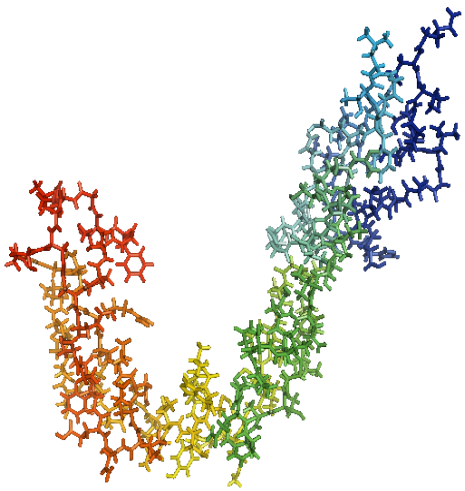
PROGRAMME



The 8th International Conference

Notch Targeting in Cancer

Grecian Park Hotel, Konnos Bay, Cyprus

20th-22nd June 2018

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Anastasis Biotec Ltd

Wednesday 20th June 2018

4.00 - 4.30pm Registration

4.30 - 4.35pm Welcome: Agamemnon Epenetos

SESSION 1 - Overview - Chairperson: Freddy Radtke

4.30 - 5.00pm Keith Brennan, University of Manchester, UK.

Highlights of the 7th Meeting, Notch Targeting in Cancer, Konnos Bay, June 2017

5.00 - 5.30pm Russell J.H. Ryan, Department of Pathology, University of Michigan, Ann Arbor, MI, USA

Notch-dependent oncogene activation in mature B-cell lymphomas

PEST domain-truncating mutations in *NOTCH1* and *NOTCH2* are recurrent in several subtypes of mature small B-cell lymphoma / leukemia, and recent studies have highlighted a likely role for similar gain-of-function Notch mutations in subgroups of large B-cell lymphoma. I will discuss our recent work using integrative transcriptional and epigenomic profiling to define the regulome of direct Notch target genes in mantle cell lymphoma models, and the relevance of these findings to other mature B-cell cancers. Significant direct Notch targets in B-cell lymphoma models include a small number of tissue non-specific canonical Notch targets, as well as apparently B-cell-specific targets that regulate signaling pathways with a

known role in lymphoma. Notch also activates *MYC* in mature B cells through a cluster of distal enhancers distinct from those used in T-lymphoblastic leukemias. I will present ongoing work directed at identifying the factors that collaborate with Notch to activate B cell-specific regulatory elements and genes, including results of high-throughput CRISPRi-based functional profiling of the multi-modular B-cell Notch-dependent *MYC* enhancer.

5.30 - 6.00pm Angélica Santiago-Gómez, Megan Thompson, Elena Spina, Francesca Chemi, Ciara O'Brien, Bruno Simões, Martin Baron, Robert Clarke
Breast Biology Group, Manchester Cancer Research Centre, University of Manchester, UK

A role for Notch4 receptor in breast cancer stem cells and their resistance to anti-oestrogen therapies

Breast cancers (BCs) typically express oestrogen receptors (ERs) but frequently exhibit de novo or acquired resistance to hormonal therapies. We have reported that short-term treatment with the anti-oestrogens tamoxifen or fulvestrant decrease cell proliferation but increase BC stem cell (BCSC) activity through JAG1-NOTCH4 receptor activation both in patient-derived samples and xenograft (PDX) tumours.

To study this mechanism further, we generated ER+ breast cancer cells with a NOTCH4 receptor gene knock out (KO). In the NOTCH4 KO cells, HEY2 was the only NOTCH target gene to be downregulated. We detected a marked increase in ER expression at both protein and mRNA levels in NOTCH4-KO cells, increased ER activation but lower BCSC activity. These results suggest that NOTCH4 signalling may regulate both ER and stemness in breast cancer cells.

A better understanding of the mechanisms that regulate NOTCH4–ER crosstalk, CSC activity and endocrine responses could help us develop new strategies to overcome endocrine therapy resistance and avoid breast cancer recurrence.

6.00 - 6.30pm R. Sierra[1], J. Trillo-Tinoco[1], E. Mohamed[1], L. Yu[2], B. R. Achyut[3], A. Arbab[3], J.W. Bradford[4], F. Hossain[2], A. A. Epenetos[5], 5B.A. Osborne[6], L. Miele[2] and P.C. Rodriguez[1]

1 H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida.

2 Louisiana State University Health Sciences Center, New Orleans, Louisiana.

3 Georgia Cancer Center, Augusta University, Augusta, Georgia.

4 Department of Biological Sciences, Augusta University, Augusta, Georgia.

5 Anastasis Biotec Ltd

6 Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts.

Jagged Monoclonal Antibodies Inhibit MDSCs and Promote Tumor Immunity

Myeloid-derived suppressor cells (MDSC) are produced in large numbers in cancer patients and are recruited to the tumor microenvironmnent, where they promote chronic inflammation and inhibit adaptive cancer immunity. MDSC are a major obstacle to cancer immunotherapy. Despite significant effort, pharmacological tools to block their immune-suppressive activities are still lacking. MDSC have been reported to have low Notch activity, due to Notch phosphorylation by Casein Kinase 2. We determined that MDSC in tumor microenvironment increase the expression of Notch ligands Jagged1 and 2, while they decrease the expression of DLL4 compared to circulating MDSC. This increase in Jagged expression is mediated by NF-kB activity, perhaps stimulated by inflammatory cytokines in tumor microenvironment. We assessed the therapeutic effect of the humanized anti-Jagged1/2-blocking antibody CTX014 on MDSC-mediated T-cell suppression in tumor-bearing mice. CTX014 decreased tumor growth, affected the accumulation and tolerogenic activity of MDSCs in tumors, and inhibited the expression of immunosuppressive factors arginase I and iNOS. Jagged monoclonal antibody treatment overcame tumor-induced T-cell tolerance, increased the infiltration of reactive $CD8^+$ T cells into tumors, and enhanced the efficacy of T-cell-based immunotherapy. Depletion of MDSC-like cells restored tumor growth in mice treated with anti-Jagged. CTX014 promoted the appearance of $CD11c^+$ MDSC-like cells that acted as antigen-presenting cells. Co-injection of MDSC-like

cells from anti-Jagged-treated mice with cancer cells delayed tumor growth. We explored the mechanism of anti-Jagged antibody effects on MDSCs. The effect does not appear to be due to Notch inhibition within MDSCs, as pre-treatment of MDSCs with cell-penetrating Notch antagonist peptide Syntana-4 increased their immune-suppressive activity, consistent with the literature on the role of Notch in MDSC. We hypothesize that anti-Jagged monoclonal antibodies suppress cis-inhibition in MDSCs, thus promoting Notch activation by DLL family ligands, and/or act indirectly by suppressing Notch activity in tumor cells, which then decreases expression of MDSC-promoting cytokines. We are currently testing this hypothesis. Our results offer preclinical proof of concept for the use of anti-Jagged1/2 to reprogram MDSC-mediated T-cell suppression in tumors, as a first-in-class anti-MDSC monoclonal antibody agent.

