2024

Poster Session Abstracts

CANCER RESEARCH KI RETREAT 2024

DINA DABAGHIE

Multi-stain modelling of histopathology slides for breast cancer prognosis prediction

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Introduction:
The disease prognosis and treatment planning of breast cancer patients are determined using the pathologic assessment of the established biomarkers using the standard hematoxylin & eosin (H&E) and immunohistochemical (IHC) stained whole slide images (WSIs). Capturing the interaction between the prognostic information from the multiple stained WSIs can potentially improve the prognosis prediction in breast cancer patients. In this study, we aim to develop a deep learning-based breast cancer prognosis prediction model using the routine H&E and IHC-stained histopathology WSIs.

Materials and Methods:
The dataset consisted of the retrospectively collected surgical resected specimens from the South General Hospital in Stockholm (N=2167). We included 1 H&E and 4 IHC (ER, PR, HER2, and Ki-67) stained sections for each patient, where the sections were retrieved from the same piece. We registered the IHC WSIs with the H&E WSI and extracted the corresponding smaller image patches for each WSI modality. Further, we will apply intermediate and late fusion of extracted features from the registered patches using the deep CNN and vision transformer (viT) models. We trained the models using the cox loss (negative partial log-likelihood loss) and recurrence-free survival (RFS) as the survival endpoint.

Results:
We observed the c-index of 0.64 for the RFS prediction using only the H&E WSIs. Next step includes the observation of predictive performance by the joint modelling of IHC stained WSIs with H&E WSI.

Conclusion:
Routine H&E and IHC stained WSIs are used to determine the established prognostic markers for breast cancer patients. The extraction of the prognostic information from the multiple stained WSIs can potentially improve the risk of recurrence prediction over the standard practice i.e. the discreet and individual assessment of each prognostic marker.

Keywords: Breast cancer, Deep learning, Prognosis, Whole Slide Images

The ROCK-1/2 inhibitor RKI-1447 inhibits N-MYC, promotes cell death, and emerges as a synergistic partner for BET inhibitors in neuroblastoma

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High-risk neuroblastoma has a poor prognosis despite intensive treatment, highlighting the need for new therapeutic strategies. Genetic alterations in activators and inactivators of Rho GTPase have been identified in neuroblastoma suggested to activate Rho/ROCK signaling. ROCK has also been implicated in therapy resistance. Therefore, we have explored efficacy of the pan-ROCK inhibitor RKI-1447 in neuroblastoma, emphasizing combination strategies. Treatment with RKI-1447 resulted in decreased growth, increased cell death, and inhibition of N-MYC in vitro and in vivo using the TH-MYCN mouse model. A combination screen revealed enhanced effects between RKI-1447 and BET inhibitors. Synergistic effects from RKI-1447 and the BET inhibitor, ABBV-075, were confirmed in various neuroblastoma models, including zebrafish. Interestingly, ABBV-075 increased phosphorylation of myosin light chain 2, the main downstream effector of ROCK, an increase that was blocked when adding RKI-1447. The combination treatment also augmented an inhibitory effect on N-MYC/C-MYC protein expression. BET inhibitors have shown preclinical efficacy against neuroblastoma, but acquired resistance has limited their therapeutic benefit. We reveal that the combination of ROCK and BET inhibitors offers a promising treatment approach that can potentially mitigate resistance to BET inhibitors and reduce toxicity.

Analyses of extracellular vesicles to reveal Ephrin and Eph signaling in non-small cell lung cancer-identification of possible by-pass signaling affecting EGFR-TKI responsiveness.

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Background: For Non-small cell lung cancer (NSCLC) driven by mutated Epidermal growth factor receptor (EGFR), resistance to EGFR Tyrosine kinase inhibitors (TKIs) is a major clinical problem. Such resistance may be due to mutations in the EGFR kinase domain that block TKI binding but also as a consequence of by-pass signaling alterations in others receptor tyrosine kinases (RTKs). Previous results have shown that EphA2 signaling may constitute such a by-pass driver in relation to 1st generation EGFR-TKIs. We earlier reported that Ephrin B3 regulate migration and invasion of NSCLC cells. Moreover, Ephrin B3 was found to bind EphA2 and was in this complex phosphorylated on Ser897, a site linked to metastasis. Here we focus on the role of Ephrin and Eph:s in osimertinib (osi) resistance in NSCLC cells and released extracellular vesicles (EVs).
Materials and methods: Ephrin B3 were silenced in the EGFR mutant cell line H1975 and its effect on EGFR-TKI erlotinib (erlo) and osimertinib (osi) sensitivity analyzed by clonogenic assays. Flow cytometry was used to characterize Ephrins and EphA:s expression levels. EVs were isolated from the H1975 cell line media and, from an osi resistant subline H1975/OR, in response to osi by size exclusion chromatography (SEC) followed by Nanoparticle Tracking Analysis (NTA) and, western blot characterization. Mass spectrometry (MS) was used for total protein profiling of the EVs cargo and data bioinformatically processed by the Qlucore® platform.
Results: In our model of H1975 and H1975/OR NSCLC cells we showed expression of Ephrin´s and Eph´s and silencing of Ephrin B3 or EphA2 with siRNA sensitized for erlo and osi. Components of the Ephrin/Eph pathway were found in EVs isolated from H1975 and H1975/OR cell culture media including EphA2, EphB3, Ephrin B1 and Ephrin B3. Qlucore bioinformatics revealed a protein signature associated with Ephrin B1, which when analyzed by the STRING platform, showed protein-protein in process related to mesenchymal cell differentiation (SEMA5A, TGFB1, LAMA5 and LOXL2) and angiogenesis (SEMA5A, HSPG2, PRCP and COL18A1).
Conclusion: Ephrin B3 silencing increases sensitivity of osi suggesting that the Ephrin B3/EphA2 pathway could be involved as a by-pass mechanism of EGFR-TKIs. Several Ephrin´s and Eph´s associated signaling networks were found in the EVs. These results should be further analyzed in context of cell-to-cell communication and pathway activation in distant metastasis and for its possible biomarker potential.
Keywords: Lung cancer and extracellular vesicles

Identifying mechanisms for development of high-risk neuroblastoma

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Text Body: Neuroblastoma is one of the deadlier and most common childhood tumors, marked by cellular and genetic heterogeneity. Identifying the cell of origin in neuroblastoma holds significant potential for targeted treatments. Recently, our Omics data on neuroblastoma tissues suggest that Schwann cell precursors (SCPs) might serve as the cell of origin for some neuroblastoma subtypes. However, current neuroblastoma models typically drive oncogene expression in adrenergic cells under the dopamine-B-hydroxylase (dbh) or the tyrosine hydroxylase (TH) promoter, with MYCN oncogene overexpression in these cells being the most common mechanism used for successfully modeling neuroblastoma. In this study, we present initial steps to establish preclinical models in induced pluripotent stem cell (iPSC) derived neural crest cells (NCC) to investigate whether overexpression of selected genes in pre-malignant SCPs can trigger neuroblastoma tumorigenesis. Leveraging this model, we have directed MYCN expression toward SCPs, which emerge earlier in the sympathoadrenal lineage development. Preliminary findings indicate that MYCN-induced differentiation of SCPs towards sympathoblasts, potentially recreates early neuroblastoma development events. Further exploration of the role of SCPs in neuroblastoma development could pave the way for targeted therapeutic strategies aimed at the early stages of this malignancy.

Keywords: Neuroblastoma, MYCN

Pseudo-mutant p53 as a targetable predictor of leukemic transformation

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Survival of acute myeloid leukemia (AML) remains dismal despite the incorporation of new drugs into treatment protocols. This is mainly attributable to the presence of chemo-resistant leukemic clones already present at diagnosis and makes prevention of AML an appealing therapeutic strategy. AML evolves following a prolonged period during which hematopoietic stem and progenitor cells (HSPCs) acquire somatic mutations, conferring cellular fitness. These cells can be identified in healthy individuals, a condition termed clonal hematopoiesis (CH). However, their malignant potential is unclear because most CH clones are not pre-leukemic (preL) and do not transform to AML. Thus, although current prediction tools for AML are sensitive, they are not specific. Some of the most common preL-HSPCs (that carry mutations in their DNMT3A gene) were recently found to harbor a misfolded, "pseudo-mutant" p53 protein (PMp53), even though their TP53 gene was intact. The goal of this research is to better define the prevalence of PMp53 in high-risk CH clones and to functionally characterize pre-leukemic cells that harbor PMp53. We have incorporated p53 conformation-specific monoclonal antibodies that were conjugated to polyadenylated oligonucleotides into the AbSeq single-cell technology (BD Biosciences) to allow simultaneous assessment of p53 folding and the transcriptional signature at the single cell level. We optimized our previously calibrated p53 intra-nuclear staining protocol for RNA preservation and tested it in OCI-AML2 cells. Our results illustrate the ability of this protocol to identify the expression of 1000 genes per cell indicating sufficient quality for transcriptional signature analyses, but further optimization is needed. We will use this new methodology to study bone marrow samples of patients with pre-leukemic conditions and hematological neoplasms in order to stratify the gene-expression profile of high-risk preL-HSPCs (that progress to leukemia) according to the conformation of their p53 protein. This project is expected to lead to biomarker- and functional-guided pre-clinical and clinical studies that will test p53-targeted therapeutic interventions to eliminate pre-leukemic cells and thereby prevent AML.

Keywords:
Pseudo-mutant p53, clonal hematopoiesis

Title: Targeting replication stress tolerance pathways in MYCN-amplified neuroblastoma

Name of presenters: Andrä Brunner, Jinjiang Chou and Olle Sangfelt

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Abstract:
Neuroblastoma (NB) is the most common cancer among infants with a survival rate of only ~50% for high-risk patients. Amplification of the MYCN gene is a critical indicator of unfavorable prognosis in NB and its overexpression has been attributed to the regulation of various biological processes including cell proliferation, apoptosis, senescence, differentiation, metabolism, DNA damage repair, and protein synthesis. Furthermore, MYCN represses neuronal differentiation genes to drive NB oncogenesis but has also been found to induce replication stress (RS). While the precise mechanisms by which MYCN governs most of these processes remain elusive, unraveling them could pave the way for innovative therapeutic approaches to combat MYCN-driven cancers.

We recently demonstrated that the FBXL12 ubiquitin ligase provides a unique protective function in cancers with high levels of RS by targeting CHK1-phosphorylated FANCD2 for proteasomal degradation (Brunner et al., 2023), indicating that FBXL12 could be an attractive target in cancers experiencing RS. Importantly, high expression of FBXL12 is a significant predictor of poor outcomes in NB patients, independent of other clinical variables. This suggests that FBXL12 may play a vital role in the progression of NB by inhibiting MYCN-driven RS. Intriguingly, depletion of FANCD2 as well as its exacerbated degradation through FBXL12 overactivation results in marked reduction of MYCN expression in NB cell lines. Ongoing research is focused on the characterization of the MYCN-FBXL12-FANCD2 axis, specifically examining its role in replication fork protection, DNA damage and the subsequent response to therapy in Neuroblastoma. Furthermore, our preliminary data suggest that the disruption of this RS tolerance pathway could activate cGAS-STING-IFN signaling. Together, these findings imply that FBXL12 may represent a novel therapeutic target in MYCN-amplified tumors.

Keywords: Neuroblastoma, MYCN, FBXL12, FANCD2, Replication Stress

Title: Development of a novel 3D Co-Culture Model for Functional Studies of Tumor Invasion in Pancreatic Cancer Liver Metastases.

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Co-Authors: Annika Viljamaa, Sara Söderqvist, Kseniya Ruksha, Natalie Geyer, Carlos Fernández Moro, Marco Gerling

Abstract:
Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive solid malignancies with a 5-year survival rate of less than 12%. Over half of all PDAC patients are diagnosed with liver metastases, which are overall the most common metastases in gastrointestinal tract cancers. Once PDAC liver metastases have developed, surgical resection is no longer considered a therapeutic option because of the dismal prognosis, and treatment is limited to palliative chemotherapy. Our preliminary data have revealed that malignant invasion triggers a pathognomic peritumoral injury response in the liver, characterized by the transdifferentiation of liver parenchymal cells into injury-induced progenitor cells. These progenitor cells physically engage with tumor cells at the forefront of tumor invasion and are gradually replaced by malignant cells, representing the primary mechanism of tumor progression in liver. Increased contacts between tumor cells and hepatocytes predicts an unfavorable outcome. In addition, our preclinical studies show that the inhibition of hepatocyte trans-differentiation suppresses tumor growth, suggesting a functional link between progenitor cell formation and tumor invasion.
Despite the clinical relevance of tumor cell-hepatocyte crosstalk, the molecular mechanisms that drive perimetastatic transdifferentiation and replacement remain poorly understood. An effective and manipulatable in vitro model would provide an excellent tool to address these complexities, but such a platform is currently lacking. To this end, we aim to shed light on the intricate interaction between PDAC cells and injury-induced parenchymal progenitor cells. In preliminary experiments we successfully isolated hepatocytes from mouse liver and released the parenchymal cells from
differentiation by the addition of a defined cocktail of signaling pathway inhibitors and growth factors, resulting in bipotent liver progenitor cells. The transdifferentiated progenitors and murine PDAC spheroids are combined in an in vitro co-culture system consisting of a core of PDAC cells surrounded by physically connected hepatocyte progenitor cells, nested in 3D collagen gel droplets. This hospitable environment provides the cells a matrix for the cells to grow and interact. The PDAC spheroids stably express a fluorescent label, allowing us to visualize tumor cell transdifferentiated cell interactions by live confocal microscopy and quantify them using established, machine-learning-augmented image analysis pipelines. Our model holds promise for unravelling the molecular mechanisms underlying the replacement of injury-induced progenitor cells in liver metastases and for identifying targeted approaches to inhibit cell replacement of tumor-adjacent parenchymal cells.

