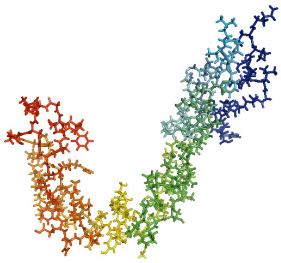
PROGRAM



The 5th International Conference
'Notch Targeting in Cancer'
Santa Marina Hotel, Mykonos, Greece
24 - 26 June 2015

WE ARE GRATEFUL TO OUR SPONSORS FOR THEIR SUPPORT

Roche

Trojantec

Wednesday 24th June 2015

4.00 - 4.30 pm **Registration**

4.30 - 4.35 pm **Welcome: Agamemnon Epenetos**

SESSION 1 Chairman: **Adrian Harris**

4.30 - 5.00 pm **Dennis Hughes**

Highlights of the 4th Meeting, Notch Targeting in Cancer, June, 2014

5.00 - 5.30 pm Kim Dale, Division of Cell and Developmental biology
College of Life Sciences, Dundee University, Dundee , Scotland

Taking it up a Notch: A novel and conserved role for Notch in priming the cellular response to Shh.

During embryonic development notochord-derived Sonic Hedgehog (SHH) is essential for dorso-ventral patterning of the overlying neural tube. Increasing concentration and duration of Shh signal induces progenitors to acquire progressively more ventral fates. We show Notch signalling augments the response of neuroepithelial cells to Shh, leading to the induction of higher expression levels of the Shh target gene Ptc1 and subsequently induction of more ventral cell fates. Furthermore, we demonstrate activated Notch1 leads to pronounced accumulation of Smo within primary cilia and elevated levels of full-length Gli3. Finally, we show Notch activity promotes longer primary cilia both in vitro and in vivo. Strikingly, these Notch-regulated effects are Shh-independent. These data identify Notch signalling as a novel modulator of Shh signalling which acts mechanistically via regulation of ciliary localisation of key components of its transduction machinery. This role for Notch is likely to affect a broad range of

other pathways reliant on ciliary localisation of signalling components in a wide variety of developmental and disease contexts.

5.30 - 6.00 pm G. Sflomos[1], V. Dormoy[1], T. Metsalu[2], R. Jeitziner[1], L. Battista[1], A. Treboux[3], J-F. Delaloye[3], M. Fiche[3], J. Vilo[2], A. Ayyanan[1], C. Brisken[1,]

[1]ISREC - Swiss Institute for Experimental Cancer Research, NCCR Molecular Oncology, School of Life Sciences, Ecole polytechnique fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.

[2]Institute of Computer Science, University of Tartu, Liivi 2, Tartu 50409, Estonia.

[3]Lausanne University Hospital, 1011 Lausanne, Switzerland.

A novel preclinical model for ERα positive breast cancer reveals the mammary epithelial microenvironment as a critical determinant of the luminal phenotype

Ninety percent of new drugs in oncology fail (Arrowsmith, 2011; Hait, 2010), largely because the preclinical models used to test them do not adequately reflect

their clinical counterparts. Breast cancer is a leading cause of cancer-related death among women worldwide and 75% of all cases are estrogen receptor positive (ER+)/luminal, yet, there are few preclinical models, in particular for the increasingly frequent lobular subtype, and ER+ patient-derived xenografts (PDXs) hardly grow. We tested a more relevant microenvironment by grafting breast cancer cell lines of different molecular subtypes into mouse milk ducts. Except MDAMB231, all cell lines grow retaining histopathological features of the original tumors. The basal-like cell lines invade quickly and give rise to palpable tumors whereas luminal lines grow, largely within the ducts, without exogenous hormone supplementation required in traditional xenografts. In this model, MCF7 cells respond to different endocrine treatments and further resemble clinical ER+ tumors in proliferative index, vascularization, micro-calcifications, tumor progression including metastasis, and molecular signatures. Gene expression analysis reveals that the fat pad microenvironment enhances EMT features, cell proliferation, and changes in cell adhesion whereas the intraductal microenvironment maintains expression of hormone receptors, and pathways associated with breast cancer metastasis. Our findings indicate that the mammary epithelial microenvironment is a critical determinant of the luminal phenotype

and open new perspectives for translational research and personalized medicine in the area of ER+ breast cancer.

