

EACR Travel Fellowship Awards

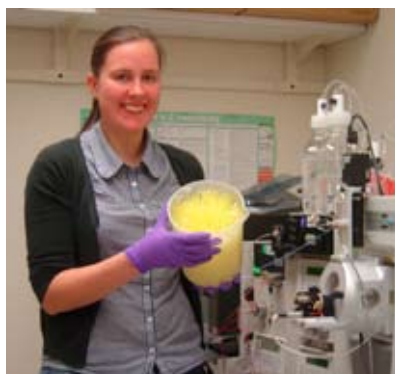
Travel Fellowships co-sponsored by EACR and AICR are awarded to 'Young' and 'Early Career' Cancer Researchers to provide additional support for their specialist research work in an appropriate centre of excellence

An Hendrix



Home Institute: Department of Medical Oncology, University Gent, Gent, Belgium

Host Institute: Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH, Bethesda, Maryland, USA



An Hendrix

I'm a second year PhD student at the Department of Medical Oncology, University of Gent, Belgium. My PhD project focuses on the role of Rab GTPases in cancer. Rabs are members of the Ras-like small GTPase superfamily of proteins and constitute the largest branch of this family with over 60 members.

Rabs are initially synthesized as soluble proteins; for membrane attachment they require a posttranslational C20-prenylation. Rab GTPases are localized to the cytosolic face of the limiting membrane of organelles, where they function as regulators of distinct steps in membrane trafficking pathways. Rabs cycle between a GTP-bound active and a GDP-

bound inactive form, assisted by GEFs (guanine nucleotide-exchange factors) and GAPs (GTPase-activating proteins) respectively. GTP-bound Rabs recruit specific effector proteins through which they regulate vesicle formation, actin- and tubulin-dependent vesicle movement and membrane fusion and fission.

We investigate the role of Rab GTPases in cancer development and progression. Our laboratory is specialized in different model systems that can be used to study invasion of cancer cells into different matrices like collagen type I and Matrigel. Using these model systems we have shown that both transient and stable Rab27 overexpression stimulate invasion of MCF-7 breast cancer cells in vitro.

The laboratory of Dr. Gahl is experienced in the study of Rab GTPases and vesicle trafficking. In a previously EACR-supported visit of Dr. Olivier De Wever to Dr. Gahl's lab, we were able to establish an interdisciplinary collaboration. By performing mutagenesis, we created different mutant forms of Rab27: two dominant negative mutants (defects in binding GTP), a constitutive active mutant (defects in GTP hydrolysis) and a geranylgeranylation mutant (mutation of COOH-terminal prenylation motif results in inappropriate vesicle membrane attachment). Studying the effect of these different Rab27 mutants in our invasion models revealed that Rab27 is only able to induce invasion of MCF-7 in a GDP/GTP dependent and geranylgeranylation dependent way.

The goals of the visit were to identify interaction partners of

Rab27 in MCF-7 and to characterize the Rab27 vesicle. Realization of these goals at the laboratory of Dr. Gahl was an excellent opportunity because the host department possesses all the necessary equipment to conduct comprehensive research: mass spectrometry, confocal microscopy and electron microscopy in particular.

To identify the different interaction partners of Rab27 in MCF-7 we performed immunoprecipitations. Since Rab27 is GFP linked we therefore used an anti-GFP antibody. Co-immunoprecipitated proteins were separated via SDS-PAGE, followed by Coomassie Blue stain. All the different protein bands were analyzed via mass spectrometry.



An Hendrix with Dawn Maynard

Setup and execution of these experiments happened in close collaboration with Dr. Dawn Maynard, the staff scientist who runs the proteomics facility in the host institute. We were able to identify different effector and motor proteins that are involved in the trafficking of the Rab27 vesicle. To confirm these results we currently perform confocal microscopy to study co-

localization between the Rab27 and the possible interaction partners. The endosomal system comprises many different specialized compartments and organelles (early endosomes, sorting endosomes, late endosomes, lysosomes, multivesicular bodies, ...). The presence of specific Rab proteins on specific endosomal compartments has been described in great detail. The localization of Rab27 has been described for specialized cells with lysosome related organelles (LRO's like platelets, melanocytes and mast cells). Dr. Wendy Westbroek is a research fellow in the Gahl lab who runs the immune-electron microscopy facility and whose expertise includes LRO's and other endosomal compartments. She introduced me to immune-electron microscopy to characterize the Rab27 vesicles in MCF-7 breast cancer cells.

I would like to express my gratitude to the European Association of Cancer research for this fellowship which represented a great professional experience to advance my knowledge and to get practical skills in mass spectrometry and immune-electron and confocal microscopy. Furthermore, EACR allowed us to continue the successful collaboration between the home and the host institute.



An Hedrix and William Gahl

I also thank Dr. Gahl and his collaborators for the friendly atmosphere in the laboratory and their assistance in the achievement of our goals.

Patryk Kambakamba



Home: Ruprecht-Karls-Universität Heidelberg

Host: IFOM MILAN
November 2008



Patryk Kambakamba & Francesco Blasi

There is rising evidence that HOX proteins and its co-operation partners play a role in the development of cancer in a variety of malignancies such as esophageal cancer, prostate cancer, breast cancer and cancer of the bladder. PREP1, a protein of 64kDa, is a member of the TALE subfamily of MEINOX proteins and represents a homeodomain encoded protein. Several publications indicate a crucial role for PREP1 in embryonic development. Down regulation of PREP1 in zebra fish brings a lethal phenotype with major homeotic defects. Additionally PREP1 and other UPA-enhancer elements are known to influence the cooperation of the PEA3 and AP1 binding sites in the UPA-R promotor. Whereas the role of the plasminogen activator system in carcinogenesis has been studied thoroughly, there is not much known about the expression of PREP1 in human solid cancer entities. Therefore our work attempts to investigate the appearance of PREP1 in human colorectal cancer.

For our study we, the department of experimental surgery Mannheim (head of department, Prof. Heike Allgayer) were supplied with colorectal cancer samples from the surgical institute at the University Hospital Mannheim. We first checked our tissue samples by immunohistochemistry using the

Avidin Biotin method for staining paraffin embedded samples. The slides have been evaluated by microscopical analysis. PREP1 has been found in the cytoplasm and in the nucleus. We further started to evaluate our slides semiquantitatively by estimating the intensity of staining and the number of stained cells for each slide. Statistics comparing the calculated values with established tumour markers will be performed with the aid of the statistical program SPSS13 (data not ready to present yet).

In order to confirm our findings we have consulted an experienced pathologist. We tried to reproduce our immunohistochemical findings in Western Blot. Therefore we investigated separately both cell compartments for the presence of PREP1.

The extraction of cytoplasmic and nuclear fraction has been performed under freezing conditions and the extracts have been aliquoted and stored at -80°C. For the detection of PREP1 by Western Blot we have used a murine monoclonal antibody produced and used by Prof. Blasi's team in Milan. This antibody recognizes the full length of human PREP1. Referring to previous publication of Prof. Blasi's working group we used HeLa cells nuclear extracts as our loading control. This cell line is well known to deliver a strong signal for PREP1 in Western blot. Nevertheless, we have not yet managed to establish the procedure for our colorectal samples, which might be due to low expression of the protein in adult human tissue.

As well, in EMSA, using an oligo which represents a transcription factor binding site on the upa-r promotor, we have seen only narrow or no bands as compared to HeLa nuclear extracts. Of course we are still trying to modify the protocol.

Further we extracted RNA using the trizol /chloroform technique. Additionally we purified our RNA-samples with the commercial cleanup Kit. Subsequently we quantified the concentration with

a nanodrop. Finally the RNA was defined as pure when the 260/280 ratio surmounted 1,8. In addition we loaded 1-2ug of our RNA-samples on a 0,5% Agarose Gel screening for the 18S and 28S subunits. Intact total RNA running on an denaturing gel gave two sharp bands with an approximate ratio of intensity 2:1 comparing the 28s unit to the 18s unit. After having exactly checked the RNA, the following idea was to amplify PREP1 by PCR. Hence we retrotranscribed the RNA into cDNA and performed an amplifying PCR. We used primers for human PREP1. An experimental attempt of the retrotranscription procedure, lacking the enzyme reverse transcriptase, functioned as a negative control in the PCR.

Although we have loaded each PCR attempt with an equal amount of cDNA we could see differences in the signal we obtained by the amplified product. This leads to the suggestion that PREP1 is expressed at variable levels in our patient collective. Now that we have achieved the extraction of a qualitative total RNA and have transcribed it into cDNA the next step will be the exact quantification of the amplified DNA fragment by qPCR in our samples. This will give a more objective evaluation of PREP1 levels in our colorectal samples as compared to our semiquantitative immunohistochemical estimation. Nevertheless we are aware that a strict correlation between both measuring methods is not mandatory, as Immunohistochemistry is giving an idea of the protein's expression and the RNA level of PREP1 is not considering any posttranslational modifications of the encoded product. Of course you will be supplied with further results at the end of my staying at IFOM.

I once more want to thank the EAC committee for supporting our project; Prof. Heike Allgayer and Prof. Francesco Blasi for giving me this opportunity to gather insights at the renowned institute and co-operate with experienced scientists.