Machine learning-based cellular interaction mapping of tumor-liver contact in colorectal liver metastases and association with patient outcomes

Name of presenter: Annika Viljamaa

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Recent work of our group has identified replacement-type growth, such that invading tumor cells replace hepatocytes, to be the main mode of tumor growth into the liver.
In contrast, a perimetastatic capsule, a strong positive prognostic factor in liver metastases, results from a reparative process caused by liver injury reaction upon impaired tumor cell invasion. To further expand these findings at the cellular level, we here aimed to test the hypothesis that a lower fraction of tumor-hepatocyte interactions is associated with a favourable outcome. To achieve this, we created a machine learning model using 4445 manual annotations of liver structures on digital whole slide images (n=21) of colorectal cancer liver metastases (CRLMs) patient samples. The resulting model is able to call hepatocyte-class proximity to invading tumors and it allowed us to profile cellular interactions in tumor-liver interface at high resolution over hundreds of digital WSIs of CRLMs patient samples. Here, we present preliminary results of cell interaction mapping at the tumor-liver interface in CRLMs and correlate these findings to the previously established proportions of encapsulated and replacement histopathological growth patterns (HGPs) as annotated manually in the same sample of patients. By characterising tumor cell and normal liver parenchyma interactions in the encapsulated and replacement HGPs of CRLM patient samples, a deeper understanding of the cell interactions and their role in the development of fibrotic rim will be gained. Ultimately, we aim to develop a convolutional neural network-based approach for improved liver metastases prognostication based on quantifying tumor-liver contacts.

Replacement of peroxidatic cysteine with selenocysteine greatly reduced the overoxidation propensity of human Peroxiredoxin 2 enzyme

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(Zsuzsanna Anna Pató, Mahendravarman Mohanraj, Beáta Biri-Kovács, Attila Kolonics, Qing Cheng, Elias S. J. Arnér)

Abstract
Human Peroxiredoxin 2 (Prx2) is an important hydrogen peroxide scavenger and regulates signaling processes via controlling the intracellular H2O2 level, which can result in either facilitating or suppressing tumorigenesis. It reduces organic peroxides and peroxynitrite also. The protein exists mostly in the cytosol as a homodimer, but also readily forms larger oligomers. Prx2 is one of the four representatives of the typical 2-Cys peroxiredoxin family containing two cysteine dependent redox active sites on the same molecule named as the peroxidatic (CP) and resolving (CR) residues. In the catalytic cycle of Prx2, the CP-SH is oxidized to sulfenic acid (CP-SOH). The CP is also sensitive to hyperoxidation and prone to form sulfinic (Cp-SO2H) and sulfonic acid (Cp-SO3H) derivatives upon exposure to higher hydrogen peroxide (H2O2) concentrations. The sulfinic acid derivative of Prx2 is catalytically inactive, but can be reduced back to sulfenic acid by Sulfiredoxin in an ATP dependent reaction, while the sulfonic acid form is irreversibly overoxidized. In the resolution step the sulfenic acid, CP-SOH forms a disulfide bond with CR-SH of another subunit, which is then reduced, mostly by Trx in the final catalytic recycling step.
In a few bacterial species, there are isoforms of peroxiredoxins with selenocysteine (Sec, U) in their active sites replacing the CP residue. In general, replacing the sulfur in cysteine with selenium results in its greater reactivity, and at the same time the molecule becomes more resistant to overoxidation. We wondered whether the CP-to-UP substitution changes the enzyme activity of Prx2, and if so, in which direction it modulates the activity.
We expressed the human Prx2 C51U in E. coli C321.ΔA strain and purified the recombinant protein to homogeneity. Characterizing the recombinant human Prx2 C51U we found, to our surprise, that the selenoprotein was about tenfold more active as a peroxidase than the wild-type enzyme when coupled to thioredoxin as the recycling system. Our preliminary results showed also that pretreatment of the selenocysteine variant with high level of H2O2 decreased only slightly its peroxidase activity, while the wild type enzyme lost its activity completely under the same condition.
The explanation of why the less active cysteine variant in human peroxiredoxin evolved over time is possibly related to the role of the enzyme in redox regulation.

Keywords: Prx2, selenoprotein

Cytotoxic effects of novel human glutathione peroxidase (GPX1, GPX2 and GPX4) inhibitors

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(Zsuzsanna Anna Pató, Mahendravarman Mohanraj, Beáta Biri-Kovács, Attila Andor, Qing Cheng, Elias S. J. Arnér)

Abstract

Selenoprotein glutathione peroxidases (GPX), such as the ubiquitously expressed GPX1 and the ferroptosis modulator GPX4, exert antioxidant activities by reducing hydroperoxides using glutathione. Overexpression of these enzymes is common in cancer and can be associated with the development of resistance to chemotherapy. Using previously developed, optimized glutathione reductase (GR)-coupled GPX assays for the biochemical high-throughput screen (HTS) of almost 12,000 compounds with proposed mechanisms of action, our group identified novel GPX1/GPX2- or GPX4-specific inhibitors. Of the selected 4 GPX1/GPX2 inhibitors, Tenatoprazole (EC50% GPX1 8.9 □M, GPX2 7.7 □M) showed only weak cytotoxic effects on selenium supplemented human lung carcinoma cell line, A549 following 3-day treatments (IC50%, 66.4 □M). However, all identified novel GPX4 inhibitors were found to be more cytotoxic than the identified GPX1/GPX2 inhibitors. Stronger cytotoxicity values were observed in other lung cancer cell line, H1975 (Table 1.).

	GPX4 EC50% (μM)	**A549 cytotoxicity** IC50% (μM)	**H1975 cytotoxicity** IC50% (μM)
Navitoclax	11.4	4.9	1.9
Venetoclax	18	16.1	9.6
Simeprevir	7.3	10.4	11.7
VUO661013	8.4	9.8	6.8
Grazoprevir	3.4	17.6	n.d.
Lusutrombopag	5.4	21.6	n.d.
Brilanestrant	6.8	47	>100
Pranlukast	6	39	n.d.

Table 1. Summary of the effects of GPX4 inhibitors on enzyme inhibition and Se suppl. A549 and H1975 cytotoxicity

Our findings support that the identified GPX4 inhibitors have potent cytotoxic effects and further characterization needs to reveal the contribution of GPX4 inhibition to cytotoxicity profile.

Keywords: GPX4 inhibitors, cytotoxicity

The Function of Microglial Arginase 1 in Glioblastoma Progression

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ABSTRACT:
Glioma cells recruit and convert microglia, resident immune cells of the brain, into tumor-supportive cells, promoting neoplasm progression. For decades, the expression of arginase 1 (ARG1), an enzyme known for its ability to catalyze the hydrolysis of L-arginine, has been used as a marker to define tumor-associated microglia. However, the function of microglial ARG1 in the context of a brain neoplasm remains unknown. We hypothesize that ARG1 could play an essential role in regulating cellular functions in microglia, and thereby contribute to the acquisition and/or maintenance of a pro-tumoral phenotype in the context of brain malignancies. To test this hypothesis, we generated in vitro models of ARG1 overexpression and knockdown in the mouse BV2 microglia cell line. Additionally, we took advantage of direct and segregated coculture systems involving rodent BV2 microglia and C6 glioma cells to identify the role of microglial ARG1 in the context of glioma cancer cells stimulation. We found that in direct coculture with glioma cells, proliferation of microglia is decreased. However, the presence or absence of ARG1 per se does not alter microglial proliferation. This finding could be interpreted as microglial ARG1 not being involved in the glioma-induced decrease in microglia proliferation. ARG1-overexpressing microglia exhibited an increased expression of Igf-1 at mRNA level and resulted in greater attraction of C6 glioma cells in a migration assay. The absence of ARG1 in microglia led to more ramified morphology and increased migration, whereas ARG1-overexpressing microglia exhibited reduced ramification and more static behavior in a scratch-wound assay. Moreover, overexpression or absence of ARG1 led to downregulation of NOS2, suggesting impaired microglial response to external signaling. Collectively, these preliminary findings indicate a potential role for ARG1 as being a key regulator of microglial functions that could be dysregulated and exploited by the tumor cells in the context of brain cancer. Therefore, enhancing our comprehension of microglial ARG1 function and its involvement in the progression of brain tumors is essential to reveal novel strategies for targeting exceptionally aggressive tumors.

Keywords: Arginase 1, microglia

Monitoring the cross talk of signaling pathways in single cells: a fluorescent reporter tool to assess NF-κB, STAT3 and Nrf2 pathways

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Monitoring the cross talk of signaling mechanisms of different transcription factors is of high importance in several diseases, including tumor development. However, tools for understanding their complex regulation within single cells and how their activities are related at a cellular context are still limited. We recently developed a new reporter (called pTRAF, for plasmid for transcription factor reporter activation based upon fluorescence) that enables simultaneous single-cell resolution monitoring of three separate redox biology related transcription factors: NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), STAT3 (signal transducer and activator of transcription 3) and Nrf2 (nuclear factor E2–related factor 2), that is a derivative of a former variant of this reporter tool (containing HIF, hypoxia-inducible factor instead of STAT3). Detection of the activation of the transcription factors is based upon measuring the fluorescence intensity of three separate fluorescent proteins linked to each transcription factors, and is measured by fluorescence microscopy, flow cytometry and live cell imaging techniques. Reporter plasmids can be applied in various studies, here we describe the analysis of TXNL1, a redox-active thioredoxin-like protein with chaperone functions, possibly related to Nrf2 signaling. The newly described pTRAF variant can be used to simultaneously monitor the impact of diverse perturbances on cancer- and redox related signaling pathways NF-κB, STAT3 and Nrf2, at single cell resolution.

Keywords: fluorescent reporter tool; single cell analysis

Joint single-cell genetic and transcriptomic analysis reveal embedded premalignant subclones and progressive cell states in human neuroblastoma

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Abstract:

Neuroblastoma is a heterogeneous embryonal malignancy assumed to arise from the multipotent neural crest where its exact cellular origin is under debate. To understand the clonal development of neuroblastoma cell states, we analyzed human tumor samples using single-cell multi-omics, including joint DNA/RNA sequencing of sorted single cells (DNTR-seq). Tumor subclones showed considerable inter-individual variation in terms of copy number profiles and transcriptional phenotype. Despite this heterogeneity, transcriptional deregulation between subclones converges on cellular processes related to axon guidance via the SLIT/ROBO pathway, proliferation, and differentiation. Genomic heterogeneity was also observed for *MYCN.* Subclones harboring 2p/*MYCN* gain (< 8 copies) co-exist with amplified subclones (> 20 copies), indicating that *MYCN* amplification may represent a non-initiating genomic event. Beyond adrenergic heterogeneity, we identify a subpopulation of abnormal cells resembling multipotent Schwann cell precursors, characterized by expression programs of proliferation, apoptosis, and a non-immunomodulatory phenotype. Their genomic profile and phylogeny suggest an ancestral, pre-malignant role in tumorigenesis. While the function of these SCP-like cells in tumor initiation remains to be established, their identification expands the reservoir of tumor cells, considering their multipotency reflecting a central neuroblastoma feature.

Keywords:

Neuroblastoma multi-omics

Clonal and cellular heterogeneity

A novel tumor-prone mouse model harboring the *Trp53*^{R210X} nonsense mutation

Name of Presenter: Charlotte Strandgren

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The *TP53* tumor suppressor gene is mutated in approximately 50% of all tumors. Around 10% of *TP53* mutations in human cancer are nonsense mutations, causing premature termination of translation and resulting in a truncated and inactive p53 protein. Given the high frequency of *TP53* mutations overall, tumors with *TP53* nonsense mutations represent a substantial number of cancer patients worldwide. The most common *TP53* nonsense mutation is p.R213X, which is also the 6th most common of all cancer-associated *TP53* mutations. To study the impact of *TP53* nonsense mutations *in vivo*, and develop therapeutic strategies for this mutation type, we generated mice harboring the *Trp53* nonsense mutation p.R210X, corresponding to human *TP53* p.R213X. Previous studies have demonstrated a high incidence of spontaneous tumors in *Trp53*-null and *Trp53*^{R172H} missense knock-in mice. Our results show that *Trp53*^{R210X} mice are initially phenotypically normal. However, the proportion of female *Trp53*$^{R210X/R210X}$ mice is dramatically reduced. Female homozygous mice are very poor breeders, and significantly smaller than female heterozygous and wildtype littermates. *Trp53*$^{R210X/R210X}$ mice start developing tumors at 2.5 months of age, and the current maximal lifespan of homozygous mice in this ongoing study is 8.5 months. *Trp53*$^{R210X/+}$ mice show tumors from around 10 months of age, and by 16.5 months of age 50% of all heterozygous mice have developed tumors. Out of 144 tumors from *Trp53*$^{R210X/+}$ mice analyzed, 59% show loss of heterozygosity. The most common tumor type in homozygous mice thus far is lymphoma, whereas heterozygous mice most frequently display sarcomas. The *Trp53*^{R210X} tumor phenotype is comparable to those previously described for *Trp53*-null and *Trp53*^{R172H} mice. Our new unique mouse model will allow further studies of the effects of *Trp53* nonsense mutation in a multi-organ system and should also be valuable for preclinical evaluation of novel therapeutic strategies for targeting *TP53* nonsense mutations in cancer. Our long-term goal is to develop more efficient treatment for tumors carrying nonsense mutant *TP53*. This may also be relevant for treatment of tumors harboring nonsense mutations in other tumor suppressor genes, such as *APC* and *PTEN*.

