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Abstract 01 - Ágota Tűzesi

The role of miR-497-5p in mediating resistance to Temozolomide in paediatric glioma stem cells

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Background: Aberrations in epigenetic mechanisms can lead to tumour occurrence. Cancer stem cells have an important role in the brain tumour glioblastoma as these cells escape traditional treatments which can lead to tumour relapse. In this study we identified microRNAs (miRNA) regulated by DNA methylation and investigated the role of specific miRNAs in mediating resistance to current treatment with Temozolomide (TMZ).

Materials & Methods: We used patient-derived paediatric glioblastoma stem cell (GSC) cultures and normal neural stem cell (NSC) cultures. DNA methylation profiling was done using Illumina DNA methylation arrays and miRNA profiling with 3D-Gene Human miRNA Oligo chip. For miRNA silencing we used siRNA or CRISPR/Cas9 and for overexpression synthetic miRNA mimics.

Results: MiRNAs with high expression in GSCs, such as miR-195-5p and miR-497-5p, showed correlation with the DNA methylation levels. Overexpression of miR-497-5p resulted in reduced cell proliferation but increased resistance to TMZ. Furthermore, overexpression induced gene expression changes in tumour dormancy-related genes.

Conclusions: MiR-497-5p plays an important role in mediating resistance to TMZ in glioma stem cells and the miRNA expression is regulated, at least partially, by its DNA methylation status. These findings could be used to improve future treatment strategies.

Abstract 02 - Ahmad Shareef

Doxorubicin is a good chemotherapy drug candidate for combination therapy with γδ T cells in colon cancer treatment

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Background: Combinations of cellular immune-based therapies with chemotherapy and other antitumor agents may be of significant clinical benefit in the treatment of cancers. γδ T cells are of particular interest for use in such combinational therapies due to their potent antitumor cytotoxicity and relative ease of generation in vitro. However, whether concurrent use of chemotherapy drugs with γδ T cell adoptive therapy can result in any toxic effects on γδ T cells is still not very clear.

Materials & Methods: Here, we investigated the effects of seven Chemotherapy drugs (Etoposide, Vincristine, Fludarabine, Melphalan, 5-Aza-2 deoxycytidine, Doxorubicin and Cladribine) on γδ T cell proliferation and apoptosis. We performed parallel experiments on mouse γδ T cells extracted from spleen and expanded Vγ9Vδ2 T cells from peripheral blood from healthy donors. γδ T cells were incubated with different concentrations of chemotherapeutic agents for 24, 48 and 72 hours. We use CMT-93 (Mouse rectum carcinoma) and LDL-1 (human colorectal carcinoma) cells as controls for the effectiveness of the chemotherapy drugs.

Results & Conclusions: Our results from human Vγ9Vδ2 T cells demonstrate that the lowest concentration of Cladrabine and Doxorubicin had effective killing on tumor cells, while minimal toxicity on γδ T cells. They are potential drug candidates for combination therapy with γδ T cells at these concentrations.

From mouse γδ T cells, the results show that the lowest concentration of Fludarabine, Doxorubicin and Vincristine had effective killing on tumour cells, while minimal toxicity on γδ T cells. Thus, combining the results from both mouse and human γδ T cells, we conclude that Doxorubicin (3.6μM) might be used concurrently with adoptive therapy with γδ T cells.

Abstract 03 - Alessandro Camponeschi

Unravelling the mechanisms underlying leukemogenesis

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Background: Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. Of pediatric ALL, B-cell precursor ALL (BCP-ALL) accounts for 80-85%, and represents the leading cause of cancer-related death in children and young adults. BCP-ALL is a heterogeneous disease with chromosomal aberrations that are used to categorize the leukemia into different subtypes. Around 25% of the cases carry the t(12:21) translocation that results in the ETV6-RUNX1 fusion gene.

Other subtypes are not as common and each contributes from 1 to 7%, e.g. TCF3-PBX1 and BCR-ABL1. However, most of these chromosomal aberrations are not sufficient to give rise to leukemia. Additional essential alterations in the physiology of the B-cell precursors (BCPs) are needed in order to lead to malignant transformation, e.g. sustaining proliferative signaling, evading growth suppressors, resisting cell death and enabling replicative immortality.

Materials, Methods, Results & Conclusions: We have performed *Gene Set Enrichment Analysis (GSEA*) of public data sets with almost a thousand BCP-ALL patient samples and compared the gene expression signatures from the different subtypes with those from healthy BCPs, e.g. pro-B and pre-B cells. We found that the subtype ETV6-RUNX1 shows a gene expression signature that resembles that of healthy pro-B cells, and vice versa, suggesting that this particular leukemia subtype is arrested at the pro-B cell stage.

We have also performed GSEA using public data sets covering BCP-ALL cell lines. One of these, REH, carries the ETV6-RUNX1 translocation and GSEA confirmed that it resembles healthy pro-B cells and ETV6-RUNX1 patient samples.

Although sharing gene expression signatures, some of the genes highly expressed in the pro-B cells are not expressed in the ETV6-RUNX1 leukemia cells, and some of those highly expressed in the ETV6-RUNX1 are not expressed in the pro-B cells.

Narrowing down the number of such genes, we have selected 19 among the top candidates. These are genes that have been implicated in sustaining proliferative signaling, evading growth suppressors, resisting cell death and enabling replicative immortality. On the basis of these preliminary data, we will explore the function of these genes, which are likely involved in the hallmark processes of cancer, and hence of critical importance to the mechanisms that underlie leukemogenesis.

Abstract 04 - Andreas Svanström

3D printed hydrogels resembles de-cellularized patient derived breast tumors by the characteristics and genetic response of reporter breast cancer cells

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Background: Breast cancer is the primary cause of cancer related deaths in women worldwide. Current platforms for cancer drug development are mainly limited to 2D cell culture systems that poorly mimics physiological environments and traditional as well as low throughput animal models. In light of novel 3D printing technology and the possibility to engineer tissues on demand, novel large scale *in vivo*-like screening assays has the ability to substitute current traditional platforms and reduce the use of animals.

The extracellular matrix (ECM) of the microenvironment has previously been shown to provide with both biochemical and mechanical cues to the inhabiting cell population. Here, we aim to create 3D printed hydrogels that affect breast cancer cells similarly as the ECM of a real breast tumor.

Materials & Methods: We have produced hydrogels with different additives via extrusion based 3D printing and compared the characteristics and genetic response of breast cancer reporter cells cultured on hydrogels, patient-derived scaffolds and 2D plates.

Results: We show that cells cultured on hydrogels and scaffolds share a 3D-specific phenotype, viability and ability to induce holoclone formation. By studying genetic markers for proliferation, differentiation, pluripotency, *epithelial*-mesenchymal transition and cancer stemness, we show that we can alter the genetic response of reporter cells cultured on hydrogels by different hydrogel additives to a response similar as of cells cultured on scaffolds, contrasting that of 2D cultured cells.

Conclusions: Our adjustable 3D-printed scaffolds provide a promising *in vivo-like* platform for large-scale screening of compounds for cancer drug development.

Abstract 05 - Anna Bäck

Optimization of dose determination and dose delivery in external radiotherapy – Focus on fast and secure establishment of treatment dose uncertainty

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Background: Radiotherapy is one of the fundaments for treatment of cancer. The latest technique for external radiotherapy, i.e. modulated technique, has the potential to reduce radiation dose to healthy tissues but involves a higher complexity and thereby a larger uncertainty in the delivered dose, i.e. larger difference between planned and delivered dose.

One treatment can be delivered in different ways of different uncertainties, but the level of uncertainty is unfortunately not known at the time when designing the treatment which prevent an active effort to minimize the uncertainty. The aim of this research project is to develop a complexity metric for a fast and secure pre-establishment of the uncertainty in the delivered dose.

Materials & Methods: Based on our earlier studies on where differences between planned and delivered dose appear, i.e. the penumbra region of the irradiation field, a theoretical complexity metric that quantifies this region is composed. The complexity score was validated by comparison to the difference between planned dose and Gafchromic film dose measurements of six treatment plans. Furthermore, the complexity scores of 30 different treatment plans were studied.

Results: The complexity scores correlated with a Pearson´s r-value of -0.96 to the measured difference between planned and delivered dose. The amount of higher complexity scores varies for treatments of different cancer diagnoses.

Conclusions: A complexity metric have been developed that correlate to the uncertainty in the delivered dose. Further studies on the different interpretations of complexity scores for different types of treatments are needed before clinical implementation.

Abstract 06 - Anna Danielsson

DNA methylation profiles of Patient-Derived Stem Cells from Paediatric Glioma focusing on radiotherapy-induced alterations

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Background: Radiation is an important therapeutic tool for children diagnosed with brain tumours, however glioma often shows treatment resistance. Such radioresistance may be intrinsic or induced by the radiation therapy itself by promoting co-evolution of genetic and epigenetic alterations. The DNA methylation pattern is strictly regulated during cell differentiation and development, allowing each cell type to acquire phenotypic changes to meet its specific functions and needs.

Materials & Methods: Five GSC cultures (four established from tumours before treatment and one after treatment) were irradiated in vitro with repeated doses of 2 or 4Gy. Radiation was given in 3, 15 or 18 fractions. Cellular phenotypic, genomic and epigenomic alterations were profiled.

Results: All except one sample exhibited a radioresistant phenotype in vitro which was consistent with clinical response. After three radiation dose fractions, cell cycle and morphological effects were prominent, but very few differentially methylated positions (DMPs) were detected. Whereas at 18 radiation fraction a large number of differentially methylated positions (DMPs) were identified. Most of these DMPs gained methylation and were frequently found in genomic regions harboring enhancers and at intergenic locations. However the DNA copy number profiles were stable with retained DNA amplifications and losses at individual tumour specifc sites.

Conclusions: Specific DNA methylation alterations of sites with regulatory functions in proliferation and differentiation identified in our models reflect cellular response to radiation stress through epigenetic reprogramming and differentiation cues.

Abstract 07 - Anna Gustafsson

Breast cancer patient derived scaffolds – an *in vivo* like screening system for cancer treatments

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Background: The tumour initiating population of cancer stem cells (CSC) are believed to promote metastasis and drug resistance, suggesting that selectively targeting CSC and their niche may be a potential beneficial therapeutic strategy. However, specific driving factors in the microenvironment influencing the CSC niche remains unknown.

Materials & Methods: To address this issue and to study the influence of specific cancer microenvironments, we have developed a novel 3D cell culture platform, using cell free scaffolds from breast cancer tumours.

Results: We demonstrate that repopulating scaffolds with ERα-positive standardized cell lines and treating them with endocrine treatments Fulvestrant and (Z)-4-Hydroxytamoxifen better recapitulate *in vivo* situation than traditional monolayer cultures. We could by RT-qPCR show that Fulvestrant clearly increased expression of cancer stem cell related genes, whilst robustly decreased proliferation and cell cycle transcripts in all the patient derived scaffolds.

Additionally, a variation in drug-response and treatment toxicity between the patient derived scaffolds was observed, not only showing the importance of a 3D culture system, but also highlighting the significance of using patient specific material to demonstrate tumour specific variation *in vitro*.

Conclusions: Our findings together suggest that patient derived scaffolds may provide a promising platform for evaluating anti-cancer drugs and potentially provide valuable insight of clinically relevant behaviors.

Abstract 08 - Anna Wenger

Profiling and targeting epigenetic mechanisms in paediatric high-grade glioma

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Background: The leading cause of cancer-related mortality among children is brain tumours and high-grade glioma (HGG) has one of the worst prognoses. One of the drivers behind tumour growth and tumour recurrence is believed to be cancer stem cells (CSC), which are resistant to chemo and radiation therapy, and these cells are therefore of extra interest to target.

Another tumour driver, and of particular importance in paediatric cancers, is epigenetics, which regulates gene expression. Epigenetic aberrations in regulation are reversible and the aberrations involved in cancer can as such potentially be treated. We therefore aimed to identify epigenetic targets in CSC derived from paediatric HGG.

Materials & Methods: We performed a pooled lentiviral CRISPR knock-out (KO) screen with an epigenetic/chromatin modifier library on patient-derived paediatric CSC lines and analysed the data to identify which genes that are lethal hits and also genes that are enriched as a result of the KO. Analysis of additional cell lines and individual validation of the hits is ongoing.

Results: 74 genes were identified as depleted and 18 as enriched. Few of these hits were shared between the cell lines, but these lines differ in K27M mutation status of *H3F3A* and it is reasonable that different targets and treatments are necessary for these subtypes. Among the shared hits were genes previously linked to tumour initiation in various cancer forms.

Conclusions: CSC in paediatric HGG can be eliminated by CRISPR KO of specific epigenetic modulators thus providing new treatment targets for children afflicted with HGG.

Abstract 09 - Anna-Karin Elf

Can SSTR2 expression in small intestinal nets predict ^{177}Lu-DOTATATE uptake and survival after peptide receptor radionuclide therapy?

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Background: Small intestinal neuroendocrine tumours (SI-NETs) often present with regional or distant metastases at diagnosis. Peptide receptor radionuclide therapy (PRRT) with radiolabelled somatostatin analogues is a systemic treatment option that may increase overall survival (OS). However, treatment response is variable and predictive factors have not been established. PRRT targets somatostatin receptor 2 (SSTR2). The uptake in tumour tissue on pre-treatment 68Ga -DOTATATE-PET correlates positively with tumour reduction upon PRRT. The immunohistochemical (IHC) SSTR2 expression in tumour samples has been reported to positively correlate with the PET uptake. Theoretically, a low SSTR2 expression in tumours could predict an inferior treatment response to PRRT.