Gilli Galore-Haskel



Home Institute - Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Host Institute - Basic Medical Sciences Division, St George's University of London, UK



Gilli Galore-Haskel

The telomerase complex, which is composed of two major subunits – hTR and hTERT, is involved in the extension and maintenance of the telomeres. Activation of this enzyme is therefore required for cells to overcome replicative senescence and obtain the ability to divide without limit [1].

Telomerase activation is observed in almost 90% of human cancers, but not in normal tissues of somatic origin and thus is believed to be a critical step for immortalization of cells and carcinogenesis. Consistent with these observations, high telomerase activity is shown in the majority of primary and metastatic malignant melanomas, the most lethal type of all skin cancers, while its common precursor lesions (i.e., dysplastic and benign melanocytic nevi) show weak or no telomerase activity at all [2].

The activation of telomerase during melanoma development is not yet understood. Further understanding may have value for therapy. Deducing from other types of cancer, one suggestion is that telomerase subunits are up-regulated by transcription factors, which bind to the promoters and activate the transcription of the subunits.

Thanks to the EACR Travel Fellowship, I worked in Prof. Bennett's lab at St. George's, University of London. Prof. Bennett is a leading scientist in the field of melanocyte molecular biology and melanoma development. Her vast knowledge in melanoma cell biology, excellent lab facilities and large melanocytic lesions cell bank allowed me to study the transcriptional regulation of telomerase in melanoma development. I investigated the correlation between the expression of MYC, a transcription factor suspected in the regulation of telomerase subunits, and the expression of hTR and hTERT in different types of normal melanocytes and melanoma cell lines. During my stay in the lab, I got the opportunity to practice cell culture techniques and acquired new skills for growing a variety of melanocytic cell lines. I have learned how to extract RNA from the different cell lines and used RT-PCR to check the expression levels of the different genes. In order to obtain more accurate results, I gained knowledge in designing, optimizing and analysing quantitative real-time PCR assays.



David Kallenberg, Anita Amadi-Myers, Prof Dorothy Bennett, Gilli Galore-Haskel, Dr Rebecca Collinson, Dr Elena Sviderskaya.

The preliminary results from this project are intriguing and will be used as a basis to continue the study of telomerase transcriptional regulation in melanoma. In addition, taking part in the lab meetings exposed me to other melanoma related research and allowed me to further learn about cell senescence and the molecular genetics of melanoma.



Gilli Galore-Haskel

I would like to express my gratitude to the EACR for this fellowship and for giving me the opportunity to gain experience and acquire new skills which I will implement at my home institute. I truly hope that my stay at Prof. Bennett's lab will lead to a future collaboration. I would also like to thank Prof. Dorothy Bennett and the rest of the team for their support and assistance throughout my stay.

*References: Counter CM et al. (1992) EMBO J. 11:1921-1929
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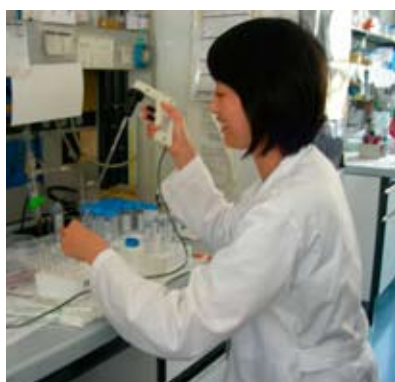
Xiaoyan Huang



Home Institute - Cancer Hospital, Fudan University, Shanghai, China

Host Institute - Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

1 June 2008 to 30 June 2008



Xiaoyan Huang

I am the resident of the breast department in the Cancer Hospital, Fudan University. My major is oncology, especially for breast carcinoma.

The incidence of breast cancer in Shanghai has increased sharply during the past few years, probably due to rapid changes in the social and environmental factors that cause breast cancer.

The Italian and Chinese populations are different for genetics and risk factors. Our Institution and Fondazione IRCCS Istituto Nazionale Tumori (INT) are collaborating to create a database containing clinical, pathologic and biologic data of breast cancer treated in the years 2005-2007 and to analyze by microarray a cohort of Chinese and Italian breast cancer samples. The final aim is to identify similarities and differences useful for management of therapies and prevention in the two countries.

Firstly, we collect the slices of immunochemistry of ER, PR, Her2/neu of the breast carcinoma from Cancer Hospital Fudan University, and blinded to the pathologists of INT. I have worked in collaboration with the Pathology Department of INT to standardize the immunohistochemical data. More than 100 slices of breast tumours analyzed for ER, PR, and Her2/neu status from Italian and Chinese hospital were compared and the concordance of results was 80%-91%.

I worked in the data integration of the breast cancer Italian/Chinese database. Clinical and biological parameters were defined. Possible variations in data sources, collection methods, data management, and quality control between the Italian and Chinese Institutions were analyzed and defined. We have finished the database from 2005-2006, the year 2007 is under integration. A web database is under construction, so that the investigators across two sites can view the information held by others and modify their own. The clinical, pathologic and biologic data will be shared.

I have finished collecting the fresh samples of 100 cases of Chinese breast carcinoma in series from

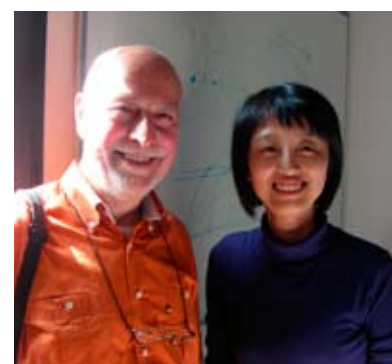
June to December 2007, including 34 cases of basal group, 64 cases of luminal A, one case of ER-/Her2+ and one luminal B case. After the hard work of shipping, the 100 cases arrived in Milan successfully. We agreed with the Italian statisticians and oncologists to select the Italian Cohort from the 2007 breast cancer samples of INT tissue bank. As of now, mRNA extraction is in progress and profiling will be performed on the Illumina platform of INT.



Xiaoyan Huang and Rosaria Orlandi

I discussed with the Italian oncologists the surgical and pharmacological management of breast cancer patients and I had the chance to visit the surgical room and statistic center of INT.

The fellows in the Fondazione IRCCS Istituto Nazionale dei Tumori are so nice, they taught me a lot, patiently, step by step. It greatly advanced my knowledge and practical skills.



Xiaoyan Huang and Romano Demicheli

I would like to thank the European Association of Cancer Research for the financial support of my travel to Milan to take part in the establishing cooperation between the two institutions, helping us to do further collaborative research in the future.

Daniel White



Home: University of Manchester,
Manchester
UK

Host: Erasmus MC
Rotterdam,
The Netherlands



Dr Stefan Meyer (visiting from Manchester) and Dan White.

Our group at the Stem Cell and Leukaemia Proteomics Lab at the University of Manchester is focused on understanding myelodysplasia (MDS) and acute myeloid leukaemia (AML) arising during childhood. The risk of developing paediatric AML is greatly increased in patients with the inherited syndrome Fanconi Anemia (FA) and FA could potentially be a paradigm to study MDS and investigate the progression to AML [1]. Currently, the mechanisms that drive disease progression to MDS and AML in FA are incompletely understood. On the one hand, mutations in the FA genes cause genomic instability that, in part, contributes to malignant transformation. On the other hand, the high risk of developing AML opposed to other malignancies suggests that the FANCD1/BRCA2 pathway has additional roles in protecting against malignant transformation during haematopoiesis.

We have been able to establish two unique AML cell lines from a patient with FA arising from biallelic BRCA2 mutations and have used them to identify acquired genetic changes that drive malignant transformation

[2]. This analysis has confirmed the importance of gains of chromosomal material and amplification of the long arm of chromosome three in disease progression and revealed that this abnormality leads to extremely high expression of the *EV11* gene [3]. *EV11* encodes a DNA-binding oncogenic transcription factor, which, when overexpressed in AML confers very poor prognosis. Currently the mechanism by which *EV11* drives progression to MDS and leukaemic transformation is poorly understood [4]. To further investigate how *EV11* functions, we carried out an in-depth investigation of the endogenous *EV11* protein by mass spectrometry. This analysis provided evidence of previously unreported post-translational modifications.

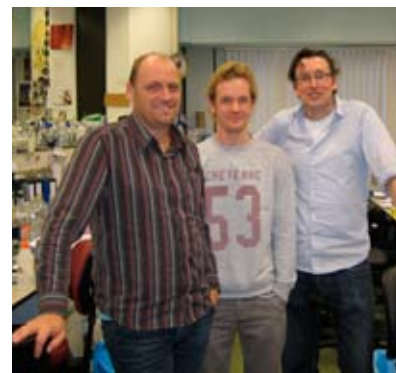
The EACR travel fellowship was used to fund a seven week visit to Ruud Delwel's laboratory at the Erasmus MC in Rotterdam. Dr Delwel's group has carried out pioneering research into AML and in particular the biology of *EV11* in AML in a clinical context and identified *EV11* as an important prognostic marker that confers extremely poor prognosis in AML. His lab has expertise in many assays used to investigate this protein.

During my visit I aimed to learn how to carry out two of these assays in order to use them for studying the novel protein modifications identified in our lab. In the first assay *EV11* is expressed in Rat fibroblast. This leads to anchorage independent growth, an indication of cell transformation. In the second assay, a portion of the *EV11* protein is fused to GST and the ability of the fusion protein to interact with DNA and other proteins is assessed.

Whilst in Rotterdam I gained experience in performing these assays and successfully generated constructs that express the modified *EV11* protein. I was able to purify the GST/*EV11* fusion proteins and assess the effect of the protein modification on its function. This generated some exciting results that suggest that post-translational modification of *EV11* may regulate its

function as a transcription factor.