8.00 - 10.00pm ***Welcome Reception Cocktail and Canapes***

Thursday 21st June 2018

SESSION 2 - Stem and progenitor cells - Chairpersons: Rob Clarke

9.00 - 9.20am **Phil Jones**, Wellcome Sanger Institute**,** Wellcome Genome Campus Hinxton, Cambridge CB10 1SA, UK

Strong evolutionary selection of NOTCH1 mutants in oesophageal pre-cancer

Somatic mutations in normal tissue form the landscape from which cancer develops. We mapped mutations in 74 cancer driver genes in normal oesophageal epithelium from 9 organ transplant donors aged 22 to 75 years. The density of mutant clones rose with age from 200/cm2 in donors in their 20's to 750/cm2 in the oldest subjects. The mutational signatures were those of aging, indeed there was no difference in the burden of mutations in smokers compared with non-smokers. We found 13 genes under genetic selection, the most frequent being NOTCH1 that colonized over 1/3 of the epithelium by 40 years of age. NOTCH1 missense mutants were clustered in EGF repeats 8-12, and include codon

alterations in amino acids that contact JAGGED1. All clones in which heterozygosity could be determined exhibited LOH. Most strikingly, the prevalence of NOTCH1 mutation in normal tissue exceeds that in squamous cell carcinoma several fold, suggesting it may decrease the likelihood of malignant transformation, challenging the assertion that NOTCH1 inactivating mutants are cancer drivers.

9.20 - 9.40am Cecilia Sahlgren, Cell Fate Lab, Åbo Akademi University, Tykistökatu 6 20520 Turku, Finland

Notch3 RAM domain phosphorylation by PIM-kinases regulates RBPJκ binding affinity and breast cancer tumorigenicity

The dichotomous modus operandi of the Notch juxtacrine signaling pathway, with both canonical and non-canonical outputs is still poorly understood. Post-translational modifications (PTMs) are emerging as important nodes for dynamic fine-tuning of Notch, and we have previously shown PIM-kinases to phosphorylate Notch1 thus regulating its nuclear localization and transcriptional activity. Here we expand on the paradigm and show implications of Notch3 phosphorylation by PIM for non-canonical Notch3 output. Notch3 is phosphorylated by PIM at the RAM domain at the -3 position from the WxP RBPJκ binding motif, and this event interferes with RBPJκ binding and transcriptional activity. The phospho-deficient mutant form of N3ICD binds RBPJκ robustly, while the binding potential of the phospho-mimicking mutant is severely compromised. Phosphorylation of Notch3 sustains cellular respiration and glycolysis and enhances cell viability. Knocking out Notch3 with CRISPR abolishes tumor formation in vivo, however, phospho-deficient N3ICD consistently lowers tumor growth while the phospho-mimicking NI3CD supports it. Our data reveals a novel PTM-mediated switch between canonical and non-canonical Notch3 signaling and demonstrate that PIM mediated phosphorylation of Notch3 supports tumor metabolism and growth.

9.40 - 10.00am Simona Hankeova[1,2], Kerstin Seidel[3], Indira V Chivukula[4], Aiman Elmansuri[1], Vitezslav Bryja[2], Urban Lendahl[5], Christian W. Siebel[3], Emma R Andersson[1,5]

[1] Dept of Biosciences and Nutrition, Karolinska Institutet, Sweden

[2] Institute of Experimental Biology, Faculty of Science, Masaryk University, CZ-625 00, Brno, Czech Republic

[3] Department of Discovery Oncology, Genentech Inc., USA

[4] Integrated Cardio Metabolic Centre, Karolinska Institutet, Sweden

[5] Dept of Cell and Molecular Biology, Karolinska Institutet, Sweden

Notch is required for liver tumor development - implications for Alagille syndrome

Hepatocellular carcinoma (HCC) is the most common form of liver cancer in adults, usually caused by infection or cirrhosis, and is increasing world-wide. In children, HCC is relatively rare, but pediatric risk factors, including infection or genetic factors dramatically up-regulate the childhood risk of acquiring HCC. Unfortunately, the 5-year survival for patients with HCC is as low as 28%, and drops rapidly to 2% with metastases, making this an area urgently in need of research to discover effective treatment options. Based on a plethora of case reports, it has been suggested that Alagille syndrome, a congenital liver disease caused by mutations in the Notch ligand *JAGGED1*, is a risk factor for developing HCC. Chronic liver disease is of course a significant risk factor for HCC, but the role of Jagged1 in this is unclear, where both up-regulation and down-regulation of *Jag1* have been described. It is therefore currently controversial what the role of Jag1/Notch in liver malignancy is.

We recently developed a mouse model for Alagille syndrome, based on a missense mutation in *Jag1*, that recapitulates bile duct paucity and cholestasis, with adult recovery. Using this mouse model, we are testing whether *Jag1* abrogation causes patients to be prone to, or protected from, developing liver tumors, and determining the underlying mechanisms driving risk. HCC development is being correlated with the Notch signature of tumors arising in the mice. Our ongoing work indicates that Notch signaling is required for liver tumor development, and

future work will aim to determine the characteristics of liver tumors most responsive to Notch inhibition.

10.00 - 10.20am Giulia Bottoni[1,2*], Atul Katarkar[2*], Andrea Clocchiatti[1,3], Sandro Goruppi[1,3], Pino Bordignon[2], Giovanna Chiorino[4], Paola Ostano[4], Fabio Tordini[4], Francesca Lazzaroni[2], Csaba Lazlo[2], Seung-Hee Jo[1,3], Thomas Lunardi[5], Joachim Lingner[5], Victor Neel[6], and G. Paolo Dotto[1,2,7+]

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[2]Department of Biochemistry, University of Lausanne, Epalinges 1066, Switzerland
[3]Department of Dermatology, Harvard Medical School, Boston, MA 02125, USA
[4]Cancer Genomics Laboratory, Edo and Elvo Tempia Valenta Foundation, Biella 13900, Italy
[5]Swiss Institute for Experimental Cancer Research, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Lausanne 1015, Switzerland.
[6]Department of Dermatology, Massachusetts General Hospital, Boston, MA 02114, USA.
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* equally contributed to this work
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Dual role of CSL and NOTCH1 in genomic stability and DNA damage response of stromal fibroblasts and tumor-promoting CAFs

Genomic instability is a hallmark of cancer. Whether it also occurs in cancer-associated fibroblasts (CAFs) is a question of importance that remains to be settled. Loss of CSL/RBPJ, the effector of canonical Notch signalling with intrinsic transcription repressive function, results in early steps of CAF activation. In parallel, we find that CSL down-modulation in stromal fibroblasts causes DNA damage,

telomere shortening and chromosome fusions that also occur in clinically-derived CAFs in which CSL is down-modulated. Inversely to CSL, the NOTCH1 gene is amplified and overexpressed in CAFs and required to suppress DNA damage-induced ATM/p53 activation and growth arrest. The findings are of translational significance, as genetic or pharmacological suppression of Notch1 activity suppresses CAF proliferation and, in an orthotopic model of skin squamous cell carcinoma, suppresses cancer/stromal cell expansion.