Materials & Methods: Using a Tissue Micro Array (TMA) consisting of samples from 412 SI-NET patients we identified a subgroup consisting of 44 patients that had received PRRT treatment during 2006-2016 at Sahlgrenska University Hospital. IHC expression of SSTR2 and Ki-67 was assessed. The uptake of 177Lu-DOTATATE uptake in 33 patients was determined. An additional subgroup of 34 patients with paired samples from 3 tumour sites was identified. SSTR2 expression was assessed in corresponding tissue samples (n=102). Data regarding OS and other treatments were collected for both groups.

Results: SSTR2 expression did not vary between tumour sites, but correlated among a patient's lesions. Patients were grouped into Low SSTR2 or High SSTR2 depending on levels of SSTR2 expression. OS based on SSTR2 expression was not significantly different. Interestingly, PRRT treated patients with low SSTR2 expression received less additional treatment compared to patients with high SSTR2 expression and had a tendency towards higher 177Lu-DOTATATE uptake.

Conclusions: The results from the present study suggest that a low SSTR2 expression should not exclude patients from PRRT.

Abstract 10 - Arman Romiani

Fractionated administration of ^{177}Lu-octreotate in CLB-BAR xenografted nude mice resulted in a better anti-tumor effect

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Background: Neuroblastoma (NB) represents 7-9% of all tumors detected in children and is one of the most diagnosed tumors in infants. NB is a neuroendocrine tumor and has various characteristics and features, with diverse outcomes. The most malignant NBs have a 5-year survival rate of only 40-50%, indicating the need for new and improved treatment options.

Since NB overexpresses somatostatin receptors ^{177}Lu-octreotate is a potential treatment option. The aim of this work was to study the therapeutic effects after fractionated administration of ^{177}Lu-octreotate in CLB-BAR xenografted mice.

Materials & Methods: 5-6 weeks old nude BALB/c mice were injected s.c. on their flank with CLB-BAR cells (2x10^6). This study included tumor volumes of 250 up to 1100 mm^3. Three groups were i.v injected with a total activity of 15MBq ^{177}Lu-octreotate.

Group A: receiving one injection with 15MBq; Group B: receiving two injections with 7.5MBq with 2-hours between each injection; Group C: receiving three injections with 5.0MBq with 1-hour between each injection.

Results: Mice in group C had the longest mean survival time of 16 days after injection. Corresponding values for group A and B were 9.6 and 14 days, respectively.

Conclusions: The fractionated administration of ^{177}Lu-octreotate resulted in a better anti-tumor effect. The most noticeable results were observed in group C. These results might be due to upregulation of receptors from previous fractions in combination with saturation of the somatostatin receptors for the higher amounts of ^{177}Lu-octreotate. Further studies are needed to recognize the mechanisms behind the results and optimize the fractionation scheme.

Abstract 11 - Belson Rugwizangoga

Early onset and poor prognosis of acute leukemia in Rwanda

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Background: Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are potentially lethal forms of hematopoietic cancer. While age-standardized incidence rate (ASR) is similar in most Western countries, such information in Sub-Saharan Africa is incomplete. This study compared demographics and survival of acute leukemia in Rwanda and Sweden.

Materials & Methods: We performed a retro- and prospective cohort study to capture all acute leukemia cases diagnosed during 2012-2017 in Rwanda. Swedish cases diagnosed in 2012-2016 were retrieved from Swedish ALL and AML registries. Demographic data were obtained from statistics centers in both countries. ASR was calculated by adjusting population demographics to the world population.

Results: During the study period 498 cases of acute leukemia were diagnosed in Rwanda (319 ALL, 147 AML and 32 non-classified) and 2,115 cases in Sweden (591 ALL and 1,524 AML). ASR (cases/100,000/year) in Rwanda and Sweden was 0.41 and 1.63 for ALL and 0.25 and 1.58 for AML, respectively. Male predominance was observed in both cohorts.

Age distribution between both countries differed in that 67% of ALL patients in Rwanda are below 15 years versus 50% in Sweden, and 58% of AML patients in Rwanda are below 30 years versus 5.8% in Sweden. 41 % of Rwandan ALL patients and 10% of AML patients were alive at 2 years after diagnosis implying poor survival compared with Western countries.

Conclusions: Acute leukemia affects younger patients in Rwandan than in Sweden. The poor survival of acute leukemia patients, particularly AML, in Rwanda merits implementation of treatment with curative intent.

Abstract 12 - Britta Langen

The relevance of age and sex bias for biomarker screening in cancer radiotherapy

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Background: Protein biomarkers are a promising tool for risk assessment in cancer radiotherapy. The accuracy and specificity of biomarker candidates, however, can be strongly biased by the age and sex of the specimen. This can lead to severe misrepresentation of tissue toxicity and, ultimately, erroneous decisions in cancer radiotherapy planning. The aim of this study was to assess age and sex bias of blood-based protein biomarkers for radiation treatment.

Materials & Methods: Male and female juvenile (7w old) and adult (18w old) C57BL/6N mice (n=4–5/group) were irradiated (whole-body) with 0.5 Gy absorbed dose (15 MV (nominal) photon beams). Matching control groups were mock-treated. Mice were killed 24h after treatment. Blood plasma samples were processed for proteomic analysis (Orbitrap Fusion™ Tribrid™). Analysis was performed using, *e.g*, R, Gene Ontology, and PANTHER/REViGO.

Results: Cluster analysis showed that the proteomic response in plasma differed strongly between the groups and did not exhibit a simple correlation with sex or age. Age and sex influenced individual protein abundance and thus created bias for accuracy and specificity of biomarker candidates. The extent of age- and sex-specific differences also depended on biological function: *e.g.*, inflammation-related responses were significantly stronger in juvenile than in adult mice; while sex-specific differences were marginal, they could vary largely in other categories (*e.g.* metabolism).

Conclusions: Sex and age can create severe bias in biomarker screening. The extent of bias appears to depend, in part, on the analytical endpoint. This work contributes to the establishment of bias-free risk assessment in cancer radiotherapy.

Abstract 13 - Britt-Marie Iresjö

Myosin Heavy Chain proteins and Amino Acid transporters in skeletal muscle tissue from cancer patients as markers for anabolism as related to medical care

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Background: Metabolic alterations leading to progressive loss of skeletal muscle tissue are common in patients with gastro-intestinal cancer; with subsequent reduction in Health related Quality of Life and physical functioning. Understanding to improve muscle mass and function would be of great benefit for patients anticipated cancer treatment.

This study focused on control of myofibrillar protein synthesis following provision of in-hospital parenteral nutrition. Amino acid transporter proteins are proposed to participate in mTOR signaling for activation of muscle protein translation at ribosomes. The present study examines if changes in Amino Acid transporter LAT3 reflects increased gene transcription of contractile myosin proteins.

Materials & Methods: 22 patients (weight loss 6±2 %) scheduled for upper GI-surgery received continuous standard TPN- (0.16 $gN \cdot kg^{-1} \cdot day^{-1}$, 30 $kcal \cdot kg^{-1}\ day^{-1}$) or saline infusion for 12 hours overnight before operation. Biopsies from the rectus abdominis muscle were taken at operation. mRNA of LAT 3 and Myosin heavy chain isoforms (MHC1, MHC2A MHC2X) were analyzed by Real-time PCR. LAT3 protein was quantified by western blot.

Results: MHC2A mRNA showed positive regression with LAT 3 mRNA ($r=0.56$; $p=0.007$), without relationships among MHC1, MHC2X and LAT3 mRNA. There was negative relationship between MHC2A mRNA and LAT 3 protein ($r=0.62$; $p=0.007$), while MHC2X mRNA showed positive correlation with LAT3 protein ($r=0.53$; $p=0.02$); without relationship between MHC1 mRNA and LAT3 protein.

Conclusions: mRNA and protein concentration of amino acid transporter LAT 3 predicted Myosin Heavy Chain 2A mRNA concentration in muscles. Lack of predictions between other Myosin Heavy chain isoforms and LAT3 suggest specific regulation of individual MHC transcripts in human muscles. These findings relate to understand the control of myofibrillar protein translation to improve Quality of Life in cancer patients.

Abstract 14 - Charlotte Andersson

The protective antioxidant rA1M allows full antitumor effects of ^{177}Lu-octreotate treatment in PDX neuroendocrine tumor models

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Background: When using radioprotectors in radiation therapy of cancer it is important to make sure that the agent protects normal cells without adversely influencing tumor control. Alpha-1-microglobulin (rA1M) is an antioxidant that has been proposed as kidney protector during ^{177}Lu-octreotate treatment of neuroendocrine tumors (NETs). In this study we investigated if co-infusion of rA1M affects the therapeutic response of ^{177}Lu-octreotate in patient derived xenograft (PDX) NET models.

Materials & Methods: Nude mice with human medullary thyroid carcinoma, GOT2 were injected with ^{177}Lu-octreotate with or without rA1M. The animals were terminated at different time points and ^{177}Lu concentration in organs and tissues were measured ex-vivo. Nude mice with human small intestine NET, GOT1, were injected with either ^{177}Lu-octreotate, a combination of ^{177}Lu-octreotate and rA1M or rA1M only. Tumor volume were followed over time and compared between the groups and with un-treated controls.

Results: The biodistribution of ^{177}Lu was similar between the two groups of GOT2-bearing mice. In GOT1-bearing mice a strong therapeutic response was observed for those treated with ^{177}Lu-octreotate, or ^{177}Lu-octreotate + rA1M. During the two first weeks, the mean tumor volume decreased to about half of the initial volume but then increased. Both the tumor remission and re-growth of the GOT1 tumors in these two groups were similar. Absence of tumor reduction was observed in both control and rA1M only groups.

Conclusions: Neither biodistribution of ^{177}Lu nor therapeutic response were negatively affected when ^{177}Lu-octreotate was combined with rA1M injection.

Abstract 15 - Diana Cervantes-Madrid

Phosphoproteome and gene expression profiling of ALK inhibition in neuroblastoma cell lines reveals conserved oncogenic pathways

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Background: Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor that is a clinical target of major interest in cancer. Mutations and rearrangements in *ALK* trigger the activation of the encoded receptor and its downstream signaling pathways. *ALK* mutations have been identified in both familial and sporadic neuroblastoma cases as well as in 30 to 40% of relapses, which makes ALK a bona fide target in neuroblastoma therapy.

Tyrosine kinase inhibitors (TKIs) that target ALK are currently in clinical use for the treatment of patients with ALK-positive non-small cell lung cancer. However, monotherapy with the ALK inhibitor crizotinib has been less encouraging in neuroblastoma patients with *ALK* alterations, raising the question of whether combinatorial therapy would be more effective.

Materials & Methods: In this study, we established both phosphoproteomic and gene expression profiles of ALK activity in neuroblastoma cells exposed to first- and third-generation ALK TKIs, to identify the underlying molecular mechanisms and identify relevant biomarkers, signaling networks, and new therapeutic targets.

Results & Conclusions: This analysis has unveiled various important leads for novel combinatorial treatment strategies for patients with neuroblastoma and an increased understanding of ALK signaling involved in this disease.

Abstract 16 - Elena Garre

Screening of FDA approved drugs for the targeting of breast cancer stem cells using the novel cancer patient derived scaffold.

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Background: Despite the many different therapeutics employed in the treatment of breast cancer, disease relapse and metastasis in treated patients remains a major challenge. The tumour initiating population of cancer stem cells (CSC) are believed to promote metastasis and drug resistance, suggesting that selectively targeting CSC and their niche may be a potential beneficial therapeutic strategy.

Another challenge, it is to bring new drugs to the market; it takes many years, and high cost and effort. The screening of FDA approved drugs, to identify potential drug candidates that could be repurposed, reduces the failure risk and the time frame for drug development.

Materials & Methods: The novel patient derived scaffolds (PDS), developed in our group, recapitulates the native microenvironment where the cancer cells originate and, promotes cancer stem cells features. We repopulated these cell free tumour scaffolds with an established breast cancer cell line, and we treated them with several FDA approved drugs.

Results: The analysis of marker genes involved in the main processes related with cancer progression properties, showed that the cells growing in the scaffolds exhibited different gene expression than monolayers cultures. Interestingly, in several cases these changes observed in treated scaffolds were more similar to *in vivo* results obtained in mice than 2D.

Conclusions: Our findings indicate that using this patient derived system; we will have a better representative model for an *in vivo* situation than with methods based on standard 2D cell culture, allowing high precision and predictability of the drug effect on tumour malignancy properties.

Abstract 17 - Elin Bernson

Cytomegalovirus Serostatus Affects Autoreactive NK Cells and Outcomes of IL2-Based Immunotherapy in Acute Myeloid Leukemia

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Background: Human cytomegalovirus (CMV) infection is reported to promote NK cell differentiation and education. The CMV-induced generation of highly differentiated adaptive-like NK cells has been proposed to affect favorably on the maintenance of remission in patients with acute myeloid leukemia (AML) after allogeneic stem cell transplantation (allo-SCT). The impact of CMV infection and adaptive-like NK cells on relapse and survival of patients with AML not receiving allo-SCT remains unknown.