I would like to thank the EACR for funding my visit to Rotterdam, where I learned very useful assays and techniques. I would like to thank Dr Delwel, all the members of his laboratory and everyone else at in the Erasmus MC Hematology department for making my time there so enjoyable.



Dr Ruud Delwel, Dan White and Dr Eric Bindels.

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AICR
Cancer knows no boundaries.
Fortunately, neither do we.

Kirill Kirsanov



Home institution: Blokhin Cancer Research Center RAMS, Moscow, Russia.

Host institution: Institute of Entomology, Biology Centre Czech Acad. Sci, Czech Republic.



Kirill Kirsanov

The development of human cancer is a multi-step process, involving the cooperation of mutations affecting mitogenic signaling, cell-cycle and cell-death pathways, as well as interactions between the tumour and its microenvironment. The effect of a mutation often depends on the context of other mutations within the same cell. To dissect the particular steps of tumourigenesis, simple animal models are needed. One of the best model organisms that have been widely studied is *Drosophila*.

New original short-term tests, based on a recessive mutation in the tumour suppressor gene *warts*, were created in the laboratory of Carcinogen screening methods (Primary investigator, Professor Belitsky). This gene has close homologues in mice (*LATS1*) and in humans (*h-lats*). Mutations in homologous genes are intimately bound with soft tissue sarcomas in mice and ovarian tumours in humans. Before finding the *wts* mutation it was impossible to induce tumour in an adult fly. As the *Drosophila warts* gene encodes a cyclin dependent kinase regulator, its mutation produces alterations in a cell cycle machinery. In the *wts* / + heterozygotes the *wts*

homozygous clones induced by somatic recombination divide more rapidly than the surrounding heterozygous tissue and give rise to tumour outgrowths easy to register in imagoes. The last feature of *wts* is notable among *Drosophila tumour* suppressors as most of them fail to develop homozygous clones in a heterozygous background mainly due to apoptosis and cell competition.

The main goal of our new assay is registration of the blastomogenic effect of xenobiotics through direct observation of tumours instead of rough estimation of somatic mutation frequency for non-tumorous markers in previous tests.

Prior to visiting the Institute of Entomology we have developed modifications of our testing system enabling us to induce *wts* tumours in a *p53*-deficient background using the *Drosophila p53^{259H.GUS}* dominant mutation. We consider this modification very useful as it increases sensitivity of the test to the mutagens inducing apoptosis in a *p53*-dependent manner and also provides a possibility to differentiate potentially carcinogenic substances inducing apoptosis in either *p53*-dependent or *p53*-independent manner, such as between oxoplatin and benzo(a)pyrene.

During my visit to the Institute of Entomology I performed experiments to advance our fly model by making it more adequate to mammals and by simplifying the detection of tumour clones. For that purpose I studied methods of introduction of human *p53* (normal and mutant alleles) to *Drosophila wts* heterozygotes. To simplify the detection of tumour clones, to distinguish the clones with similar morphology as well as to distinguish them from morphoses, I was mastering the art of labelling tumour clones with a visible marker – the green fluorescent protein (GFP). We mastered also the techniques of microinjection in *Drosophila* embryo and generation of transgenic strains, using a semi-automate microinjector. These skills are of

great importance to Laboratory of Carcinogen screening methods as it has the equipment for microinjection. Also we recloned human *p53* with mutation of binding domain from the original pCMV vector into the pUAST *Drosophila* vector.

During the visit we also obtained the *wts*, the *p53*, the UAS-GFP heterozygotes for further mutagenizing and analysis, and mastered skills in dissections of the 3rd instar larvae imaginal discs. Now we perform experiments to induce tumorous clones in these strains.

All the investigations were performed in collaboration with Dr. M. Zurovec and Dr. R. Sidorov.

I would like to thank European Association for Cancer Research committee for the support of my project. It was a great pleasure to work in the laboratory of Dr. Zurovec. I would like to say thanks to all researchers of Dr Zurovec's laboratory for very useful discussions, interesting ideas, and help with the techniques. I hope our collaborative work will continue and remain fruitful in the future. And we are very grateful to the Russian Academy of Medical Science for assistance through collaboration with the Czech Academy of Science.



Michael Zurovec and Kirill Kirsanov

Sebastian Carotta



Home Institute: The Walter & Eliza Hall Institute of Medical Research
Melbourne, Australia

Host Institute: "Research Institute of Molecular Pathology" and "Center for Integrative Bioinformatics Vienna", Vienna, Austria



Sebastian Carotta and Ricardo de Matos Simoes

"PU.1 and IRF-8 act together as tumour suppressors in B lymphocytes"

I am interested in how certain transcription factors direct lineage specification from immature, uncommitted cells and how mis-regulated expression of these transcription factors can lead to the development tumour formation.

The transcription factors PU.1 and IRF-8 are essential regulators of haemopoiesis. Both are expressed in early multipotent haemopoietic progenitors as well as in myeloid and lymphoid committed precursors. The deletion of either PU.1 or IRF-8 in adult haemopoiesis results in the expansion of granulocytes and development of myeloid leukaemia.

While the role of PU.1 and IRF-8 as tumour suppressors is well established in myeloid lineage, it is currently not known whether they also function as tumour suppressors in other lineages, such as lymphocytes. We tested this for the B cell lineage by inactivating PU.1

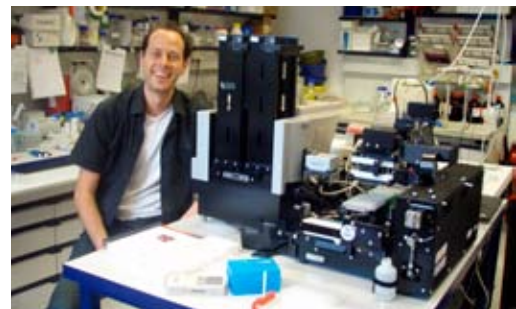
and IRF-8 in B-lymphocytes: By 6-months most PU.1/IRF-8-deficient mice developed a B cell leukaemia characterised by enlarged spleen and axial lymph nodes. Leukaemic cells could also be found in bone marrow, peritoneal cavity and peripheral blood. PU.1 and IRF-8 act as classical tumour suppressor genes in the B cell lineage as the re-introduction of either gene by retroviral expression inhibited the formation of leukemia in secondary recipients.

The major aim of this project now is to identify the mechanisms by which PU.1 and IRF-8 regulate B cell development and prevent B cell leukaemia formation. To do so, we were interested to compare the gene expression of control leukemic cells over-expressing with GFP with leukaemic cells over-expressing either IRF-8 or PU.1 using microarray technology.

Microarray experiments generate an enormous amount of data points, which are impossible to analyse properly without expert statistical knowledge. With the support of the EACR Fellowship I was able to travel to Vienna to visit Dr. Peter Steinlein at the "Research Institute of Molecular Pathology (IMP)" and Prof. Arndt von Haeseler "Center of Integrative Bioinformatics Vienna (CIBIV)" and attend their tutorial "Statistical and bioinformatical analysis of microarray data". During the 10-day course I gained knowledge about microarray experiments, R-programming and the basics of statistical analysis of microarray data. With the expert help of Ricardo de Matos Simoes, a PhD student in Prof. Haeseler's group, we analysed which genes are differentially expressed in the presence of the tumour suppressors PU.1 or IRF-8 and generated a list of interesting target genes.

Being back in Melbourne again, the next step is now to validate these target genes in an independent experimental setting and to test if modulation of their activity can lead to the suppression of the leukaemia. It has been a great opportunity to

spend two weeks at the impressive Biocenter Cluster in Vienna that



Sebastian Carotta

consist of the IMP, CIBIV and IMBA and to present and discuss my data there. We will also continue to collaborate together to maximize the output of the generated data.

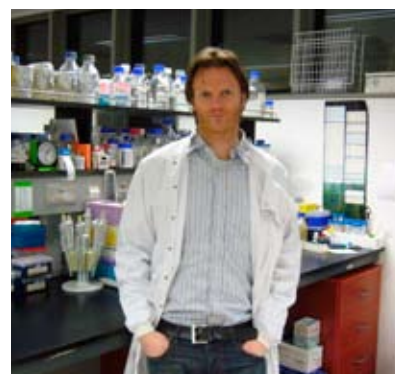
I am very grateful to the European Association for Cancer Research for this great opportunity. Thanks a lot!

Darran O'Connor



Home Institution: Conway Institute, University College Dublin, Dublin, Ireland.

Host Institution: NKI-AVL, Amsterdam, The Netherlands.



Darran O'Connor

Functional Genomic Interrogation of DUB Regulation of ER Transcriptional Activity

Breast cancer presents great emotional, social, economical and clinical problems for Europe, and there is a crucial need to develop more targeted therapies for the disease. At present, treatment relies on surgery combined with

adjuvant radiotherapy and systemic medical therapy, including both chemotherapy and endocrine therapy. The use of anti-estrogens (most commonly, tamoxifen) arose from a specific understanding of the role estrogen plays in the development of the mammary gland and the promotion of breast cancer growth. However, anti-estrogen resistance has since emerged as a significant problem in the management of breast cancer, and further understanding of the mechanisms of hormone-dependent breast cancer progression hold the key to the development of the next generation of therapeutics. Before commencing my research visit, a genome-wide RNAi screen for mediators of tamoxifen resistance was proposed. However, a prior attempt to carry out the screen implicated the deubiquitinating enzyme family (DUBs) as playing a key role in controlling the growth of hormone-responsive breast cancers. Therefore, rather than simply repeating the previous screen, it was felt that it would be more beneficial to focus on a targeted approach in the hope of further advancing this theme.

