE
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	CEINGE
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	UGENT
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	UGENT
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	DKFZ-Tumour Genetics
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	DKFZ-Tumour Genetics
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	DKFZ-Tumour Genetics
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	UGENT, DKFZ-Tumour Genetics
ANR Conference, Chiba, Japan, 21-24.05.2008	poster presentation	research international	all	500	UGENT, UKE
Doctorate program Paediatric and Clinical Research Programme, 5.06.2008	Oral presentation	Italian research	Italy	<50	CEINGE
Biology and biotechnology. - Medicine and human health, San Diego, CA, USA 16-June-2008	Exhibition at Conference:	Industry / research international	North America	thousands	BIOFLAG
German Society for Paediatric Haematogy and Oncology June, 2008	oral presentation	medical and scientific oncologists	Germany	100	UKE
German Society for Paediatric Haematogy and Oncology June, 2008	oral presentation	medical and scientific oncologists	Germany	100	UKE
Functional and Structural Genome Research, 9. July 2008	conference	research national	Germany	60	DKFZ-Theoretical Bioinformatics
AACR Conference on Metastasis Vancouver, Canada 2.8-2008	Poster presentation	research international	all	Hundreds	CEINGE
09.08.2008	Oral presentation	Research staff and faculty of Jozef Stefan International	Slovenia	50	JSI

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
		Postgraduate School			
9th International Conference on Systems Biology, Gothenburg, Sweden, 25.08.2008	Poster presentation	research international	all	>100	JSI
The 2nd International Workshop on Machine Learning in Systems Biology (MLSB), Brussels, Belgium, 13-14.09.2008	Poster presentation	research European	Europe	80	JSI
The Second International Workshop on Machine Learning in Systems Biology, 13-14 September 2008, Brussels, Belgium	Conference / Publication	research international	all	Thousands	JSI
The 16th Euroconference on Apoptosis and 5th Swiss Apoptosis Meeting, Bern, Switzerland, 6-9.09.2008	Poster presentation	Research/Industry/ Higher education	Europe	1000	UZH
40th Congress of the International Society of Paediatric Oncology (SIOP), Berlin, Germany, 2-6.10.2008	oral presentation	research international	all	800	UKE
SIOP Meeting, Berlin, Germany, 2-6.10.2008	poster presentation	research international	all	800	UKE
Benelux qPCR symposium, Ghent, Belgium, 6 Oct 08	oral presentation	Research	international	300	UGENT
NCRI Cancer Conference, Birmingham, UK, 05-08.10.2008	oral presentation	Research	international	>500	CCRI
Public Forum: Understand Cancer - Save Lives, Vienna, 20.11.2008	Public Forum	High school students	Austria	300	CCRI
Bridging innovation and translation in pediatric oncology, Vienna, 21-22.11.2008	oral presentation	Research International	all	300	CCRI
Bridging innovation and translation in pediatric oncology, Vienna, 21-22.11.2008	poster presentation	Research International	all	300	CCRI
Bridging innovation and translation in pediatric oncology, Vienna, 21-22.11.2008	poster presentation	Research International	all	300	CCRI
Bridging innovation and translation in pediatric oncology, Vienna, 21-22.11.2008	oral presentation	International	all	300	UKE
Bridging innovation and translation in pediatric oncology, Vienna, 21-22.11.2008	poster presentation	International	all	300	UKE
Bridging innovation and translation in pediatric oncology, Vienna, 21-22.11.2008	oral presentation	International	all	300	CEINGE
Int J Cancer 122 (2008) 699-704.	publication	Research international	all	Thousands	UKE
BMC Genomics 2008 Jan 29;9:52.	publication	research international	all	Thousands	DKFZ-Tumour Genetics