Materials & Methods: We assayed CMV IgG serostatus to determine past CMV infection in 81 nontransplanted AML patients who received relapse-prevention immunotherapy comprising histamine dihydrochloride and low-dose interleukin-2 (HDC/IL2; NCT01347996).

Results & Conclusions: CMV seropositivity correlated negatively with leukemia-free and overall survival of patients receiving HDC/IL2, but did not correlate with outcomes in a contemporary control cohort. Inhibitory killer-cell immunoglobulin-like receptors (iKIRs), binding to cognate HLA, play a significant role in regulating NK cell cytotoxicity. Many individuals have a mismatch between KIRs and corresponding HLA, which may lead to the generation of NK cells expressing only iKIRs to non-self HLA (NS-iKIR). We have earlier reported NS-iKIR NK cells to be protective during HDC/IL2 immunotherapy in AML.

Analysis of outcome after stratification of patients based on concordant or discordant KIR and HLA genotypes implied that the negative impact of CMV seropositivity was restricted to patients lacking a ligand to iKIRs. Previous CMV infection was also associated with fewer NK cells expressing only NS-iKIR. We propose that CMV-driven NK cell education depletes the population of NS-iKIR NK cells, which in turn reduces the clinical benefit of relapse-preventive immunotherapy in AML.

Abstract 18 - Elinor Bexe Lindskog

Pharmacokinetics of leucovorin and methyltetrahydrofolate in plasma as a function of administered dose

Elisabeth Odin, Helena Taflin, Bengt Gustavsson, Yvonne Wettergren and **Elinor Bexe Lindskog**

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Background: 5-fluorouracil is administered in combination with the folate leucovorin (LV, natural and unnatural form) to improve the chemotherapy effect in colorectal cancer. Methyltetrahydrofolate (MTHF) is the main metabolite from LV in plasma. High plasma folate concentrations may correspond to high folate concentrations in tumor. This study was designed to gain an understanding of how a single intravenous infusion of LV affects plasma folate concentrations.

Materials & Methods: Thirty colon cancer patients were randomized to receive 60, 200 or 500 mg/m² LV given as a two-hour infusion. Blood was sampled from patiens at predose, during infusion at 10 and 30 min and at 2, 4 and 24 hours after start of infusion. Plasma levels of LV and MTHF were quantified by LC-MS/MS.

Results: The LV plasma concentration reached its maximum at 120 min, at the time of infusion stop. The highest concentration of MTHF was reached at 240 min, indicating a two-hour conversion rate. LV and MTHF AUC increased significantly with increasing dose and varied greatly within each dosage group. There was a positive correlation between LV and MTHF AUC at 60 and 200, but not at 500, mg/m^2.

Conclusions: The high variation in plasma folate concentration indicates that some patients need an adjusted dose of LV. The lack of correlation found at 500 mg/m^2 LV indicates saturation or inhibition, possibly due to the unnatural form of LV. Future studies will show if plasma folates reflect tissue folate concentrations.

Abstract 19 - Emma Aneheim

Poly-L Lysine based Approaches for Pretargeted Radioimmunotherapy with Astatine-211

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Background: Pretargeted radioimmunotherapy (PRIT) can increase activity uptake in tumors relative to normal tissue compared with conventional radioimmunotherapy. In PRIT there is preferably a three step administration of pretargeting agents. Firstly the pretargeting molecules are administered allowing for maximum tumor targeting.

Secondly, a clearing agent is injected in order to remove unbound pretargeting molecules from the blood. Thirdly, a radiolabeled effector molecule with high affinity for the pretargeting molecule is administered. In this work, different approaches using Poly-L Lysine within PRIT with astatine-211 are explored.

Materials & Methods: Two different pretargeting approaches are the (strept)Avidine(SA)<->Biotin system and the Tetrazine(Ttz)<->Tetracyclooctane(TCO) system. In either case the pretargeting molecule is functionalized with one of the ingoing components while both the clearing agent and the effector molecule is functionalized with the other. Poly-L Lysine is a polymer that is available in different chain-lengths allowing for change in circulation and clearance properties. One of the best alpha particle emitters for curative therapies is astatine-211 that have a 7.2 h half-life and one alpha emission per decay.

Results: Poly Lysine can be used as clearing agent by functionalization with galactose amine to steer clearance to the liver and succinic anhydride for charge modification. Charge modification is also necessary for use as effector molecule where the poly Lysine in addition is functionalized with N-succinimidyl-3-(trimethylstannyl)benzoate to allow for incorporation of astatine-211.

Conclusions: Poly-L Lysine is a versatile scaffold that can be modified in order to function both as effector molecule and clearing agent within pretargeted radioimmunotherapy using astatine-211.

Abstract 20 - Emma Jonasson

Heterogeneity and cell fate decisions in cancer development

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Background: Cellular and genetic studies have revealed large variability within tumors and recent advances have proven existence of cancer stem cells (CSCs) that are important for metastasis and treatment resistance. Our aims are to identify subpopulations of cancer cells and to define regulatory pathways that control cell transitions and further define key steps in identified pathways and evaluate their potential as drug targets.

Materials & Methods: The development of single-cell analysis has improved the possibilities of studying subpopulations of tumor cells. We have developed a toolbox were we can combine different qPCR-based methods and RNA sequencing on single cells. Using staining methods and functional assays, we have collected and analyzed cells from different stages regarding stemness, differentiation and proliferation from two different cancer types, breast cancer and myxoid liposarcoma.

Results: Using information about the cells received from sorting combined with their molecular expression signature, we have been able to identify processes involved in the transitions between different cell states as well as markers specific for different cellular subpopulations. Furthermore, we have developed a method for measuring the total transcriptome amount in single cells which we can connect to biological functions.

Conclusions: Using combinations of functional cell assays and gene expression analysis we have defined processes and genes important for different cellular subpopulations.

Abstract 21 - Emma Persson

Analysis of secreted proteome in an in vivo like human 3D model of breast cancer

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Background: Breast cancer is the most common form of cancer amongst women and affects millions worldwide each year. A key biological process in cancer progression and disease outcome is the tumor micro environment of which a highly important factor is the secreted proteome including secretion of cytokines, chemokines and other proteins.

Both autocrine and paracrine secretion influence cancer cell characteristics such as stem-like properties, proliferation and metastatic capacity. Due to the importance of the tumor micro environment with cancer cell signaling and cell to cell communication, a 3D environment was used whereby new signaling pathways and new possible drug targets can be investigated.

Materials & Methods: 54 human breast cancer tumors were decellularized to form scaffolds whereafter repopulated with either MCF7 or MDA-MB-231 breast cancer cell lines. Cells were grown on scaffolds for 21 days. After 16 days, cell media was changed and at day 21 of culture, conditioned media was collected for secretome analysis. Multiplex Proximity Extension Assay (OLINK, Uppsala Sweden) was performed on conditioned media and 184 proteins were analyzed for each sample.

Results: Both MCF7 and MDA-MB-231 cells secreted higher amount of proteins in total when cultured in the human 3D model compared to 2D cultivated cells. MDA-MB-231 cells secreted higher amount of proteins compare to MCF7 cells, in both 3D and 2D conditions. The analyzed secreted proteins were divided into three groups; proteins exclusively secreted in 3D cultivation; proteins that are highly secreted in 3D compare to 2D cultures (2-fold increase) and proteins equally secreted in 2D and 3D culture.

Out of 184 analyzed proteins, 39 proteins were exclusively secreted in 3D for MCF7 and 38 for MB-MDA-231. For MCF7 cells, 20 proteins had greater secretion in 3D compared to 2D and 53 for MB-MDA-231 cells, 11 proteins were secreted similarly for MCF7 and 30 for MB-MDA-231 and 113 proteins were not secreted at all for MCF7 and 62 for MB-MDA-231. Further, highly secreted proteins from different conditions were applied to 2D growth cultures to assess changes in gene expression and stem cell functionality.

Conclusions: Our results show that a human in vivo like 3D culture environment alter the secretome of different breast cancer cell types which highlights the importance of cultivating cells in an in vivo like 3D model when studying tumor micro environment. We hypothesized that proteins found to be exclusively or highly secreted in 3D compared to 2D cultures are, at least in part, responsible for cancer progression.

Abstract 22 - Emman Shubbar

The effect of timing and sequence of gemcitabine administration and ionizing radiation for more efficient treatment of medullary thyroid carcinoma

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Background: Medullary thyroid carcinoma (MTC) is a neuroendocrine tumour which arises from calcitonin-producing C cells. MTC is difficult to treat as it is not susceptible to radioiodine therapy. We recently demonstrated that gemcitabine, a nucleoside analogue, which inhibits DNA synthesis and repair significantly enhanced tumour radio-response of a patient-derived MTC (GOT2) animal model treated with a single-dose of external beam radiotherapy (EBRT). However, the ideal timing of gemcitabine administration with irradiation has not been studied.

Therefore, the aim of this study was to determine whether the time course and sequence between gemcitabine administration and irradiation is essential to enhance tumour suppressive response.

Materials & Methods: Balb/C-nu mice with GOT2 tumours were injected with a single-dose 60 $mgkg^{-1}$ gemcitabine, either 72h before, 0.25h before, 0.25h after, or 72h after irradiation with a single-fraction of 3Gy. Tumor size was measured twice a week, using a digital caliper**.**

Results: When gemcitabine was administrated before or after EBRT, a significant tumour regression was observed during the first 19 and 26 days, respectively, compared with untreated animals. The effect of the combination treatments was found to be additive, irrespective of time interval between gemcitabine and EBRT administration. No statistically significant difference was observed among the combination groups.

Conclusions: Combining EBRT with the nucleoside analogue, gemcitabine resulted in significantly enhanced tumour growth inhibition compared to either agent, alone. The results should be used for future development of optimal treatment planning schedules for this type of combination therapy.

Abstract 23 - Ezgi Uckun

“BioIDentification” of ALK-associated signaling complexes by proximity labeling

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Background: Alterations of the Anaplastic lymphoma kinase (ALK) such as point mutations, intragenic deletions, genomic rearrangements and gene amplifications have been described in human cancers including familial and sporadic neuroblastoma. ALK-associated downstream pathways are highly diverse and it is not clear how ALK oncogenes become wired to different downstream effectors. Analysis of receptor-associated signaling complexes in the context of wild-type and oncogenic ALK activation would provide essential information for the development of co-therapeutic approaches to target ALK-driven cancers.

Materials & Methods: Proximity-dependent biotin identification (BioID) is a powerful method for mapping protein-protein interaction networks. We employed BioID to identify and characterize novel components of ALK-signalling in both *Drosophila melanogaster* and human cells. BirA* and two new BirA* enzyme variants, MiniTurbo and TurboID, were introduced into the endogenous *Alk* locus of *Drosophila* by CRISPR/Cas9-mediated genome editing. Biotinylated proteins were isolated by streptavidin pull-down and further identified by mass-spectrometry.

Results: *ALK::BirA*HA*, *ALK::MiniTurbo.HA* and *ALK::TurboID.HA* transgenic flies were validated by molecular and immuno-fluorescence analyses. As BioID, miniTurbo and TurboID have different enzymatic activities and labeling radii, the interactome-profiles obtained with these constructs were compared in different tissue lineages as well as in the context of wild-type and activated ALK-signalling.

Conclusions: Employing this proximity labelling approach enables reconstruction of the ALK-signalling interactome in a tissue-specific context in a comparative manner. We are now applying the BioID system to human neuroblastoma cells to further validate our findings in our *Drosophila* models.

Abstract 24 - Fredrik Westerlund

Optical mapping of ultra large DNA molecules for detection of structural variations and epigenetic marks within the human genome

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Background: Optical DNA mapping (ODM) has proven to be an important complement to DNA sequencing for detecting long range structural variations of complex genomic regions. Moreover, ODM has allowed for epigenetic profiling of the human genome, providing insights beyond the DNA sequence. Traditionally ODM is based on enzymatic labeling but we here present a simple and enzyme-free approach for ODM of human DNA, based on competitive binding.

Materials & Methods: The competitive binding ODM assay consists of 4 main steps. 1) Isolation of large DNA molecules from cells using agarose gel plugs. 2) If desired, enzymatic labeling of epigenetic marks and/or DNA damage 3) Formation of an emission intensity profile along DNA, based on the underlying DNA sequence, by addition of two molecules, YOYO-1 and netropsin. 4) Visualization of the intensity pattern, and potentially the enzymatic labels, by stretching DNA in nanofluidic channels and imaging using a fluorescence microscope.

Results: Experiments on human DNA extracted from blood samples, in combination with *in-silico* simulations, demonstrated that at least 97% of the human genome is mappable at a DNA size of 330 kilo base pairs. The competitive binding assay also proved to be compatible with visualization of damage sites on the DNA caused by a cytotoxic drug.

Conclusions: We demonstrate that it is possible to precisely map a DNA fragment to its corresponding position within the human genome using competitive binding. The assay can be used to aid assembly of complex genomes, characterize structural variations, as well as mapping DNA damage throughout the human genome.