10.20 - 11.00am Coffee Break

SESSION 3 - Notch, development, and differentiation - Chairpersons: Jon Aster

11.00 - 11.20am Diana Bellavia[1], Saula Checquolo[2], Rocco Palermo[1], Isabella Screpanti[1].

[1]Department of Molecular Medicine, Sapienza University of Rome - Italy;

[2]Department of Medico-Surgical Sciences and Biotechnology, Sapienza University of Rome – Italy

Multifaceted targeting of Notch3: an added value to cancer therapy

Notch signaling is considered a rational target in the therapy of several cancers, particularly those harbouring Notch gain of function mutations, including T-cell acute lymphoblastic leukemia (T-ALL). Although among Notch receptor paralogues Notch1 is mainly reported bearing activating mutations in either T-ALL and different solid tumors, more recently Notch3 expression and activity deregulation has been recognised to drive oncogenic signals in cancer cells of various origins, including breast, lung, ovary and colon, as well as T lymphoid cells. Notably, while several data have been reported in literature demonstrating that Notch1 can directly activate *NOTCH3* expression in T-ALL cells, in which *NOTCH1* often displays activating mutations, the same has not been clarified for solid tumors (i.e. lung and

breast cancers), where *NOTCH1* activating mutations are rarely observed, despite the overexpression and/or the constitutive activation of Notch3.
In addition, after our first observation that constitutive activation of Notch3, sustained by the enforced expression of the constitutively active intracellular domain of Notch3, *N3ICD*, was able to induce an aggressive T-ALL in mice, a number of reports suggested a role for Notch3 in human T-ALL. More importantly, activating mutations of *NOTCH3* have been recently identified in primary T-ALL patient-derived xenografts, suggesting the possible existence of a human T-ALL cluster characterized by *NOTCH3* mutations, and in a number of cell lines derived from different human solid cancers. In addition, Notch3 expression deregulation has been identified as a key factor in sustaining uncontrolled Cancer Stem Cell self renewal and/or expansion and in characterizing poor prognosis and chemoresistance in several solid tumors.
Together these observations suggest that deregulated expression and/or activating mutations of *NOTCH3* may behave as either oncogenic drivers or supporters of drug resistance and suggest the targeting of Notch3 as a compelling additional anticancer therapeutic possibility to overcome chemoresistance and to reinforce conventional therapies.
In our past and recent studies we approached this issue by characterizing the role of Notch3 and interconnected pathways in development and progression of T-ALL and utilizing direct and indirect Notch3 targeting. During the presentation, our most recent findings concerning the role of Notch3 targeting in T-ALL and some solid tumors will be discussed.

11.20 - 11.40am Mateusz Antonszweski[1], Ute Koch[1], Delphine Harduin, Linlin Cao, Nadine Zangger[1], Antoine Chabloz[1] Christelle Dubey[1] & Freddy Radtke[1]

[1] Ecole Polytechnique Fédérale de Lausanne, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland

Notch signalling in T-ALL and CLL

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological cancer caused by the malignant transformation of immature thymocytes. The malignancy occurs more frequent in children then adults. *Notch1* gain-of-function mutations are present in >50% T-ALL cases resulting in the activation of Notch1 intracellular domain (N1ICD), leading to aberrant Notch1 signalling in thymocytes. Another hematological malignancy in which Notch gain-of-functions are found is Chronic lymphocytic leukemia (CLL). CLL is the most common adult B-cell malignancy in the Western World. Notch1 gain-of-function mutations are found in 10-15% of CLL patients at diagnosis and increase to 30% in relapse and/or refractory patients. It is currently unclear why overall survival of patients with Notch-gain-of-function mutations is reduced compared to patients with wild-type Notch alleles. Whether Notch deregulates growth in these two different hematological malignancies through similar mechanisms is currently unknown.
Using mouse models for T-ALL and CLL we will try to address some of these issues. Moreover, we will discuss potential resistance mechanisms of Notch inhibitors in T-ALL.

11.40 - 12.00pm Eva Zacharioudaki, Julia Falo SanJuan, Sarah Bray
Department of Physiology Development and Neuroscience, University of Cambridge, UK

Mechanisms limiting the progression of Notch induced neural stem cell derived tumours.

Many solid tumours (e.g glioblastomas, neuroblastomas) are comprised of heterogeneous cell populations, which include cancer stem cells (CSCs). These drive tumour growth by deploying programs normally used by stem cells for tissue development. However, the mechanisms underlying CSC emergence during tumorigenesis remain elusive. *Drosophila* neural stem cells, NSCs, are a simple model to study the onset of tumorigenesis, dividing asymmetrically so that one daughter cell retains NSC identity and the other is routed to differentiate. Perturbations like excessive Notch signalling result in aberrant NSC proliferation,

ultimately driving hyperplasia and tumour formation. Examining the evolution of hyperplasia over time reveals that there is a significant delay in the emergence of the CSCs. Using NSC cultures, we have monitored live the expression of an early NSC marker in NSCs and their progeny in normal conditions and at the onset of Notch driven hyperplasia. Our results reveal that the CSCs arise from a reactivation of the NSC programme in progeny, rather than an expansion of the NSCs themselves. This suggests that the normal process of NSC fate attenuation is initiated but subsequently suppressed. Screening for factors that overcome this attenuation (focusing on genes that are frequently mutated in glioblastoma and neuroblastoma), we find a critical role for a chromatin remodelling complex and show that in its absence, Notch activity drives the NSC daughter cell towards the CSC fate. These results illustrate the importance of "enhancer decommissioning" in the stem cell progeny and highlight ways that second site mutations can exacerbate signal addiction in tumour cells.