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Klin Padiatr. 2008 May-Jun;220(3):137-46	publication	research international	all	Thousands	DKFZ-Tumour Genetics
Carcinogenesis 2008 Oct; 29(10):1869-77. Epub 2008 Jun 19	publication	research international	all	Thousands	DKFZ-Tumour Genetics
Oncogene 2008 Mar 27; 27(14):2035-44. Epub 2007 Oct 8.	publication	research international	all	Thousands	CURIE
02/2008 (TechNotes AB, Vol 15, Number 1)	Publication	Research	All	Thousands	UGENT
Cancer Lett 2008 Sep 28; 269(1):111-6. Epub 2008 Jun 13.	Publication	Research	All	Thousands	UGENT
BMC Genomics. 2008 Jan 29;9:52.	publication	research international	all	thousands	DKFZ-Tumour Genetics
Klin Padiatr. 2008 May-Jun;220(3):137-46.	publication	German-speaking research	Germany, Austria, Switzerland	thousands	DKFZ-Tumour Genetics
Carcinogenesis. 2008 Oct;29(10):1869-77. Epub 2008 Jun 19.	publication	research international	all	thousands	DKFZ-Tumour Genetics
Oncogene. 2008 May 22;27(23):3329-38. Epub 2007 Dec 17.	publication	research international	all	thousands	DKFZ-Tumour Genetics, DKFZ-Theoretical Bioinformatics, UKE
Clin Cancer Res. 2008 Oct 15;14(20):6590-601.	publication	research international	all	thousands	DKFZ-Tumour Genetics, DKFZ-Theoretical Bioinformatics
Genome Biol. 2008 Oct 13;9(10):R150.	publication	research international	all	thousands	DKFZ-Tumour Genetics, DKFZ-Theoretical Bioinformatics, UGENT
01.12.2008 Encylopedia of Cancer, 2nd Edition, Springer	publication	research international	all	thousands	DKFZ-Tumour Genetics
01.12.2008 Encylopedia of Cancer, 2nd Edition, Springer	publication	research international	all	thousands	DKFZ-Tumour Genetics
Genome Biol. 2008 Oct13;9(10):R150.	Publication	research international	all	Several thousand	DKFZ-Tumour Genetics, DKFZ-Theoretical Bioinformatics
Cancer Res. 2008 Sep 1;68(17):7100-9.	Publication	research international	all	Several thousand	CCRI
Nucleic Acids Res. 2008 Dec;36(21):e143. Epub 2008 Oct 21.	Publication	Research	international	thousands	UGENT
The 3rd Internation Workshop on Machine learning in Systems Biology, Ljubljana, Slovenia, 5-6.09.2009	Poster prenentation	MLSB 2009, Workshop, Ljubljana, Slovenia, research audience	all	80	JSI
The 3rd Internation Workshop on Machine learning in Systems Biology, Ljubljana, Slovenia, 5-6.09.2009	Oral presentation	MLSB 2009, Workshop, Ljubljana, Slovenia, research audience	all	80	JSI
Symposium on Targets for Cancer Prevention and Therapy, Zurich, Switzerland, Feb 2009	Poster presentation	Research/Industry/ Higher education	all	1000	UZH
The American Association of Cancer Research, 100th annual meeting April 2009, Denver (CO)	Poster presentation	Research/Industry/ Higher education	all	20'000	UZH
Invited talk at Sheba Medical Center, Israel, 01.01.2009	oral presentation	Research International	all	40	CCRI
Beshg conference, Brussels, Belgium, 13 Feb 2009	poster presentation				UGENT

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Beshg conference, Brussels, Belgium, 13 Feb 2009	poster presentation				UGENT
conference - miRNAs in human disease and development (Boston, USA), Mrz 09	oral presentation	Research	international		UGENT
conference - MC-Gard Meeting (Edinburgh, Schotland), 1-5 April 09	poster presentation				UGENT
18-22/04/09, 100th AACR Annual Meeting, Denver, USA	poster presentation	Research International	all	>500	CCRI
18-22/04/09, 100th AACR Annual Meeting, Denver, USA	poster presentation	Research International	all	>500	CCRI
18-22/04/09, 100th AACR Annual Meeting, Denver, USA	poster presentation	Research International	all	>500	CCRI
Invited talk at SIOPE-hosted discussion to form a network of excellence for paediatric ongology, Brussels, Belgium, 29.04.2009	oral presentation	research european	Europe	30	UKE
workshop - CNIO integrative genomics 2009 (Madrid, Spain), July 09	poster presentation				UGENT
A novel and universal method for microRNA RT-qPCR data normalization, Jun 09	press release	Research	international	thousands	UGENT
41st Annual Meeting of the International Society of Pediatric Oncology (SIOP) Meeting, Sau Paulo, Brazil, 4-9.10.2009	Oral presentation	Research international	All	Hundreds	UKE, UGENT
SIOP Meeting, Sau Paulo, Brazil, 4-9.10.2009	Oral presentation,	Research international	All	Hundreds	UKE, UGENT
SIOP Meeting, Sau Paulo, Brazil, 4-9.10.2009	oral presentation	Research international	international	Hundreds	UGENT
SIOP Meeting, Sau Paulo, Brazil, 4-9.10.2009	conference	International	Brasil	Hundreds	CEINGE
SIOP Meeting, Sau Paulo, Brazil, 4-9.10.2009	oral presentation	Research International	all	>500	CCRI
NGFNplus Meeting, Berlin 26 – 28.11.2009	Poster presentation,	German research	Germany	100	UKE, UGENT
NGFNplus Meeting, Berlin 26 – 28.11.2009	Poster presentation,	German research	Germany	100	UKE, UGENT
TGiN Meeting 3, NIH, Bethesda, MD, 12-13.11.2009	oral presentation	research international	international	<100	UKE
TGiN Meeting 3, NIH, Bethesda, MD, 12-13.11.2009	oral presentation	research international	international	<100	UGENT
Mol. BioSyst, 2010, DOI: 10.1039/b913690h	Publication	research international	all	Thousands	JSI
Machine learning in systems biology: proceedings of the Third International Workshop (2009) ISBN 978-952-10-5699-4. p. 115-124	Publication	research international	all	Thousands	JSI
Current Cancer Drug Target 2009 Mar;9(2):176-88.	Publication	research international	all	thousands	UZH
Cancer Lett. 2009 Nov 18;285(1):99-107. Epub 2009 Jun 3.	publication	research international	all	thousands	DKFZ-Tumour Genetics
Oncogene 2009 May 14;28(19):2015-23. Epub 2009 Apr 13.	Publication	research international	All	Thousands	UKE, CEINGE