Abstract 25 - Ganesh Umapathy

EML4-ALK variant E6a/b;A20 positive NSCLC cell lines are associated with growth upon blocking MEK-ERK pathway

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Background: The protein kinase B (PKB/AKT) and RAF/MEK/ERK signaling pathways are activated in a wide range of human cancer types. In many cases, concomitant inhibition of both pathways is necessary to block proliferation and induce cell death and tumor shrinkage. Several feedback mechanisms have been described in which inhibition of one pathway leads to activation of a parallel signaling pathway, thereby decreasing the effectiveness of targeted monotherapies.

Materials & Methods: In order to determine which signalling core should be blocked for combinatorial treatment, we treated a panel of EML4-ALK positive lung cancer cell lines using MEK-ERK inhibitors. Resazurin assay was performed to evaluate cell viability. Protein levels were determined using western blotting.

Results: In this study, we describe a feedback mechanism in which MEK-ERK inhibition leads to increased activation of AKT signaling in EML4-ALK variant (E6a/b;A20) positive NSCLC cell lines. Interestingly, EML4-ALK variant (E13;A20) NSCLC cell lines responds to MEK-ERK inhibitors and shows synergism when combined with ALK tyrosine kinase inhibitors.

We found that feedback response in EML4-ALK variant (E6a/b;A20) positive NSCLC cells was mediated by the mammalian target of rapamycin complex 2–associated protein SIN1, resulting in increased survival and proliferation that depended on AKT signaling.

Conclusions: Taken together, these results elucidate an important feedback network and contraindicate the use of MEK inhibitors as effective therapeutic strategy in EML4-ALK variant (E6a/b;A20) positive NSCLC.

Abstract 26 - Georg Wolfstetter

Genetic screening for *Alk* signaling modulators in *Drosophila* identifies an essential role for *Wallenda* (*MAP3K/DLK*) in *JNK*-mediated cell competition

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Background: Anaplastic lymphoma kinase (ALK) is an oncogenic driver in a subset of human cancers. Chromosomal aberrations are the most common genetic alterations of human ALK and induce ectopic expression of oncogenic fusion proteins in tissues that are normally ALK-naïve. In addition, kinase domain mutations of the full-length receptor lead to inappropriate activation of ALK.

Materials & Methods: In order to investigate underlying mechanisms of oncogenic Alk signaling we conducted a genome-wide deficiency screen in a *Drosophila melanogaster* model, ectopically expressing Alk in the developing eye.

Results: We identified multiple genomic loci encoding for components of the Ras/Raf/ERK1,2-, PI3K-, and STAT-signaling pathways to be important for Alk-signaling. We further identified subunits of the *TFIID*-complex indicating alterations in cell proliferation and differentiation state.

In addition, *tailless* (*tll*) the *Drosophila* homolog of *NR2E1/TLX*, *Delta* (*DLL1*) and the transcription factors *eyeless* (*PAX6*), *sine oculis* (*SIX1,2*) and *eyes absent* (*EYA*) were identified suggesting that ectopic Alk signaling is capable of reprogramming the affected cells. Most interestingly, Alk-expressing cells gain enhanced fitness by inactivating the JNKKK Wallenda (MAP3K/DLK) which allows them to escape apoptosis and proliferate at the expense of their wild-type neighbors.

Conclusions: We identified many factors previously associated with oncogenic ALK signaling as well as novel components suggesting that this approach offers a valuable contribution to dissect molecular mechanisms underlying ALK-driven cancer. The discovery that enhanced ALK signaling leads to Wallenda inactivation is an interesting finding highlighting the importance of cell-cell interactions between oncogenic cells and their wild-type neighbors during early tumor formation.

Abstract 27 - Gustav Johansson

Considerations and quality controls when analyzing cell-free DNA

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Analysis of circulating cell-free DNA (cfDNA) is a promising biomarker in cancer, prenatal testing, transplantation medicine and beyond. Ultrasensitive technologies enable detection of low (< 0.1%) allele frequencies, a pre-requisite to fully utilize the potential of cfDNA for these applications. Reliable cfDNA analysis requires that the entire liquid biopsy workflow is carefully optimized.

Here, we illustrate important considerations for cfDNA detection in plasma. We show how each experimental step can easily be evaluated using simple quantitative PCR assays, including detection of cellular DNA contamination and PCR inhibition. Furthermore both DNA fragmentation and the target sequence is shown to affect cfDNA assay performance.

Finally, we show that quantitative PCR is useful to estimate the required sequencing depth and to monitor DNA losses throughout the workflow. The use of quality control assays enables the development of robust and standardized workflows that facilitate the implementation of ctDNA analysis into clinical routine.

Abstract 28 - Hannah C. Sonnenberg

The role of Biniou and Bagpipe transcription factors in Alk signaling during *Drosophila* embryogenesis

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Background: Amplifications and mutations of the Anaplastic lymphoma kinase (Alk) are connected to tumor growth and Alk is a high-risk factor in several cancer types, amongst others the pediatric cancer neuroblastoma as well as non-small-cell lung cancer. Our lab employs *Drosophila melanogaster* models to investigate Alk signaling and its downstream effects *in vivo* in a highly controlled genetic background. In *Drosophila*, Alk has a well-known function in the visceral mesoderm (VM).

My project aims to elucidate the involvement of two transcription factors, Biniou (Bin) and Bagpipe (Bap), in Alk signaling during *Drosophila* embryogenesis. Like Alk, the FoxF transcription factor Bin as well as the NK-like homeobox transcription factor Bap are essential for VM development. However, it is not known if either or both transcription factors are downstream targets of Alk signaling.

Materials & Methods: We employ genetic interaction studies to investigate Bin and Bap protein localization and/or expression levels in the context of *Alk* loss and gain of function. ChIP-seq analysis with endogenously-tagged Bin and Bap proteins to identify transcriptional targets of both factors during VM development have been performed to investigate the role of Alk signaling on Bin and Bap function. Our analyses compares ChIP-seq data obtained from embryos with *wild type*, *Alk* mutant and ectopically activated Alk backgrounds to identify changes in chromatin occupancy and gene expression.

Results: Endogenous tagging of either Bin or Bap transcription factors with CRISPR/Cas9 techniques results in genetically modified alleles that are homozygous viable and functional. Both modified proteins show a wildtype expression throughout the VM. Results from ChIP-seq analysis in backgrounds of variable Alk activation will be presented.

Abstract 29 - Helena Kristiansson

Analysis of cell-free tumor DNA using SiMSen-Seq

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Background: Analysis of circulating cell-free tumor DNA (ctDNA) in liquid biopsies offers new means for early cancer detection, real-time monitoring of treatment efficiency, and the discovery of relapse. Despite its potential use, ctDNA remains challenging to detect and to quantify as it represents only a small fraction of total plasma circulating cell-free DNA.

Materials & Methods: We have developed an ultrasensitive sequencing technology, SiMSen-Seq, that allows allele frequencies < 0.1% to be detected, using several kilobases of DNA. SiMSen-Seq is simple to perform, flexible in multiplexing and requires minimal DNA input. SiMSen-Seq allows detection of variant alleles with easy customization of library content and a protocol that can be implemented in any molecular biology laboratory.

Results & Conclusions: Here, we present how SiMSen-Seq can be implemented in a liquid biopsy workflow, including sample collection, cell-free DNA extraction, sequencing and finally bioinformatics to quantify extremely lowly prevalent disease-specific mutations.

Abstract 30 - Joachim Tetteh Siaw

Clinical response of the novel activating ALK-I1171T mutation in neuroblastoma to the ALK inhibitor ceritinib

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Background: Tumors with anaplastic lymphoma kinase (ALK) fusion rearrangements are highly sensitive to ALK tyrosine kinase inhibitors (TKIs). Although mutations in ALK are heavily implicated in childhood neuroblastoma, response to ALK TKI crizotinib has been disappointing. Embryonal tumors in patients with DNA repair defects such as Fanconi anemia (FA) often have a poor prognosis, because of lack of therapeutic options.

Material & Methods: Patient: A 16-mo-old boy patient was examined and MIBG scintigraphy, CT scans, and MRI revealed a metastatic neuroblastoma with metastases in bone, lungs, and intracranial.

Genomics profile with SNP array and parallel DNA sequencing were carried out followed by preclinical studies with neuroblastoma cell culture models.

Results: We report a child with underlying FA and *ALK* mutant high-risk neuroblastoma. Conventional chemotherapy treatment caused severe, life-threatening toxicity. Genomic analysis of initial biopsy identified germline *FANCA* mutations and novel *ALKI1171T* variant. ALK-I1171T generates potent gain-of-function mutant, as measured in PC12 neurite outgrowth and NIH3T3 transformation. Pharmacological inhibition profiling of ALK-I1171T in response to various ALK TKIs identified an 11-fold improved inhibition of ALK-I1171T with ceritinib when compared with crizotinib. Ceritinib was therefore selected for treatment in this child. Ceritinib monotherapy was well tolerated and resulted in normalized catecholamine markers and tumor shrinkage.

After 7.5 months treatment, residual primary tumor shrunk, was surgically removed, and exhibited hallmarks of differentiation together with reduced Ki67 levels. Clinical follow-up after 21 months treatment revealed complete clinical remission including all metastatic sites.

Conclusions: Ceritinib therefore, presents a viable therapeutic option for ALK-positive neuroblastoma.

Abstract 31 - Joanna Szydzik

The ALK/ROS1/TRK inhibitor, repotrectinib (TPX-0005), effectively reduces growth of ALK driven neuroblastoma cells.

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Background: Neuroblastoma is the most frequently diagnosed extracranial tumor in children, it can arise anywhere along the sympathetic nervous system however the majority of the patients develop the disease in the adrenal glands.

The first line of patient treatment is surgery following chemotherapy and/or radiotherapy causing tremendous side effects and approximately half of the patients with high risk neuroblastoma survive only up to 5 years.

Genetic analysis identified that *ALK* is involved in several cancer forms and it is presence in more than 50% of neuroblastomas relapses. *ALK* expression is high during foetal development and its expression decrease following birth which makes it a powerful target for treatment.

Different ALK inhibitors have been developed and approved by FDA for clinical use. Recently, a new ALK inhibitor, repotrectinib was developed and we tested it in neuroblastoma.

Materials & Methods: In the present study we investigated the effects of repotrectinib in a neuroblastoma setting *in vitro* and *in vivo*. Neuroblastoma cells were treated with repotrectinib to test if the drug can inhibit ALK and to determine its effect on proliferation. PC12 cells transfected with different ALK mutant variants indicated the efficacy of repotrectinib to block ALK activation/signaling. We tested the *in vivo* effect of repotrectinib in a neuroblastoma xenograft model.

Results: Our results show that repotrectinib is capable of inhibiting signaling activity of a range of ALK mutant variants and importantly it exhibits strong antitumor effects in a xenograft model of neuroblastoma.

Conclusions: Repotrectinib is a new potent ALK inhibitor for neuroblastoma.

Abstract 32 - Johan Bourghardt Fagman

p62/SQSTM1 accumulation is associated with poor patient survival in resected pancreatic ductal adenocarcinoma

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Background: Autophagy is a catabolic quality control process that promotes cellular homeostasis. Its impairment causes accumulation of the signal adaptor protein p62/SQSTM1 which has been shown to drive progression of pre-malignant lesions to pancreatic cancer and maintained malignancy in mice. However, the link between p62 accumulation and survival in PDAC patients is not known.

Materials & Methods: Immunohistochemical staining for p62 was performed on formalin-fixed paraffin-embedded tissue sections from 31 surgically resected patient tumors. Staining intensity and percentage of positive tumor cells were evaluated and the combined histoscore was examined for associations with clinicopathological characteristics and survival.

Results: High p62 protein expression in tumor cells was significantly associated with shorter overall survival (high p62: 7.9 months vs. low p62: 29.1 months, P = 0.044, log rank test). Hazard ratio for death in patients with high p62 protein expression in tumor cells compared with low p62 was 2.22 (95% CI: 1.01-4.90, *P* = 0.049, Cox regression). In multivariate analysis, where regional lymph node metastasis and p62 score were included, neither presence of regional lymph node metastasis nor high p62 protein expression score remained as significant independent prognostic factors for overall survival (*P* = 0.094 and *P* = 0.113, Cox regression).

Conclusions: Our results show that high p62 protein expression in tumor cells is associated with reduced survival following pancreatic tumor resection. This supports a role for p62 in predicting clinical outcomes and as a potential target for development of targeted therapy.

Abstract 33 - Junko Johansson

Isolated limb perfusion with melphalan may trigger the CXCL10/CXCR3 axis to induce tumour regression in patients with metastatic melanoma

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Background: Patients with melanoma metastases confined to the limbs may be treated with hyperthermic isolated limb perfusion with melphalan (M-ILP), with a complete response (CR) rate of ~60%. The melphalan-induced killing of melanoma cells triggers the release of tumour cell-derived constituents that may stimulate transcription of interferon-stimulated genes (ISGs). Here we explored the effects of M-ILP on ISG responses with focus on the chemokine CXCL10 and its receptor CXCR3.

Materials & Methods: Peripheral blood from melanoma patients was obtained before and 1 month after M-ILP. Samples were analysed for chemokines and chemokine receptors. To define the mechanism of ISG induction, an *in vitro* model was established where melphalan-exposed melanoma cells were cultured alone or in the presence of immune cells.

Results: Patients achieving CR after M-ILP showed higher serum levels of CXCL10 prior to M-ILP ($p<0.05$) and higher levels of the ISGs CCL2 and PD-L2 after M-ILP ($p=0.01$ for both). The levels of PD-L2 increased following M-ILP ($p=0.01$) with a similar trend for CXCL10 and CCL2.