Keywords: p53, nonsense mutation

Investigating Single-Cell Transcriptomic Heterogeneity and Its Link to Neuroblastoma Drug Resistance

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Neuroblastoma intratumoral heterogeneity is associated with disease progression, metastasis, and drug resistance. Previous studies have primarily focused on quantifying genetic heterogeneity, leaving epigenetic heterogeneity less explored. In this study, we developed an in-silico sampling method to generate data across a spectrum of heterogeneity levels. We subsequently assessed various population diversity measurement metrics to determine their effectiveness in quantifying heterogeneity using single-cell RNA sequencing data. The most effective metric, the Coefficient of Variation, was selected to assess epigenetic heterogeneity levels across different sample types. Our results indicate that neuroblastoma tumor samples exhibit significantly higher heterogeneity compared to neuroblastoma cell lines. Moreover, MYCN-amplified tumors show greater transcriptomic heterogeneity than MYCN-non-amplified tumors. This method allows for the quantification and comparison of intratumoral heterogeneity across various cancer types and correlates these findings with drug resistance and clinical outcomes.

Keywords: Neuroblastoma, Transcriptomic Heterogeneity

CANCER CORE EUROPE – An alliance committed to advance cancer research to improve cancer care

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Founded in 2014, this bottom up initiative - Cancer Core Europe (CCE) - is a consortium of seven leading cancer centres that have committed their own resources and come together to collaborate and create a sustainable, high level, shared research platform (expertise, technologies, personnel, resources, know-how). The overarching aim is to perform translational cancer research for the benefit of new innovative therapy/ care for patients, through personalised cancer medicine (PCM). Furthermore, CCE contributes to increase Europe's competitiveness as a place to conduct cutting-edge research which then can be translated into the clinic.

Work is performed in four main pillars:
I. Clinical trials and translational cancer research: CCE is running the Basket of Baskets (BoB) trial, a multicentre study to evaluate targeted agents in molecularly selected populations with advanced solid tumours. Its primary objective is to evaluate the antitumour activity of each matched therapies in small patient populations as a signal finding study. Within the frame of BoB, KI researchers have developed the Molecular Tumour Board Portal (1), a clinical decision support system, which now is being further developed and applied in other trial settings. CCE is also working on creating its own academic Clinical Research Organisation (aCRO), a 'One Stop Shop', as well as a costing tool that can be applied in the startup of new clinical trials.
II. Virtual data centre (VDC): Data sharing is key to progressing and developing PCM. CCE is establishing a common virtual platform for this purpose, aiming at a European multi-source and federated oncology data network.
III. Education and Training: While also assuring the sustainability of the consortium, the aim of this pillar is to educate & train the next generation of cancer researchers and clinicians in PCM. Activities include among others CCE's educational program 'TRYTRAC', the CCE lecture series, and the long time running Summer school in translational cancer research. The latter provides centres and staff outside the CCE an opportunity to access the CCE knowledge sharing.
IV. Prevention and Early detection: CCE's most recent pillar which is underway to outline its roadmap during Autumn 2024.
In addition, CCE has Taskforces on Phase 1 trials, Genomics, Imaging, and Pathology. To handle ethical, legal and financial questions the ELFI taskforce was established and holds representatives from all CCE partners.

Keywords: European collaboration, personalized cancer medicine

1. Tamborero, D., Dienstmann, R., Rachid, M.H. et al. Support systems to guide clinical decision-making in precision oncology: The Cancer Core Europe Molecular Tumor Board Portal. Nat Med 26, 992–994 (2020)

Deoxyguanosine analogues allosterically modulate the dNTPase activity of chemoresistance factor SAMHD1

Name of presenter: Christopher Dirks

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SAMHD1 is a deoxynucleotide hydrolase (dNTPase) that regulates cellular dNTP levels and has recently been implicated as a resistance factor to nucleoside analogue antimetabolite chemotherapy. Antimetabolites are important in the treatment of many cancer types, but tumour-specific factors can negatively affect therapy outcome. Nucleoside analogues enter the cell as prodrugs and, once activated, disrupt the cellular dNTP pool or cause direct damage to the genome. As a dNTPase, SAMHD1 cleaves the active metabolites of these drugs and is therefore an attractive target for the development of inhibitors to improve the effectiveness of nucleoside analogue therapy. Compounds that modulate SAMHD1 activity could also serve as experimental tools to further understand the enzyme's biological roles.
Catalytically competent SAMHD1 requires nucleotide binding at two distinct allosteric sites (AS1 and AS2) to induce tetramerization, with AS1 being specific for guanine nucleotides. Guanine nucleotide analogues, such as the triphosphate metabolite of the antiviral Acyclovir, act as allosteric activators of SAMHD1. Interestingly, in the absence of their triphosphate moiety, both Acyclovir and the endogenous nucleoside deoxyguanosine inhibit the dNTPase activity of SAMHD1 in vitro. However, the mode of inhibition was not yet described.
In the present study, we investigate these as yet unknown modes of SAMHD1 activity modulation by deoxyguanosine analogues. Using a pipeline of biochemical and biophysical studies including enzyme activity assays, competition binding and in-vitro protein crosslinking, we are studying the affinity of different nucleoside analogues for SAMHD1's allosteric sites, as well as their effect on the enzyme's dNTPase activity and oligomerisation status. Preliminary results suggest that deoxyguanosine analogues inhibit SAMHD1 by inducing an inactive dimeric state that is unable to progress to catalytically active homotetramer. The triphosphate metabolites of deoxyguanosine analogues, acting as allosteric activators, induce the formation of a tetrameric and catalytically competent SAMHD1 with inherently reduced hydrolase activity.
Altogether, this study aims to diversify the modes of inhibition towards the chemoresistance factor SAMHD1 and provide a starting point to rationally develop allosterically targeting molecules.

Keywords: Nucleoside analogues, SAMHD1

Effect of circulating inflammatory proteins on outcome in melanoma patients receiving immune checkpoint inhibitors

Name of presenter: Cissi Liu

Authors: Cissi Liu, Hildur Helgadottir, Suzanne Egyhazi Brage

Background: The introduction of immunotherapies with immune checkpoint inhibitors (ICIs) has led to a significant improvement in the overall survival for patients with metastatic cutaneous malignant melanoma (CMM), but only a subset of the patients has durable benefit from this treatment. Our group (Karlsson et al. Cancer Res 2021, Costa Svedman IOTECH 2024) recently proposed a panel of inflammatory proteins that has the potential of being used for stratification of CMM patients for ICI therapy. It is thus important to further validate these potential biomarkers that may be used to predict therapy response and avoid exposing patients to unnecessary toxicity.
Aim: To validate whether the inflammatory circulating cytokines CXCL10 (IP-10), IL-6 and IL-8 could be used to monitor and predict clinical benefit from ICIs in patients with metastatic CMM.
Methods: Thirty-six patients with stage IV CMM who received anti-PD-1 at Karolinska University Hospital, Stockholm, were included in this retrospective study between August 2015 and June 2019. Sequential plasma samples were collected prior and during treatment (10-64 days after treatment start) to determine the circulating cytokine levels. CXCL10 levels were measured by ELISA and IL-6 and IL-8 levels by ProQuantum Immunoassay. The cytokine levels were related to response, progression free survival (PFS) and overall survival (OS) with follow-up of at least 4.5 years.
Results: We used ROC (receiver operating characteristic) curve estimates to evaluate the predictive capacity of these three potential biomarkers regarding discrimination between patients with disease control and non-responders, with or without progression of disease at 1 year and dead or alive at 2 years after ICI therapy. IL6 on-treatment/pre-treatment ratio levels showed the best predictive performance regarding response and PFS at one year, AUC value 0.81 (95% CI .067-0.96; p=0.0021) and AUC value 0.71 (95% CI 0.55-0.88; p=0.029), respectively. Furthermore, IL8 pre-treatment levels showed the best predictive performance regarding OS at 2 years, AUC 0.75 (95% CI 0.58-0.91; p=0.012). However, CXCL10 had no discriminating capacity regarding response, PFS or OS.
High IL6 on-treatment/pre-treatment ratio levels (HR 4.05; 95% CI 1.73–9.46; p=0.001) and high IL8 pretreatment levels (HR 2.94; 95% CI 1.10–7.89; p=0.032) significantly correlated with shorter PFS and OS, respectively. Further analysis of sequential plasma samples from additional patients receiving ICI therapy is ongoing.
Conclusions: IL-8 pre-treatment and IL-6 on-treatment/pre-treatment ratio plasma levels may provide prognostic value and predict clinical outcome for patients with CMM receiving anti-PD-1 therapy.

Keywords: immunotherapy, biomarkers

Exploring the combinatorial potential of the ROCK2-specific inhibitor KD025 for neuroblastoma Therapy

Name of Presenter: Conny Tümmler

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Background:
High-risk neuroblastoma remains a clinical challenge as the long-term survival rates for patients with recurrent disease are below 10%. Novel drug combinations are therefore needed to support existing therapies, improve survival, and reduce late effects. Signaling mediated by the Rho family of GTPases, and the downstream Rho kinases (ROCK), regulates important developmental processes and our research group has previously demonstrated that the Rho/ROCK signaling pathway is a promising therapeutic target in neuroblastoma.
Aim:
To explore novel drug combinations for neuroblastoma therapy with focus on the ROCK2-specific inhibitor KD025 (Belumosudil, Rezurock™).
Methods:
Drug combination screening was performed using KD025 together with a cancer drug library containing 528 drugs. Cell viability, clonogenicity, and protein expression/ phosphorylation were evaluated in neuroblastoma cell lines and IncuCyte® LiveCell analysis was used to assess tumor spheroid growth and cell death. Additionally, the Agilent Extracellular Flux Analyzer was used to investigate metabolic changes in response to drug treatment. The in vivo efficacy of KD025 was evaluated in 9464D allografts and the transgenic TH-MYCN mouse model. RNA-sequencing and gene signaling enrichment analysis were applied to study transcriptomics.
Results:
KD025 impaired cell viability and growth of neuroblastoma cell lines, decreased N-myc levels, and increased phosphorylation of p38 and Akt. Additionally, we observed that KD025 impaired tumor growth of 9464D allografts and in homozygous TH-MYCN mice but did not completely suppress it. RNA-sequencing of TH-MYCN tumors proposed downregulation of genes associated with metabolic processes. A drug combination screening revealed several synergistic combination partners for KD025 including TIC10 (ONC201) and the p97 inhibitor NMS-873. Synergistic effects of these drug combinations were confirmed in neuroblastoma cell lines grown in monolayer and multicellular tumor spheroids. Furthermore, we observed changes in glycolytic parameters in neuroblastoma cell lines treated with KD025, TIC10, and NMS-873, suggesting metabolic effects as one of the potential underlying molecular mechanisms of action.
Conclusion:
Combination treatments using the ROCK2-specific inhibitor KD025 together with p97 inhibition or TIC10 may be promising therapeutic approaches for neuroblastoma.

Keywords: pediatric cancers, combination therapy

Modulation of c-MYC structure and activity by targeting a conformational switch

Name of presenter: Dilraj Lama

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Abstract
The structural heterogeneity of Intrinsically Disordered Proteins (IDP) facilitates interaction with multiple binding partners, but presents a unique challenge to target them for therapeutics. In this regard, we have recently demonstrated a strategy using the disordered c-MYC oncoprotein, that can be employed to modulate the disorder-function relationship of IDPs (Lama, D. et al, Nat Commun, 15, 1865, 2024). In this study, we initially designed peptide derivatives of c-MYC and subjected them to probe-based molecular dynamics simulations. This led to the identification of an epitope (termed coreMYC for COnformational REgulator of c-MYC) within the transactivation domain (TAD) of c-MYC, which undergoes a ligand-induced conformational shift from a predominantly extended state to a more compact configuration. AlphaFold modelling indicated that the high-energy extended structure of coreMYC represent an active module for protein recognition, while the low-energy compact conformation is the inactive state. These observations were then verified using Ion mobility mass spectrometry, where incubation of the recombinantly produced coreMYC with a small molecule epigallocatechin gallate (EGCG), propagated the compact conformation and impeded its interaction with c-MYC TAD binding partners such as TRRAP and TBP. Finally, employing in situ proximity ligation assay, we saw that treatment of cells with EGCG indeed inhibited interaction of c-MYC with both the endogenous co-factors, thereby establishing a potential druggable feature of coreMYC. We have further investigated coreMYC: EGCG interaction with NMR spectroscopy and utilized the knowledge to screen a more specific small molecule interactor of coreMYC, which we are currently characterizing. Together, our research presents a blueprint for the systemic identification and characterization of ligand binding interfaces in IDPs. It also opens new avenues towards the development of shape-shifting compounds that could be the hallmark of targeting disordered proteins like c-MYC regarded as undruggable.