7.00 - 9.00 pm
Welcome Reception Cocktail

Thursday 25th June 2015

SESSION 2 Chairman: Gian-Paolo Dotto

9.30 - 10.00 am Reyhaan A. Chaudhri[1], Thaned Kangsamaksin[1,2], Henar Cuervo[1], Jing Du[1], Zhuangzhuang Cong[1], Aino Murtomaki[1], Natalie M. Kofler[1], Ian W. Tattersall[1], Carrie J. Shawber[3], Jan Kitajewski[1,4,5]

[1]Department of Obstetrics/Gynecology, Columbia University Medical Center, Columbia University, New York, New York
[2]Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand
[3]Department of Surgery, [4]Department of Pathology and Cellular Biology, [5]Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, Columbia University, New York, New York

The Notch decoys: Targeting tumor angiogenesis by unique mechanisms with ligand-specific Notch blockade to inhibit tumor growth

As a key regulator of both developmental and tumor angiogenesis, the Notch signaling pathway offers an attractive target for therapeutic intervention. Several current approaches for targeting the Notch pathway include gamma secretase inhibitors (GSIs), Notch antibodies, or Notch ligand antibodies. Unfortunately,

with many of these approaches, especially with GSIs, severe toxicity has been observed. While several approaches to modulate current therapeutics are ongoing, novel therapeutics are still needed to inhibit Notch in tumors without eliciting the severe toxicities of these current therapies. Based on the mammalian Notch1 extracellular domain, which contains 36 EGF-like repeats, we were able to develop ligand-specific Notch1 inhibitors that we have named the Notch1 decoys. Using an assay developed to induce ligand-specific NOTCH activation by culturing cells on tissue culture polystyrene plates tethered with recombinant Dll or Jagged ligands, a Notch1 decoy comprised of EGF-like repeats 1-13 fused to human Fc ($N1_{1-13}$Fc) specifically inhibited Dll4-mediated NOTCH activation, while a Notch1 decoy comprised of EGF-like repeats 10-24 ($N1_{10-24}$Fc) specifically inhibited Jagged1-mediated NOTCH activation. Interestingly, $N1_{1-13}$Fc decoy and $N1_{10-24}$Fc decoy exhibited differential effects on in vitro, retinal, and tumor angiogenesis indicating unique mechanisms of DLL4/NOTCH and JAG1/NOTCH signaling on angiogenesis. Inhibition of Dll4-mediated Notch signaling by $N1_{1-13}$ decoy in tumors led to paradoxical hypersprouting of a nonfunctional tumor vasculature, while inhibition of Jagged1-mediated Notch signaling by $N1_{10-24}$ decoy led to reduced angiogenic sprouting in tumors; however, both decoys were able to reduce tumor growth in several mouse tumor models. As for adverse effects, Notch1 decoys exhibited markedly reduced gut toxicity in mice when compared to GSI treatment, and while Dll-specific Notch1 inhibition with $N1_{1-13}$ decoy demonstrated signs of minor sinusoidal dilation in the liver, Jagged-specific Notch1 inhibition with $N1_{10-24}$ decoy showed no signs of adverse effects in the liver. In addition, none of the Notch1 decoys exhibited appreciable effects on renal histomorphology. Therefore, we conclude that ligand-specific Notch inhibition by the Notch1 decoys represents effective therapeutics to inhibit tumor angiogenesis and progression by targeting Notch signaling.

10.00 - 10.30 am Kai Wang[1], Qin Zhang[1], Danan Li[1], Cathy Zhang[1], Keith Ching[1], Paul Rejto[1], James Christensen[2], Peter Olson[1]

[1] Pfizer Global Research and Development, Oncology Research Unit, 10724 Science Center Dr., La Jolla, CA, 92121;
[2] Mirati Therapeutics Inc., 9393 Towne Center Drive, Suite 200, San Diego, CA, 92121

Disparate Mutations in Notch Receptors Comprise on Oncogenic Driver Class in Triple Negative Breast Cancer Sensitive to a Gamma Secretase Inhibitor

While the Notch pathway is reportedly activated in breast cancer, the molecular mechanisms leading to hyperactivation remain poorly understood, hampering the optimal clinical development of Notch targeted therapies. To identify predictive biomarkers for the gamma secretase inhibitor PF-03084014, we sequenced patient-derived xenograft models and mined The Cancer Genome Atlas. We uncovered an array of alterations in the extracellular and PEST domains in multiple Notch receptors that activated the pathway and were sensitive to drug. These data define a novel oncogenic driver class that may respond to Notch inhibitors.