Regulation of transcription via posttranslational protein modification with ubiquitin and ubiquitin-like moieties has recently emerged as a key mechanism controlling gene expression. While there are numerous links between receptor turnover and the transcriptional activity of ER, the precise role of specific ubiquitination events, and the enzymes involved in catalyzing the process remain unidentified. Here, we adapted the NKI genome-wide shRNA library to investigate the role of DUBs in regulating the transcriptional activity of ER. Four shRNA constructs targeting all known DUBs were assembled into a knockdown panel comprising 108 individual genes (432 shRNA hairpins in total). shRNA hairpins for each gene were pooled and used for an arrayed screen to measure effects of DUB knockdown on ER activity. Estrogen-sensitive ZR-75-1 were plated in 12 well plates and the following day transfected with

an estrogen-responsive element (ERE)-luciferase reporter construct, plus a CMV renilla luciferase construct to balance transfection efficiencies, and pooled shRNA hairpins for an individual DUB gene. The following day, transfected cells were switched to hormone-stripped, phenol red-free media, and two days later stimulated overnight with estradiol. The following day cells were washed, harvested, and ER transcriptional activity measured by firefly luciferase expression.

Following the initial screen, DUBs whose knockdown super-activated or repressed ER activity were further validated by repeating the screen in triplicate (where DUBs whose knockdown had no effect on ER activity served as controls), both in the presence and absence of estradiol. Knockdown of individual DUBs was then confirmed by qRT-PCR and finally, individual hairpins targeting each ER-modulating DUB were used to confirm the specificity of the phenotypes observed. The screen successfully identified a number of DUBs that when knocked-down by shRNA either potentiated ER-mediated transcription, or repressed it. Indeed, the top and bottom hits identified in the screen both target the same protein, and the protein itself has been previously implicated in the development of breast cancer. Therefore we believe that we have identified a novel mechanism of ER regulation which we are currently exploring further.

Overall I would rate my time spent at the NKI with Prof Bernards and his team as extremely fruitful and immensely stimulating. It was an absolute honour to work for a time with such a visionary scientist, and in addition to the successful work that I carried out there, exposure to the various other projects ongoing in the lab provided me with a well rounded view of the utility and power of functional genomics screens. Indeed, the reason I chose Prof Bernards and his group at the NKI as hosts for my visit was due to their pioneering work in the field over the last number of years.

I would also like to express my gratitude to all those involved in the EACR Travel Fellowships scheme for facilitating this research project. It was an extremely worthwhile and rewarding experience, which has allowed me to develop significantly as a scientist.

Jacqueline A Hall 

Home Institute: McGill Centre for Bioinformatics, Montreal, Quebec, Canada

Host Institution: DKFZ, Heidelberg, Germany



Jacqueline A Hall (right) and Anne Mai Wassermann

Although breast cancer is a common female malignancy, we still cannot accurately predict clinical outcome nor response to treatment in this disease. Many gene expression profiling studies have attempted to classify breast cancers into discrete subtypes with distinct molecular and clinical characteristics to aid the clinical management of patients. These studies recapitulated the three main types of clinically recognized breast cancer, estrogen receptor (ER) positive, HER2 positive and triple negative disease (ER, progesterone receptor (PR) and HER2 negative). The aim of deriving these subtype specific molecular signatures is to identify the different pathways activated in the different tumour types. Emerging evidence suggests the interaction between the epithelial derived cancer cells with the surrounding

breast stoma also contributes to this heterogeneity, but little is known about the respective contribution of the epithelium and the stroma.

During my visit to the Division of Theoretical Bioinformatics at the DKFZ I was able to explore and develop a novel method for the classification and description of the heterogeneity observed in breast cancer. I developed a new algorithm to order breast cancer patients by the degree of pathway activation. The major finding was that a transition in estrogen signalling occurs between estrogen receptor positive and HER2/neu positive tumours and that estrogen signalling and growth factor signalling are strongly inversely correlated. This transition was correlated with grade and clinical outcome in our dataset as well as 8 other datasets, demonstrating the transition has clinical relevance. In contrast, no such transition between ER positive and triple negative disease was observed. These results suggest two main types of breast cancer exist those that arise from a common precursor of ER and HER2 positive disease and a those arising from the precursor for triple negative disease. The remaining heterogeneity observed in breast cancer arises from the degree of activation of different pathways that can drive the growth of the tumour.

These results stimulated the development of a novel approach to deconstructing signalling pathways and processes using the idea of a transition between ER positive and HER2 positive disease. I applied this method to both gene expression profiling data from the tumour epithelium and expression data from the stroma.



Jacqueline A Hall

The ER to HER2 transition was strongly associated with numerous pathways implicated in breast cancer progression and aggressiveness, such as hypoxia, motility, adhesion molecule signalling, and genes associated with prognosis in breast cancer. Further development of this software could lead to a method for targeting therapy to the tumour type that would most benefit.

My internship at the DKFZ provided an excellent opportunity to combine a biological understanding with computational methods development, working with scientists of diverse training and backgrounds. This cross-disciplinary experience has been extremely valuable in my career development. I would like to thank the EACR their support for this unique opportunity and I look forward to applying this new found knowledge to my postdoctoral fellowship.

Roy
van der
Meel



Home Institute: University of Utrecht, Holland
Supervisor: Dr. Annette Byrne

Host institute: UCD Conway Institute, Dublin, Ireland



Roy van der Meel

Project background

Angiogenesis is the sprouting of new blood vessels from pre-existing ones. It is well accepted that angiogenesis is required for tumour progression. Rho GTPases are small proteins that function as a

molecular switch in the cell. These proteins regulate a broad array of cellular functions but are studied mostly as their role in regulating the actin cytoskeleton and cell migration. Recent literature indicates a role for the Rho GTPases in angiogenesis. We therefore proposed that Rho GTPases are a promising target of angiogenesis therapeutics. The aim of this project was to use siRNA mediated silencing to knockdown Rho GTPases in vitro and in vivo.

Host Institution

The UCD Conway Institute was chosen because of the already established collaboration between this institute and the Utrecht Institute of Pharmaceutical Sciences (UIPS). Last year, I performed an internship at UIPS. I'm a student of the masters program Drug Innovation and we are stimulated to perform an internship abroad. It was a very good chance for me to experience living abroad, and to gain research experience in an oncology group.

Achievements

The results obtained in this study showed that knockdown of the Rho GTPase Rac1 in endothelial cells resulted in disruption of tube formation, cellular migration and invasion. Furthermore, treatment of mice bearing B16 melanoma tumours resulted in a decrease in tumour growth when compared to mice treated with siRNA against luciferase. Also, knockdown of the Rho GTPases Cdc42 and RhoG in endothelial cells resulted in inhibition of tube formation and cellular migration. These results indicate various Rho GTPases as targets for interference with angiogenesis. The results obtained in this study were used to in a grant application for a follow-up study. Furthermore, a manuscript describing our results is in preparation in collaboration with UIPS.

Personal impact and home institute

This project has been very important to me regarding research experience as well as personal experiences. I was able to perform most of the experiments by myself and this experience will benefit



Roy van der Meel, Back row, 2nd left

me without a doubt when I start a PhD next year. This successful project has strengthened the already established collaboration between the UCD Conway Institute and UIPS. The two groups have different backgrounds but can supplement each other. At UIPS, the focus is on drug target and delivery, the group in the UCD Conway Institute focuses on oncology. A great example of our collaboration was the pilot study in vivo that was performed for this project. This study was performed at UIPS because of their experience with drug delivery. I'm sure this successful project will give more students chances to perform an internship at the UCD Conway Institute.

Tim Waterboer



Home: German Cancer Research Center (DKFZ), Heidelberg, Germany

Host: Queensland Institute of Medical Research (QIMR) in Brisbane (Australia)



Tim Waterboer

The role of Human Papillomaviruses in the development of non-melanoma skin cancers

Non-melanoma skin cancers (NMSC), mainly composed of cutaneous squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), are the most common cancers in white-skinned populations worldwide. Besides ultraviolet (UV) radiation, infections with several skin-infecting Human Papillomavirus (HPV) types have been implicated as risk factors for SCC development.

In an international multi-centre study, data of almost 2000 NMSC cases and controls was collected and exposure to HPV was measured by analyzing viral DNA in hair follicles (viral presence and load) and serum antibodies to HPV antigens.

The aim of my project was to analyze the correlation between these markers for HPV infection, particularly between viral loads and antibodies. I performed these analyses in the Cancer and Population Studies Group (headed by Prof. Adèle Green) at the Queensland Institute of Medical Research (QIMR) in Brisbane (Australia). This group is the main data coordinating centre of the consortium performing the international multi-centre study and one of the most eminent epidemiology groups in skin cancer research worldwide.

Serological and viral load data was available for eight different cutaneous HPV types. On a HPV type-specific level, there was only little correlation between viral loads and antibodies, which is probably because antibodies may also reflect past exposure to HPV, while viral DNA can only be found in acute infections. However, supported by the statisticians at the QIMR, I generated cumulative viral loads (after transformation of the viral load data to standard normally distributed variables) and compared these with cumulative serology. This analysis

revealed that higher cumulative loads for HPV correlate with higher antibody titres. The combination of different markers for HPV infection will help us understanding the natural history of HPV and its role in the development of NMSC.