Keywords: Disorder, coreMYC

Exploring the Therapeutic Potential of eIF4A3 RNA Helicase within the Exon-Junction Complex for Acute Myeloid Leukemia.

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Co-authors: Sophia Miliara, Elisabetta Cozzi, Xiangfu Zhong, Isaac Chan, Karl Ekwall, Sören Lehmann, Andreas Lennartsson, Jiri Bartek & Dimitris C. Kanellis.

Abstract
Acute myeloid leukemia (AML) is a severe hematologic cancer with poor prognosis in both adults and children, with long-term survival rates below 25%. Despite existing therapeutic options, AML patients often experience poor outcomes due to acquired resistance or the intolerable cytotoxicity of current treatments. Recent research has indicated that targeting aberrant post-transcriptional regulation can be an effective strategy in cancer therapy. The exon-junction complex helicase eIF4A3 plays a critical role in various post-transcriptional processes downstream of RNA polymerase I and II. While eIF4A3 has garnered attention for its prognostic and therapeutic potential in solid tumors, its role in blood cancers remains underexplored. Using a combination of computational and biochemical methods, we demonstrate that high eIF4A3 expression in AML is linked to deregulated RNA metabolism, impacting processes such as ribosome biogenesis and splicing. Additionally, we show that depleting eIF4A3 induces cell death through both p53-dependent mechanisms (via activation of the Impaired Ribosome Biogenesis Checkpoint) and p53-independent pathways. Our findings suggest that AML is highly dependent on eIF4A3, presenting a potential targetable vulnerability for AML therapy.

BET inhibitors as a precision medicine for the treatment of cervical carcinoma.

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Cervical carcinoma (CC) is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer-related death in women. Infection by the human papillomaviruses (HPVs), specifically HPV 16 and 18, is responsible for 50% of high-grade clinical pre-cancers. There are three types of CC, where the most common is squamous cell carcinoma (80-90%), while 10-15% are adenocarcinomas, and other rare types (5%). Importantly, these tumors express high levels of the *MYC* oncogene.
Our Lab has previously shown that inhibition of MYC or MYCN causes metabolic reprogramming manifested by lipid droplet accumulation in neuroblastoma and clear cell renal cell carcinoma. To address whether this is a general effect among cancers, we performed a screening in 60 human cell lines of different cancer types treated with the first-generation BET (bromodomain and extra-terminal) domain inhibitor JQ1, which among other genes inhibits *MYC*, as well as with two MYC-MAX inhibitors. Our data showed an accumulation of cytoplasmic neutral lipids and morphological changes in CC cell lines, which we further investigated.
We explored the initial observations in CC cell lines with different *p53*, *RB*, and/or HPV status following treatment with JQ1 and the second-generation BET inhibitor IBET762. Our data showed downregulation of MYC levels upon BET inhibitors treatment together with reduced cell proliferation. In addition, we observed morphological changes in HPV-positive cell lines, indicating that BET inhibitors could impact the cytoskeletal organization in association with virus-carrying tumors.
To further unravel the effects of BET inhibitors in CC, we performed proteomics following treatment for 24 hours or seven days with IBET762. We found that the most significantly deregulated proteins were involved in lipid metabolism and cytoskeleton organization. Two of these proteins were the nerve growth factor receptor (NGFR) or $p75^{NTR}$, and Ras Homolog Family Member A (RhoA). The latter plays crucial roles in cytoskeleton organization, migration, and cell division. Importantly, the p75 NGF receptor has been described to modulate RhoA, making this axis a potential responsible for the morphological changes we observed after treatment with BET inhibitors. To further unravel these first results, we analyzed protein expression, cellular morphology, lipid droplet accumulation, metabolic changes, and tumorigenic properties by Western blot, immunofluorescence, seahorse metabolic profiling, and invasion assays.
Based on our data, BET inhibitors could potentially serve as an effective and innovative approach for treating CC. Third-generation BET inhibitors including ABBV-075 are currently being optimized for clinical use, aiming to minimize adverse effects, and may be an attractive advance for CC treatment.

Keywords: Cervical carcinoma, MYC, BET inhibitors, cytoskeleton, precision medicine

mPGES-1 inhibitor enhances the cytotoxic effect of vincristine in PDAC multicellular tumor spheroids

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The stroma surrounding the tumor is usually characterized by the presence of fibroblasts. Cancer cells can activate fibroblasts to perform various functions, ultimately leading to a tumor microenvironment that actively promotes tumor growth. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX) enzymes, resulting in decreased synthesis of prostaglandins (PGs). This not only has an anti-inflammatory effect, but also reduces tumor growth by inhibiting the synthesis of the tumor-promoting factor prostaglandin E2 (PGE2) and thus its signal transmission via prostaglandin E2 receptors (EP1-4). To avoid various adverse effects of COX inhibitors associated with non-selective PG reduction, inhibition of microsomal PGE synthase-1 (mPGES-1), which specifically blocks PGE2 formation, could be an interesting target. In this study, we investigated the effect of inhibiting mPGES-1 in combination with the cytotoxic drug vincristine on multicellular tumor spheroids (MCTS). We tested the effect of this combined treatment on pancreatic ductal adenocarcinoma (PDAC) cells (PaTu-8988T and Capan-2) co-cultured with human dermal fibroblasts (HDF) in MCTS models. Cell viability was assessed using an ATP-based cell viability assay and high-affinity nucleic acid staining. Combined treatment with mPGES-1 inhibitor 934 and vincristine resulted in significantly increased cell death in both PaTu-8988T and Capan-2/HDF MCTS compared to mono-treatment with vincristine. In addition, we tested combination treatment with celecoxib, EP2 and/or EP4 antagonists in both types of MCTS. The combination of vincristine and celecoxib or EP antagonists did not result in significantly higher cell death compared to vincristine treatment alone. We optimized Capan-2/HDF MCTS for high-throughput screening and advanced cell imaging analysis to thoroughly investigate the combination of mPGES-1 inhibitors with various other cytotoxic drugs. The initial results confirm previous findings that inhibition of mPGES-1 enhances the efficacy of vincristine, so the use of mPGES-1 inhibitors could be a new therapeutic approach for PDAC.

Keywords: Pancreatic cancer, prostaglandin E2

Evaluating IMPDH inhibitors in the treatment of T-cell acute lymphoblastic leukemia

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CO-AUTHORS: Sean G. Rudd

ABSTRACT: While much progress has been made in the treatment of pediatric leukemia, patients with T-cell acute lymphoblastic leukemia still have a 5-year overall survival below 80%. This poor survival indicates the need for newer treatment options. Nelarabine, a deoxyguanosine analogue, is the newest approved drug for the treatment of T-ALL, but with limited preclinical combination therapy data. IMPDH is the rate-limiting enzyme in the production of endogenous (deoxy)guanosine. In the present study, we evaluate IMPDH as a therapeutic target in T-ALL and evaluate several drugs targeting IMPDH for antileukemic activity in combination with the nelarabine prodrug ara-G. Frontline T-ALL therapy containing thiopurines can result in resistance via an activating mutation in NT5C2, the enzyme responsible for breakdown of thiopurines and (deoxy)guanosine. While this results in resistance to thiopurines, it increases sensitivity to IMPDH inhibitors. So next, we rationalize that combined therapy consisting of IMPDH inhibitors together with nelarabine could have enhanced antileukemic activity in T-ALL carrying the activating NT5C2 mutation, and we subsequently evaluate this combination in several T-ALL cell lines. Taken together, this study further underscores the potential of drugs targeting IMPDH in the treatment of leukemia and provides data on a rationally designed combination chemotherapy that warrants further investigation.

Keywords: IMPDH, T-cell acute lymphoblastic leukemia

Breaking Barriers in Breast Cancer Research with Self-Supervised Artificial Intelligence Models

Name of presenter: Francisco J. Peña

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In cancer research, the extensive costs and labor required to acquire annotated data significantly hinder the development of artificial intelligence models. Recent advancements in self-supervised learning offer a promising alternative by enabling the use of large unlabelled datasets to develop models that generalize to varied diagnostic tasks. Yet, despite its potential to alleviate major bottlenecks, the application of this approach in cancer pathology, particularly for large-scale foundational model training, remains limited.
Despite the potential benefits, most previous studies in cancer pathology have utilized relatively small datasets for both pre-training and evaluating model performance. This project sets a new benchmark by developing the largest academic foundation model for breast cancer pathology, leveraging a dataset composed of 100,000 whole slide images, which total around one billion tiles. This is orders of magnitude larger than any existing single-organ dataset. We aim to use advanced self-supervised learning algorithms to train this model and assess its effectiveness across a variety of clinical tasks specific to breast cancer, thereby reducing the reliance on extensive annotated datasets.
The project employs a diverse array of self-supervised learning strategies, such as contrastive learning and knowledge distillation, which are specially adapted to this expansive dataset. By focusing solely on breast cancer imagery, we aim to determine if a model trained on this specific organ can surpass the performance of those trained on more diverse medical datasets. Initial evaluations will measure the model's ability to generalize across various diagnostic tasks, offering a significant step forward in precision medicine for breast cancer.
Early results are promising, indicating that models pre-trained on pathology-specific data achieve superior diagnostic accuracy compared to those trained on generic datasets. This advancement not only pushes the frontiers of computational pathology but also paves the way for more precise, efficient, and accessible diagnostic tools in breast cancer care. The implications of this research extend beyond immediate clinical applications, potentially setting
new standards for the integration of artificial intelligence in medical diagnostics.

Keywords: breast cancer, artificial intelligence.

CBK79: a copper-binding compound that inhibits the ubiquitin-proteasome system and autophagy
Name of Presenter: Hélder Pereira

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Cells rely on the ubiquitin-proteasome system (UPS) to maintain protein homeostasis (proteostasis) [1]. Cancer cells have ever so demanding proteostasis requirements due to their hyperactive state. Drugs that can impair the UPS have emerged as a therapeutic strategy to target malignant cells [2]. We performed a high-content screening for inhibitors of the UPS, which led to the development of CBK79 [3]. Interestingly, CBK79 not only inhibits the UPS but also impairs autophagy through the induction of non-canonical lipidation of the autophagy marker LC3. Analysis of the transcriptome of cells exposed to CBK79 revealed a profile that was very similar to the one observed in response to a known proteasome inhibitor with a prominent exception: CBK79, in contrast to the proteasome inhibitor, induced expression of metallothioneins – a family of proteins involved in metal ion homeostasis [4]. Subsequent analysis showed that CBK79 interacts with Cu(II) ions. As preloading of CBK79 with Cu(II) strongly enhanced its ability to inhibit the UPS as well as its toxicity towards cancer cells, this set of data suggests that copper metabolism plays an important role in the mode-of-action of CBK79.

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Keywords*:* Cancer, Proteostasis

Validation of SMUG1 and UNG2 as potential targets for treatment of colorectal cancer
Name of Presenter: Helge Gad

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Replication stress is a major cause of genomic instability and can be induced by alteration of the DNA bases in the genome. Many anti-cancer drugs including PARP inhibitors, Chk1 inhibitors and 5-fluoro deoxyuridine (FdU) induce replication stress by different mechanisms including trapping of PARP on DNA, inhibiting signalling from stalled forks induced by DNA damage and increasing genomic uracil. A hallmark in replication stress is the induction of single-stranded DNA (ssDNA) gaps that if left un-repaired leads to toxic lesion and cell death.

Genomic uracil can be induced by either mis-incorporation of uracil during DNA replication or deamination of cytosine, e.g. by the APOBEC3A/B protein, but the mechanism that leads to replication stress and toxicity is poorly understood. Uracil in DNA is primarily repaired by the BER pathway in which the excision of the uracil base is mediated by the Uracil DNA glycosylase protein family including UNG2 and SMUG1. There is conflicting evidence to the role of hSMUG1 and UNG2 in the repair of uracil and how the loss of these enzymes would lead to replication stress. We have developed MTHFD1/2 inhibitors that induce genomic uracil and replication stress, but the how this would lead to cell death is still largely unknown and studies in SMUG1 and UNG deficient cells could clarify the mechanism of action.

In viability assays using dialyzed serum, we could show that the SMUG1 KO cells were hypersensitive to PARPi (Talazoparib and Olaparib), Chk1i and FdU, while UNG KO cells were hypersensitive to PARPi, FdU and MTHFD1/2i. SMUG1 KO cells had increased levels of replication stress (pRPA/yH2AX foci) and genomic uracil after PARPi and Chk1i treatment, compared to wildtype (WT) cells. The toxicity of FdU and MTHFD1/2i, but not PARPi and Chkl1i, could be rescued by a low dose of thymidine and dUTPase inhibition led to further sensitivity of SMUG1 KO cells to FdU and UNG KO cells to PARPi, Chk1i and MTHFD1/2i. Using the novel U-DNA sensor assay, we could show that treatment with MTHFD1/2i specifically induce uracil in SMUG1/UNG double KO cells or cells expressing the bacterial Uracil DNA glycosylase Inhibitor (UGI) but not in single KO cells.