12.00 - 12.20pm Elena Parmigiani, Verdon Taylor and Claudio Giachino
Department of Biomedicine, University of Basel, Switzerland

Notch signalling inhibition in glioma cells alters the tumour microenvironment and disease progression

Notch signalling is believed to be oncogenic in glioma, primarily by virtue of its stem cell promoting activity. However, surprisingly, inactivating mutations in Notch pathway components have been identified in glioma subtypes (Bai et al., 2015; Brat et al., 2015; Suzuki et al., 2015) and Notch inhibition dramatically accelerates tumour progression in mouse models of some forms of the disease (Giachino et al., 2015).
Here, we investigated the mechanisms underlying the tumour suppressive function of Notch in glioma. In order to identify pathways that are involved in gliomagenesis and affected by Notch, we combined conditional genetics in mouse models of glioma with fluorescent activated cell sorting, and established RNA-seq

gene expression profiles of Notch-signalling-depleted tumours. We found that Notch inhibition in tumour cells downregulates expression of genes associated with quiescent neural stem cells and releases expression of genes associated with stem cell activation and cell cycle progression, thereby promoting an active proliferative state. Unexpectedly, blocking Notch also downregulates genes associated with recruitment and activation of immune cells, resulting in impaired microglia activation and a hampered immune response. Interestingly, individual Notch receptors have distinct functions during glioma development, and only specific Notch receptors or receptor combinations can activate a tumour suppressive signal.
Our data indicate that Notch suppresses glioma formation by regulating both tumour cell proliferation and interaction with the tumour microenvironment, and that distinct Notch receptor paralogues are differently engaged in glioma progression.

12.20 – 12.40pm Aleksandra Filipovic, Imperial College London, UK and Puretech ,Boston, USA

Puretech open innovation model of bringing life to science: venture creation of breakthrough academic science

Abstract awaited

12.40 – 2.30pm Lunch Break

2.30 – 3.30pm Open Air workshop: Co-ordinators Jon Aster and Freddy Radtke
Topic: The Yin and Yang of Notch in Cancer

SESSION 4 - Biomarkers and Consequences of Notch Activation in Cancer - Chairperson: Rajwinder Lehal

4.00 - 4.20pm **Juan Rodriguez-Vita and Andreas Fischer,** Vascular Signaling and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany.

Endothelial Notch signaling as a therapeutic target in Cancer.

Endothelial cells (ECs) do not only form new blood vessels but also provide angiocrine factors orchestrating tumor progression. We have recently shown that Notch1 receptors are frequently activated (N1ICD) in ECs of human carcinomas and melanoma, and in ECs of the pre-metastatic niche in mice. EC N1ICD expression in melanoma correlated with shorter progression-free survival. Sustained N1ICD activity induced EC senescence, expression of chemokines and the adhesion molecule VCAM1. This promoted neutrophil infiltration, TC adhesion to the endothelium, intravasation, lung colonization and postsurgical metastasis. Thus, sustained vascular Notch signaling facilitates metastasis by generating a senescent, pro-inflammatory endothelium.
Consequently, treatment with Notch1 (provided by Dr. Christian W. Siebel, Genentech) or VCAM1-blocking antibodies prevented Notch-driven metastasis, and genetic ablation of EC Notch signaling inhibited peritoneal neutrophil infiltration. This shows that the inhibition of endothelial Notch activation would be an interesting therapeutic strategy to explore. However, recent studies from our group showed that inhibition of EC Notch signaling by genetic ablation of Rbp-jκ led to reactivation of angiogenesis in adult mouse hearts. In addition, EC Notch inhibition diminished expression of fatty acid transport genes and thus impaired the breakdown of triacylglycerols and transendothelial transport of long-chain fatty acids to muscle cells. The attenuated supply of cardiomyocytes with long-chain fatty acids was accompanied by higher glucose uptake, mTOR-S6K signaling and eventually resulted in cardiac hypertrophy and heart failure. The latter was also observed upon treatment of mice with Dll4 blocking antibodies (provided by Dr. Minhong Yan, Genentech).

Lastly, we aimed to overcome this limitation. Displacing glucose as an energy substrate by feeding a ketogenic diet improved heart function and prolonged survival of EC-specific Rbp-jκ-mutant mice.

4.20 - 4.40pm **Jon Aster,** Department of Pathology, Brigham and Women's Hospital Boston, USA

Biomarkers of Notch Expression and Activation in Cancer

Although Notch has diverse context-dependent roles in cancer, it is notable that responses to Notch pathway inhibitors in preclinical models and clinical trials have been restricted to tumors with strong gain-of-function mutations in Notch receptor genes. These experiences emphasize the need to develop and implement reliable biomarker tests in order to identify cancers that are likely to respond to Notch-directed therapeutics. Challenges to sensitive and specific test development include the diverse variety of genetic aberrations that produce oncogenic Notch signaling and the highly context-dependent nature of Notch target genes, even among cancers in which Notch signaling is oncogenic. This talk will update the current state of biomarker tests directed at Notch and will discuss new approaches that aim to fill gaps in our diagnostic armamentarium.

4.40 - 5.00pm **Armelle Logié-Dishington, Daniel Ciznadija, Mike Ritchie**
Champions Oncology, Inc., Hackensack, New Jersey , USA

Leveraging Champions Tumorgraft™ models to assess the activity of Notch targeted agents.

While patient-derived xenografts (PDXs) offer a powerful modality for translational cancer research, very few in vivo models have been identified to assess the activity of agents targeting the Notch pathway. The purpose of this study was to select

appropriate models in the Champions Oncology bank of >1,000 patient-derived xenograft (PDX) models to help guide Notch targeting drug development strategies.

Tumors obtained from surgical or biopsy procedures from over 1000 cancer patients with a variety of solid tumors were implanted into immunodeficient mice. Whole-exome sequencing, RNA sequencing and immunohistochemistry was carried out to analyze the expression level, mutational status and localization of Notch 1 protein. Through the use of these analyses, three categories of Notch PDX models, were identified: expression of all four receptors, expression of less than four receptors and models showing little to no expression of any NOTCH receptors. We also identified mutations and copy number variations in NOTCH receptor family across major tumor cohorts. Finally, IHC data were generated in a small subset of models showing various level of expression of Notch 1.

Subsequent pharmacology studies, assessing the *in vivo* activity of these Notch agents, are planned and will aid in the development of clinical stratification strategies for Notch-targeting therapies.

5.00 - 5.15pm **BRIEF POSTER PRESENTATION**

6 minutes presentation

- **Title and Introduction**
- **Methods and Results**
- **Summary and conclusion**

4 minutes Q+A

Megan Thompson, Angélica Santiago-Gómez, Martin Baron, Robert Clarke,
The University of Manchester, Manchester Cancer Research Centre, 555 Wilmslow Road, Manchester, M20 4GJ

Investigating Notch4 signalling mechanisms to better inhibit stem cell activity in endocrine therapy-resistant breast cancer

Breast cancer stem cell (BCSC) activity is enhanced following anti-estrogen treatment of oestrogen receptor-positive (ER+) breast cancer, potentially leading to endocrine therapy resistance. Notch4 receptor signalling is highly activated in these BCSCs, linking Notch4 activity to endocrine therapy resistance. In Drosophila, the Notch Ax(E2) mutant causes a switch from ligand-dependent to ligand-independent Notch activation. The human Notch4 possesses the same residue change, diverging from Notch1/2/3. It is hypothesised that further Notch4 Ax class-like mutations selected during endocrine treatment will enhance ligand-independent Notch trafficking, increase the BCSC population and drive endocrine resistance.