The data generated during my stay in Brisbane will be published in a paper on which I will be the lead author. Therefore, I will personally greatly benefit from my project in Australia. In addition, my group at the German Cancer Research Center (DKFZ) in Heidelberg will profit from the application of the statistical and epidemiological methods I learned in Australia.

Travel Fellowships

The EACR Travel Fellowship scheme is intended to assist cancer researchers to advance their research.

They support research on the basis of technical exchange, including participation in workshops and courses.

The value of each award is now up to 2,500 Euros or 4,000 Euros if match funded.

There are three deadlines for the submission of applications each year



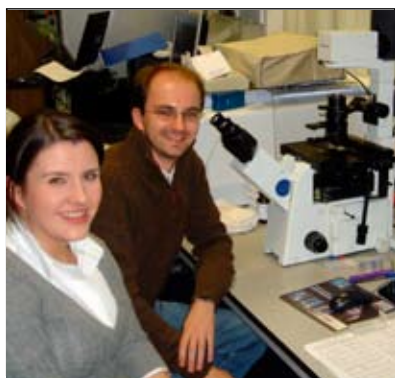
visit www.eacr.org for further information

Suzanne McFarlane



Home Institution: Centre for Cancer Research & Cell Biology
Queen's University Belfast

Host Institution: Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin



Suzanne McFarlane with Paul Tibbits

I am currently a post-doctoral researcher at Queen's University in Belfast. My research has concentrated on investigating the significance of the cell surface glycoprotein receptor CD44 to breast and prostate cancer metastasis. Although most studies would agree that expression of CD44s (standard form) is increased in breast cancer, there have been conflicting results about the clinical implications of the protein as increased CD44s expression has been associated with both poor prognosis and increased survival. These contradictory findings necessitated the further investigation of CD44s expression in tumour tissue. Therefore I used the EACR travel fellowship to initiate and conduct a collaborative study with Prof Elaine Kay and Dr Leonie Young (Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin) to undertake a retrospective immunohistochemical analysis of CD44 expression in breast cancer biopsy tissue.

Our initial study was conducted on a breast cancer tissue microarray established by Dr Leonie Young containing biopsy tissue taken from 459 patients. Statistical

analysis correlated the expression of CD44s with large tumour size, node-positive tumours and with distant recurrence and thus with a poorer prognosis. Given these initial findings, we have extended our research programme to conduct a similar analysis on two additional and geographically distinct breast cancer tissue samples, using cohorts from Sweden and the West of Ireland. These studies will permit further validation of the correlation of CD44s expression with clinico-pathological features and importantly, permit an extensive analysis of CD44 expression against important molecular parameters and determinants of breast cancer response.

The second aspect of the immunohistochemical study was to demonstrate the clinical relevance of my *in vitro*, cell-based research. We have previously demonstrated that CD44 signaling promotes adhesion of breast and prostate cancer cells to bone marrow endothelium *in vitro*. In currently unpublished studies, we have also described a novel mechanism by which CD44-induces increased 1-integrin expression/activation in order to promote this arrest and firm adhesion. In these *in vitro* studies, our data indicated that the 41 integrin was important in underpinning the CD44-initiated adhesion. However, there is currently limited knowledge pertaining to the expression of the 41 integrin receptor in breast cancer tissue. Therefore, during my secondment to the Beaumont Hospital, I used breast and prostate cancer tissue microarrays to demonstrate that the 4 subunit of the 41-integrin heterodimer is expressed in breast and prostate cancer.

I am very grateful to the EACR for the award of the Travel Fellowship as I have had the opportunity to gain practical experience in the preparation, staining and evaluation of breast cancer tissue microarrays, techniques in which I had no prior training or expertise. More importantly receipt of this funding has enabled me to determine the

clinical relevance of my *in vitro* work which would not have been possible within my home institution. In addition this EACR Travel Fellowship has helped to strengthen the collaboration between the two institutions and has led to new projects that are continuing at present.

Ehud Segal



Home institution: Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

Host institution: Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah, USA.



Ehud Segal

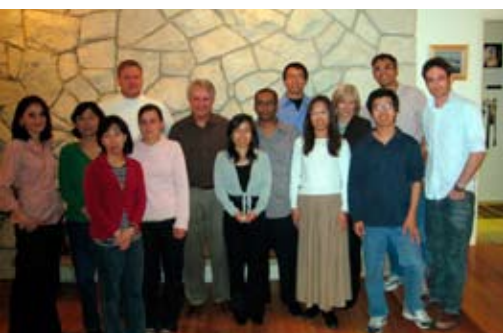
“Novel combined targeted antitumor and anti-angiogenic polymer therapeutics”

There is a crucial need for novel therapies for osteosarcomas and bone metastases. The combination of drug delivery systems with angiogenesis inhibitors is a new approach to cancer therapy. Our main goal in developing synthetic conjugates is to target selectively and efficiently osteosarcomas and bone metastases while decreasing the side effects.

The fellowship awarded by the European Association for Cancer Research gave me the opportunity to spend time at Kopecek's laboratory in Utah University. Prof. Jindrich (Henry) Kopecek is one of the leading scientists in the field of targetable polymeric

anticancer drugs. His research group members implement innovative interdisciplinary approaches to explore the relationship between the structure of synthetic macromolecules and their association with living tissue.

During this time, we synthesized and characterized several different conjugates consisting of different anti-angiogenic drugs linked through a degradable linker, cleaved by cathepsin K, a cysteine protease overexpressed in osteosarcoma and bone metastases. We labeled the conjugates using fluorescein isothiocyanate (FITC) for detection and *in vivo* biodistribution studies. To achieve control of the conjugates molecular weight and polydispersity, we used an advanced polymerization technique named reversible addition-fragmentation chain transfer (RAFT). By using this unique technique, we also achieved optimal drug loading percent of the drugs on the copolymers. We found that this novel conjugate has homogenous size distribution and is capable to internalize into the cytoplasm of HUVEC and Saos-2 human osteosarcoma cells via endocytosis in a similar pathway to the transferrin receptor. *In vitro* studies demonstrated the anti-angiogenic potential of the conjugate by inhibiting human umbilical vein endothelial cells (HUVEC) proliferation, migration and capillary-like tube formation.



In addition, we found that Saos-2 human osteosarcoma cell proliferation was similarly inhibited *in vitro* by the conjugate and by the combined free drugs demonstrating that the bound drugs retained their antitumor activity following polymer conjugation.



Huai-Zhong Pan with Ehud Segal

Thanks to the European Association for Cancer Research we are now capable of expanding the *in vivo* evaluation of our novel conjugate, and to progress the polymer chemistry techniques at my home institute at Tel Aviv University. I was honored to receive the prestigious European Association for Cancer Research travel fellowship and I appreciate your confidence in me and willingness to contribute to my future research. Your support enabled me to improve my knowledge and experience in the latest techniques of synthesis and characterization of targetable polymeric conjugates which is essential to the progress of my work and to my contribution to cancer research.

Aisling
O'Connor



Home Institute: UCD Conway Institute, University College Dublin.

Venue: University of Milan, Italy

Project: Novel agents for Photodynamic Therapy-Based Treatment of Cancer: Pre-clinical and Mechanistic Studies

Photodynamic therapy (PDT) is an established treatment modality for a range of diseases including cancer. We have developed a new class of non-porphyrin PDT agent, the BF₂-chelated tetraaryl-azadipyromethenes (ADPMs). The focus of my PhD is to investigate the mechanism of action of ADPM06, a member of the ADPM family of

photosensitizers which has been specifically designed for therapeutic application. *In vivo* efficacy and mechanistic studies are also being performed as part of my research. Due to the fluorescent nature of ADPM06, as well as the use of luciferase-tagged cancer cells, much of this *in vivo* work is being carried out employing the use of optical imaging. Additionally, a number of knock-out reporter cell lines are currently being developed in order to further examine key mediators of cell death induced by ADPM06-mediated PDT.

The EACR Travel Fellowship gave me the opportunity to travel to the University of Milan for a 5-day training course entitled "Reporter Animals as novel Tools for Drug Discovery: A Practical Approach". The training course employed the use of imaging technologies for *in vivo* pharmaco-toxicological studies in drug discovery using reporter mice. The course consisted of lectures as well as practical work which covered animal handling, drug delivery by various routes, dissection of tissues, organs and brain areas, optical imaging procedures, *ex vivo* assays and data analysis and interpretation. The training course was very relevant to the work I am carrying out in my PhD at present and has helped me immensely in designing and completing various *in vivo* studies. Moreover, attendance at this course allowed me to discuss my findings with exceptional individuals in the field. I would like to sincerely thank the EACR for awarding me this prestigious fellowship which gave me the opportunity to gain invaluable experience in modern techniques in optical imaging.



Aisling O'Connor (Front -3rd from right)

Simona Coco



Home Institution: Translational Pediatric Oncology, National Cancer Research Institute (IST), Genoa, Italy.

Host Institution: Klinikum der Universität zu Köln Zentrum für Kinderheilkunde und Jugendmedizin Kinderonkologie und –Hämatologie, Köln, Germany.