SUMMARY: Our results suggest that SMUG1 and UNG1/2 could be potential drug targets for combination therapy with PARPi, Chk1i and FdU. However, SMUG1 and UNG2 have distinct functions in uracil repair and this project will delineate these mechanisms. We are currently developing SMUG1 inhibitors and have generated inhibitors with an IC50 in vitro in the micomolar range and will start testing these in cellular assays comparing the efficacy of SMUG1 inhibitors in WT and SMUG1 KO cells to assess the specificity.

Keywords*:* Replication stress, PARP inhibitors

Exploring activation of the DNA glycosylase NTHL1 in DNA damage repair
Name of Presenter: James Haslam

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Damage to DNA is a recurring feature of several diseases. Failing or inefficient DNA repair is implicated in ageing, inflammatory or neurodegenerative disease. Alleviating oxidative stress to the genome by activating function of enzymes involved in its repair is a potential therapeutic strategy.
Our laboratory has previously demonstrated that the repair of a common oxidative base lesion, 7,8-dihydro-8-oxoguanine (8-oxo-G) can be increased through activation of the base excision repair protein DNA glycosylase OGG1. Another DNA glycosylase involved in the recognition and repair of oxidative damage, specifically oxidised pyrimidines, is endonuclease-III-like protein 1 (NTHL1).
In this work we aim to explore the concept of activating NTHL1. We combined knowledge of the shared rate limiting step of OGG1 and NTHL1 and the crystal structures of their active sites to rationalise the position of a potentially activating mutation in the active site of NTHL1. Potentially activating mutants were generated and re-expressed as fluorescently tagged proteins in a NTHL1 knock-out cell line. Using multiple live cell microscopy assays, we uncover distinct differences in protein recruitment, dynamics and mobility between the mutant proteins and wild-type in response to DNA lesions induced by laser microirradiation or hydrogen peroxide. Furthermore, there may be a functional relevance to these differences as some of the mutant NTHL1 expressing cells are sensitised to hydrogen peroxide treament. Further work will quantify the levels of activation or substrate preference by *in vitro* kinetic analysis of the purified proteins on a panel of damaged DNA substrates. This work provides the first evidence of functional effects of activating the catalytic activity of NTHL1 during DNA damage repair.

Chromaffin to neuroblast cell state transitions define cell plasticity in paraganglioma and neuroblastoma
Name of Presenter: Jiacheng Zhu

Jiacheng Zhu, Wenyu Li, Peng Cui, Maria Arceo, Petra Bullova, Oscar Bedoya Reina, Monika Plescher, Catharina Larsson, Arthur Tischler, Christofer Juhlin, Susanne Schlisio

Introduction:
Intratumor heterogeneity and high plasticity account for therapy resistance and poor clinical outcomes in neuroblastoma patients. The causes of plasticity cell state transition during tumor progression remain poorly understood. Here, we deleted tumor suppressors *Kif1bβ* and *Nf1* in the embryonic mouse sympatho-adrenal lineage and observed pheochromocytoma (PCC), neuroblastoma (NB), and composite tumors arising in aged mice. Deep single-cell RNA sequencing combined with immunofluorescence (IF) and RNAscope revealed chromaffin-neuroblast cell state transitions embryonically and postnatally driving tumor plasticity accounting for invasive behavior. Leveraging Spatial Transcriptomics, we illustrated the heterogeneity of tumors at spatial level and revealed the potential location and morphology of the transitioning regions. Combined with bulk and single-nuclei RNA sequencing (snRNA-Seq) data of human paraganglioma and pheochromocytoma (PPGL), we showed that this chromaffin-neuroblast cell state transition event is prognostic relevant to metastatic PPGL.

Methods:
We collected mouse post-natal wild type (Adrenal Gland) AG, *KIF1Bβ* KO AG, *NF1* KO and DKO tumor samples, and processed them with SMART-seq2 protocol. We annotated the single cell data based on the classical cell type markers. According to the single cell data, we selected 104 cell type enriched genes to and applied In-Situ Sequencing (ISS) to detect the transcripts on the original sample/tumor sections. We then segmented our ISS data into hexagonal bins with radius of 250 or 300 pixels. The hexagonal bins were analyzed by using Giotto. Then we performed deconvolution for the hexagonal bins by applying RCTD. The annotated single cell data were used as reference for deconvoluting the ISS data. The human PPGL snRNA-Seq data was obtained from a parallel project in our group, which was processed with the SMART-seq2-Nuc-Seq protocol. The bulk RNA-Seq data for human PPGL was obtained from the study conducted by *Calsina et al*. The deconvolution of bulk-RNA-Seq data was down by using DWLS and BayesPrism.

Results:
Loss of *KIF1Bβ* potentiated the oncogenic activity of *NF1* loss causing the development of large, bulky, and locally invasive masses that arose in the adrenals. Histopathology revealed pheochromocytoma, neuroblastoma and composite tumors in aged mice. We found chromaffin tumor cells obtain a neuroblastic feature postnatally (3 month or older) and continue to form NB, PCC and composite tumors of both types. Meanwhile, these tumors have a remarkable heterogeneity. In early tumor development at 3-month-old, a distinctive three segment structure has been found breaking through the cortex from medulla, which shows the transitional state of chromaffin cells to neuroblasts. It is consistent with the single-cell RNA velocity prediction. Such newly discovered lineage transitions suggest important implications for understanding neuroblastoma and pheochromocytoma heterogeneity.
However, the lack of cell spatial information is challenging us to locate this heterogeneity. ISS gives an overall profile of the tumor. By analysing the distribution of 104 selected genes', signals, we located the neuroblastoma, pheochromocytoma, and composite tumor cell status areas on the sections in situ. We observed gene expression patterns changes in specific tumor areas, suggesting switches of cell states.
Within the snRNA-Seq data, we mapped the tumor cells to a normal adrenal gland reference dataset and re-labeled them with normal adrenal medulla cell type names based on their transcriptomes. By integrating this data with a large bulk RNA-Seq PPGL cohort, we discovered that cells labeled as Embryonic_Cycling_Neuroblast and Embryonic_Early_Chromaffin_Cell exhibit higher enrichment in the metastatic group compared to the non-metastatic group. Conversely, Postnatal_Chromaffin_Cells show the opposite trend. This suggests the prognostic relevance of the chromaffin-neuroblast state transition in PPGL metastasis.

Impact of inhibition of the epigenetic regulator Enhancer of Zeste Homolog 2 in childhood medulloblastoma malignancy.
Name of Presenter: Jiansheng Wang

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85% of children diagnosed with cancer are alive at least five years after diagnosis due to medical advances. However, long-term survival is also accompanied with late or long-term effects, both physical and psychological. Therefore, there is an urgent need for novel effective treatment. Childhood cancers carry few genetic mutations compared to adult cancers, and some of these tumors develop due to epigenetic alterations, demonstrating that these processes play a vital role in the development and progression of childhood cancers.
Medulloblastoma (MB) is one of the most common malignant brain tumors in children. Histone methyltransferase Enhancer of Zeste Homolog 2 (EZH2), a catalytic subunit of Polycomb Repressive Complex 2 (PRC2), is a crucial epigenetic regulator that mediates transcriptional repression through trimethylation of lysine residue K27 in histone H3 (H3K27me3). However, the role of EZH2 in MB is not fully understood yet.

We selected three well-known EZH2 inhibitors UNC1999, GSK126, and GSK343, and observed significant induction of lipid droplet accumulation induced in UW2283 cells upon treatment. Lipid droplets can be formed either due to an increase in *de novo* fatty acid synthesis or alterations in their oxidation. To this end, we studied the levels of fatty acid synthase (FASN), the rate limiting enzyme of lipogenesis, by Western blot and observed that the FASN protein was upregulated after EZH2 inhibition. Interestingly, we observed that FASN inhibitors including UB006 and Orlistat, prevented the lipid accumulation caused by EZH2 inhibitors in UW2283 cells, indicating a potential increase in fatty acid synthesis upon targeting EZH2. Moreover, we demonstrated that the combo treatment of EZH2 and FASN inhibitors resulted in decreased UW2283 cell proliferation compared to single treatments using CyQUANT assay. Notably, we found that lipid deprivation led to a lower degree of lipid droplet accumulation as well as decreased cell proliferation in UW2283 cells. Together, our findings revealed that dysregulated lipid metabolism may diminish the antitumor effects of EZH2 inhibitors.

Our results showed that both FASN inhibition as well as lipid deprivation resulted in decreased lipid droplet formation and cell proliferation upon UNC1999 treatment. Future experiments will focus on studying the mechanism by which inhibition of acid synthesis results in decreased cell proliferation. We will also examine the increased sensitivity of UW2283 cells to EZH2 inhibitors by suppression of fatty acid synthesis and/or lipid droplet formation in more detail. In addition, we will use lipolytic agents or lipid-decreasing drugs currently used in a clinical setting for analysis of possible synergistic effects together with EZH2 inhibitors, providing a putative a therapeutic strategy for precision medicine in MB.

Keywords: Medulloblastoma, EZH2, lipid metabolism, FASN, cancer treatment

Mechanisms and Resistance of Proteasome Inhibition in Multiple Myeloma: Insights into Dynamic Protein-Protein Interaction Networks and Translocation
Name of Presenter: Jiawen Lyu

Affiliation: Karolinska Institutet, Oncology-Pathology
Authors: Jiawen Lyu, Pär Nordlund

Proteasome inhibition have been a cornerstone of therapeutic strategy for multiple myeloma (MM) since the first proteasome inhibitor, bortezomib, was approved by FDA in 2003. Despite the successful development of additional compounds, including FDA approved drugs carfilzomib and ixazomib for MM and other hematological cancer, the mechanisms underlying proteasome inhibitor(s) are still not fully understood. A fundamental question remains: how does the accumulation of excess protein due to proteasome inhibition can eventually lead to cancer cell death? It is obvious that a variety of proteins must have reciprocally participated into this signal cascade of drug action.
Using our in-house developed Integrated Modulation of Protein Interaction States—Cellular Thermal Shift Assay (IMPRINT-CETSA) and pulse quantitative proteomics, we are the first to systematically investigate the cellular activities under protein inhibition in two MM cell models. Rather than simply determining differential expressed genes, we have established a data panel, including temporal traces of protein homeostasis, dynamic protein-protein interaction variations, spatial observations of subcellular protein translocation, all reported on a proteome-wide scale. Leveraging these multidimension data, we firstly discovered an acute relocation of proteasome 20S core in response of drug exposure, while the 19S regulatory particle remains static, which indicates that the 26S proteasome giant complex are modulated differentailly upon inhibition, referring to its different functions of subcompartments. Furthermore, the interaction network of many proteasome co-factor proteins has been altered, which guide us to link proteasome inhibition to many stress responses, for example ER stress, oxidative stress. These co-factors reciprocally regulate proteasome assembly, which explains the differential modulation of 19S and 20S. Beyond protein degradation, another aspect of proteostasis quality control, biogenesis machinery is also affected by impaired UPS system. Ribosome flunctuation and spliceosome perturbation suggest an arrest of new protein biosynthesis, which is later transduced to nucleus and leads to nuclear apoptosis response.
A parallel study has been conducted on two resistant cell lines tolerant to bortezomib and calfilzomib, respectively, which enable us to decipher the mechanism of PI resistance. While studies attributed the resistance to point mutations that prevents drug binding, our research clearly shows that both bortezomib and carfilzomib can bind to their target region, and lead to the translocation and disassembly of proteasome 20S core particle, as same as in the corresponding sensitive parental cells. We suggest alternative hypothesis that multiple pathways contributed to the resistance, including proteasome function enhancement, drug excretion enhancement by ABCB1 overexpression, alteration of stress response, etc.
Diving in the vast data, we composed a comprehensive mechanism study of proteasome inhibition in MM cells, elucidated a cascade of proteins orchestrate MM cell death, and also shed light on novel therapeutic approach development for refractory and relapse MM.

Keywords: proteasome inhibition mechanism, multiple myeloma resistance

Targeting Peroxiredoxin 6 as a Differentiation Inducing Strategy for Neuroblastoma
Name of Presenter: Judit Liaño-Pons

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Neuroblastoma (NB), an embryonal tumor of the sympathetic nervous system, accounts for 7% of all childhood cancers. It is heterogeneous, spanning from tumors that spontaneously regress to others that are invasive and metastatic. The MYCN oncogene is amplified in 40% of the high risk cases and is correlated with an undifferentiated phenotype and poor patient outcome. Amplification of MYCN drives the metabolic reprogramming in NB, with high rates of oxidative phosphorylation, elevated fatty acid dependent respiration, and increased expression of antioxidant enzymes. Here, we explored the potential of using the antioxidant systems as targets for NB treatment. We found robust effects when targeting peroxiredoxin 6 (PRDX6), a moonlighting enzyme involved in ROS scavenging and lipid metabolism. Inhibition of PRDX6 led to apoptosis, abrogation of cell proliferation, decreased MYC/MYCN levels and induced neural differentiation, especially in MYCN-amplified NB cell lines. Cells accumulated lipid droplets, which were crucial for acquiring a differentiated phenotype. The effects were potentiated both in vitro and in vivo when targeting PRDX6 together with another antioxidant enzyme, Glutathione S-transferase P (GSTP1). We confirmed the specificity of the treatments by double knockdown and overexpression experiments. When analyzing single-cell RNA-seq data of the developing murine adrenal gland, we found high levels of PRDX6 and GSTP1, suggesting a key role of these enzymes in maintaining redox homeostasis during development.
In patients, elevated expression of PRDX6 and GSTP1 was found in high-risk, MYCN-amplified NBs, in association with undifferentiated tumors and poor prognosis. Notably, there were no LowPRDX6+GSTP1 MYCN-amplified patients in any of the cohorts. GSTP1 had already been described as a MYCN direct target gene, and here we show that MYCN also regulates PRDX6 by direct binding to its promoter.
Together, our results provide insights into the role of PRDX6 and GSTP1 in NB tumorigenesis and reveal the potential of targeting PRDX6 as a precision medicine strategy to induce neural differentiation and reactivate cellular pathways that drive cancer cells to transition into a less aggressive phenotype.