Analysis of online genomic databases of breast cancer has identified 34 Notch4 mutations in primary breast cancers (0.95% of samples analysed) and 30 in metastatic breast cancers (2.04%), with 20 exclusive to metastatic. Investigation of BCSC activity in a Notch4 CRISPR knockout cell line has shown decreased BCSC activity, with a more pronounced effect after endocrine therapy. Interestingly, the Notch4 knockout cell line shows increased proliferation in estrogenic conditions, suggesting an interaction between the Notch4 receptor and ER signalling.

We aim to explore Notch4 receptor signalling mechanisms using Notch decoys, a Notch4 therapeutic antibody and the knockout/overexpression of Notch4 and trafficking proteins. Exploration of Notch4 trafficking will be carried out using immunofluorescence to observe the ligand dependent and independent activation pathways. The involvement of the four human Deltex proteins in the ligand independent pathway will also be explored. Notch4 mutations in breast cancer and their effects on trafficking will be investigated using CRISPR gene modification and insights from Drosophila studies. The ultimate aim is the identification of a therapeutic target to reduce Notch4 activity to be used in combination with endocrine therapy.

8.30pm until late Conference Dinner

Friday 22nd June 2018

SESSION 5 - Ligand-Receptor Interactions - Chairpersons: Penny Handford

9.00-9.20am **Martin Baron,** School of Biological Sciences, University of Manchester, Manchester, UK

Shaping Notch signalling responses through an endocytic regulatory network

Understanding the connections between genotype and phenotype remains an important and challenging research problem in biology and medicine today. The *Notch* gene in *Drosophila,* encodes a highly modular developmental signalling receptor with widespread roles in development and adult tissue homeostasis. In *Drosophila,* historical studies have revealed the complex genetic architecture of *Notch*, comprising a diverse allelic series with intriguing tissue specificities and varying degrees of Notch loss or gain of function. More recently this genetic complexity is being recapitulated by 100's of human Notch mutations, uncovered

through cancer genome sequencing. This diverse collection of mutations now provides an important resource to probe Notch structure/function and regulation. However much of this diversity is not easily interpretable at a structure/function level in terms of straightforward alterations to the core canonical Notch activation mechanism. Work in my group, combining genetic, cell biological and computational approaches, has revealed how a network of endocytic trafficking routes, including novel ligand-independent signal activation mechanisms, acts to tune Notch signalling levels in response to environmental and other signalling inputs by directing Notch to different endocytic destinations associated with its activation or down regulation. We have further found that different Notch misense mutants "postcode" Notch to different endosomal subdomains, helping to provide some new mechanistic understanding of their different phenotypic outcomes.

9.20 - 9.40am Qianqian Ming[1], Elliot Medina[1], Byoung Choul Kim[2,3], Chenghao Ge[4], Suzanne Furuyama[5], Paulo C. Rodriguez[1], Irwin D. Bernstein[5], Cheng Zhu[4], Taekjip Ha[2,3], K. Christopher Garcia[3,6], Vincent C. Luca[1]

[1]Moffitt Cancer Center, Department of Drug Discovery, Tampa, FL, USA.
[2]Johns Hopkins University, Department of Biomedical Engineering, Baltimore, MD, USA.
[3]Howard Hughes Medical Institute, Baltimore, MD and Stanford, CA, USA.
[4]Georgia Institute of Technology, Department of Biomedical Engineering, Atlanta, GA, USA.
[5]Fred Hutchinson Cancer Research Center, Department of Pediatric Oncology, Seattle, WA, USA.
[6]Stanford University School of Medicine, Department of Molecular & Cellular Physiology, Stanford, CA, USA.

Modulating Notch activity through structure-guided protein engineering

Individual Notch receptors may have carcinogenic or tumor suppressive roles depending on cellular context, necessitating the development of specialized biologics with exquisite specificity. Furthermore, Notch activation is critical for T cell development and has been shown to improve the outcome of T cell-based cancer immunotherapy. We are targeting Notch receptors using sophisticated

engineering strategies that incorporate structure-guided protein design and directed evolution. We hypothesize that the structural and biophysical parameters governing Notch receptor-ligand interactions determine their downstream effector functions and that these properties can be tuned using ligands with altered affinity and selectivity profiles. By investigating the molecular requirements for Notch activation, we hope to pave the way for the generation of a diverse "toolbox" of engineered ligands for use as cancer therapies.

Essential to developing an understanding of Notch signaling is the structural characterization of receptor-ligand interactions. Until recently, the intrinsically low binding affinity between receptors and ligands prevented reconstitution of stable complexes for structural studies. We overcame this obstacle by using in vitro evolution to affinity-mature the interaction between Notch receptors and ligands, allowing us to capture the complexes for co-crystallization**.** Our approach led to the determination of crystal structures of the interacting domains of Notch1-DLL4 and Notch1-Jagged1, which provided us with unprecedented access to the molecular details of the interface. The success of our strategy puts us in a unique position to study Notch activation by additional ligands, and to use our newly acquired structural information as a template for rational ligand engineering. Through these studies, we hope to address the following questions: (1) how do the biophysical properties of DLL and Jagged ligands regulate their function? (2) how can biologics be designed to antagonize Notch signaling on specific cell types? (3) what is the optimal strategy for activating Notch to enhance the antitumor activity of adoptively transferred T cells?

9.40 - 10.00am Boguslawa Korona[1], Yao Meng[1], Richard Suckling[2], Pat Whiteman[1], Thomas Rowntree[1], Christina Redfield[1], Susan Lea[2] and Penny Handford[1]

1 Department of Biochemistry & 2 Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, UK

NOTCH RECEPTOR/ LIGAND ARCHITECTURE AND INTERPLAY WITH LIPID.

Recent data have increased our knowledge of the molecular basis of the Notch receptor/ligand interaction. It is now known that Notch EGF 11-12 interact with the DSL and C2 domains of ligand to form a complex stabilized by protein-protein, as well as protein-O-glycan interactions, and additional ligand-specific interactions occur along the longitudinal axis of the complex. New structures of the N-terminal region of hJagged-2, and hDll-4 together with previously published structures of hJagged-1, rat Dll-4 and hDll-1, suggest that membrane interactions with the C2 domain are likely to be important in optimizing the Notch signal. We demonstrate that the Delta and Jagged ligand families show different preferences for ganglioside- or sphingomyelin-rich liposomes, and ligand mutations associated with genetic and acquired disease can affect membrane recognition and reduce Notch activation in cell-based assays. These data suggest that lipid binding is important for optimizing ligand-dependent Notch signaling, and changes to the membrane environment have the potential to modulate the Notch signal in normal and pathological states.