We observed that the expression of the receptor CXCR3 was higher on NK cells and T cells from melanoma patients than from healthy controls ($p<0.05$). Analysis of supernatants from *in vitro* cultures revealed that melphalan-exposed melanoma cells triggered a massive production of CXCL10 from immune cells, and that T cells and NK cells from these cultures expressed higher levels of CXCR3.

Conclusions: Our results suggest that constituents released from melphalan-exposed melanoma cells stimulate the CXCL10/CXCR3 axis, which may contribute to anti-tumoural efficacy of M-ILP.

Abstract 34 - Karoline Berger

High Progranulin expression is associated with poor prognosis in ERα positive and lymph node positive breast cancer patients

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Background: Progranulin is involved in various biological processes such as proliferation, tumorigenesis and inflammation. Progranulin and its associated receptor sortilin are known to be overexpressed in subgroups of breast cancer and are further associated with clinically aggressive properties. The aim of this study was to determine if progranulin could be used as a biomarker for breast cancer prognosis and treatment prediction.

Materials & Methods: We used tissue microarrays from a randomized trial including 564 premenopausal breast cancer patients receiving either tamoxifen treatment or no adjuvant treatment. The data were analyzed using immunohistochemistry and stained for progranulin and sortilin expression to determine their associations with clinicopathological data and breast cancer specific survival (BCSS).

Results: High Progranulin expression was linked to decreased BCSS in untreated lymph-node positive (LN+) breast cancer patients (p=0.004). In this group, we found significant associations between progranulin expression and tumour grade (0.375**), sortilin (0.167*), HIF (0.328**), Ki67 (0.286**), ERα (-0.389**) and PR (-0.270**). Interestingly, high progranulin combined with high sortilin expression further defined a highly malignant subgroup of patients. Also, in ERα+ patients, high progranulin expression was associated with decreased BCSS independent of treatment.

Conclusions: In this study we showed that high progranulin expression was associated with poor survival in ERα+ and LN+ breast cancer patients. We also identified a highly malignant subgroup expressing both progranulin and sortilin. These data suggest that high progranulin expression can be used as a prognostic biomarker in ERα+ breast cancer and that the high-risk groups could potentially be targeted by anti-sortilin based therapies.

Abstract 35 - Kathrin Pfeifer

Analysis of neuroblastoma-derived *ALK* mutations in *Drosophila melanogaster*

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Background: The receptor tyrosine kinase (RTK) anaplastic lymphoma kinase (ALK) belongs to the insulin receptor superfamily of RTKs. ALK amplifications and various point mutations within the kinase domain have been identified in neuroblastoma (NB). We employ *Drosophila* as a “NB-model” to gain further molecular insights into the contribution of patient derived Alk point-mutations in a stable genetic background.

Materials & Methods: CRISPR/Cas9 mediated homology directional repair has led to the successful generation of *ALK-F1251L* (human F1174L) and *ALK-Y1355S* (human Y1278S) gain-of-function (GOF) within the endogenous locus.

Results: In both mutants we find increased neurogenesis resembling ALK gain-of-function phenotypes described in mice. In case of *ALK-Y1355S* ectopic pERK in the visceral mesoderm (VM) can be detected similar to ligand over expression. Closer examination of the VM showed an ectopic distribution of the Alk ligand Jeb compared to the wild type. In *ALK-Y1355S,* Jeb activates more ALK positive cells leading to increased ALK signaling. Over expression of dominant negative Rab5 in the VM phenocopies the *ALK-Y1355S* phenotype.

Conclusions: This finding leads to the assumption that ALK GOF point mutations might not get negatively regulated by the endosomal/lysosomal compartment raising the possibility for the ligand to engage more cells and increase ALK signaling.

Abstract 36 - Kristell Barreau

Single-cell bisulfite sequencing to determine DNA methylation alterations after fractionated irradiation of pediatric glioma stem cells

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Background: Tumor recurrence of pediatric high-grade glioma (pHGG) brain tumors is common despite aggressive treatments including surgery, radiotherapy and chemotherapy. The recurrence is thought to be driven by the presence of cancer stem cells (CSCs) which are resistant to current therapies. Epigenetic aberrations in CSCs could be involved in mediating the resistance to therapy. It is therefore critical to define heterogeneity within CSCs in order to identify new therapeutic approaches.

Materials & Methods: We use manual single-cell isolation and bisulfite sequencing (sc-BS-seq) to determine the DNA methylation profiles of pediatric glioma CSCs, and how these profiles alter during therapy in individual cells.

Results: The optimized sc-BS-seq protocol provides good mapping efficiency (45-49%) and 10% CpG coverage as expected. We have isolated cells with different morphologies following fractionated irradiation (5x 2 Gy), without contamination from the surrounding suspension. Ongoing experiments will determine the DNA methylation of the cells using our optimized pipeline.

Conclusions: sc-BS-seq can be used to investigate DNA methylation profiles in single cell from pediatric HGG cancer stem cell cultures. Single-cell methylation profiling allows us to answer questions on therapy acquired alterations vs therapy induced selection of subpopulations.

Abstract 37 - Louis Szeponik

Regulatory T cells suppress the cytotoxic phenotype of T cells in intestinal tumors of APCmin/+ mice

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Background: The presence of activated T cells in colorectal cancer (CRC) tissues is a strong predictor of patient survival. Our previous research have shown that regulatory T cells (Treg) are able to reduce T-cell transendothelial migration in vitro and accumulation of effector T cells in intestinal tumors in vivo in a murine model for intestinal adenomas ($APC^{Min/+}$).

Materials & Methods: In this study we investigated the effect of Treg depletion on the cytotoxic potential of conventional αβ T cells and γδ T cells in intestinal adenomas. We used the $APC^{Min/+}$ \DEREG mouse model, which harbour a high affinity diphtheria toxin receptor under the control of the FOXP3 promoter, to deplete Treg in tumor bearing mice.

Results: We found that the number of CD8αβ T cells per mg tissue in the lamina propria was significantly increased in the Treg depleted intestinal adenomas in comparison to the non-depleted tumors. We could confirm this finding with IHC staining in tissue sections. The number of CD8αα T cells and γδ T cells remained unchanged.

Furthermore, ex vivo frequencies in the lamina propria of Granzyme B^+ CD8αβ T cells were increased in the Treg depleted intestinal adenomas. Following in vitro stimulation of lymphocytes from lamina propria with PMA/ionomycin, there was a trend towards a higher frequency of IFNγ $^+$ CD8αβ T cells in Treg depleted adenomas, but frequencies of CD107a$^+$ CD8αβ T cells were decreased.

Conclusions: The results indicate that the modulation of Treg in colorectal cancer could have a positive effect on T cell cytotoxicity.

Abstract 38 - Lukas Lundholm

Non-invasive assessment of tumour microstructural environment using VERDICT evaluation of diffusion magnetic resonance imaging data

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Background: Non-invasive methods for tumour microenvironment characterization would facilitate prediction and early assessment of therapy response and personalized treatment planning. A promising method that should be examined for such purposes is to use the VERDICT model based on diffusion weighted magnetic resonance imaging (DWI). VERDICT provides quantitative estimates of microstructural parameters such as cell size, and intracellular, extracellular and vascular volume fraction, with sub-millimetre spatial resolution. However, the robustness and interpretation of these parameters need to be evaluated and results compared with histological methods.

The aim of this study was to optimize DWI for VERDICT based assessment of radiotherapy response in a mouse model of small-intestine neuroendocrine tumour (GOT1).

Materials & Methods: Balb/C nude mice with s.c. GOT1 xenografts were either irradiated externally by 5 MeV photons or not irradiated (controls). The mice were imaged *in vivo* using the 7T preclinical MR system at Gothenburg University before and after irradiation, using a DWI-method that we optimized for VERDICT evaluation. Parametric tumour maps of VERDICT estimates were created. After last imaging, the mice were euthanized and the tumour harvested for histological analyses.

Results: VERDICT maps of cell radius, intracellular, extracellular and vascular volume fractions demonstrated intra-tumour heterogeneity. Analyses of the histological sections are not finalised. So far VERDICT estimates agree well with histological parameters from previous studies on GOT1 model.

Conclusions: The results so far demonstrate the feasibility of using VERDICT for characterization of tumour microstructure. We anticipate the results of the radiotherapy experiments to evaluate VERDICT for treatment response prediction.

Abstract 39 - Mahmood Faraz

LRIG1 gene copy number analysis by droplet digital PCR and correlations to clinical factors in breast cancer

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Background: Leucine-rich repeats and immunoglobulin-like domains 1 (*LRIG1*) copy number alterations are reported to occur in breast cancer. Importantly, *LRIG1* loss was recently shown to predict early and late relapse in stage I-II breast cancer.

Materials & Methods: We developed droplet digital PCR (ddPCR) assays for the determination of relative *LRIG1* copy numbers and used these assays to analyze *LRIG1* in twelve healthy individuals, 34 breast tumor samples previously analyzed by fluorescence *in situ* hybridization (FISH), and 423 breast tumor cytosols.

Results: Four of the *LRIG1*/reference gene assays were found to be precise and robust, showing copy number ratios close to 1 (mean, 0.984; standard deviation, +/- 0.031) among the healthy control population. The correlation between the ddPCR assays and previous FISH results was poor, possibly because of the different normalization strategies used. One in 34 breast tumors (2.9%) showed an unbalanced *LRIG1* recombination event.

LRIG1 copy number ratios were associated with the breast cancer subtype, steroid receptor status, *ERBB2* status, tumor grade, and nodal status. Both *LRIG1* loss and gain were associated with unfavorable metastasis-free survival; however, they did not remain significant prognostic factors after adjustment for common risk factors in the Cox regression analysis. Furthermore, *LRIG1* loss was not significantly associated with survival in stage I and II cases.

Conclusions: Although *LRIG1* gene aberrations may be important determinants of breast cancer biology, the results of this study do not verify an important role for *LRIG1* copy number analyses in predicting the risk of relapse in early-stage breast cancer.

Abstract 40 - Malin Larsson

Long-term effects in thyroid and plasma after internal low dose exposure with ^{131}I in rat

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Background: ^{131}I-labelled (iodide) tumour targeting agents are used to treat various types of cancer, including thyroid cancer where ^{131}I may be released and accumulated in the thyroid. During the Chernobyl accident, ^{131}I was released resulting in the induction of thyroid cancer in children. The aim of this study was to identify biomarkers for long-term effects of ^{131}I exposure in the thyroid, related to thyroid function and cancer, using rats as a model system.

Materials & Methods: Male Sprague Dawley rats (5w) were i.v. injected with 0.5- 500 kBq ^{131}I ($D_{thyroid}$ ca 0–1 Gy). Rats were killed after 9 months, and the thyroid and blood samples were collected. Microarray analysis was performed using RNA extracted from thyroid tissue and associated GO terms were identified, followed by protein expression analysis using LC-MS/MS on thyroid tissue and blood samples. Ingenuity Pathway Analysis (IPA) was used to analyse canonical pathways, function and diseases and upstream regulation.

Results: The Significantly regulated transcripts *Afp* and *RT1-Bb* were found in all test groups and are suggested biomarkers for ^{131}I exposure. Proteins with dose-dependent expression were identified (LDHA, APRT, NOL3, YBX3, MYBPC3, HADHA, TGM3 and DSG4). Also, thyroid function and cancer related proteins thyroid peroxidase and thyroglobulin were seen.

Conclusions: Exposure and absorbed dose-related effects on gene and protein expression were observed, with several genes related to thyroid function or cancer. The results describe a biological basis for carcinogenesis in thyroid tissue after ^{131}I irradiation.

Abstract 41 - Malin Lindén

FET fusion oncoproteins bind the SWI/SNF chromatin remodeling complex

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Background: The FET family of fusion oncogenes carry one of the three genes, *FUS, EWSR1* or *TAF15,* as 5'-partners juxtaposed to one of many different DNA binding transcription factor genes as 3'-partners. The FET oncogenes are in many cases the only mutation present in the tumor cells indicating that they alone have the capacity to induce tumor development. We recently discovered that FET fusion oncoproteins bind to, and affect the ~2 MDa SWI/SNF chromatin remodeling complex thus providing a direct link to epigenetic mechanisms and global transcriptional effects. The aim of the present study is to further investigate the FET proteins' binding to SWI/SNF.

Materials & Methods: We used immunoprecipitations followed by mass spectrometry or western blot to extensively characterize the interaction between FET fusion oncoproteins and the SWI/SNF complex.

Results: We show that the N-terminal parts of normal and oncogenic FET proteins bind core components of the SWI/SNF complex. In contrast to normal FET proteins, major fractions of FET oncoproteins bind SWI/SNF, indicating a dysregulated interaction. Overexpression of FUS-DDIT3 did not affect the amount of normal FET proteins bound to SWI/SNF. This lack of binding competition between normal and oncogenic FET proteins suggest that they bind different sites on SWI/SNF or different variants of SWI/SNF.

Conclusions: The discovery that FET fusion oncoproteins bind the SWI/SNF complex provides a unifying explanation for tumorigenesis for the large group of tumors caused by FET fusion oncogenes thus providing an opportunity for a new mutual treatment strategy.