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Int J Cancer 2009 May 15;124(10):2488-94.	Publication	research international	All	Thousands	UKE
Cancer Cell 2009 Jan 6;15(1):67-78	Publication	research international	All	Thousands	UKE
Cancer Lett 2009 Feb 8;274(1):10-5. Epub 2008 Jul 17	Publication	research international	All	Thousands	UKE
Cancer Lett. 2009 Sep 8;282(1):55-62. Epub 2009 Apr 5	Publication	research international	All	Thousands	UKE
PLoS One. 2009;4(4):e5415. Epub 2009 Apr 30	Publication	research international	All	Thousands	CCRI
PLoS One 2009;4(3):e4998. Epub 2009 Mar 24. 2009	Publications	Research	All	Thousands	CEINGE, CURIE
Genome Biol. 2009;10(6):R64. Epub 2009 Jun 16.	publication	research international	all	thousands	DKFZ-Tumour Genetcs, UGENT
Lancet Oncol 2009 Jul;10(7):663-71. Epub 2009 Jun 8	publication	research international	all	thousands	UGENT, CURIE
Future Oncol. 2009 Jun;5(5):625-39.	publication	research international	all	thousands	DKFZ-Tumour Genetics
Clin Cancer Res. 2009 Jan 1;15(1):91-9.	publication	research international	all	thousands	DKFZ-Tumour Genetics
Clin Cancer Res. 2009 Mar 15;15(6):2085-90. Epub 2009 Mar 10.	publication	research international	all	thousands	DKFZ-Tumour Genetics
Oncogene. 2009 Nov 30. [Epub ahead of print]	publication	research international	all	thousands	DKFZ-Tumour Genetics, UGENT, UKE
Neuron. 2009 Sep 10;63(5):585-91.	Publication	research international	all	Several thousand	DKFZ-Theoretical Bioinformatics
Oncogene. 2009 Nov 9. [Epub ahead of print].	Publication	research international	all	Several thousand	DKFZ-Tumour Genetics, DKFZ-Theoretical Bioinformatics
Molecular targeted therapies for childhood cancer, Günzburg a.d. Donau, Germany, 28-30.01.2010	oral presentation	Research International	all	50	CCRI
AACR Cell Death Mechanism and Cancer Therapy, San Diego (CA), 1-4.02.2010	Poster presentation	Research International	all	thousands	UZH, UKE, UGENT, CEINGE, DKFZ-Tumour Genetics
Beshg conference, Ghent, Belgium, 26 Feb 2010	Poster presentation	Research national	Belgium	300	UGENT
BIO 2010 Conference, Chicago, IL, 03 May 2010	Exhibit	Industry / research international	all	thousands	BIOFLAG
101st Annual AACR Meeting, Washington D.C., USA, 18-22.04.2010	Poster presentation	Research international	All	Thousands	UKE
101st Annual AACR Meeting, Washington D.C., USA, 18-22.04.2010	poster presentation	Research International	all	>500	CCRI
101st Annual AACR Meeting, Washington D.C., USA, 18-22.04.2010	poster presentation	Research International	all	>500	CCRI
101st Annual AACR Meeting, Washington D.C., USA, 18-22.04.2010	poster presentation	Research International	all	>500	CCRI
Bull Cancer 2010 Jan;97(1):37-45.	Publication	Research international	all	thousands	CURIE
Molecular Cancer Therapeutics 2010 Jan;9(1):167-79.	Publication	research international	all	thousands	UZH

Planned/actual dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
In revision, Cancer Res (2010)	Publication	Research International	all	thousands	CCRI
Submitted, Nucleic Acids Research	publication	research international	All	Thousands	UKE, UGENT
Submitted, Molecular Cancer	Publication	research international	All	Thousands	UKE, UGENT
submitted Jan 2010, BMC Genomics	Publication	Research international	all	thousands	UGENT
Department of Knowledge Technologies, Jožef Stefan Institute, Technical Report, Ljubljana, Slovenia, 2010.	Publication	research international	all	Thousands	JSI
Department of Knowledge Technologies, Jožef Stefan Institute, Technical Report, Ljubljana, Slovenia, 2010.	Publication	research international	all	Thousands	JSI

Section 3 - Publishable results

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