10.30-11. 30 Coffee Break

SESSION 3 Chairman: Dennis Hughes

11.30 - 12.00 noon Fabian Junker, Antoine Chabloz, Ute Koch and Freddy Radtke, Ecole Polytechnique Fédérale de Lausanne, School of Life Sciences, Swiss Experimental Cancer Research Institute, Lausanne, Switzerland

Dicer1 imparts essential survival cues in Notch driven T-ALL via miR-21 mediated tumor suppressor Pdcd4 repression

The modulatory function of individual miRNAs in Notch driven T-ALLs has recently been established. Although pro-tumorigenic and tumor-suppressive miRNAs are implicated in disease onset in murine models of Notch-driven T cell leukemia, whether Dicer1-processed miRNAs are essential for Notch-driven T-ALL is currently unknown. Here we used conditional and inducible genetic loss of function approaches to test whether the development and maintenance of Notch-driven T-ALL was dependent on *Dicer1* function. Mice with specific inactivation of both *Dicer1* alleles in the T cell lineage did not develop Notch-driven T-ALL. In contrast, loss of one functional *Dicer1* allele did not significantly perturb T-ALL onset and tumor progression. Inducible inactivation of *Dicer1* in early stage polyclonal T-ALL cells was sufficient to abrogate T-ALL progression in leukemic mice whereas late stage monoclonal T-ALL cells were counter-selected against loss of *Dicer1*. Lineage tracing experiments revealed that *Dicer1* deficiency led to the induction of apoptosis in T-ALL cells whereas cell cycle progression remained unaltered. Through microarray-based miRNA profiling, we identified miR-21 as a previously unrecognized miRNA deregulated in both mouse and human T-ALL. Herein, we demonstrate that miR-21 regulates T-ALL cell survival via repression of the tumor suppressor *Pdcd4.*

12.00 - 12.30 pm Marianna Prokopi[1,2] PhD, Christina A. Kousparou[1] PhD, , Konstantinos K. Kapnisis[2] PhD, Andreas S. Anayiotos[2] PhD, Costas Pitsillides[2] PhD, Agamemnon A. Epenetos[1, 3*] FRCP, PhD

[1] Trojantec Ltd, The Bank of Cyprus Oncology Centre, Nicosia, Cyprus

[2] Department of Mechanical Engineering and Materials Science & Engineering, Cyprus University of Technology, Limassol, Cyprus

[3] Imperial College London, St Bartholomew's Hospital and the Harley Street Oncology Clinic, London, UK

Therapeutic miRNAs delivered systemically using normal stem cell derived microparticles .

The objective is to develop a micro RNA based cancer targeted to tumors d via microparticles (MPs) derived from normal umbilical cord mesenchymal stem cells (MSCs). These MPs are loaded with specific miRNA molecules impacting the activity of genes associated with oncogenesis, metastasis and drug resistance.The proposed Hypothesis is that the engineered MPs home selectively to tumor sites via specific chemokine receptors, fuse with tumor cell membranes and incorporate miRNA directly into the target cancer cells exerting their therapeutic effect.

Genomics technologies in combination with innovative imaging techniques *in vivo* flow cytometry, fluorescence and bioluminescence based reflectance imaging and computational modelling are used to conduct pharmacokinetics and pharmacodynamics and define the mode of action, safety and efficacy of the therapeutic technology.

We are currently optimizing the MP-miRNA technology through components and techniques such as: a) the standardization of the isolation and production of MSC-derived MPs, b) the optimization of MP size to achieve maximum delivery efficiency through experimental investigation of the size-dependent micro-particle kinetics and bio-distribution, c) validation of the miRNA-loaded MPs as targeted cancer therapy in appropriate preclinical animal cancer model and d) the advancement of *in vivo* imaging modalities needed to monitor the therapeutic potential of the therapeutic technology in animal models of disease.

Data thus far, demonstrate the ability of MP-mi RNA to enter cancer cells in vivo and exert a biological effect.

The MP-MiRNA technology is a new and potentially breakthrough development in the area of targeted cancer therapies. The proposed project may lead to the development of specific MP-miRNA complexes that are capable of selectively targeting malignant cells in cancer patients.

1.00 - 2.30 pm **Lunch**

2.30 - 3.30 pm **Open Air workshop:**
Notch Based Companion Diagnostics:
Freddy Radtke/ Adrian Harris

SESSION 4 Chairman: Keith Brennan

4.00 - 4.30 pm G. Paolo Dotto
Department of Biochemistry, University of Lausanne, CH

Multistep process of cancer associated fibroblast (CAF) determination under combined CSL-p53 control

The vast majority of epithelial cancers is limited to *in situ* lesions that, for internal organs like breast, prostate or lung, can remain undetected for the whole life of an individual. The reason(s) why only a minor fraction of these lesions progresses into malignancy is not understood. In fact, many if not most of genetic changes found in invasive and metastatic tumors can be already present in pre-malignant lesions, raising the question of whether such changes are of primary causative significance or merely permissive for later cancer-spreading events.

Changes in tumor stroma are most frequently viewed as secondary to changes in the epithelium. However, recent evidence indicates that they may play a primary role. Such a possibility would help explain not only dormancy of most epithelial cancers, but also *field cancerization*, a condition of major clinical significance linked with multifocal and recurrent tumors and broader tissue changes beyond areas of tumor development.