Abstract 42 - Malin Nilsson

Genetic and pharmacological inhibition of NOX2 delays the expansion of murine BCR-ABL1$^+$ leukemia

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Background: Reactive oxygen species (ROS) produced by the myeloid NADPH oxidase (NOX2) are central effectors in the immune defense against microbes, but may also be harmful to cell components upon imbalance between their generation and detoxification. Elevated ROS production and concomitant genomic instability have been described as common features in malignant cells of patients with BCR-ABL1$^+$ chronic myeloid leukemia (CML), but further studies are required to define the role of ROS in leukemogenesis. Here, we utilized genetically engineered murine hematopoietic cells to evaluate the importance of NOX2-derived ROS for the *in vivo* expansion of BCR-ABL1$^+$ cells.

Materials & Methods: Irradiated C57BL/6J mice received transplants of wild-type (WT) or *Nox2* knockout (*Nox2*-KO) hematopoietic cells transduced with GFP-labeled human *BCR-ABL1*. Peripheral blood was drawn biweekly for fluorimetric quantification of BCR-ABL1$^+$ cells. To determine the effects of pharmacological inhibition of NOX2 on leukemic expansion, the NOX2 inhibitor histamine dihydrochloride (HDC) was administered to mice carrying WT BCR-ABL1$^+$ transplants (1.5 mg 3 times/week i.p.).

Results: The *in vivo* expansion of *Nox2*-KO BCR-ABL1$^+$ cells was reduced compared to that of WT BCR-ABL1$^+$ cells, which translated into prolonged survival of mice carrying *Nox2*-KO transplants. In the HDC treatment experiment, only 2/5 HDC-treated mice showed BCR-ABL1$^+$ cell expansion in blood during the study period, compared to 6/7 mice in the untreated cohort. Initial experiments using primary human cells revealed increased expression of NOX2-related genes in CD34$^+$CD38$^-$ leukemic stem cells vs. cord blood stem cells.

Conclusions: Our results implicate NOX2 as a conceivable therapeutic target in BCR-ABL1$^+$ leukemia.

Abstract 43 - Maria Carmen Leiva

Scaffolds derived from breast cancer as a model to study drug response

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Background: Breast cancer is a heterogeneous disease regarding cellular composition, compartments and cancer cell subtypes including cancer stem cells, which have been associated with metastasis, tumor relapse and treatment failure. Traditional 2D cell growth lacks the complexity of proper cell-cell contact and the interaction with the extracellular matrix (ECM) that is known to influence cell proliferation, migration, differentiation and drug response.

Materials & Methods: Our research group has developed a novel 3D model based on tumors from breast cancer patients that have been decellularized by sequential detergent washes. The resulting scaffolds were repopulated with a standardized breast cancer cell line and treated with front line chemotherapy agents. Gene expression and cytotoxicity were analyzed.

Results: The cells growing in scaffolds presented a more undifferentiated phenotype and an increased robustness in comparison to the 2D counterpart. In addition, treatment with 5-FU and DOX influenced the expression of genes related to epithelial-mesenchymal transition, proliferation, differentiation and pluripotency, suggesting different effects on various cancer cell subpopulations. Cell death at higher doses triggered a diverse behavior in DOX with inhibition of the cancer stem cell low proliferative population, whereas little differences were seen in 5-FU effect.

Conclusions: Our scaffolds recreate the human tumor microenvironment, providing information about the influence of the ECM in drug response. Additionally, scaffolds may be used in clinics as a tool for complementary breast cancer diagnosis and in cancer research for screening of potentially new antitumor drugs.

Abstract 44 - Mikael Elvborn

Fractionated Lu-177-octreotate treatment lead to prolonged survival of female BALB/c mice with small-intestinal neuroendocrine tumors GOT1

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Background: Many neuroendocrine tumors have a high somatostatin receptor (SSTR) expression and can be treated with radiolabeled somatostatin analogs. Although promising, SSTR mediated radionuclide therapy needs to be personalized due to individual differences in tumor uptake, inherent radiosensitivity and response to treatment.

Materials & Methods: GOT1-bearing BALB/c nude mice were intravenously injected three times with 24 h apart. The mice received totally 120 MBq ^{177}Lu-octreotate, either as 1x120, 2x60 or 3x40 MBq ^{177}Lu-octreotate with saline at the remaining occasions. Tumor volume was determined twice per week. Animals were killed when tumor weight exceeded 10% of body weight.

Results: Overall, fractionated treatment resulted in different tumor regression than single treatment. Mean relative tumor reduction was similar for the three groups but the time to regrowth was almost doubled for mice treated with three fractions. The maximal mean tumor volume reduction was 97% after 4 weeks for mice treated with 3 fractions of ^{177}Lu-octreotate, while it was 92% at 3 weeks for the other schedules. The fractionated treatments prolonged the overall survival by 25% compared with single administration.

Conclusions: The study showed longer time to regrowth and overall survival after three fractions of ^{177}Lu-octreotate compared with single administration. The mechanisms behind these findings should be investigated further, in order to improve the treatment schedules clinically used today.

Abstract 45 - Mikael Montelius

Multiparametric magnetic resonance imaging for characterization and assessment of response after 177Lu-octreotate therapy in a human neuroendocrine tumor model

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Background: ^{177}Lu-octreotate-treatment show promise for patients with inoperable small-intestine neuroendocrine tumor (NET), but requires optimization. Multiparametric MRI (mpMRI) enables non-invasive tumor tissue characterization for response assessment, but choice, timing and interpretation of mpMR-derived tissue parameters needs evaluation. The aim was to evaluate mpMR for characterization of siNETs receiving ^{177}Lu-octreotate.

Materials & Methods: Mice (n=21) bearing human siNETs (GOT1) received ^{177}Lu-octreotate (15MBq) day0. mpMRI were conducted at 7T (animal MRI, Sahlgrenska academy) days -1,1,3,8,13, followed by harvesting of tumor tissue for proteomics. In-house developed post-processing algorithms enabled extraction of mpMR-parameters for correlation with response (tumor shrinkage) and proteomics.

Results: Several statistically significant correlations were found between MR-parameters, response and protein levels, including early (day3) increased tissue water diffusion in peripheral tumor, response and CATA levels (Catalase, encoded by *CAT,* previously associated with oxidative stress, proliferation, cell cycle arrest, and apoptotic cell death); pre-treatment (day-1) rapid uptake of contrast media, reflecting redistribution-rate of nutrients and oxygen between interstitial and vascular space, response and CCD89 (Coiled-Coil Domain Containing 89, encoded by *CCDC89* (DNA damage & repair, proliferation, cell cycle arrest)).

Five mpMR-parameters were sensitive for response assessment only when evaluated regionally (60-80% of tumor centre-to-periphery distance). Twenty-four MR-parameters required evaluation as delta-values (relative to earlier measurement) between day-1 and day1 (Δday-1:1). Corresponding number for Δday-1:3 and Δday3:8 were 4, 11 and 3, respectively.

Conclusions: mpMRI shows great potential for tumor characterization and early response assessment in animal models of siNET treated with ^{177}Lu-octreotate, but requires spatiotemporal evaluation to account for tumor spatial and temporal heterogeneity.

Abstract 46 - Muhammad Wasi Alam

Alectinib, an anaplastic lymphoma kinase inhibitor, abolishes ALK activity and growth in ALK-positive neuroblastoma cells

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Background: Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor, belongs to insulin receptor (IR) family, which has been implicated in numerous solid and hematologic cancers. Approximately 9% of initially diagnosed neuroblastoma harbor ALK mutations, however, in the relapsed patient population it has recently been reported that up to 20-25% of tumors contain ALK mutations suggesting that ALK may be a more important factor in the development of neuroblastoma than previously thought.

Crizotinib, the first clinically approved ALK inhibitor for the treatment of ALK-positive lung cancer has had less dramatic effect in neuroblastoma. Alectinib, is a second-generation ALK inhibitor and showed a dramatic effect in ALK-positive lung cancer.

Materials & Methods: We analyzed alectinib activity in ALK-driven neuroblastoma models in vitro and in vivo.

Results: In vitro kinase assays and cell-based experiments examining ALK mutations of increasing efficacy show that alectinib as an effective inhibitor of ALK with greater activity towards ALK neuroblastoma mutants. Administration of alectinib showed efficient tumor reduction in vivo, in subcutaneous xenograft, in comparison to crizotinib. Dramatic inhibition of ALK activity was observed in vitro in different constitutively active ALK variants in biochemical assays.

Conclusions: These results suggest that alectinib is an efficient inhibitor of ALK kinase activity in ALK addicted neuroblastoma and should be considered as a potential future therapeutic option for ALK-positive neuroblastoma patients alone or in combination with other treatments.

Abstract 47 - Patrik Sundström

Tumor-infiltrating Mucosa-Associated Invariant T (MAIT) cells express cytotoxic effector molecules and kill target cells

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Background: Mucosa-associated invariant T (MAIT) cells all express a semi-invariable T cell receptor recognizing microbial metabolites presented on the MHC class I-like molecule MR1. Upon activation, they rapidly secrete cytokines and increase their cytotoxic potential. We showed recently that MAIT cells accumulate in human colon adenocarcinomas, but that their ability to produce IFN-□ upon polyclonal stimulation is compromised.

Materials & Methods: Here, we investigated the cytotoxic potential of MAIT cells in colon adenocarcinoma patients, and to what extent it may be affected by the tumor microenvironment. MAIT cells were identified by flow cytometry and analyzed for their expression of cytotoxic effector molecules and degranulation.

Results: Polyclonal, T cell receptor-, and cytokine-mediated activation of MAIT cells from tumors induced increased Granzyme B, while degranulation was mainly seen in response to cognate antigen recognition. The cytotoxic potential of tumor-associated MAIT cells was similar to that of MAIT cells from unaffected colon. Furthermore, tumor infiltrating pre-activated MAIT cells killed antigen-presenting target cells. MAIT cells were also identified by immunofluorescence in direct contact with tumor cells in sections from colon cancer specimens.

Conclusions: Taken together, our data demonstrate that tumor-associated MAIT cells from colon tumors have potent cytotoxic function and are not compromised in this regard compared to MAIT cells from the unaffected colon. We conclude that MAIT cells may contribute significantly to the protective immune response to tumors, both by secretion of Th1-associated cytokines and by direct killing of tumor cells.

Abstract 48 - Peter Micallef

The adipokine C1qTNF3 is increased in breast cancer-associated adipose tissue and pushes M2-type macrophages towards an M1-like phenotype

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Background: Breast cancer tumors originate in an adipose tissue-rich environment, implying that local interactions between adipose tissue-resident cells and cancer cells may be of particular importance for the malignant progression of these cancers. We have identified the adipokine C1qTNF3 as one of the most upregulated secreted proteins in breast cancer-associated adipose tissue. Previous research suggests that C1qTNF3 affects both mitochondrial and immunological function. Thus, we hypothesize that increased adipose tissue C1qTNF3 levels affects breast cancer progression via immunometabolic reprogramming.

Materials & Methods: To test this hypothesis, we orthotopically injected obese C57BL/6 female mice with the triple-negative breast cancer cell line E0771 and compared the effect of C1qTNF3-neutralizing and isotype control antibody treatment on leukocyte infiltration (FACS analysis of spleen, tumor and mammary adipose tissue) and tumor growth. The effect of C1qTNF3 on macrophage metabolism (respiration and glycolysis) and polarization was analyzed in bone marrow-derived macrophages using respectively, Seahorse technology and qPCR.

Results: C1qTNF3-antibody treatment reduced the number of M1-macrophages in tumors and mammary adipose tissue, while tumor size remained similar between groups. In cultured macrophages, C1qTNF3 treatment decreased respiration and increased glycolysis associated with increased expression of M1-markers, thus providing a plausible mechanism underlying the observed reduction of M1-macrophages in tumor and adjacent adipose tissue in vivo.

Conclusions: Infiltration of oxidative M2-macrophages increase in late stage tumors and can stimulate tumor progression. Based on our data, we propose that breast cancer-induced increase in adipose tissue C1qTNF3 levels play a role in tumor rejection through its immunometabolic effects on infiltrating macrophages.

Abstract 49 - Roberta Kiffin

Wounding-induced inflammation facilitates murine melanoma metastasis via activation of NOX2

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Background: The trauma associated with cancer surgery may result in release of tumor cells into the blood stream along with a systemic inflammation that hampers cellular immunity. These factors may enhance the risk of establishment of distant metastases. During inflammation and cancer, myeloid cells produce enhanced levels of NOX2-derived reactive oxygen species (ROS), which trigger dysfunction and apoptosis in adjacent anti-neoplastic lymphocytes.

In this study, we explored the role of NOX2-derived ROS in surgery-induced immunosuppression and metastasis formation *in vivo* by using a wounding model.

Materials & Methods: To mimic post-surgical inflammation, sterile PVA sponges were implanted s.c. to WT and NOX2-deficient (*Nox2*$^{-/-}$) mice one week before i.v. injection of B16F10 melanoma cells.

Results: The presence of sponges increased the number of pulmonary metastases formed from the injected melanoma cells in WT mice ($p=0.0015$, $n=19$), but not in corresponding *Nox2*$^{-/-}$ mice ($p>0.5$, $n=5$). Treatment with the NOX2-inhibitor histamine dihydrochloride (HDC) significantly reduced metastases induced by surgical inflammation in WT mice ($p<0.0001$, $n=10$).