Keywords: Neuroblastoma; differentiation-inducing therapy.

Gene expression landscape of long-term tamoxifen resistance in premenopausal ER+ breast cancer in the STO controlled randomized clinical trial with >20-year follow-up

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Background
The endocrine agent tamoxifen is a cornerstone treatment for estrogen receptor positive (ER+) breast cancer (BC). Long-term risk of recurrence is a distinguishing trait of ER+ BC, particularly in premenopausal patients. Late recurrences of ER+ BC despite tamoxifen treatment pose a considerable challenge in the clinic, but the underlying mechanisms remain poorly understood.
Methods
Secondary analysis of 406 premenopausal ER+, HER2- invasive BCs randomized to 2 years of tamoxifen treatment or control in the Stockholm Tamoxifen 5 (STO-5) trial, with 20 years complete follow-up. Tumor expression of ~21.5K unique genes (Agilent Technologies) was analyzed in relation to distant recurrence-free interval (DRFI) in Cox proportional hazards models restricted to 10-20 years post-diagnosis. Genes with a tamoxifen-interaction term *p* value (p_i)<.01 were further analyzed. Gene sets identified by k-means clustering were subjected to GO enrichment analyses with Benjamini-Hochberg adjustment to generate *q*-values. Correlations were evaluated using Pearson correlation.
Results
With a cutoff at p_i<.01, 188 genes were associated with tamoxifen effect on late recurrences: 58 with sensitivity, 130 with resistance. These genes did not include *ESR1* or any gene strongly correlated to *ESR1* expression (correlation constant >|.5| and *p*<.01), and only 50% were previously described in BC. Clustering the 188 genes in a heatmap, sensitivity and resistance genes were found to be separately co-expressed, suggesting that treatment sensitive tumors and treatment resistant tumors compose distinct phenotypes. Functionally, the clusters represented a wide range of GO-terms (*q*<.05); resistance-related enrichment terms included e.g. export from the nucleus, while sensitivity-related functions included e.g. regulation of transcription and translation.
Conclusion
Among the genes related to long-term tamoxifen benefit in this patient subset, co-expression patterns indicated distinct phenotypes for benefit and resistance. The lack of predictive indication of *ESR1* suggests that in this setting, tamoxifen resistance may be attributed to indirect tamoxifen effects rather than tumor ER. Our data supports both the need and the feasibility of a novel gene expression-based resistance panel for clinical utilization.

Keywords: breast cancer, treatment

Reprogramming of TAMs anti-tumoral phenotypes by targeting MNK2.

Name of Presenter: Kaveri Banerjee

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Abstract: Tumor-associated macrophages (TAMs) exhibit remarkable cellular plasticity, ranging from anti-tumor to pro-tumor phenotypes. These versatile cells can regulate vessel functionality, acting as gateways for hematogenous dissemination. Within TAMs, the intricate interplay between mitogen-activated protein kinase (MAPK) interacting protein kinase (MNK)1 and MNK2 plays a pivotal role. These kinases selectively modulate mRNA translation by impacting eIF4E phosphorylation, thereby facilitating reshaping of the proteome without altering the abundance of corresponding mRNAs. Our recent research has uncovered the crucial role of selective mRNA translation changes as a central hub regulating immunosuppressive functions of macrophages. Our latest study demonstrates that targeting MNK2, rather than MNK1, effectively modulates eIF4E activity in TAMs. This targeted approach results in reprogramming of immunosuppressive TAMs, effectively impeding the growth of mammary tumors. Moreover, we have observed that these reprogrammed TAMs adopt an angiostatic/anti-metastatic phenotype. Firstly, they induce tumor blood vessel normalization, facilitating the intratumoral recruitment of cytotoxic T and natural killer (NK) cells while impeding mammary metastatic spreading. Additionally, blocking MNK2, but not MNK1, skews immunosuppressive TAMs towards an immunostimulatory phenotype, thereby enhancing the activity of cytotoxic T and NK cells. We thereby propose that targeting MNK2 represents a highly efficient strategy to reprogram TAMs into an anti-tumoral phenotype.

Keywords: Reprograming Tumor-Associated Macrophages; Breast cancer metastases.

Delineating genome instability dynamics in breast cancer by time-course single-cell copy number profiling

Name of Presenter: Konstantinos L. Georgiadis

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Hormone receptor positive (HR+) breast cancer (BC) is the most frequently diagnosed type of breast cancer consisting almost 70% of breast cancer cases. Copy number alterations (CNAs) are a hallmark of aggressive HR+ breast cancer and have been proposed as prognostic biomarkers. However, the dynamics of CNAs during the course of BC therapy remain poorly understood. Here, we present our ongoing work on the characterization of CNA dynamics in BC patients undergoing neoadjuvant treatment with chemotherapy, anti-HR agents and the CDK4/6 inhibitor palbociclib in the context of PREDIX Luminal B clinical trial (NCT: 02603679). This is a phase II study where patients were randomized to either neoadjuvant chemotherapy or endocrine therapy and palbociclib with crossover halfway through the course of neoadjuvant treatment. Research biopsies were collected and stored fresh frozen at -80°C at baseline, before treatment switch and at surgery. A total of 180 patients were enrolled until 2021 and follow-up is ongoing. We utilized biopsies before and after treatment from 19 patients and optimized methods to achieve low-cost, high-throughput singe-cell whole genome sequencing to generate high quality copy number profiles from thousands of nuclei. Library preparation is performed by deploying a tagmentation based protocol using in-house produced Tn5. Miniaturization and automation of reactions utilizing the robotic nanodispenser I.DOT (Dispendix) results in scalability of library preparation workflow. So far, 16,000 single nuclei from 13 different patients have been sequenced. Preliminary results demonstrate the feasibility of our method and further bioinformatics analysis is ongoing. The analytical approach we plan to implement to reconstruct the dynamics of CNAs and possibly more complex genomic rearrangements will be outlined. Ultimately, utilizing CNA profiles, we aim to describe the evolution of tumor clonal landscape during treatment. This could provide insight into development of resistance to therapy aiding with designing new treatment approaches in the future.

Targeted inhibition of WIP1 and histone H3K27 demethylase activity synergistically suppresses neuroblastoma growth

Name of Presenter: Kristina Ihrmark Lundberg

Diana Treis, **Kristina Ihrmark Lundberg**[1], Conny Tümmler, Emma Åkerlund, Adena Pepich, Brinton Seashore-Ludlow, Kazuyasu Sakaguchi, Per Kogner, John Inge Johnsen, Malin Wickström
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Neuroblastoma is the most common cancer of infancy. It arises in the developing sympathetic nervous system. High-risk disease is often characterized by segmental gains or losses of chromosomes of which gain of 17q is the most frequent and the strongest correlated to adverse disease. The gene *PPM1D*, located on 17q, encodes for the protein WIP-1 which is a regulator of p53, DNA repair and apoptosis. WIP-1 inhibition has previously been shown to suppress neuroblastoma growth *in vitro* and *in vivo*. However, to improve the cytotoxic efficacy of the drug, a combination screening was performed on neuroblastoma cell lines using WIP-1 inhibitors together with a drug library with 527 different drugs. This identified a synergistic effect on viability when combining the WIP-1 inhibitor SL-176 and the H3K27 demethylase JMJ3D inhibitor GSK-J4. Interestingly, immunoblotting and qPCR showed an elevated expression of p53 downstream targets PUMA and p21, and RNA sequencing verified an enrichment of DNA damage response pathways. An *in vivo* experiment with zebrafish xenografts confirmed our findings. Four different groups of transplanted embryos were exposed to either DMSO, SL-176, GSK-J4 or a combination of both. The group exposed to the combination treatment had significantly reduced tumor growth, and no obvious toxicity was seen. We conclude that the combination of SL-176 and GSK-J4 synergistically induces cytotoxicity in neuroblastoma cells both *in vitro* and *in vivo*.

Keywords: WIP-1, Zebrafish xenografts

The role of CD73 in CAF-mediated immunosuppression
Name of Presenter: Laia Gorchs

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Abstract
Pancreatic ductal adenocarcinoma (PDAC) has a 5-year survival rate of just 12%. PDAC exhibits a highly fibrotic tumor microenvironment dominated by an heterogenous population of cancer-associated fibroblasts (CAFs), limiting T cell infiltration. CD73, a membrane-bound 5' nucleotidase, is highly expressed in PDAC, and correlates with poor prognosis. In concert with CD39, CD73 suppresses anti-tumor immune responses by converting ATP into immunosuppressive adenosine. Preclinical models of PDAC, have shown that CD73 inhibitors can enhance immune responses, and ongoing clinical trials are assessing their safety and efficacy in combination therapies. However, the specific role of CD73-expressing CAFs in mediating T cell suppression is yet unknown. This study investigates the role of the adenosinergic pathway in CAF-mediated T cell suppression. Primary pancreatic CAFs and healthy donors PBMCs were cocultured and activated for five days. Preliminary data suggest that blocking CD73 activity by the small molecule APCP promote a less exhausted T cell phenotype, as evidence by reduced expression of PD1, TIM-3 and LAG-3. These finding highlight the potential of targeting CD73 in CAFs to alleviate T cell exhaustion and improve anti-tumor immune responses in PDAC.

Spatial Insights into the Tumor Microenvironment of Merkel Cell Carcinoma Preceding Immune Checkpoint Inhibition

Name of Presenter: Libuše Janská

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Immune checkpoint inhibition (ICI) has transformed the possibilities of cancer treatment by boosting anti-cancer immunity. Its clinical use has been particularly successful in metastatic Merkel Cell Carcinoma (MCC), one of the rarest but deadliest skin cancers. Nevertheless, only half of patients respond to treatment, and many develop resistance. MCC, often caused by viral integration into the host genome, is highly immunogenic and, thus, a good model for studying cancer-immune interactions. However, the underlying reasons for ICI resistance remain unclear. Leveraging single-cell and spatial transcriptomics, we investigate how the tumor microenvironment (TME) shapes ICI response. Spatial transcriptomic samples from pre-treatment MCC patients reveal the heterogeneity and spatial patterning of different immune, stromal, and cancer-related gene programs. The added power of single-cell transcriptomics elucidates the cell-type prevalence and spatial distribution, highlighting the microenvironmental niches formed. Correlating these findings with ICI treatment responses, we validate the identified patterns in bulk RNA-seq from a larger cohort of ICI-treated patients. Our study aims to unravel mechanisms contributing to ICI failure in the MCC TME, offering insights into enhancing ICI efficacy across cancers.

Keywords: Tumor microenvironment, Spatial and single-cell transcriptomics

Impact of the estrogen receptor beta (ERβ) and the TNFα signaling on circadian genes in colitis-induced colorectal cancer development

Name of Presenter: Lina Stepanauskaite

Lina Stepanauskaite, Linnéa Lindquist, Madeleine Birgersson, Rajitha Indukuri, Linnea Hases, Amena Archer, Cecilia Williams

Circadian rhythm is a natural endogenous oscillation of regulatory genes that can respond to environmental cues. This system controls many biological processes, including hormone secretion, glucose homeostasis, and metabolism. The core clock resides in the brain and mainly responds to photic cues. Peripheral tissues have their own peripheral circadian clocks that can control the rhythmicity independently and are susceptible to ambient influences such as feeding schedules or body temperature changes.

The circadian clock impacts the expression of nearly half of all protein-coding genes in the organism. Estrogenic, sex-dependent regulations and proinflammatory factors, such as NFκB signaling, have been suggested as the modulators of the circadian clock. Additionally, circadian rhythmicity is essential for the colon, controlling the inflammatory response, gut permeability, cell proliferation, colonic motor activity, and gut microbiota composition. Subsequently, it has been observed that circadian clock dysregulation is frequent in inflammatory diseases and cancer, where it has been linked to the metastatic capacity of colorectal cancer (CRC) tumors.

In our previous work, we demonstrated, *in vivo* and *in vitro*, that estrogen receptor beta (ERβ) suppresses inflammatory and tumorigenic mechanisms. We showed that ERβ modulates the TNFα/NFκB- p65 signaling pathways and influences p65 binding to the regulatory regions of key circadian genes. Now, we show *in vivo* that the lack of ERβ in the colon significantly impacts the expression levels and the cycling patterns of circadian genes during colitis. We also found *in vitro* that the re-expression of ERβ in CRC cell lines can modulate the effect of TNFα on clock genes.

Our work shows that estrogen signaling might have a protective effect against CRC partially through the regulation of circadian rhythmicity in the colon.

Keywords: circadian rhythm, inflammation

Novel Image Spatial Analysis Identifies Multi-Marker-Defined Colorectal Cancer Peri-Endothelial and -Vascular Environments Associated with Macrophage Density and Prognosis

Name of Presenter: Linglong Huang

Introduction

The vasculature of the CRC consists of several different types of structural cells, such as pericytes, and vascular smooth muscle cells. The impact of these peri-endothelial cells on prognosis and their association with surrounding immune cell environments remains poorly characterized.