In this presentation, I will overview our recent and ongoing work in this area, with a specific focus on a novel functional and physical cross-talk between the CSL and p53 proteins at the basis of cancer associated fibroblast (CAF) determination.

4.30 - 5.00 pm Daniel Antfolk[1,2], Marika Sjöqvist[1,2], Cecilia Sahlgren[1,2,3]

[1]Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, FI-20520 Turku, Finland

[2]Department of Biosciences, Åbo Akademi University, FI-20520 Turku, Finland

[3]Department of Biomedical Engineering, Technical University of Eindhoven, 5613 DR Eindhoven, the Netherlands

Vimentin regulates Notch signaling during angiogenesis

The Notch pathway coordinates endothelial cell behavior during angiogenesis. Dll4/Notch signaling inhibits, whereas Jagged/Notch signaling, promotes sprouting. We demonstrate that Vimentin, a type III intermediate filament, balances Notch signaling in angiogenesis. For decades Vimentin has been considered a marker of dynamic cellular processes and a necessary structural component providing the cell with mechanical stability. However, recent data indicate a modulatory role in many processes including cell adhesion, wound healing, inflammation, epithelial to mesenchymal transition (EMT) and cancer cell migration and invasion. The vasculature in Vim-/- embryos with surrounding yolk sacs is underdeveloped with thinner vessels and reduced branching. Endothelial cells lacking Vimentin display increased Notch activity linked to enhanced routing to late endosomal-lysosomal compartments facilitating proteolytic processing and receptor activation. By contrast, the trans-activation capacity of Jagged is compromised, and despite elevated Jagged levels and efficient receptor binding,

non-functional ligands accumulate at the cell membrane. Notch activation by recombinant Jagged ligands rescues sprouting angiogenesis in aortic rings from Vim-/- mice and in endothelial 3D sprouting assays. Our data reveal that vimentin restricts Dll4/Notch activity, but is required for efficient activation of Notch by Jagged and that in the absence of Vimentin Dll4/Notch signaling dominates favoring suppression of angiogenesis.

8.00 pm until late
Conference Dinner

Friday 26th June 2015

SESSION 5 Chairman: Freddy Radtke

9.30 - 10.00 am Dennis P. M. Hughes, MD, PhD
Department of Pediatrics – Research, the MD Anderson Children's Cancer Hospital, Houston, Texas, and the Graduate School of Biomedical Sciences of the University of Texas at Houston.

Non-Notch targets of gamma-secretase inhibition

Regulation of cell signaling is a complex process, with regulation and control mechanisms active at many levels, including transcription of receptors and ligands, control of ligand exposure and release, proteolytic activation, proteolytic inactivation, trafficking, ubiquitination and degradation in the proteasome. One important regulator of cell signaling is the □-secretase complex, an integral membrane enzyme complex that serves to cleave susceptible proteins through the transmembrane domain. Minimally, □-secretase consists of 4 protein subunits: Presenillin, Nicastrin, APH-1 and Pen-2. Presenillin, which is the active subunit of the complex, is an aspartyl protease that functions as a homodimer following protein target recognition by nicastrin, generating a somewhat variable cleavage of the target in the transmembrane domain. For most receptors, □-secretase activity must be preceded by shedding of the extracellular domain of the protein, with nicastrin stabilizing the target. While most investigators focus on

its role in the plasma membrane, □-secretase also can be found in the membranes of the endoplasmic reticulum and the golgi.

Interest in the □-secretase complex first arose because of its vital role in generating the □-amyloid associated with Alzheimer's disease. Multiple pharmaceutical approaches were developed to inhibit the complex, hoping to develop a preventative medicine for this devastating disease. Unfortunately, early studies of □-secretase inhibitors (GSI) were disappointing, with minimal effect on the course of disease and some severe toxicities.

The Notch pathway is important in many types of cancer, and □-secretase also is necessary for Notch activation. Because of the vital role Notch plays as an oncogene in many cancers, interest shifted to repurposing the GSIs under development for oncologic use. Clinical development of GSIs has been slowed both by the minimal effect seen in monotherapy trials and the high side-effect profile observed in many studies. One bright spot has been the GSI PF-03084014, which was better tolerated in phase I studies and showed a strong effect against desmoid tumor and possibly other sarcomas.