Email address: penny.handford@bioch.ox.ac.uk; **Keywords:** EGF domain, C2 domain, Lipid, O-glycans,

10.00 - 11.00am Coffee Break

SESSION 6 - Immuno-oncology. Chairpersons: Aleksandra Fillipovic

11.00 - 11.20am **Keith Brennan, Abigail Edwards,** University of Manchester, Manchester, UK

Targeting IL-1 signalling in Notch-driven breast cancer

Despite significant advances in recent years, there remains considerable demand for the development of novel breast cancer therapeutics. Current treatment options are especially limited for triple negative breast cancer patients, and for patients with therapy resistant breast cancer. The Notch signalling pathway has been found to be aberrantly activated in breast cancer, and is correlated with poor prognosis and therapy resistance. Therapeutic inhibition of the Notch signalling pathway is an attractive approach in breast cancer treatment, however pan Notch inhibition is associated with significant side effects. As more specific Notch inhibition has been shown to reduce the risk and severity of these side effects, there is a rationale for identifying and targeting signalling events downstream of the Notch receptor. Our group has found that Notch signalling induces the expression of the cytokine IL-1α in breast cancer epithelial cells, which results in the autocrine activation of pro-survival Akt signalling. IL-1 signalling inhibitors are well tolerated and have an extensive safety record in the clinic, presenting an opportunity for the re-appropriation of IL-1 inhibitors to target Notch/IL-1α signalling in breast cancer treatment. Here I present data which demonstrates the importance of IL-1 signalling in the breast cancer cell phenotype, including in apoptosis resistance, invasion and xenograft tumour formation, which supports the hypothesis that Notch/IL-1 signalling could be therapeutically targeted in the treatment of breast cancer.

11.20 - 11.40am Deniz A. Ucar-Bilyeu[1,2], Margarite D. Matossian[3], Van T. Hoang-Barnes[4], Fokhrul M. Hossain[1], Mohit Gupta[4], Hope E. Burks[2], Thomas D. Wright[4], Jane Cavanaugh[5], Patrick Flaherty[5], Matthew E. Burow[3], Lucio Miele[1,2].
[1]Louisiana Cancer Research Center, [2]Department of Genetics, New Orleans LA,
[3]Tulane University School of Medicine, Department of Medicine, New Orleans LA
[4]National Cancer Institute, Laboratory of Cell and Developmental Signaling, Frederick, MD, [5]Duquesne University, Mylan School of Pharmacy, Department of Pharmacology, Pittsburgh, PA

Targeting Notch From One Notch Above

Triple negative breast cancer (TNBC) is a molecularly heterogeneous, clinically aggressive disease group that is highly prevalent among African-Americans and younger patients. Standard chemo/radio therapy often produces clinical responses, but recurrence and metastasis are unfortunately common. Metastatic disease is generally incurable. Chemo/radiotherapy has been shown to induce EMT and enrich a chemo-resistant cancer stem cell-like (CSC) population in TNBC. CSCs are thought to drive disease recurrence. Notch signaling, particularly Notch1, is critical for maintenance of CSC in many TNBCs. Expression of Notch1 and its ligand Jagged1 are correlated with poor prognosis. Efforts to pharmacologically target Notch directly have been impaired by the systemic toxicity of the Gamma Secretase Inhibitors (GSI) used, and by the fact that Notch1 also plays a key role in anti-tumor adaptive immunity. Therapeutic agents that target Notch signaling in breast cancer cells indirectly and selectively are a potentially attractive strategy. However, no such target has been identified to date. We have found that the MAPK5-ERK5 kinase pathway, which contains at least two druggable targets, functions as a master regulator of Notch signaling in TNBC cells. ERK5 knockout TNBC cells have dramatically decreased expression of Notch receptors, ligands and targets. In vivo, these cells form barely detectable tumors that do not metastasize and express nearly undetectable levels of Notch1 and its ligand Jagged1. Re-expression of Notch1-IC in ERK5 knockout cells restores the CD44hi/CD24lo CSC population. Using in silico screening, we have identified a small molecule compound, SC-181, that targets MAP2K5 (MEK5) and decreases phosphorylation of MAPK7 (ERK5). Expression of ERK5 is associated with poor prognosis in TNBC. Consistent with results in ERK5KO cells, suppression of ERK5 phosphorylation decreased the amount of Notch1 and Jagged1 proteins and mRNAs. More importantly, SC-181reversed EMT and reduced the $CD44^{hi}/CD24^{lo}$ CSC population in TNBC cells without suppressing T-cell proliferation. Treatment with nanomolar concentration of this compound decreased the number and size of mammospheres in a dose-dependent manner. Our preliminary results suggest that targeting the MEK5-ERK5 pathway is a promising strategy to selectively target Notch signaling in TNBC CSC without systemic Notch inhibition.

11.40 - 12.00 Hong Qiu[1], Patrick M. Zmina[3], Alex Y. Huang[2], David Askew[2], Barbara Bedogni[1,3*]

[1]Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106; [2]Department of Pediatrics, Case Western Reserve University, Cleveland, Ohio 44106; [3]Department of Dermatology, Miller School of Medicine, Miami, FL 33136.

Inhibiting Notch1 enhances immunotherapy efficacy in melanoma by preventing Notch1 dependent immune suppressive properties

We have previously shown that Notch1 plays a critical role in modulating melanoma tumor cell growth and survival. Here we show that Notch1 also contributes to an immune-suppressive tumor microenvironment (TME). Notch1 inhibition reduces immune suppressive cells (i.e. MDSCs and Tregs) while allowing the recruitment of functional CD8(+) T cells, leading to a decrease in the Tregs/CD8(+) ratio, a key parameter in assessing positive responses to immune-checkpoint inhibitors. Inhibition of Notch1 improves the antitumor activity of nivolumab and ipilimumab, particularly when given in combination. Mechanistically, tumor-associated Notch1 regulates the expression of several chemokines involved in MDSCs and Tregs recruitment. Among them, CCL5, IL6 and IL8, or MIP2 in mouse, were consistently reduced by Notch1 depletion in several human and mouse melanoma cell lines. Notch1 controls the transcription of IL8 and IL6; and the secretion of CCL5 likely by inhibiting the expression of SNAP23, a member of the SNAREs family of proteins involved in cell exocytosis. Inhibition of SNAP23 decreases CCL5 secretion similarly to Notch1 inhibition. Hence, targeting Notch1 would affect both melanoma intrinsic growth/survival properties, and provide an immune-responsive TME, thus improving immune therapy efficacy.