Simona Coco

Project Title: Identification of tumour genetic somatic abnormalities in infant neuroblastomas with high-risk factor or adverse outcome: a detailed genome-transcriptome analysis.

Neuroblastoma (NB) is the most common extracranial solid tumour in childhood and it is responsible for approximately 15% of all childhood cancer deaths. The clinical behavior of NB is unique and enigmatic ranging from spontaneous regression to fatal tumour progression. The main goal of my project was to study whole-genome in infant patients with metastatic disease combining high resolution a-CGH and gene expression profiling. I hypothesized that comparing genome profile obtained from tumours of patients at stage 4 with favourable and unfavorable clinical courses could improve discrimination of patients' risk and identify putative genes to understand better the mechanism of progression of metastatic NB.

Thanks to the EACR travel fellowship to study, I went to Children's Hospital in Koln headed by Prof Berthold to study the genome profiles of disseminated NBs. Prof Berthold is an expert oncologist and a leader in neuroblastoma field. Furthermore researchers of the host Institute have a great expertise in microarray technologies and statistical analysis applied to Neuroblastoma. At first, I had the opportunity to discuss with Prof Berthold the patients with metastatic disease to be enrolled in the study. I observed that most of the patients under six months of age at diagnosis have tumours at stage 4S (Special). Stage 4S NB is a metastatic tumour, characterized by spontaneous regression with little or no therapy. On the contrary, the frequency of infants at stage 4 increases from the sixth month of age and infants with stage 4 NB have a more favourable prognosis compared to those over 18 months of age at diagnosis.

Therefore, we decided to include in the study patients at stage 4S and identified three groups of metastatic NBs: i) Group 1: NBs of patients at stage 4S; ii) Group 2: NBs of infants at stage 4, ≤ 18 months of age, with good prognosis; iii) Group 3: NBs of patients at stage 4, >18 months, with progression and death of disease. Comparing three such groups may help to clarify the molecular mechanism of spontaneously regressing stage 4S and progressing stage 4 NBs.

First of all, I studied the genome aberrations using 44K microarray (Agilent Technologies) in 132 NBs (Group 1: 50 cases, Group 2: 32 cases, Group 3: 50 cases). Group 1 tumours were prevalently characterized by gains and losses of whole chromosomes, in particular 74% of cases showed only numerical aberrations; Fifty-six per cent of group 2 NBs had both numerical and structural aberrations and 16% of tumours had only segmental alterations; Eighty per cent of Group 3 tumours showed both numerical and segmental changes and 20% of cases had

only structural alterations. Although all metastatic tumours were characterized by aberration patterns prevalently focused on already known prognostically relevant chromosomes (loss 1p, loss 3p, loss 11q and gain 17q), the proportion of segmental chromosome aberrations increased in the three Groups: Group 1 < Group 2 < Group 3, indicating that chromosomal structural changes are associated with a more aggressive phenotype.

Then, the host Institute allowed me to perform gene expression profiling of the 3 Groups of tumours by a custom 11K oligonucleotide microarray, designed by Koln researchers according to NB transcriptome information from various published expression studies. Such expression analysis demonstrated that metastatic groups differ in specific gene expression profiles, providing gene signatures able to distinguish favourable and progressing stage 4 tumours. The SAM analysis identified 14 genes (*ADRB2*, *CAMTA1*, *CGNL1*, *EPB41L3*, *MAP7*, *NRCAM*, *NTRK1*, *PGM2L1*, *PVRL3*, *SCG2*, *SLC18A2*, *VAMP3*, *WNT4*, *EPB41L4A*) that are up-regulated in Group 1 and 2 compared with Group 3 NBs, suggesting their putative role in aggressive NB.

I would you like to thank the EACR for giving me the opportunity to carry out my study on NB at the Children's Hospital in Koln. A special thank to Prof. Frank Berthold and Host Institute researchers for fruitful discussions, suggestions and technical support.



Simona Coco and Host Lab Researchers

Lenka Oplustilova



Home Institution: Laboratory of Growth Regulators, Department of Cell Cycle Regulators, Palacky University in Olomouc, Czech Republic

Host Institution: Danish Cancer Society, Institute of Cancer Biology, Department of Cell Cycle and Cancer, Copenhagen, Denmark



Lenka Oplustilova

Background of the project

Defects in DNA damage repair processes are frequent features of various types of cancer. Targeting the remaining, still operational pathways of DNA repair represents a strategy for selective killing of such cancer cells. Inhibition of poly(ADP-ribose) polymerase-1 (PARP-1), a key enzyme of DNA single strand break (SSB) repair, prevents successful repair of such lesions. During cell cycle progression, SSB are converted to DNA double strand breaks (DSB). Those cells which cannot cope with accumulated genotoxic stress are determined to cell cycle arrest or apoptosis.

It has already been shown that inhibition of PARP-1 profoundly increases cell death in the cells deficient for BRCA-1 protein. BRCA-1 is important for DNA double strand breaks repair by homologous recombination (HR), one of the two basic DSB repair mechanisms.

Aim

The primary aim of the project was to examine various cancer cell lines

for deficiency of BRCA-1 and for Mre11, Rad50 and Nbs1 proteins forming complex (MRN complex) essential for functional HR, and whether deficiency in any of MRN complex components correlates with sensitivity to PARP-1 inhibitor.

Host laboratory

The Department of Cell Cycle and Cancer at the Danish Cancer Society, headed by Prof. Jiri Bartek, has made several significant contributions to the current knowledge of cell cycle control mechanisms and DNA damage response pathways in normal and cancer cells.

Achievements

My results obtained during the fellowship period in the Copenhagen laboratory showed profound sensitivity to PARP-1 inhibition in BRCA1 deficient human cancer cell lines. Combined defects of BRCA1 and MRN complex proteins even increase the cellular sensitivity to PARP-1 inhibitor. However, cancer cell lines deficient in proteins of MRN complex alone do not respond uniformly to the treatment with PARP-1 inhibitor.

We are going to continue with this project, still in collaboration with Danish Cancer Society, after my return to my home laboratory in the Czech Republic. In the case of MRN deficient non-sensitive cell lines we will test a combination treatment of PARP1 inhibitor and other DNA damaging agents and investigate whether a particular drug combination might have an intended synergic killing effect on such cells.



The data collected so far indicate that PARP-1 inhibitor as a single

drug treatment represents useful therapeutic strategy for a variety of cancer cells bearing BRCA1 mutation. Optimal treatment of cancer cells with the MRN complex deficiency is a challenge for our further experiments.



Martin Mistrik and Lenka Oplustilova

Impact of the fellowship for the awardee and the home institute:

My Ph.D. project is focused on designing of an optimal treatment with PARP-1 inhibitor applicable on wider spectrum of cancers with common characteristics. It will be finalized at the home institute in Olomouc, based on, and extending, the data from my EACR fellowship at the Danish Cancer Society in Copenhagen. The EACR travel fellowship provided me a great opportunity to enhance my research experience. I mastered several new methods, including skills in confocal microscopy techniques and biochemical assays. I gained a lot of knowledge from discussions with top researchers in my field.

My stay, financially supported by EACR, also significantly contributed to mutual collaboration between Palacky University in Olomouc and Department of Cell Cycle and Cancer in Copenhagen.

Finally, the results obtained during my fellowship project will be submitted for publication in an international journal, and the generous support from EACR will be acknowledged. Similarly, I have presented the data as an oral presentation at the 2nd EU - IP DNA Repair Workshop for young scientists in Porto, Portugal and at the Palacky University in Olomouc.

Juan Manuel Rosa



Home : Human Cancer Genetics Programme
Spanish National Cancer Centre (CNIO), Spain

Host: Cold Spring Harbor Laboratory (CSHL), USA



Juan Manuel Rosa

Project: “High throughput sequencing technology applied to candidate regions identified by linkage analysis: Another step in the quest for Breast Cancer susceptibility genes, will it be the finishing line?”

I am a third year PhD student at the Human Genetics Group at the Spanish National Cancer Research Centre (CNIO). The main objective of my PhD project is to find new breast cancer susceptibility genes, by performing linkage analysis in multiple-case families.

Breast cancer (BC) is the most frequent malignant tumour among women with approximately one million new cases per year around the world. About five percent of all BC cases are considered to be due to the segregation of a germinal mutation within a family (FBC). The two major BC susceptibility genes, *BRCA1* and *BRCA2*, are estimated to be involved in 20% of FBC, whereas mutations in other high susceptibility genes, such as *PTEN*, *STK11*, *P53* or in moderate BC susceptibility genes as *CHK2*, *PALB2*, *BRIP1* explain only around 5% of FBC. Thus, the majority of

familial breast cancer cases remain unexplained, and are named as non-*BRCA1/2* families.

In our group, we have performed two different linkage analyses in familial breast cancer during the last few years. In our first linkage analysis performed in 19 non-*BRCA1/2* families (Gonzalez-Neira et al. 2007), we identified 5 chromosomal regions candidate to harbour breast cancer susceptibility genes. After this first study, we performed a second study in 41 Spanish BRCAX families, a national collaborative linkage study, identifying other 3 candidate regions.

The purpose of the short term stay was the culmination of the study of a candidate region within 11q13, which is a candidate region to carry a breast cancer susceptibility gene obtained in the first linkage study. Only one family was linked to this region from the first study, although the linkage signal was very important. From the second linkage study we observed that two other families could be linked to the same region. We delimitate a final region of 3 Mb within 11q13, containing more than 70 genes. The complete sequencing of these 70 genes could represent a very expensive work (in time and money) in the traditional way. However, a new generation of high throughput sequencing technologies allowed the sequencing of several fragments at the same time in a single chip.