Results

Based on single cell RNA-seq and in-situ multi-antibody staining two types of perivascular cells were identified; B1 (*MCAM+*, MYH11-) and B2 (*MCAM+*, MYH11+). Both sub-groups displayed a mixture of PDGFRB-negative and -positive cells. B1 showed expression of pericyte markers like RGS5, whereas B2 cells showed high expression of ACTG2 and muscle cell markers, such as Desmin.

An initial explorative pilot study on 6 colon cancer cases found strong associations between peri-endothelial environments (markers MCAM, MYH11, Desmin) and surrounding peri-vascular densities of macrophage subsets (markers CD68, CD11c, CD163). However, no differences regarding densities of perivascular T-cell subsets (markers CD3, CD8, FoxP3) were detected when peri-endothelial environments were contrasted.

To extend these findings, image-analyses were performed to quantitate at case-level the composition of PDGFRB-, M-CAM-, MYH-11-defined areas in peri-endothelial areas (5 micrometer from CD34-defined vessels) and in peri-vascular areas 5, 15 and 45µm from the peri-endothelial areas. Additionally, density of macrophage subsets was quantitated in peri-endothelial and peri-vascular areas. 124,876 segmented vessels in the central, peripheral, and metastatic regions of the TMA from a Swedish population-based CRC cohort were analyzed. A total of 19654 (approximately 15.74%) segmented vessels were selected from 104 cases for prognostic analysis. For each case value, the mean value of perivascular cells was derived by covering all vessels.

Higher density of peri-endothelial- and -vascular (45µm) MCAM+/MYH11- or peri-endothelial PDGFRB+ MCAM+/MYH11- areas were associated with better prognosis. Also, higher density of peri-endothelial and -vascular (all expansion areas) MCAM+MYH11+ areas was associated with favorable survival outcomes. This signal was also detected when the PDGFRB+ subgroup of this combination was analyzed. In contrast, higher density of peri-vascular (all expansion areas) MCAM-MYH11+ areas were strongly associated with worse prognosis.

Regarding macrophages, high peri-endothelial density of M0 and M2 and low density of M1/M2 was associated with poor prognosis. Also, peri-vascular M1/2 (5µm) and peri-vascular M2 (45µm) were associated with good and bad prognosis, respectively.

Summary

The novel workflow uncovers previously un-recognized survival associations of peri-endothelial environments and also suggests peri-endothelial environments as functional determinants of macrophage exudation.

Targeting MYC induces lipid droplet accumulation by upregulation of HILPDA in clear cell renal cell carcinoma

Name of Presenter: Lourdes Sainero-Alcolado

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Metabolic reprogramming is crucial during clear cell renal cell carcinoma (ccRCC) development, manifested by accumulation of lipid droplets (LDs). This process is mainly governed by the constitutive activation of the hypoxia inducible factors (HIFs) due to loss of the *von Hippel-Lindau* (*VHL*) gene, and upregulation of MYC signaling. Lipid droplets are organelles rich in triglycerides and sterol esters, surrounded by a phospholipid monolayer. In recent years, they have gained recognition as emerging regulators of tumorigenesis, yet the mechanisms and factors regulating their biogenesis remain poorly described.

Here, we studied the molecular mechanism underlying lipid droplet accumulation in ccRCC after MYC inhibition. Using a combination of lipidomics and metabolic tracing, we found that constitutive HIF expression combined with MYC inhibition induces reprogramming of glutamine metabolism, which was directed towards accumulation of triglycerides, the main component of LDs. Importantly, concomitant inhibition of both MYC and glutamine metabolism reduced tumor burden and impaired LD accumulation in vivo. Using RNAseq analysis, we identified the hypoxia inducible lipid droplet associated protein (HILPDA) as the key driver for MYC inhibition-derived LD accumulation, and we identified the hypoxia-inducible lipid droplet–associated protein (HILPDA) as the key driver for induction of MYC-driven LD accumulation and demonstrated that conversely, proliferation, LD formation, and tumor growth are impaired upon its downregulation. Finally, single-cell RNAseq analysis of ccRCC tumors from patients and healthy renal control samples, postulate HILPDA as a specific biomarker for ccRCC. Currently, we are focusing on understanding the role of HILPDA in ccRCC tumorigenesis and exploring how cancer cell-derived LDs influence other components of the tumor microenvironment.

Together, our study characterizes the molecular interplay between hypoxia and MYC signaling resulting in LD accumulation with HILPDA as a novel target for precision medicine. These discoveries provide an attractive approach for the development of new therapeutic interventions for treatment of ccRCC.

Keywords: MYC, clear cell renal cell carcinoma, lipid droplets, HILPDA

BET inhibition sensitizes cancer cells to viral dsRNA mimics and boosts recognition by tumor-specific CD8+ T cells

Name of Presenter: Lucas Baldran-Groves

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Abstract
While immunotherapies have revolutionized cancer treatment, most patients do not derive durable clinical benefits, in large part due to tumor-intrinsic resistance mechanisms. Of interest is the dsRNA sensing pathway RIG-I/MDA5 that has been shown to overcome immunotherapy resistance. However, the role of oncogenic BET proteins in the context of dsRNA signaling pathways is still poorly understood. Previous research in our group revealed that the epigenetic drug JQ1, a prototype BET inhibitor, can sensitise tumor cells for T cell-recognition by enhancing IFN signalling and antigen presentation by HLA-I to CD8+ T cells. Interestingly, proteomic analysis of JQ1-treated melanoma cells suggested the effects of JQ1 are due to amplification of antiviral signalling in tumor cells. Therefore, we investigated the role of BET proteins in regulating dsRNA sensing pathway and whether their pharmacological inhibition could potentially improve the efficacy of cancer immunotherapy.

Human melanoma cell lines were treated with the prototype BET inhibitor JQ1 and subsequently exposed immunogenic dsRNA. The latter was achieved by transfecting tumor cells with the viral dsRNA mimic polyinosinic-polycytidylic acid (poly(I:C). Flow cytometry and qPCR were used to analyze treated tumor cells for expression changes in key immunogenicity markers. Functional assays were done by co-culture of JQ1- and/or poly(I:C)-treated tumor cells with autologous tumor-infiltrating lymphocytes (TIL) to assess T cell-activation.

We found that prolonged (at least 72h) JQ1 treatment strongly boosted the immunogenic-enhancing effects of dsRNA signaling, as indicated by a stronger induction of key cytokines involved in type I interferon signaling such as CXCL10 and IFNβ, antigen presentation by HLA class I, and markers of immunogenic cell death. Importantly, the molecular changes observed in tumor cells treated with JQ1 and dsRNA were associated with strongly improved tumor recognition by autologous CD8+ tumor-infiltrating lymphocytes, as indicated by enhanced cytokine production and degranulation. Overall, our data suggest that BET proteins epigenetically silence dsRNA sensing pathways in melanoma and that BET inhibition can be therapeutically exploited for enhancing the efficacy of cancer immunotherapies that aim to activate tumor-specific CD8+ T cells.

A novel assay for determination of glutathione peroxidase isoenzyme-specific activities in crude cell lysates

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Cancer cells rely heavily on glutathione peroxidase (GPX) antioxidant enzymes, which reduce potentially harmful hydrogen peroxide and lipid hydroperoxide species. These redox enzymes have emerged as attractive targets for treating cancer as they have been linked to treatment resistance and are overexpressed in some cancer types. There are 8 GPX isoenzymes, 5 of which are selenoproteins. Of special interest are ubiquitously expressed GPX1, and GPX4 counteracting ferroptosis. Potent and specific GPX1/2 and GPX4 inhibitors Auranofin and Lusutrombopag, respectively, were recently discovered, showing close to 100% inhibition of the pure recombinant enzymes (PMID: 37244126). To date, there are no facile yet specific GPX1/GPX2 and GPX4 activity assays for crude lysates. Here we present such an assay based upon cumene hydroperoxide reduction using glutathione (GSH) recycled by glutathione reductase (GR) and spectrophotometric determination of NADPH consumption, combined with Auranofin and Lusutrombopag as isoenzyme-specific inhibitors. Recombinant GPX1 and GPX4 standard curves are used to calculate active isoenzyme activity levels (corresponding to ng active GPX/µg lysate protein). Validation with sodium selenite-supplemented cells or immunoblots for expression levels were also done. The assay can be easily employed for determinations of GPX isoenzyme activities in lysates from cells treated with diverse inhibitors, or with GPX1, GPX2 and GPX4 knockout (KO) cell lysates. The method provides a bridge from biochemical small molecule inhibitor discovery to the cellular context, a new avenue for understanding GPX redox interactions in cells and allows for facile determination of active GPX isoenzyme profiles across various cell or tissue samples.

ERβ as a potential diagnostic biomarker in granulosa cell tumors

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Granulosa cell tumors (GCTs) are rare ovarian tumors, classified into juvenile GCT which occurs at a very young age, or the more prevalent adult GCT which mainly affects peri- and postmenopausal women. Both subtypes are understudied and are thus often treated as general epithelial ovarian cancer despite the distinct molecular differences. This is the only subtype that expresses estrogen receptor β (ERβ/ESR2). In this study, we aimed to better understand the biology of GCTs, and to investigate the role of ERβ as a potential biomarker and therapeutic target. Using tissue microarrays of formalin-fixed paraffin-embedded GCTs (n=50) and non-tumor ovaries (n=16), we evaluated ERβ as a potential biomarker for GCTs using a highly validated antibody (PPZ0506). We also performed RNA-sequencing of fresh-frozen samples (n=6). We found that 90% of the GCT samples expressed ERβ, where 80% of adult GCTs had a high protein expression. The expression of ERβ in adult GCTs was also positively correlated with current clinical GCT markers and FOXL2. Studying the transcriptome, we found distinct differences in the transcriptomes between the two subtypes, including an enrichment of cell proliferation in juvenile GCT. Moreover, investigating the different ERβ splice variants, we identified an upregulation of ERβ2 (ERβ_cx) and ERβ4 in the tumors. ERβ4 was further shown to inhibit the ligand-dependent transactivation by ERβ1 (WT), indicating the splice variant could play a role in GCT. With these results, we propose ERβ as a diagnostic biomarker for GCT and a potential therapeutic target.

Keywords: Granulosa cell tumor, Estrogen receptor β

Differential Long-Term Benefit of 2-Year Adjuvant Tamoxifen Therapy for Luminal-Type Breast Cancer: Insights from a 20-Year Follow-Up Analysis of the STO Trials

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Background: Patients with estrogen receptor (ER)-positive breast cancer have a long-term risk of distant recurrence, but the underlying reasons remain unclear. Therefore, studies with long-term follow-up are essential to understand endocrine therapy benefit. Here, we investigate tamoxifen therapy benefit in Luminal-type breast cancer as defined by the 80-gene and Genomic Risk signatures in the Stockholm tamoxifen (STO)-trials with 20-year follow-up.

Methods: Secondary analysis of the STO-trials including both pre- and postmenopausal patients of high and low clinical risk with a complete 20-year follow-up. The enrolled patients were randomly assigned to at least 2 years of adjuvant endocrine therapy or no endocrine therapy (control). In this study only patients assigned to 2 years of tamoxifen or no endocrine therapy were included. Patients with ER-positive and Luminal-type tumors (n=822), as assessed with the BluePrint® and Genomic Risk signatures, were included. Long-term (20 years) distant recurrence-free interval (DRFI) was assessed by Kaplan-Meier, Cox regression, and time-varying analyses.

Results: Luminal A-type patients had a significant long-term tamoxifen therapy benefit (Kaplan-Meier: Treated 74% vs. Control 61%, log-rank P=0.0047; Multivariable: DRFI HR=0.49; 95% CI [0.33-0.72]), whereas Ultralow (Kaplan-Meier: Treated 77% vs. Control 74%; log-rank P=0.5; DRFI HR=0.62; 95% CI [0.19-1.99]) and Luminal B-type patients had no significant benefit (Kaplan-Meier: Treated 54% vs. Control 50%; log-rank P=0.39; Multivariable: DRFI HR=0.86; 95% CI [0.56-1.32]). Time-varying analysis revealed a long-term treatment benefit for Luminal A-type patients up to 20-years (HR= 0.43, 95% CI [0.23-0.81]), but not for Luminal B-type patients.

Conclusions: Patients with Luminal A-type breast cancer had a long-term benefit from 2 years of adjuvant Tamoxifen up to 20 years beyond primary diagnosis. No significant benefit was seen for Ultralow or Luminal B-type patients. This highlights the importance of extended follow-up to understand treatment benefit and the importance of individualized management for ER-positive patients.