In blood drawn from sponge-bearing WT mice one week after implantation, there was a significant increase of $CD11b^{+}Ly6C^{+}$ inflammatory monocytes ($p<0.0001$, $n=18$) and their ROS production ($p=0.01$, $n=10$). No increase of inflammatory monocytes was noted in sponge-bearing *Nox2*$^{-/-}$ mice ($p>0.5$, $n=3$). HDC treatment completely prevented the increase of inflammatory monocytes in wounded WT mice ($p<0.0001$, $n=10$).

Conclusions: These data suggest that surgical inflammation may augment $NOX2^{+}$ inflammatory monocytes in blood that, in turn, may reduce immunosurveillance and enhance metastasis formation.

Abstract 50 - Roumiana Chakarova

Optimization of absorbed dose determination and dose delivery in external beam radiotherapy- Focus on dosimetry effects of lung tissue interpretation

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Background: The usage of respiratory gating techniques, such as deep inspiration breath hold (DIBH), rapidly increases because of dose sparing effects on organs at risk. Lung densities much lower than the usual inhale ones may appear and the lung interpretation needs more investigation. The aim of this work was to study the impact of lung tissue segmentation on the determination of the lung dose using high energy photon beams for breast cancer treatment under DIBH.

Materials & Methods: One hundred consecutive patients receiving radiation therapy under DIBH with plans generated in Eclipse system were selected. Monte Carlo (MC) method was applied to determine absorbed dose distributions. Two methods for tissue segmentation were implemented with different air-lung boundaries and slopes of the CT calibration curve. Results were compared with AcurosXB calculations in Eclipse. Measurements in air-lung phantom were performed by thermoluminescent dosimeters and diamond detector.

Results: Differences in the lung dose distributions determined by the two segmentation methods were found in 10% of the patients. The deviations were larger for densities close to air; 0.7%, 3.6% and 9.6% for DVH estimates V_{16Gy}, V_{8Gy} and V_{4Gy}. One of the methods complied better with AcurosXB showing deviations up to 4.5% (V_{4Gy}). The lung in this case was presented as a mixture of air and lung in both MC and AcurosXB.

Conclusions: Dose uncertainties for low density lung tissues should be considered in the plan evaluation criteria. Accurate calculation of dose to air becomes important in the cases of DIBH.

Abstract 51 - Saumyaa

Heterogeneity of Natural Killer and Innate Lymphoid Cells in Human Head and Neck Squamous Cell Carcinoma

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Background: The presence and function of innate lymphoid cells (ILC), including natural killer (NK) cells, within tumors has been poorly characterized. Here we have assessed the heterogeneity of NK and ILC populations through single-cell RNA sequencing (scRNAseq) of hundreds of individual NK cells and ILCs within human head and neck squamous cell carcinoma (HNSCC) and matched peripheral blood.

Materials & Methods: Fresh tumor specimens and blood were obtained from patients undergoing surgical resection of HNSCC at the Stanford Medical Center (Stanford, CA) after informed consent, in accordance with an Institutional Review Board (IRB)-approved protocol. Tumor samples were digested mechanically to obtain single-cell suspensions. Single-cell suspensions were stained with antibodies and sorted as single cells into 96-well plates using flow cytometry. Libraries were prepared using the Smart-Seq2 protocol. Samples were sequenced and analyzed using the Seurat R package.

Results: Unsupervised clustering by t-SNE revealed heterogeneous clusters of NK cells and ILC subsets in HNSCC tumor tissue and blood. NK cells and ILCs from blood showed a transcription signature different than those from tumors. Within the tumor, we observed significant heterogeneity, with distinct subsets showing conventional NK, ILC1-like, ILC2-like and ILC3-like profiles. We further observed transcriptionally diverse subpopulations within the ILC1-like clusters.

Conclusions: Here, we have, for the first time, characterized NK cells and ILCs at a single cell level in HNSCC. We have identified 7 distinct gene expression clusters of these cells, within the HNSCC tumor tissue.

Abstract 52 - Simona Salerno

3D Printed Scaffolds (3DPS) as a Model of Breast Cancer Tumor Microenvironment in Cancer Drug Discovery

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Background: Cancer stem cells (CSC) constitute a subset of a breast tumor cell population, which underlies tumor initiation and resistance to drug therapy. The extra cellular matrix has been shown to regulate the transition of cancer cells into a stem-like phenotype and play an important role on the onset of breast cancer, shedding light on the challenge to develop predictive *in-vitro* drug screening platforms.

In this study, we used a hydrogel based 3DPS with editable architecture and material composition resembling patient-derived scaffolds (PDS), obtained by decellularizing patient primary tumors, to use as *in-vitro* CSC enriching platform for cancer drug screenings.

Materials & Methods: Using an extrusion-based 3D-printer we produced a hydrogel-based scaffold in which we could incorporate cues from the tumor microenvironment. MCF7 breast cancer reporter cells cultured on 3DPS, PDS and 2D were treated with increasing concentrations of doxorubicin and 5-Fluorouracil. The genetic response of the reporter cells to the cytotoxic drugs was evaluated via qPCR.

Results: Both drugs induced a similar antiproliferative effect in both 2D, PDS and 3DPS cultured cells, although more moderately in PDS and 3DPS even with higher doses. Cells cultured on 3DPS and PDS acquired resistance to the drugs compared to 2D cultured cells. Importantly, reporter cells had a scaffold specific drug response in marker genes related to stemness, differentiation and epithelial-to-mesenchymal transition.

Conclusions: We present a proof-of-concept of using a hydrogel based 3DPS as a model of tumor microenvironment for breast cancer drug discovery.

Abstract 53 - Sofia Saadati

Radiolabeled somatostatin analogue binding and internalization in human tumor cell lines

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Background: Targeting somatostatin receptor abundant on neuroendocrine tumors (NETs) with the radiolabeled somatostatin analogues enables tumor visualization and therapy. Systemic treatment with ^{177}Lu-octreotate has shown exciting results for disseminated gastroenteropancreatic NETs. The aim of this study was to find other tumor types with human origin that also could benefit from this type of treatment.

Materials & Methods: Human tumor cell lines from lung (h1975, h2228) and breast (BT474, MCF-7, MDA-MB-231, MDA-MB-361, T47D, ZR-75-1), and neuroblastoma (CLB-BAR, IMR-32) were studied. Internalization was studied for two concentrations of ^{177}Lu-octreotate at 24h and/or 48h. The amount of unbound, membrane-bound and internalized ^{177}Lu was measured by a gamma counter.

Results: Neuroblastoma cell lines IMR-32 (58% internalized, 9.4% membrane-bound) and CLB-BAR (26% internalized, 3.4% membrane-bound) showed highest binding and internalization after 24h. A majority of the breast cancer cell lines also showed specific uptake (*e.g.* 3.1% internalized, 0.5% membrane-bound in MDA-MB-361). Values were compared with those of primary cell culture from 2 gastrointestinal stromal tumors (5.8-8.0% internalized, 0.45-0.99% membrane-bound). In small-intestine NET GOT1 cell line around 0.5% was internalized and 0.01% was membrane-bound after 6 h. No specific binding was seen in lung adenocarcinoma cell lines.

Conclusions: In this study we demonstrate high uptake in several of the studied tumor cell lines with various origin. The results suggest that this type of treatment could be applied also for other tumor types that those in clinical practice today.

Abstract 54 - Soheila Dolatabadi

The role of the fusion oncogenes and cancer stem cells in myxoid liposarcoma

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Background: Myxoid liposarcoma (MLS) is characterised by the *FUS-DDIT3*, or the less common *EWSR1-DDIT3* fusion oncogene and is the second most common type of liposarcoma. The fusion oncogenes encode chimeric transcription factors that are causal factors in tumourigenesis. Notwithstanding continuous progress in treating MLS patients, existing therapies suffer from a major flaw as they do not target the cancer stem cells (CSCs).

Unique features of CSCs include self-renewal, tumour initiating capacity and increased resistance to radiotherapy- and chemotherapy-induced cell death. MLS displays extensive intratumoural heterogeneity with distinct subpopulations of tumour cells but little is known about their features or roles in the tumour. The aims of this project were to define the role of fusion oncogenes in tumourigenesis and to define signalling pathways controlling CSC features in MLS.

Methods: To define CSCs properties and therapy resistance in MLS we have employed functional cell assays, single-cell gene expression profiling, western blot, immunohistochemistry and flow cytometry analysis using cell lines and clinical samples.

Results: We investigated the regulatory mechanisms, expression levels and effects of *FUS-DDIT3* in detail, and showed that *FUS-DDIT3* was uniquely regulated at both transcriptional and post-translational level. Furthermore we showed that MLS harbour subpopulations of cells with CSCs properties and that their number is controlled by JAK-STAT signalling. Our single-cell data showed that individual cultured MLS cells expressed different amounts of canonical JAK-STAT transcripts.

Conclusions: Our findings concerning *FUS-DDIT3* function and CSCs have increased our molecular understanding of tumour development and therapy resistance in MLS that will facilitate development of specific treatment strategies.

Abstract 55 - Stefan Filges

Ultrasensitive mutation detection in FFPE tissue and circulating tumor DNA of metastatic breast cancer patients

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Background: Breast cancer is the most common malignancy in women and one in eight to ten women will be diagnosed with breast cancer during their lifetime. For metastatic patients the outcome remains poor and there is great need to optimise treatment strategies to prolong survival and increase quality of life.

Here, we evaluate the effect of additional angiogenesis inhibiting therapy to endocrine treatment in patients with an endocrine resistant breast cancer to discover new predictive markers and potential markers for monitoring treatment effect.

Materials & Methods: The patients (n = 12) received persistent endocrine therapy in combination with low dose continuous cyclophosphamide, capecitabine and bevacizumab. We identified mutations in the FFPE tissue of the primary tumor using a custom, targetted sequencing panel, covering the most commonly mutated genes in breast cancer, such as *PIK3CA*, *TP53*, *PTEN*, *AKT* and *KRAS*.

Blood samples were taken at baseline, end of treatment courses 1 and 2 and at progression. Cell-free DNA (cfDNA) was extracted using the Qiasymphony robotic extraction system and sequenced using patient-specific SiMSen-Seq panels covering known mutations from the primary tumor and expeced resistance mutations common in endocrine resistant patients.

Results & Discussion: We identified *PIK3CA* H1047R mutations in the primary tumor of 50% of patients. We continued to sequence the plasma cfDNA of 8 patients, 6 of which were *PIK3CA* H1047R positive in the primary and we find the same mutation in the blood of 3 patients at baseline (50%). We additionally identified an *ESR1* D538G mutation associated with endocrine resistance in all blood samples of a patient whose primary mutation was unknown.

Despite our limited sample size, patients with high circulating tumor DNA (ctDNA) levels in the blood seem to be associated with earlier progression and reduced response, indicating the clinical value of monitoring ctDNA. SiMSen-Seq efficiently determined even rare mutations in challenging sample types, such as FFPE tissue or cfDNA and offers an inexpensive, minimally-invasive method for monitoring treatment efficency and determination of drug resistance development in cancer. Analysis of more patients and increased sensitivity of mutation detection in the future will greatly increase our ability to further demonstrate the clinical utility of ctDNA sequencing.

Abstract 56 - Stéphanie Blockhuys

Probing the role of copper chaperone Atox1 in breast cancer migration.

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Background: Breast cancer progression is associated with increased copper (Cu) levels in the patient´s serum and cancer tissue. Cu is known to be essential for at least three hallmarks of cancer; proliferative immortality, angiogenesis and metastasis. However, it is unclear which Cu-binding proteins are involved in these processes and how they obtain Cu.

Earlier, we found that the Cu-chaperone anti-oxidant protein 1 (Atox1) is upregulated in breast cancer, and that Atox1 accumulates at the lamellipodia borders of migratory breast cancer cells, suggesting a new role of Atox1 in breast cancer cell migration.

Methods & Results: To establish the role of Atox1 in breast cancer cell migration, we mainly work with the MDA-MB-231 metastatic breast cancer cell line. Using 2D video microscopy and cell tracking analysis, we observed that Atox1 silencing reduces the migration speed and Euclidean distance of the breast cancer cells, and that Cu treatment enhances the Euclidean distance of cells with silenced Atox1 expression. Immunofluorescent stainings indicate the expression of Atox1, ATP7A, IQGAP1, Rac1 and LOXPP at the lamellipodia borders, and the proximity ligation assays suggest *in situ* interactions of Atox1 with ATP7A, IQGAP1, Rac1 and LOXPP.

Conclusions: Our study suggests new molecular mechanisms related to the role of Atox1 in breast cancer cell migration, which in extension may result in improved diagnosis and treatments for metastatic breast cancer patients.

Abstract 57 - Stig Palm

Establishing accurate radiation dosimetry for optimizing novel targeted alpha-particle radiation therapies.

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Background: The use of alpha-particle emitting radionuclides for therapy of primarily disseminated cancers has been proposed for at least half a century. The last few years, this modality has been introduced into clinical practice. Today, Xofigo (Ra-223) is commercially available and clinical trials with the alpha-emitters At-211, Bi-213, Th-227, Pb-212 and Ac-225 have been initiated. With the approval of an alpha-emitting radiopharmaceutical, and several others in clinical pipeline, improvements in clinical alpha-particle dosimetry are urgently needed.