As clinical develop of GSIs continues, it will be important to recognize that □-secretase has many targets within cancer cells, and while GSIs are often referred to as "Notch inhibitors," observation of a laudable clinical response to a GSI does not necessarily indicate that the effect was due to Notch inhibition. Among the targets of □-secretase are CD44, which functions as a receptor for hyaluronic acid, MMPs and other proteins, N- and E-Cadherins, important in epithelial-to-mesenchymal transition (EMT), and LRPs, important in Wnt signaling. The Eph/Ephrin families are important for regulation of motion control, contact inhibition and repulsion, and Ephrin B2 at least is a target of □-secretase. Some isoforms of the Her-4 protein, a member of the ERBB family of receptor tyrosine kinases, can be cleaved by □-secretase to generate an 80 kD fragment with a nuclear localization sequence. This p80 fragment of Her4 contains multiple PPXY motifs capable of binding WW domains of multiple intracellular proteins, including Yap and Stat5, often stabilizing and augmenting the function of these proteins. Additional □-secretase substrates identified by proteonomic profiling include dystroglycan, DNER, DSG2 and PLXDC2.

In this presentation we will review the non-Notch targets of □-secretase that may be important in tumors, discussing the possible impact GSIs might have by acting on each target. While improved outcomes for any cancer as a result of □-secretase inhibitors would be a great benefit, it is important for translational researchers of determine, if possible, which □-secretase target is most responsible for the effect.

10.00 - 10-30 am Ahmet Acar*, Ana Hidalgo-Sastre*, Giovanna M. Collu, Michael K. Leverentz, Christopher G. Mills, Simon Woodcock, Charles Streuli, Andrew Gilmore, Martin Baron & Keith Brennan

Faculty of Life Sciences, University of Manchester, Manchester, UK

*** these authors contributed equally**

Should the crosstalk between the Notch and Wnt signalling pathways influence our treatment of cancer?

Notch and Wnt are two essential signalling pathways that help to shape animals during development and adult tissue homeostasis. Although, they are often active at the same time within a tissue, they typically have opposing effects on cell fate decisions. In fact, crosstalk between the two pathways is important in generating the great diversity of cell types that we find in metazoans. In addition, altered signalling through both pathways has been linked to the initiation and progression of human cancer. In this talk, we will explore the molecular basis of these different mechanisms in vertebrate cells and the implications that the crosstalk mechanisms may have on our targeting these two signalling pathways in cancer.

10.30 - 11.30 am **Coffee Break and Poster Review**

SESSION 6 Chairman: **Robert Clarke**

11.30 -12.00 noon Tara Sugrue[1*], Ute Koch[1*], Christelle Dubey[1] & Freddy Radtke[1]

[1] Ecole Polytechnique Fédérale de Lausanne, School of Life Sciences, Swiss Institute for Experimental Cancer Research, CH-1015 Lausanne, Switzerland

[*] These authors contributed equally to this work.

T cell factor-1 - a key mediator in Notch1-driven T-ALL

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive cancer caused by the malignant transformation of immature thymocytes. *Notch1* gain-of-function mutations are present in >50% T-ALL cases and result in the constitutive activation of Notch1 intracellular domain (N1ICD) in mutated cells. The mechanisms by which deregulated Notch1 signalling leads to T-ALL development are currently unresolved and stringently investigated. T cell factor-1 (TCF-1) is a transcription factor encoded by *Tcf7*. Not only playing crucial roles in Wnt signalling, *Tcf7* is (i) a direct Notch1 target gene and (ii) required for driving early T lineage commitment of hematopoietic progenitors. Together, these findings suggest that *Tcf7* may play a role in Notch1-driven T-ALL. Herein, we show that the conditional loss of *Tcf7* in hematopoietic progenitors prevents T-ALL development in both genetic- and retroviral-based mouse models of N1ICD over-expression. However, T-ALL development was unaffected by conditional loss of the Wnt signalling mediator, *β-catenin.* Interestingly, conditional *Tcf7* deficiency partially restored the B lineage potential of N1ICD over-expressing bone marrow progenitors. Similar to *Tcf7*$^{-/-}$ mice, conditional *Tcf7* deficiency in bone marrow progenitors abrogated T cell development in adult thymi. However, unexpectedly, *Tcf7*-deficient hematopoietic progenitors adopted a B cell fate in these thymi, a phenotype that was found to be comparable to conditional loss of *Notch1*. Overall, our results indicate that *Tcf7* is an important mediator of Notch1-driven T-ALL. Furthermore, our data suggests that, in addition to promoting T lineage

commitment, *Tcf7* may also function as a Notch1-dependent transcriptional repressor of B lineage commitment in adult hematopoiesis. We now aim to identify the molecular players involved in *Tcf7*-mediated lineage repression using RNAseq and CHIP_Seq.