We began collaborative work to perform the high throughput sequencing of some of our candidate regions. We sequenced 10 members from the 3 candidate families by using Solexa technology. We also sequenced some control samples to have the possibility of comparing the variations found after the analysis of the data. I realised that this technology is very different to traditional direct sequencing, in concept as well as in results. We are used to see the four colours sequences and with this technology you obtain huge files with millions of small sequences in faster format.

Thus, the use of pipettes is replaced by the use of computers.

We obtained a huge amount of data (approx. 1.5 million reads per sample) that had to be analysed. Data quality was excellent, obtaining an average depth of 40 (the depth parameter is the number of times that every base is found to be sequenced; the first estimation was that a depth of 20 could be enough to detect heterozygous variants).

We also have to develop bioinformatics tools to be able to analyse all the variation data obtained from the sequencing process. Another positive point was that I had to learn programming in Perl language, and now I am working in the development of some bioinformatics tools. At that moment, we have not confirmed any of the candidate variations, but we hope that all this effort will give us good results.

I would like to express my gratitude to the EACR and to Greg Hannon for giving me the opportunity to travel to Cold Spring Harbor and to be involved in the development of this project.

I wish also to thank the lab staff, especially Emily Hodges for her patience and good teaching. I also want to thank to Andres Canela, who obtained his PhD in the CNIO, but whom I really knew in CSHL by sharing both good and not so good moments. Thanks to Oli, Gordon, Michelle, Vasily, Kata, Alexei, Nik, Antoine, Marek, Hana, and the rest of these really nice people who make a great group.



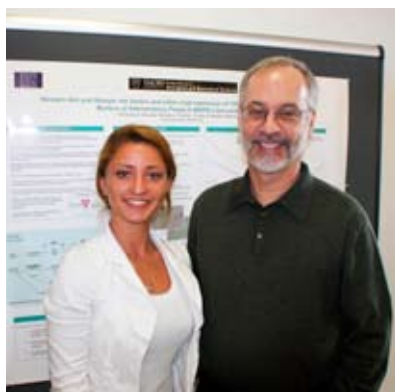
Juan Manuel Rosa

Gvantsa Khizanishvili



Home institution: National Cancer Center of Georgia, Tbilisi, Georgia

Host institution: Emory University, Rollins School of Public Health, Atlanta, GA, USA



Gvantsa Khizanishvili with Robert Bostick

Study the role of calcium and vitamin D as chemopreventive agents against colorectal cancer.

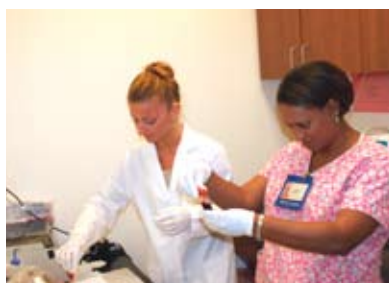
The aim of my visit to Dr. Robert Bostick's laboratory was to study the role of calcium and vitamin D as chemopreventive agents against colorectal cancer. International ecologic and migration studies clearly point out the importance of environmental exposures – especially diet and physical activity – in the etiology of the disease.

There is strong biologic plausibility and animal experimental evidence for protection against colorectal cancer by calcium and vitamin D.

A preliminary, randomized, double-blind, placebo-controlled, 2 x 2 factorial chemoprevention clinical trial (n = 88; 22/treatment group) was conducted of supplemental calcium (2,000 mg elemental calcium as calcium carbonate) and vitamin D3 (800 IU), alone and in combination vs. placebo over 6 months in patients with recent removal of incident sporadic colorectal adenomas, in order to estimate the efficacy of, and the variability of response to, these

agents on the individual components and aggregate profile of a molecular phenotype panel of biomarkers of risk for colorectal cancer. Although the duration of the entire project was 6 months, I participated in this research project during 6 weeks. During this time I learned the new methods and techniques which I will use during my work on a similar project that I conduct after my return to my home country, Georgia.

I participated in recruitment of patients to chemoprevention trials, ascertaining eligibility, interviewing, conducting placebo run-in trials, randomization procedures, immunohistochemistry, statistical analysis of data, PCR-RLFP. Once the patient was determined to be eligible to participate in the trial, three scheduled visits, including the final eligibility and randomization visit and one- and six-month follow-ups were scheduled. During these visits I performed rectal biopsies. After the biopsy was performed, immunohistochemical analyses was conducted using a DAKO Automated Immunostainer and Leica H&E Autostainer.



Based on recent advances in understanding the molecular basis of colorectal carcinogenesis, Dr. Bostick's laboratory has developed a panel of newer, reliable biomarkers that describes molecular phenotypes of the normally appearing colorectal epithelium. The panel includes biomarkers of: 1) inflammation (COX-2); 2) the expression of genes involved in the normal structure and function of the colorectal epithelium that have been found to be altered early in the two major colorectal carcinogenesis pathways (APC pathway – APC, - catenin, and E-cadherin; Mismatch repair pathway - MSH2, MLH1); 3) colorectal epithelial crypt cell cycle

events (MIB-1, p21 bcl-2, bax, bak); and 4) autocrine / paracrine growth factors (TGF , TGF). The biomarkers study and this important data suggests that vitamin D and calcium may work together to reduce the risk of colorectal neoplasm.

The knowledge gained on the modern methods of cancer epidemiology and medical statistics helps me to plan and conduct my scientific study independently, choose in a right way the optimal model of epidemiology study, explore modern methodology of medical statistics and consequently raise the level of scientific research, increase confidential quality of received results and the objectivity of conclusions.

I am very grateful to the EACR for the Travel Fellowship and would like to thank committee, EACR members and fellowship coordinator for supporting this project. I would like to thank Dr. Bostick and everyone in Rollins School of Public Health for the invitation and the time spent on me. I am looking forward to using my knowledge and experience in my home institution and during my future research career!

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AICR
Cancer knows no boundaries.
Fortunately, neither do we.

Samantha Larkin



Home institution: Pharmacy and Biomedical Sciences, University of Portsmouth

Host institution: Research and Development Support Unit, Medical School, University of Southampton



Samantha Larkin

The main objective of the workshop I attended, courtesy of the EACR travel fellowship, was to learn how to perform statistical analysis of large datasets that have been produced during my PhD. This particular part of my PhD has utilised qPCR to compare gene expression between indolent and aggressive cases of prostate cancer. This has been carried out for 96 genes and so has resulted in a large complicated data set which I had little idea how to analyse. Additionally, my supervisor hoped that the knowledge I obtained may be used to teach undergraduate project students and new PhD students entering our group.

Prostate cancer is a disease with two faces. Generally it is a slow growing disease that is present in many men over the age of 80. Sometimes, however, it is an aggressive disease that can progress and metastasise very quickly. Once it has metastasised there are few curative options available. The aim of my research is to study serum and tissue from prostate cancer patients to identify genes and proteins that are associated with prostate cancer progression. Through an extensive

literature search and with the use of artificial neuronal networks (courtesy of a colleague), I identified 96 genes (including 5 housekeeping genes) that may be involved in progression of the disease. Using Taqman® Low Density Arrays (TLDA – Applied Biosystems), qPCR was performed and the data produced. Initial analysis of the data showed that I didn't know enough about statistics to analyse the data and the only workshop that would give me this insight was quite costly.

The EACR travel fellowship enabled me to attend a week of statistics workshops that have resulted in a clear understanding of the techniques needed to analyse my data. Under the guidance of the University of Southampton medical statisticians, I have learnt about linear regression, logistic regression, ROC curves and much more. I have since completed analysis of my data (using logistic regression and ROC curves) and now feel much more comfortable with statistical analyses.

I would like to extend my gratitude to the EACR for providing me with the opportunity to attend these workshops. They enabled me to learn and understand the statistics required to finish my PhD and teach future researchers joining our cancer genetics group.

Nino Kartvelishvili



Home: National Cancer Centre of Georgia, Georgian Cancer Study Group

Visit: EORTC Course: One-Day Introduction to EORTC Trials.

Visit: Jules Bordet Institute Brussels, Belgium,

The EORTC training Course was recommended for anyone who is new to the clinical research area or has recently started working with EORTC protocols. The purpose of this introductory workshop was to give guidance for participating in EORTC clinical



Ann Marinus, Nino Kartvelishvili (right)

trials activities. Participants received information about the functioning of the EORTC and about Trials methodology, investigator / site quality requirements and control, patient safety management, adequate data collection and pitfalls for reliable data. On-line registration /randomization process and remote data capture system was demonstrated. I also had the opportunity to visit the EORTC Headquarters and to have informal discussions with the Headquarters staff.

At the Jules Bordet Institute I visited Senology Department: Medical Oncology Department with leadership of Prof. Ahmad Awada, Surgery Department with leadership of Prof. Jean-Marie Nogaret and BC Screening Department with leadership of prof. André Grivegne. The multidisciplinary breast team at the Jules Bordet Institute is dedicated to clinical management and research in the area of breast cancer. A full diagnostic unit (radiology including MRI, pathology) allows the diagnosis. Patients are treated by an effective multidisciplinary team acquainted of all the leading treatments: there is close co-operation among the specialists working in the Screening, Surgery, Radiotherapy, Chemotherapy, Nuclear Medicine, Rehabilitation and Anatomopathology services.