Materials & Methods: For dosimetry on single cells and microtumors, Monte Carlo-based microdosimetry was developed *in-house*. For preclinical (in-vivo) experiments, organ-specific radiation dosimetry was established. On the 12 patients enrolled in our clinical trial on intraperitoneally administered ^{211}At-MX35 F(ab')$_2$ for therapy of disseminated ovarian cancer, retrospective dosimetry was based on biodistribution data from blood and intraperitoneal fluid sampling; urine collection; and scintigraphy.

Our pre-clinical and clinical data on biodistribution of intraperitoneally infused radiolabeled antibodies were used to construct mathematical models for antibody distribution and cellular binding.

Results: Improved methods result in more accurate and patient-specific radiation dosimetry for disseminated cancer (single cells and micrometastases) and on critical healthy tissues. Mathematical models, based on our pre-clinical and clinical findings, allow predictions of outcome and therefore provide guidance in future optimizations of targeted alpha-particle radiation therapies.

Conclusions: Dosimetry can and should be used to predict therapeutic effect, but also for estimating possible risks. Such benefit/risk estimates can then be used to further optimize the various alpha-particle therapies that are now being introduced in the clinic.

Abstract 58 - Sture Lindegren

Realizing Clinical trials with Astatine-211: The Chemistry Infrastructure

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Background: There are a consensus around the clinical potential of astatine-211 (^{211}At), but only a limited number of research facilities work with the nuclide. There are three main reason for this which all are related to the chemistry infrastructure:

- Despite the fairly straight way of producing the rare alpha emitting element astatine-211 (^{211}At), the production is scarce.
- After cyclotron production there are no systems available for converting astatine into a chemical useful form and this is likely the biggest hurdle for widespread ^{211}At research.
- Another hurdle to overcome is the ^{211}At chemistry. Appropriate chemical synthesis methods for stable bonds between ^{211}At and the tumor specific vector has to be established.

Herein we like to present chemical strategies for overcoming these hurdles in research and clinical trials with ^{211}At.

Materials & Methods: Custom made automatic system for work up of ^{211}At and synthesis of ^{211}At labelled compounds developed. Prefabricated conjugated molecules has been synthesized to enable At-211 labelling.

Results: To increase the chemical infrastructure for At-211 research and clinical trials an automatic system for work up of At-211 and synthesis of At-211 labelled compounds has been developed. To simplify the synthesis of At-211-radiopharmaceuticals prefabricated conjugated molecules has been synthesized. This strategy reduce reaction times, increase radiochemical yields and can effortless be adopted for automatic radiochemical synthesis.

Conclusion: By providing a chemistry infrastructure for work up and chemical synthesis ^{211}At and ^{211}At-radiopharmaceuticals, the main obstacles concerning research and clinical trials of this element could be met and research significantly enhanced.

Abstract 59 - Tobias Hofving

SMAD4 haploinsufficiency in small intestinal neuroendocrine tumours

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Background: Small intestinal neuroendocrine tumours (SINETs) have frequently metastasised at the time of diagnosis. Large-scale sequencing studies have only identified recurrent mutations in a single gene, *CDKN1B*, present in less than 1/10 tumours. They however often harbour recurrent chromosomal number alterations (CNA). Here, we investigate if *SMAD4*, located on chromosome 18q21.2, function as a haploinsufficient tumour suppressor in SINET and as such provide a mechanism by which CNAs contribute to SINET progression.

Materials & Methods: Copy-number profiling was performed on 132 SINETs from 116 patients. Both the copy-number status and protein expression of *SMAD4* was investigated in a well-characterised patient cohort including 739 SINETs from 359 patients by fluorescent in situ hybridisation and immunohistochemistry respectively. mRNA expression was investigated in 33 SINETs. CgA^{+} endocrine cells of *SMAD4*$^{wt/null}$ mice was described and quantified.

Results: Loss of chromosome 18 occurs in close to 70% of SINETs, being the by far most common genetic event in these tumours. Hemizygous loss of *SMAD4* was strongly associated with a corresponding decrease in both *SMAD4* mRNA and SMAD4 protein expression. Importantly, we found that low SMAD4 protein expression in SINET primary tumours was associated with both a worse overall patient survival and with the co-occurrence of tumour metastasis. Monoallelic inactivation of *Smad4* was however not alone sufficient to induce endocrine cell hyperplasia in *SMAD4*$^{wt/null}$ mice.

Conclusion: We have showed that loss of SMAD4 is associated with lower SMAD4 protein expression and several phenotypic traits, including overall patient survival and tumour metastasis, suggesting a novel mechanism of SINET tumourigenesis.

Abstract 60 - Tzu-Po Chuang

Molecular characterization of ALK fusion genes in non-small cell lung cancer (NSCLC)

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Background: ALK first entered the field of NSCLC in 2007, when two groups simultaneously reported the presence of ALK fusion proteins in lung tumours. Although ALK fusions occur in only around 8% of NSCLC cases, the number of new occurrences per year and worldwide is estimated to 40000 (around 300 cases/ year in Sweden). Today, more than 15 ALK fusion partners have been found in patients suffering from NSCLC.

However, treatment of EML4-ALK NSCLC is currently decided without considering which fusion partner is present. We believed that there is a clinical need for a deeper dissection of the various ALK fusion variants, allowing the treatment plan to reflect an individual patient's variant.

Materials & Methods: PC12 and NIH3T3 cell were transfected with different EML4-ALK variants (Variant 1-Variant 7) and neurite outgrowth and foci formation were measured. We generated stable cell lines bearing different EML4-ALK variants in NL-20 using PLVX-tetone system (tet-on). Functional assays including soft agar, migration, invasion, cell cycle, and cell growth assays were performed with/ without ALK inhibitor using stable lines. The expression profile of each variant was analyzed by RNAseq.

Results: We observed that all ALK-fusion variants can contribute to neurite outgrowth and cell transformation to different degree indicating that some variants possessed stronger oncogenic potential. On the other hand, NL-20 bearing different EML4-ALK variants exhibit increased cell growth and cell motility which reflect patients with specific variants present worse clinical prognosis.

Conclusions: Investigation of how these fusion partners affect pretreatment clinical characteristics, disease responsiveness to target therapies or later arising acquired resistance will pave a new way in treating ALK positive NSCLC patients.

Abstract 61 - Vandana Singh

Single Molecule Imaging of Ionizing Radiation and Hyperthermia Induced DNA Damage

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Abstract: Ionizing radiation and hyperthermia are part of cancer treatment to control or kill malignant cells. Here, we adapted a new method, the Direct DNA Damage assay (D3 assay), to determine the amount of single stranded DNA damage caused by ionizing radiation. We show that the D3 assay directly determines the amount of damage and that the amount of damage increases with prior hyperthermia treatment before administering gamma irradiation.

Background: Cancer leads to the formation of abnormal cells that grow beyond their usual boundaries. Hyperthermia and radiotherapy are designed to kill cancer cells by inducing DNA damage. Detection of single strand breaks (SSBs) is cumbersome, gel-based, and error-prone. Previous methods used to assess DNA damages are for example the comet assay and nick-end labeling assay. Notably, in 2014 Zirkin *et al.* studied the repair dynamics in response to UV irradiation utilizing single molecule labeling and microscopy, the D3 assay. Here we used the D3 assay to visualize the effect on SSBs induced by ionizing radiation and hyperthermia.

Materials & Methods: Lymphocytes were isolated from blood samples and treated with radiation and/or hyperthermia. The D3 assay was performed with 500 ng of DNA, 100 µM of dATP, dGTP, dCTP, 10 µM dTTP and 10 µM Aminoallyl-dUTP-ATTO-647N in 1X nick translation buffer with a cocktail of DNA repair enzymes.

Results & Discussion: SSBs increased around 2.5 times with radiation dose ranging from 0.5 Gy to 2.5 Gy. Approximately, 57.8% of the damages caused by gamma irradiation persist for 45 min, indicating the lethality of the damage. Cells treated prior with 42°C hyperthermia for 30 min and 2 Gy radiation showed around 5.8 times increase in the number of SSBs as compared to the control sample. Inhibition of DNA repair, as well as the formation of SSBs, explains the additive effect of radiation and hyperthermia treatment. APE1 is the key player in repair of ROS induced damage. APE1 increased our ability to detect the SSBs. SSBs increased around 1.2 times when the enzymatic cocktail is supplemented with APE1 enzyme in case of 2 Gy dose, and much more at higher doses.

Conclusion: D3 assay shows an increase in the number of SSBs with increase in temperature, γ-rays, and the hyperthermia-γ-ray treatment. This demonstrates the usefulness of the D3 assay for determining DNA damage and ability to give mechanistic details of biochemical processes concerning cancer therapy.

Abstract 62 - Viktor Sandblom

Tyrosine kinase inhibitors can potentially increase the effect from radiation therapy on medullary thyroid cancer

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Background: Medullary thyroid cancer (MTC) originates from the C-cells of the thyroid and many MTCs overexpress somatostatin receptors. These receptors enables systemic radionuclide therapy with radiolabelled somatostatin analogues, *e.g.* ^{177}Lu-octreotate. Few patients achieve complete response following ^{177}Lu-octreotate therapy and optimisation is needed. Co-administration of another substance can increase the therapeutic effect from radiation.

Recently, two tyrosine kinase inhibitors (TKIs), vandetanib and cabozantinib, were approved for treatment of MTC. The aim of this study was to investigate the potentially increased therapeutic effects of combining radiation therapy with these TKIs for treatment of MTC.

Materials & Methods: BALB/c nude mice xenotransplanted with patient-derived MTC (GOT2) were treated with external beam radiotherapy (EBRT) and/or TKIs (vandetanib/cabozantinib), or were mock-treated as control. To enable detection any increased effect from combination therapy, the absorbed dose and amount of TKI was chosen to give moderate effect as monotherapy. After treatment start, tumour volume was measured twice weekly.

Results: Combination therapy resulted in the largest tumour-volume reduction and the longest time to progression. However, also as monotherapy, both EBRT and TKI treatment resulted in clear anti-tumour effects. For example, after two weeks, the tumour volume for the combination therapy animals was reduced by about 70-75% compared with control, with corresponding values of about 50-65% for the monotherapy animals.

Conclusions: The therapeutic effect of radiation therapy of MTC-bearing nude mice can be increased by co-treatment with TKIs. Future studies should evaluate the potential of using ^{177}Lu-octreotate in combination with TKIs for treatment of patients with MTC.

Abstract 63 - William Rodin

Potent anti-tumor effector functions in tumor-infiltrating γδ T cells isolated from colon cancer patients

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Background: In colon cancer, tumor progression and patient outcome is affected by the cytokine balance in the tumors. IFNγ, TNFα and Granzyme B expression are associated with favorable patient outcome, while high IL-17 expression is associated with accelerated tumor progression. However, knowledge of the regulation and activation of unconventional T cell subsets in colon tumors is limited.

The aim of this study was to characterize unconventional T cells in colon tumors and unaffected tissue, determine their capacity to produce cytokines affecting tumor progression as well as the *In vitro* cytotoxic capabilities of γδ T cells.

Materials & Methods: In this study we use flow cytometry together with polyfunctional stimulation assays to both characterize and assess the potential anti-tumor properties of tumor infiltrating γδ T cells.

Results: In this study we show that the frequencies of γδ T cells are reduced in the tumor epithelium. Using polyclonal stimulation, we also show that tumor infiltrating γδ T have an increased expression of IFNγ, TNFα and Granzyme B. IL-17 expression was also elevated in tumour infiltrating γδ T cells, but at lower levels than the T_H1 -associated cytokines.

Conclusions: Altogether, this study shows that γδ T cells contribute to the cytokine balance in colon tumors with a TH1 – dominated profile and that they have potent cytotoxic capacity, which may reduce tumor progression and improve patient outcome.

Abstract 64 - Xiaolu Zhang

Role of the *C. elegans* copper chaperone, CUC-1, in cell invasion

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Background: Copper ions (Cu) play a key role in regulation of cell growth and many enzymes that are essential in humans with the need of Cu as a cofactor. Antioxidant protein 1 (Atox1) is a human Cu chaperone that plays a fundamental role in human Cu homeostasis as it transports Cu in the cytoplasm, from the plasma membrane to Cu transporting ATPases ATP7A and ATP7B located in the Golgi apparatus. Cu levels are often higher in cancer tissue as compared to normal tissue. New data show that Atox1 also appears to play a role in cancer cell migration.

Materials & Methods: To investigate the putative role of Atox1 in cancer cell invasion (which involves cell migration), *C. elegans* was selected as a model organism in which this can be specifically studied. In *C. elegans*, *cuc-1* expresses a protein CUC-1 that corresponds to human Atox1 and it has been reported to function as a Cu chaperone also in worms.

Results & Conclusions, by CRISPR-Cas9 technology, the *cuc-1* gene was successfully knocked out in *C. elegans* and we found that the resulting worms survive. A basement membrane GFP reporter and an anchor cell mCherry reporter were crossed into the *cuc-1* knocked out *C. elegans.*

This setup allows for fluorescence microscopy studies of invasion in worms with and without CUC-1 protein. Various phenotypes, such as distal tip cell migration and anchor cell invasion, are in progress to be analyzed and the results will be presented on the poster along with additional findings.