12.00 - 12.30 pm Bruno M. Simões, Ciara S. O'Brien, Rachel Eyre, Andreia Silva, Ling Yu, Aida Sarmiento-Castro, Denis Alferez, Kath Spence, Angélica Santiago-Gómez, Francesca Chemi, Ahmet Acar, Ashu Gandhi, Anthony Howell, Keith Brennan, Lisa Rydén, Julia Gee, Andrew H. Sims, Elisabetta Marangoni, Gillian Farnie, Ahmet Ucar, Goran Landberg, Sacha Howell and Robert B. Clarke

Breakthrough Breast Cancer Unit, University of Manchester, UK

Anti-estrogen resistance in human breast tumours is driven by JAGGED1/NOTCH4-dependent cancer stem cell activity

ER-positive breast cancers frequently exhibit *de novo* or acquired resistance to hormonal therapies. We observe that a 14-day in vivo 'window' treatment of ER-positive patient-derived xenograft (PDX) tumours using anti-estrogens tamoxifen or fulvestrant decrease cell proliferation but increase breast cancer stem cell (BCSC) activity. BCSC activity was determined by mammosphere formation efficiency, ALDH enzymatic activity and tumour initiation in secondary mice after treatment.

We found that increased numbers of ALDH-positive cells were correlated to increased expression of Notch target genes (HEY1 and HES1) and that JAGGED1 ligand and NOTCH4 receptor expression are key for this Notch activation. Subsequently, we used a NOTCH4 inhibitor, the gamma-secretase inhibitor RO4929097, to reverse NOTCH4 activity and abrogate the increase in CSC activity seen with anti-estrogens.

To further address the requirement for JAGGED1 and NOTCH4 genes in the acquisition of anti-estrogen resistance we used CRISPR-Cas9 technology to disrupt

these genes in MCF7 cells. Genomic alterations were confirmed and clones with either JAGGED1 or NOTCH4 gene disruption were demonstrated to have reduced BCSC activity in response to anti-estrogens using MS and ALDH assays. In support of this novel mechanism of resistance, both ALDH1 immunostaining of 322 women with ER+ breast cancer taking part in a randomised trial of tamoxifen versus no systemic treatment as well as a NOTCH4/HES/HEY gene signature in 2 ER+ patient cohorts predict for a poor response/prognosis to tamoxifen treatment.

Our findings establish that BCSC activity driven by JAGGED1/NOTCH4 predicts both *de novo* and acquired tamoxifen resistance and combining endocrine therapy with targeting NOTCH4 can potentially overcome resistance in human breast cancers.

1.00 - 2.30 pm **Lunch**

2.30 - 3.30 pm **Open Air workshops:**
Notch Therapeutics Freddy Radtke/Rob Clarke

SESSION 7 Chairman: Agamemnon Epenetos

3.30 - 4.00 pm Madonna McManus, Yanwen Yang, Jared Mortus, Rocio Rivera-Valentin, Dennis Hughes, The Children's Cancer Hospital at MD Anderson Cancer Center ,Houston, TX, USA

A novel role for Hes4 in osteosarcoma: maintenance of a pre-osteoblastic state

Osteosarcoma (OS) is the most common primary bone cancer in childhood. Like many other tumor types, OS hijacks normal cellular machinery to promote a tumorigenic phenotype. One such mechanism relies on the maintenance of a stem-like state, while avoiding terminal differentiation. The Notch signaling pathway is a well-known mediator of differentiation, and is a crucial component in normal bone development that is implicated in a number of various cancers. In some model systems, it has been shown that the Notch downstream target gene Hairy/Enhancer of Split 1 (Hes1) is associated with a more invasive and metastatic phenotype. In an OS-specific patient derived cDNA database, however, the less studied Notch target gene Hes4 was shown to be the major Notch target gene that significantly correlated with a higher incidence of metastasis in OS patients. Here we discuss a novel role of Hes4 in OS by describing its contribution to tumor progression via its regulation of differentiation.

To increase the expression of the Notch target gene Hes4, OS cells (HOS, CCHD, CCHO, SaOS2) were either transduced with a bicistronic retroviral vector encoding Hes1 plus GFP (compared to GFP only), or were stimulated with plate bound ligand (Jag1 or Dll4). To inhibit Hes4 expression, OS cells were either transduced with shRNA to Hes4, or genome-wide knockout was achieved using CRISPR/Cas9 technology. The impact of Hes4 on cell proliferation was assessed using an automated cell counter and with competitive proliferation assays. Differentiation was measured using alizarin red staining, and by quantifying changes in markers of bone stemness and differentiation (NANOG, SOX2, OCT4, podoplanin, RunX2, osterix, alkaline phosphatase, sclerostin, osteocalcin, osteopontin and ID4) using qRT-PCR. The effect of Hes4 on the formation and progression of OS *in vivo* was assessed using OS tumor xenograft models injected with either cells transduced with GFP or GFP-Hes4.

Altering the expression of Hes4 in HOS, CCHD, CCHO and SaOS2 cells does not change the *in vitro* proliferation relative to cells transduced with GFP alone.