12.00 - 12.20pm **Laurence Cohen**, Longacre Funding Partners LLP, London UK

Exploitation of inventions and developments

Background- I spent 40 years as a solicitor in private practice dealing with the exploitation of IP in major law firms. After retiring at the end of 2014, I have run a consultancy which is linked to a fundraiser for funding new developments across the Phase 2 clinical trials which is commercially where most truly promising developments flounder for lack of funding.

In my presentation I review the life cycle of a new biotech invention (and what I say is equally applicable to pharma and medical devices). It starts with making the invention, and procuring ownership. Often those seeking help do not have the proper rights. Without that, serious money will not touch it.

Then what comes next. The only question for investors (who despite what they may say) are rarely truly altruistic. Will it make money? What is the market opportunity ? Is there a real commercial gap in the market ? What is the market size ? Is it worth the effort ? Orphan drugs are often interesting because of speed to market, but unless they morph into mainline treatments they often fail to command large premiums. Best is meeting a significant unmet need.

Next is assembling the team. Running a development program for a biotech product is not all about the science- it is about running a business. The development program needs to be clear, but so do the commercial aspects. A well run corporate vehicle inspires confidence; a poorly run organization shows, and will tend to get funded at a discount. Hence, even if they are not full time, get lawyers and accountants, and regulatory experts to help you. Especially in the early days, you can get these services for free, but for stock or shares later. There is huge goodwill towards getting biotech developments off the ground. Some of this will come from established firms, but much can come from retirees, who have huge underused skills.

Patents are vital to protect the development. Patent coverage needs to be comprehensive and is applied for by outside vendors. It has to be financed in the early days, usually from your own resource. While it is important to be sensible about the scope of patent protection and the countries where patent protection is required, skimping on patent protection is not advisable.

Finally, choose your associates wisely. In the early stages they will need to be altruistic and not look to make every penny or cent from your work. Track records are important. Snake oil salesmen abound.
Getting a new invention off the ground requires persistence and some luck, and some skill. But it is possible and as the inventor you will need to learn fast about many things that were outside your experience.

12.20 – 2.30pm Lunch break

2.30 - 3.30pm Open Air workshop: Co-ordinators: Rajwinder Lehal and Lucio Miele. Topic: Notch Therapeutics

SESSION 7 - Novel Therapeutics. Chairperson: Agamemnon Epenetos

4.00 - 4.20pm Massimo Masiero[1†], Demin Li[1†], Pat Whiteman[2], Carol Bentley[1], Jenny Greig[1], Tasneem Hassanali[1], Sarah Watts[1], Stephen Stribbling[1], Jenna Yates[1], Ji-Liang Li[3], Chandramouli Chillakuri[2], Devon Sheppard[5], Manuel Sarmiento-Soto[4], James Larkin[4], Nicola Sibson[4], Susan M. Lea[5], Penny A. Handford[2], Adrian L. Harris[3‡], Alison H. Banham[1‡]

[1]NDCLS, Radcliffe Department of Medicine, OX3 9DU, [2]Department of Biochemistry, OX1 3QU, [3]CRUK Department of Oncology, Weatherall Institute of Molecular Medicine, OX3 9DS [4]CRUK/MRC Oxford Institute for Radiation Oncology, Department of Oncology, OX3 7DQ,[5]Sir William Dunn School of Pathology, OX1 3RE, University of Oxford, Oxford, United Kingdom.

Development of therapeutic anti-JAGGED1 antibodies for treatment of TRN breast cancer

The role of Notch signalling and its ligand JAGGED1 (JAG1) in tumour biology have been firmly established, making them appealing therapeutic targets for cancer treatment. Here we report the development and characterization of human/rat-specific JAG1-neutralizing monoclonal antibodies. Epitope mapping identified their binding to the JAG1 Delta/Serrate/Lag2 domain, where E228D substitution prevented effective binding to the murine Jag1 orthologue. These antibodies were able to specifically inhibit JAG1-Notch binding *in vitro*, downregulate Notch signalling in cancer cells and to block the heterotypic JAG1-mediated Notch signalling between endothelial and vascular smooth muscle cells. Functionally, *in vitro* treatment impaired 3D growth of breast cancer cell spheroids, in association with a reduction in cancer stem cell number. *In vivo* testing showed variable effects on human xenograft growth when only tumour-expressed JAG1 was targeted (mouse models) but a more robust effect when stroma expressed Jag1 was also targeted (rat MDA-MB-231 xenograft model). Importantly, treatment of established triple receptor negative breast cancer brain metastasis in rats showed significant reduction in neoplastic growth. MRI imaging demonstrated that this was associated with a substantial improvement in blood-brain-barrier function and blood perfusion. Lastly, JAG1-targeting antibody treatment did not cause any detectable toxicity, further supporting its clinical potential for cancer therapy.

4.20 - 4.40pm Rajwinder Lehal[1], Charlotte Urech[1], Michele Vigolo[2], Jelena Zaric[2] , Maximilien Murone[1], Freddy Radtke[2].
1.Cellestia Biotech AG, Basel, Switzerland
2. Ecole Polytechnique Fédérale de Lausanne, Switzerland

Characterization and profiling of CB-103, a novel small molecule protein-protein interaction inhibitor of targeting the NOTCH transcription complex.

NOTCH signalling is a key development pathway whose aberrant activation is known to play a role in multiple human cancers. In human tumors the NOTCH pathway can be activated by various genetic lesions such over expression of ligands/receptors, GOF mutations in NOTCH receptors, including protein stabilizing

mutations in the PEST domain of NOTCH, chromosomal translocations, or loss-of-function mutations in the E3 ubiquitin ligase FBXW7 and other negative regulators of the pathway (SPEN, NUMB). Activation of signalling due to above mentioned mechanisms can be addressed in part using blocking antibodies against NOTCH ligands/receptors or small molecule inhibitors of the gamma secretase enzyme (GSIs). However, in human tumors where NOTCH signalling is constitutively activated due to chromosomal translocations in the NOTCH receptors (NOTCH1 and 2), none of the above-mentioned strategies will be effective. Moreover, due to on-target and off-target toxicities associated with blocking antibodies and GSIs, these anti-NOTCH agents failed to advance in clinical trials, although some of them showed signs of clinical efficacy. Given the role of NOTCH signalling in human tumors, there is a need to identify novel targets in the NOTCH pathway and develop new and more selective anti-NOTCH agents. To inhibit pan-NOTCH signalling in human tumors independently of the mechanisms of NOTCH activation, and in the most downstream part of the pathway, we have previously reported the discovery of a new class of small molecules able to target the NOTCH transcription complex enabling the specific inhibition of NOTCH target gene expression (e.g. cMYC, HES1, DTX1, CCND1). These small molecules act as protein-protein interaction inhibitors, and thereby compromise the assembly of functional NOTCH transcription complex. Here we present further in vitro and in vivo characterization of the lead molecule CB-103. The anti-cancer activity of CB-103 was extensively profiled in several human cancer cell lines representing NOTCH positive solid tumors, leukemias and lymphomas. Moreover, CB-103 responsiveness of these cell lines correlates with a downregulation of the NOTCH signature following treatment with CB-103. Specifically, we will present data outlining the in vivo pharmacokinetic and pharmacodynamic properties of CB-103.