I also attended a very interesting Monthly Seminar at Institut Jules Bordet: 'Growth factors and oncogenic receptor tyrosine kinases: Signal transduction mechanisms and targets for cancer therapy'. It was presented by Yosef Yarden.

I would like to thank to EACR for support and encouragement. The fellowship gave me the chance to visit one of the best scientific organizations - EORTC - and one of the comprehensive oncology institutes in Europe - Institut Jules Bordet. The visit has had a great influence on my professional development and I had opportunity to realize deeply how patients and investigators from different continents cooperate by participating in extensive clinical trials. The Georgian Cancer Study Group in collaboration with the National Cancer Centre is working very hard to implement research work in oncology. Implementation of protocol medicine will increase quality of patients care, educational and practical level of medical doctors, and will improve quality of health care system in the Country. The knowledge and experience, I have gained from this scientific visit will be very useful for my home institute. The friendship and relationships that I developed will help us to collaborate in future, and will impact very positive on the improvement of cancer research management in Georgia. I returned with many new ideas and offers. In a short time I had opportunities to observe and participate in the routine medical life of Institut Jules Bordet. They were very friendly and attentive and tried to help me to acquire practical information and new technologies. I will never forget their efforts to help me.

Our enthusiasm to join with international research groups is really compelling. As EORTC has always been dedicated to innovative clinical research designed to improve the prognosis of cancer we plan to continue the collaboration in the near future.



Nino Kartvelishvili (centre)

Paola Larghi



Home institution: Istituto Clinico Humanitas, Milan

Host institution: Keystone symposia, Snowbird, Utah



Paola Larghi

Background:

Tumour associated dendritic cells (TADC) and tumour associated macrophages (TAM), are important immune components of the tumour stroma. Under normal conditions, dendritic cells (DC) in particular, but also macrophages, play a crucial role in triggering inflammation and promoting adaptive immune response, through the presentation of antigens to T cells. Nevertheless, DC present in tumours have been often associated with tolerogenic functions, a phenotype likely induced by the tumour microenvironment. In this regard, while DC maturation was reported to require NF- κ B activation, several line of evidence demonstrated that this pathway is strongly impaired in infiltrating leukocytes, including TADC and TAM.

In the attempt to further elucidate the mechanism driving defective NF- κ B activity in tumour infiltrating leukocytes, we have recently reported that TAM display massive nuclear localization of the p50 NF- κ B inhibitory homodimer. The presence of these homodimers strongly correlates with the tolerogenic phenotype that has been extensively described in TAM and the functional significance of this

observation was demonstrated in p50 NF- κ B deficient mice, in which TAM display a phenotype more similar to that observed in normal macrophages. As a consequence, tumour growth is significantly reduced in p50 KO mice and, based on bone marrow chimera experiments, we were able to show that this reduction was determined by myeloid derived cells.

Taken together these results, suggest that tolerogenic mechanisms may target other compartments of the immune system, therefore we decided to characterize the role of p50 NF- κ B in dendritic cells.

Our data indicate that p50 NF- κ B homodimer has inhibitory functions in bone marrow derived DC, since its depletion enhances their inflammatory and co-stimulatory properties, both in vitro and in vivo. Moreover, preliminary results suggest that inhibition of p50 NF- κ B homodimer formation in TADC significantly abrogates their tolerogenic properties.

Interestingly, we also observed modulation of one of the most studied genes involved in tolerance: INDO.

In conclusion, our study suggests that targeting microenvironmental signals and pathways leading to upregulation of p50 NF- κ B homodimer in TAM and TADC may represent a novel strategy to restore antitumour functions in vivo. It was therefore of great importance for me to attend a meeting on inflammation and cancer together with world scientists in the field of tumour immunology.

One of the central topics of this congress was angiogenesis. Many important scientists are working on this with exciting results. Dr Claire Lewis (Sheffield, GB) for example, has focused her presentation on TEM (Tumour Associated Macrophages expressing Tie2) which are recruited by the tumour and cooperate with the stabilization of tumour vessels; the same do

hemangiocytes described by Dr Rafii (New York, USA) which are cells depicted to the stabilization of tumour vessels and could be macrophages, dendritic cells or monocytes. Again, Dr Fu (New Haven, USA) has described the role of transcription factor STAT3 in endothelial cells showing that mice lacking STAT3 in the endothelium display a reduced tumour growth and, most importantly, less metastasis.

Regarding this aspect, one of the most interesting talks was the one given by Michael Karin (La Jolla, USA), the organizer of the congress. He focused his work on the impact of inflammation on the metastatization process, in particular he focused on the events that occur in the tumour such as necrotic tumour cells which are able to activate inflammatory cells in the tumour microenvironment. The receptor which is involved in this process seems to be TLR2, since TLR2 KO mice are resistant to metastasis.

Finally, this was a great experience that encouraged me to deepen and continue with my work and I thank very much the EACR organizers who decided to give me this opportunity through the travel fellowship.

AICR have sponsored the EACR Travel Fellowships for many years. With the rapid increase in the number and quality of applications AICR have joined EACR in increasing the level of funding available in 2009.



Debbie Wheelans (AICR) is pictured centre with colleague Helen Ironside at EACR-20 in Lyon presenting a cheque to Kathryn Wass (EACR)

AICR International Fellowships

As a young cancer researcher it's difficult to establish yourself and run your own lab. That's why AICR, the Association for International Cancer Research, doesn't fund only distinguished scientists; it is particularly interested in funding promising young researchers. Currently the charity, which is based in St Andrews, Scotland, distributes over £10m to support research into the causes, prevention, detection and treatment of cancer. AICR is currently supporting 231 projects in 23 different countries.

The AICR International Fellowships are specifically designed to support outstanding young investigators for up to six years. Every year, AICR offers one talented individual the chance to establish his/her first research group. The research must have a fundamental or translational focus and the fellowship includes salaries for the fellow and two other research staff, as well as money for consumables, equipment and travel. It's a fantastic opportunity, open to anyone across the world. Details on the application process are available from their website. Closing date for applications is 30th June 2009.

AICR also provides funding for three-year project grants, typically funding the salary of a post-doc, or perhaps a PhD student, plus money for consumables. Operating two grant rounds per year (April and October) they are quite flexible in what they will consider: their only firm restriction is that they do not support clinical research. Obviously, there are different definitions of clinical research: AICR draws the line at intervention studies, such as clinical trials. Its current funding portfolio includes a lot of molecular cell biology, but also epidemiology, drug development, virology, immunology and diagnostics development.

AICR receives around 300 applications for each round, but is only able to award 25-30 grants. The ratio of grants to applications is lower than for many other funding bodies, but this is an inevitable consequence of being an international organisation. With national funding agencies, each application is in competition with the best from that country. At AICR, it is in competition with the best in Europe and, to some extent, the world. This ensures that AICR funds very high quality science.

If you are interested in finding out more about the Fellowships, or indeed our project grants, please contact Debbie Wheelans, AICR's Grants Manager on +44(0)1334 477910 or email debbie.wheelans@aicr.org.uk or visit their website, www.aicr.org.uk.

AICR 30th Anniversary Conference

For nearly 30 years, AICR has given over £120 million of funding to cutting-edge cancer research throughout the world. We're celebrating this achievement with a special 30th Anniversary Conference in the historic town of St Andrews in April 2010, 7th-9th.

The meeting aims to examine how advances in basic cancer research and the exploitation of improved model systems can stimulate innovative translational research. Entitled 'Today's Research - Tomorrow's Therapies,' delegates will be able to hear from a range of eminent speakers from around the world, including Sir Tim Hunt, the 2001 Nobel Prizewinner in Physiology or Medicine, Professor Allan Balmain, San Francisco, Professor Stephen Neidle, London, Professor Christer Betsholtz, Stockholm and Professor Steve West, London, to name but a few. There will, of course, be plenty of opportunity too to meet with others in the field.

The conference is being held in the Fairmont St Andrews, complete with spa, golf courses and fantastic views of St Andrews and the Fife coast.

For further information on the AICR 30th Anniversary Conference visit

www.aicrconference2010.org.uk



ASSOCIATION FOR INTERNATIONAL CANCER RESEARCH
30th ANNIVERSARY
CONFERENCE

Today's Research – Tomorrow's Therapies

Wednesday 7 to Friday 9 April 2010
Fairmont Hotel, St Andrews, Fife, Scotland

Speakers and Sessions

Anniversary Lecture: Sir Tim Hunt, UK

Basic Science: Professor Stephen West - UK, Professor Hans Clevers - Netherlands, Professor Christer Betsholtz - Sweden, Professor Guido Kroemer - France

Model Systems: Dr Owen Sansom - UK, Professor John Collard - Netherlands, Professor Allan Balmain -USA, Professor Terry Rabbitts - UK

Translational Research: Professor Richard Marais - UK, Professor Robert Brown - UK, Professor Stephen Neidle - UK, Professor Frances Balkwill - UK

Aims of the Conference

Examining how advances in basic cancer research and the exploitation of improved model systems can stimulate innovative translational research.

Registration Details

Abstract Deadline: 15 December 2009

Early Registration Deadline: 31 January 2010

Travel Grant Deadline: 31 January 2010

Cancer knows no boundaries
Fortunately, neither do we

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For further information visit the website
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