Hes4 expression did, however, significantly inhibit differentiation. Hes4 expression also significantly increased genes related to the maintenance of mesenchymal stemness and the maintenance of an immature osteoblastic state (OCT4, RunX2, and osterix). *In vivo*, mice injected with GFP-Hes4 expressing osteosarcoma cells developed significantly larger tumors and significantly more metastases than cells expressing GFP alone. **Based on this evidence, we hypothesize that Hes4 promotes OS tumor progression, primary tumor growth, and the development of metastases by inhibiting differentiation and maintaining an immature pre-osteoblastic state.**

4.00 - 4.30 pm Sofia N Santos[1], Helen Sheldon[2], Emerson S Bernardes[1*], Adrian L Harris[2*],

[1]Nuclear Energy and Research Institute, São Paulo/SP, Brazil.

[2]Weatherall Institute of Molecular Medicine, Oxford, UK

Galectin-3 induces tumor angiogenesis by increasing Jagged-1/Notch activation and reducing DLL4 signalling

* Equal senior authors.

Endothelial cell proliferation and vessel growth is a coordinated process tightly regulated by the activation/deactivation of the Notch signaling pathway in a time, space and context dependent manner. Therefore, the identification of novel molecular regulators of Notch signaling pathway has direct implications for

the development of strategies aimed at controlling tumor angiogenesis. Galectin-3, a glycan-binding protein, is a proangiogenic molecule, which regulates endothelial cell migration, proliferation and differentiation. Because of the complexity of the angiogenic process, the molecular mechanisms behind the galectin-3 proangiogenic activity have not been fully exploited yet. Here we demonstrate for the first time that galectin-3 binds to Notch ligands Jagged1 and DLL4 in a carbohydrate- and concentration-dependent manner and enhances Notch signaling activation. Although important for the angiogenic process, VEGFR signaling is not required for the galectin-3-promoted Notch activation in endothelial cells. Interestingly, galectin-3 controls the ratio of Jagged-1 and DLL4 expression by both extending the half-life of Jagged-1 and reducing DLL4 expression. Accordingly, galectin-3-induced angiogenic sprouting is totally abrogated by Jagged-1 silencing and only partially affect by DLL4 expression. Finally, we show that hypoxia-driven changes induces the secretion of galectin-3 by tumor cells and alters the glycosylation pattern of tumor and endothelial cells, thus favoring the binding of galectin-3 in the later. As a consequence, tumor-released galectin-3 promotes Jagged1/Notch activation and enhances angiogenic sprouting. Our data reveal a novel mechanism by which galectin-3 modulates tumor angiogenesis and may thus have important functional implications for control of tumor growth.

4.30 -5.00 pm Marco Loddo[1] and Gareth H Williams[1]
Oncologica UK Ltd, Suite 15, The Science Village, Chesterford Research Park, Cambridge, UK

Companion Diagnostics for Targeted Therapies using Semiconductor Next Generation Sequencing

The linking of specific cancer genetic alterations to molecular targeted therapies is driving a new era of personalised medicine. Here we discuss the implementation of cancer precision medicine into European Cancer Networks using the Next Generation Ion PGM™ System and the Oncomine Comprehensive Panel (OCP) to detect actionable driver mutations in all major tumour types. The OCP is an integrative NGS-based assay used to detect a predefined catalogue of clinically relevant solid tumour somatic genome variants (gain-of-function or loss-of-function mutations, high-level copy number alterations, and gene fusions) coupled to a bioinformatics pipeline to specifically link these variants to a knowledge base of related potential treatments, current practice guidelines, and open clinical trials. Here we will also discuss harnessing this semiconductor "targeted" sequencing technology to develop companion diagnostics for newly developed therapies. The case example presented refers to Oncologica's Cdc7 drug development programme which targets the highly evolutionary conserved DNA replication machinery in somatic cells. The specificity of this intervention strategy is the result of abrogation of a DNA origin activation checkpoint which is dependent on several tumour suppressors, FoxO3a, Dkk3, p53, Hdm2, $p21^{Cip1}$, $p14^{ARF}$ $p15^{INK4B}$, $p27^{Kip1}$ and Rb. We are now exploring variants of these tumour suppressor genes as predictors of response to Cdc7 targeting agents using the Ion PGM™ System

5.00 -5.05 pm **Agamemnon Epenetos - Adjourn to 2016**

POSTER PRESENTATIONS

These will be displayed in the Hall area during the whole period of the conference

Helen Sheldon[1], Esther Bridges[1], Evelyn Ramberger[2], Esther Kleibuker[3], Massimo Maseiro[4] and Adrian L Harris[1]

[1]Department of Medical Oncology, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, [2]The University of Applied Sciences, FH Campus Wein, Vienna, Austria, [3]VU University Medical Centre,

Amsterdam, The Netherlands, [4]Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospital, Oxford, United Kingdom.