4.40 - 5.00pm Samarpan Majumder[1], Judy Crabtree[1], Fokhrul Hossain[1], Maximilian Murone[2], Rajwinder Lehal[2], Freddy Radtke[2,3] and Lucio Miele[1]
[1]LSUHSC Department of Genetics, New Orleans, Louisiana
[2]Cellestia, Inc.
[3]Ecole Polytechnique Federale Lausanne, Switzerland

A novel, first in class Notch transcriptional inhibitor, CB-103 has activity on luminal breast cancer stem cells in combination with fulvestrant

The Notch signaling pathway plays a central role in cellular differentiation, growth and stem cell maintenance. Expression and activation of Notch pathway receptors and ligands have differential outcomes depending on the tissue, localization and cell type. When Notch pathway is aberrantly activated by genetic lesions, it can be a major driver for Notch-dependent cancers and can cause resistance to standard of care treatment. We and others have shown that in Estrogen Receptor (ER)-positive breast cancers, estrogen deprivation caused by endocrine therapy results in Notch1 and Notch4 activation. In turn, Notch1 stimulated ER-dependent transcription in the absence of estrogen, causing endocrine resistance. Combinations of Notch inhibitors and endocrine therapy are effective in preclinical models of ER-positive breast cancer and have shown promising signals in early clinical trials.

Cellestia's lead development candidate CB-103 is a small molecule, first-in-class, oral pan-Notch inhibitor. CB-103 selectively blocks Notch pathway activation-related gene transcription through binding to a Notch specific protein in the transcription factor complex. The blockade occurs by protein-protein interaction inhibition with a binding site critical for the assembly of the Notch transcription complex. This is a unique mode of action, which allows blocking Notch signaling regardless of the genetic lesions which have activated the pathway.

We have performed mammosphere assays to test the potency and efficacy of this compound on stem cell ability to form sphere. We used two different doses of CB-103; either alone or in combination with a fixed dose of Fulvestrant (30nM), a SERD, in our mammosphere assays. Two different ER^+ luminal, endocrine resistant cell lines were tested and compared with their parental controls. From our data, it's apparent that there is a synergistic effect when using CB-103 in combination with Fulvestrant. It's also evident that the efficacy of CB-103 is maximal maximizes at the lowest concentration tested in our assays. The combination was effective in 3 out of 4 models. However, the effect of CB-103 on MCF7-TAM^R either as a single agent or in combination was not statistically significant.

Cellestia has received regulatory approval to start clinical development with CB-103 in a first-in-human study Phase l – lla study investigating safety (Ph l) and preliminary single agent efficacy (Ph lla) of CB-103 in patients with advanced solid

cancers and haematological malignancies. Our data support the notion of testing this agent in ER-positive breast cancer in combination with SERDs

5.00pm Farewell Agamemnon Epenetos

POSTER PRESENTATIONS

Megan Thompson, Angélica Santiago-Gómez, Martin Baron, Robert Clarke
The University of Manchester, Manchester Cancer Research Centre, 555 Wilmslow Road, Manchester, M20 4GJ

Investigating Notch4 signalling mechanisms to better inhibit stem cell activity in endocrine therapy-resistant breast cancer

Breast cancer stem cell (BCSC) activity is enhanced following anti-estrogen treatment of oestrogen receptor-positive (ER+) breast cancer, potentially leading to endocrine therapy resistance. Notch4 receptor signalling is highly activated in these BCSCs, linking Notch4 activity to endocrine therapy resistance. In Drosophila, the Notch Ax(E2) mutant causes a switch from ligand-dependent to ligand-independent Notch activation. The human Notch4 possesses the same residue change, diverging from Notch1/2/3. It is hypothesised that further Notch4 Ax class-like mutations selected during endocrine treatment will enhance ligand-independent Notch trafficking, increase the BCSC population and drive endocrine resistance.
Analysis of online genomic databases of breast cancer has identified 34 Notch4 mutations in primary breast cancers (0.95% of samples analysed) and 30 in

metastatic breast cancers (2.04%), with 20 exclusive to metastatic. Investigation of BCSC activity in a Notch4 CRISPR knockout cell line has shown decreased BCSC activity, with a more pronounced effect after endocrine therapy. Interestingly, the Notch4 knockout cell line shows increased proliferation in estrogenic conditions, suggesting an interaction between the Notch4 receptor and ER signalling.
We aim to explore Notch4 receptor signalling mechanisms using Notch decoys, a Notch4 therapeutic antibody and the knockout/overexpression of Notch4 and trafficking proteins. Exploration of Notch4 trafficking will be carried out using immunofluorescence to observe the ligand dependent and independent activation pathways. The involvement of the four human Deltex proteins in the ligand independent pathway will also be explored. Notch4 mutations in breast cancer and their effects on trafficking will be investigated using CRISPR gene modification and insights from Drosophila studies. The ultimate aim is the identification of a therapeutic target to reduce Notch4 activity to be used in combination with endocrine therapy.

Abigail Edwards Manchester University , Manchester, UK

Targeting IL-1 signalling in Notch-driven breast cancer

Despite significant advances in recent years, there remains considerable demand for the development of novel breast cancer therapeutics. Current treatment options are especially limited for triple negative breast cancer patients, and for patients with therapy resistant breast cancer. The Notch signalling pathway has been found to be aberrantly activated in breast cancer, and is correlated with poor prognosis and therapy resistance. Therapeutic inhibition of the Notch signalling pathway is an attractive approach in breast cancer treatment, however pan Notch inhibition is associated with significant side effects. As more specific Notch inhibition has been shown to reduce the risk and severity of these side effects, there is a rationale for identifying and targeting signalling events downstream of the Notch receptor. Our group has found that Notch signalling induces the

expression of the cytokine IL-1α in breast cancer epithelial cells, which results in the autocrine activation of pro-survival Akt signalling. IL-1 signalling inhibitors are well tolerated and have an extensive safety record in the clinic, presenting an opportunity for the re-appropriation of IL-1 inhibitors to target Notch/IL-1α signalling in breast cancer treatment. Here I present data which demonstrates the importance of IL-1 signalling in the breast cancer cell phenotype, including in apoptosis resistance, invasion and xenograft tumour formation, which supports the hypothesis that Notch/IL-1 signalling could be therapeutically targeted in the treatment of breast cancer.