RHOQ – an essential mediator of the Dll4/Notch regulation of angiogenesis

Notch signalling is an essential pathway in angiogenesis. The endothelial specific ligand, Dll4 activates the Notch receptor and enables tip cell/stalk differentiation during the early stages of vessel formation. Notch signalling regulates the transcription of a number of target genes and we are interested in identifying novel targets to establish their role in vascular biology and their interactions other angiogenic signalling pathways. Using deep sequence analysis of RNA extracted from Human Umbilical Vein Endothelial Cells (HUVEC) stimulated with Dll4, we identified RHOQ as a gene which is regulated by Notch signalling. RHOQ is a member of the Rho family of small GTPases, which switch between inactive GDP-bound and active GTP-bound states and function as molecular switches in a number of signal transduction pathways. Rho proteins promote reorganization of the actin cytoskeleton and regulate cell shape, attachment, and motility. RHOQ has been shown to play a significant role in insulin-stimulated exocytosis of glucose transporters, possibly acting as the signal that turns on the membrane fusion machinery. It has also been described in neurite outgrowth and membrane expansion in developing neurons. The role of RHOQ is currently unknown in endothelial cells, so to assess its function we have knocked out RHOQ and over-expressed it using lentivirus. Although RHOQ is a Notch target gene it is also appears to be involved in the signalling process. Loss of RHOQ in HUVEC inhibited the cells ability to signal in response to Dll4. The Notch receptor is still able to cleave but its translocation to the nucleus is impaired. RHOQ co-localises with NICD and EXOC7, a member of the exocyst complex, which plays a critical role in vesicular trafficking targeting post-Golgi vesicles to the plasma membrane. Together these proteins appear to be crucial in trafficking NICD to the nucleus. Over-expression of RHOQ enabled the cells to react quicker to Dll4 stimulation with Notch target genes increasing as early as 2 hours after plating the cells onto the ligand. Consistent with a role in Notch signalling overexpression of RHOQ leads to fewer, longer sprouts whereas loss of RHOQ leads to hyper-sprouting in a hanging drop assay *in vitro*. The cells have increased VEGFR2 activity and the hypersprouting observed can be blocked with sunitinib, a VEGFR2 inhibitor. *In vivo* matrigel plugs impregnated with siRNA targeting RHOQ had a reduced vessel density compared to siControl plugs and lentiviral treatment of vessels in the

Chick Chorioallantoic Membrane (CAM) assay also resulted in dysfunctional angiogenesis. These results suggest that RHOQ may have a central role to play in Notch mediated angiogenesis and as such be a potential target in Notch inhibition strategies.

Sebastian K.-J. Landor, Niina M. Santio**, Laura Vahtera, Elina Paloniemi, Susumu Y. Imanishi, Garry Corthals, Markku Varjosalo, Ganesh babu Manoharan, Asko Uri, Urban Lendahl, Cecilia Sahlgren# and Päivi Koskinen#**

**** These authors contributed equally to this work # Shared correspondence**

Turku Centre for Biotechnology, Turku , Finland

Aberrant signaling output from key signaling pathways is linked to cancer, and deregulated Notch signaling is connected to an expanding range of tumor conditions. How Notch signaling cross talks with established drivers of tumor formation is however only partially understood. In this study, we unravel a novel interaction between Notch receptors and Pim kinases leading to Notch receptor-specific downstream effects. PIM kinases, which comprise a family of serine/threonine kinases implicated in several forms of cancer, phosphorylate Notch1 and Notch3, but not Notch2, and phosphorylation of Notch1 and Notch3 furthermore occurs in distinct regions of their intracellular domains (ICDs). Pim-mediated phosphorylation enhances nuclear localization and transactivation of Notch1, while inhibiting transactivation of Notch3. Notch1 and Pim kinase synergize in tumor progression in a cell-type dependent manner. In prostate and estrogen-dependent breast cancer cells, Pim kinase-mediated induction of cancer cell motility, a switch to a glycolytic phenotype and increased tumor growth is dependent on Notch1 phosphorylation and tumor progression in vivo is efficiently blocked by combined inhibition of Pim and Notch. Underscoring Notch receptor specificity,
Notch1 and 3 differ in their tumor-promoting abilities: Notch1 enhances tumor migration and growth, while Notch3 does not and functions as a tumor suppressor in vivo. Notch3 mediated tumor suppression in breast cancer cells is

counteracted by Pim phosphorylation. Collectively, the data demonstrate an important link between Notch and Pim kinase function, shed new light on Notch receptor-specific modulation and function, and open up new vistas for combinatorial and more targeted tumor therapy.