Thioredoxin-like protein-1 TXNL1/TRP32 links p62 and NRF2 pathways during oxidative stress

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Thioredoxin-like protein 1 (TXNL1), known as thioredoxin-related protein of 32 kDa (TRP32), is a member of the thioredoxin family. It is ubiquitously expressed in eukaryotes but has yet essentially unknown roles in relation to cellular redox homeostasis. Earlier studies have shown that TXNL1 has a strong correlation to development of many types of cancers and oxidative stress diseases. Here, we found that treatment with auranofin (AF), an FDA-approved thioredoxin reductase inhibitor, very rapidly (within hours) downregulates TXNL1 in a time- and dose-dependent manner, while AF had no such effects on thioredoxin 1 (Trx1, TXN1) protein levels. Pre-treatment of A549 cells with the proteasome inhibitor Bortezomib reversed the effect of AF on TRP32 levels, but a ubiquitin activating enzyme inhibitor (TAK-243) did not, suggesting that TXNL1 is degraded via the proteasome in a ubiquitin-independent manner. Furthermore, immunofluroescence-staining analysis showed that TRP32 is co localized with p62. Interestingly, CRISPR-CAS9 knockout of TXNL1 in 293T cells resulted in a significant decrease of p62 levels and its monomer compared to WT-cells under non-reducing condition, indicating increased p62 aggregation and/or sequestration in the absence of TXNL1. Moreover, TXNL1 knockout had impaired NRF2 activation in response to AF compared with WT cells. Over all, these results suggest that TXNL1 is involved in regulation of p62 and a major target in AF-triggered proteasomal degradation, possibly providing a functional link between NRF2 and the ubiqutin–proteasome system in responses to oxidative stress in human cancer cells.

Keywords: TXNL1/TRP32, P62

GAMMA DELTA T CELL CLONAL EXPANSION AND THEIR IMMUNOTHERAPY POTENTIAL IN HUMAN NEUROBLASTOMA

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Background

Gamma delta (γδ) T cells are considered the bridge between innate and adaptive immunity. The expression of γδ T cell receptors and NK cell activating receptors make that activation of γδ T cells is independent of MHC class I molecules. Neuroblastoma tumors exhibit particularly low MHC class I expression and therefore γδ T cells might be an attractive cell type to study and potentially to use for immunotherapeutic approaches in human neuroblastoma.

Aims

Here we aimed to elucidate gd T cell clonality, respective ligand recognition and possible therapeutic potential for gd T cells in human neuroblastoma.

Methods

We ran flow cytometry identifying gd T cell subtypes in 14 human neuroblastoma patient samples. In addition, we performed scRNA/VDJ-seq experiments to characterize clonal composition and functional states of ab and gd T cells infiltrating neuroblastoma for 11 patients from which 4 with matched blood.

Results

Flow cytometry analysis revealed that the gd T cell compartment in human neuroblastoma tumors consists of the same subtypes and varies in proportion between individual patients with a large proportion of the detected infiltrating cells being Vd1+ cells. scRNA/VDJ-seq experiments revealed prominent clonal expansion of both ab and gd T cells varying between patients. Clonal expansion was particularly prominent for gd T cells in one patient sample where nearly half of the cells represented one of the two top expanded clones with private Vd1 and Vd3 TCRs. Interestingly, we detected Vd1/ Vd3 expanded clones with both a clear cytotoxic signature and with a possible wound healing phenotype.

Conclusions

Drastic clonal expansion of gd T cells suggests that these cells may recognize antigens present in the tumor environment providing possibilities for immunotherapy.

Keywords: neuroblastoma, gamma delta T cells

Group 2 innate lymphoid cells in primary myelofibrosis

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Primary myelofibrosis (PMF) is one type of myeloproliferative neoplasm that leads to progressive and irreversible bone marrow fibrosis consisting of the excessive accumulation of fibrotic connective tissue, caused by chronic inflammation. Mouse models of BM fibrosis show a prominent role for type 2 responses, as IL-13 expression accelerates fibrotic onset. Group 2 innate lymphoid cells (ILC2) are innate lymphocytes present throughout the organism which are highly responsive to IL-33 and have the capability to produce IL-13. ILC2s are involved in pathological fibrotic responses in different tissues, through the expression of IL-13. Given the dynamic interplay of IL-33, IL-13, and fibrosis, we aim to understand the contribution of LC2s to the characteristic PMF medullary microenvironment alterations.

We have reanalyzed single-cell RNA sequencing data from a mouse model of bone marrow fibrosis at early and late disease stages. We identified ILC2s defined by high mRNA expression levels of Gata3, Il1rl1, Arg1 and Ccr9. In this model, BM ILC2s expanded at the onset of the fibrotic stage, suggesting an active role in disease progression. Furthermore, we wanted to investigate type II immunity in the context of PMF generating an alternative bone marrow fibrosis model, where we also observed ILC2s expansion compared to control mice. To decipher the role of ILC2s in the PMF intricate mechanism, we are modelling bone marrow fibrosis using ILC2-deficient mice. Finally, we will also analyze the abundance and activation of human ILC2s in peripheral blood and bone marrow aspirates from fibrotic patients and controls.

Altogether, our project aims to elucidate the contribution of ILC2s to the pathogenesis of bone marrow fibrosis.

A study on cell-free human papillomavirus (HPV) DNA in plasma for better follow-up and treatment of HPV positive head and neck cancer

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Background: Human papillomavirus (HPV) is a risk factor for oropharyngeal squamous cell carcinoma (OPSCC) and HPV16 is found in ~90% of HPV-positive (HPV^+) OPSCCs in Sweden. HPV^+ OPCC is rising epidemically in incidence and will continue to do so for decades until the effects of HPV vaccination will be of help for younger generations. Today's chemoradiotherapy (CRT) comes with severe acute and chronic side effects and though 80% are cured, CRT has not generally improved the survival rate of previously given radiotherapy alone. Clearly new treatments are needed and possibilities to de-escalate therapy would be of use for those who respond readily.

Specific aims: The study aims to measure cell free (cf) HPVDNA in plasma in patients with HPV^+ OPSCC before treatment and after treatment as a follow-up method. The hypothesis was that cfHPVDNA should exist in plasma in most patients at diagnosis and should disappear upon successful treatment. If it re-appeared this could indicate a recurrence, while if it did not disappear this could indicate that treatment was unsuccessful.

Materials and methods: Patients with HPV^+ OPSCC diagnosed and treated in Stockholm from 2024 have been asked to participate in the study. Blood samples have soon been taken at diagnosis from almost 25 patients. Samples are also taken at follow ups, which presently are at: three weeks after initiation of treatment; then every 3 months the first 2 years post treatment and then every 6 months 3-5 years after treatment. Plasma is separated from whole blood; frozen at –80 °C until cf DNA is extracted and the presence of HPV16 DNA measured by droplet digital PCR. As a positive control the household gene albumin is used in parallel.

Results: Of the initial samples taken at diagnosis 12/14 were cfHPV16DNA positive; one sample was not $HPV16^+$ and not analyzed further; and cfDNA could not be extracted from the remaining other sample. Of the samples taken three weeks after treatment 2/3 showed a considerable decrease in cfHPV16DNA compared to the diagnostic sample, while in the remaining sample cfDNA could not be extracted successfully.

Conclusions: At diagnosis, cfHPV16DNA was detected in 12/12 patients with HPV16 positive cancer, where cfDNA could be extracted. Moreover, there was a decrease in cfHPVDNA compared to the diagnostic sample in 2/2 patients with HPV16 cancer three weeks after treatment was initiated, however in the 3rd sample cfDNA could not be extracted. The data are promising and indicate that cfHPVDNA in plasma should be analyzed in additional samples. Hopefully more data will be available in the autumn of 2024 since the study is still ongoing.

Keywords: Cell free HPV DNA in plasma, oropharyngeal cancer

INTRACELLULAR DELIVERY OF MACROMOLECULAR THERAPEUTICS USING PHASE-SEPARATING PEPTIDES

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Effective drug delivery remains a crucial challenge in precision oncology, particularly concerning the efficient passage of therapeutic agents across cellular membranes to reach their intended targets. Cell-penetrating peptides (CPPs) have emerged as promising delivery vectors due to their ability to facilitate the transport of various cargoes into cells with low cytotoxicity (1). In recent years, the development of CPPs with pH- and redox-responsive properties has expanded the potential for intracellular delivery of macromolecular therapeutics, offering new avenues for targeted drug delivery in various diseases, including cancer (2, 3). Neuroblastoma, a common childhood malignancy, presents significant clinical challenges despite therapeutic advancements, highlighting the need for innovative treatment approaches. MYC overexpression plays a pivotal role in neuroblastoma pathogenesis, driving the disease's molecular complexity and influencing treatment decisions (4, 5). Here we propose an innovative strategy wherein phase-separating peptides, particularly those forming pH- and redox-responsive coacervate microdroplets, can efficiently deliver diverse macromolecular therapeutics such as Omomyc, a dominant-negative mutant blocking the MYC activation, into the cytosol of neuroblastoma and other cancer cell models. This approach bypasses endosomal entrapment and enables controlled release through redox activation. Leveraging recent advancements in peptide-based therapeutics, the study aims to investigate the potential of coacervates derived from phase-separating peptides as innovative carriers for intracellular delivery of MYC-targeting therapeutic compounds in various cancer cell models, especially neuroblastoma. Our results demonstrate the efficacy of coacervate microdroplets in delivering macromolecules into cells with low cytotoxicity and enhanced therapeutic effects, warranting further validation studies to elucidate mechanisms and optimize treatment strategies. This research represents a significant step towards advancing precision medicine approaches and improving therapeutic outcomes in neuroblastoma and other cancer types.

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Keywords: coacervates, Omomyc

Spatial profiling of the colitis- and sex-associated immune landscape of the mouse colon

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Inflammatory intestinal conditions are a major disease burden and spatial characterization of the colonic immune microenvironment is lacking. In this study, we use the novel COMET platform for multiplex immunofluorescence to spatially profile the colonic infiltration of nine immune cell populations at homeostasis and during colitis in mice of both sexes (N=16). Unsupervised clustering and spatial image analysis of tissues (SPIAT) analysis profiled the response to colitis and identified close interactions between immune cell populations, while manual quantification of infiltration in the whole colon and along the proximal-distal axis identified sex differences and regional differences. The distal colon was the most affected region during colitis, especially in males, who exhibited an increase in B cell infiltration during colitis. Sex differences in the homeostatic colon included higher T helper cell numbers in females. Our results provide a background for studies of inflammatory intestinal conditions.

Keywords: spatial proteomics, immune cells, colitis

Microglia activation is essential for peripheral macrophage recruitment in *IDH1* wildtype glioma.

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Tumor-associated brain resident microglia, as well as peripherally-recruited bone marrow-derived macrophage (BMDM), constitute a significant proportion of the cells from a glioma tumor mass. Interestingly, glioma carrying *IDH1/2* mutations (MT) (e.g., astrocytoma/oligodendroglioma) have a better prognosis than *IDH1* wildtype (WT) glioma (e.g., glioblastoma) and exhibit reduced BMDM infiltration. BMDM and microglia have distinct transcriptomes and are likely to exert different functions in the tumor microenvironment. However, TAMs have been mostly studied as a single population. Here, we study them as two separate entities, assessing their communication with one another, as well as their unique functions. We hypothesized that microglia acquire specific phenotype depending on the *IDH1* mutational status of glioma tumor, that could in turn play an essential role in the recruitment of peripheral macrophages. We found that there is a striking increase in the number of TAM macrophages in *IDH1* WT glioma, which correlates with a worsened prognosis. RNA sequencing of microglia exposed to *IDH1* WT*,* or *IDH1* MT tumor cell revealed minor differences in their gene expression profiles. To assess whether these changes in microglial phenotype could drive macrophages recruitment, we performed co-cultures between microglia and *IDH1* WT or *IDH1* MT glioma cells followed by macrophage migration assays. These results showed that microglial presence drives macrophage recruitment in *IDH1* WT glioma but not in *IDH1* MT glioma. We identified three candidates that may be responsible for macrophage recruitment*, Cavin1*, *S100a6* and *Mmp14*. Among these three genes, silenced *Mmp14* in microglia induced reduced macrophage migration. Blocking these essential macrophage recruitment factors may represent a novel treatment strategy for *IDH1* WT gliomas as macrophages presence is linked with a worsen prognosis.

Keywords: microglia, *IDH1* WT glioma.

Exploring the role of experimental anti-cancer therapies in managing rheumatoid arthritis

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Background: Rapidly proliferating cells, such as cancer cells and T cells, fuel their increased need for nucleotide supply by upregulating the folate-cycle enzymes MTHFD1 and MTHFD2, associated with the survival of pathogenic proliferative cells and the release of T cell immunogenic responses in T-cell mediated diseases. MTHFD1/2 inhibition with novel compounds has shown therapeutic potential in cancer by selectively inducing apoptosis in cancer cells.
Objective: We delve into the involvement of MTHFD1/2 in rheumatoid arthritis (RA), exploring its therapeutic potential through the utilization of MTHFD1/2 inhibitors, which have demonstrated efficacy in cancer treatment.
Methods: Bioinformatic analysis of publicly available transcriptomic data from immune cell populations of the blood and synovial fluid of treatment-naive RA patients and healthy controls to reveal the association of MTHFD1/2-related molecular signature with specific immune cell populations. Immunofluorescence microscopy of patient-derived T cells to investigate the differential expression of MTHFD1/2 before and post standard-of-care treatments in the clinic. *Ex vivo* studies with exposure of activated patient-derived T cells to novel MTHFD1/2 inhibitors (MTHFD1/2i) generated by the Helleday lab and investigation of immune responses to explore MTHFD1/2i as potential therapy in RA. *In vivo* studies with exposure of a RA murine model to MTHFD1/2i to assess the therapeutic potential of the novel targets.
Results: Transcriptomic analysis and immunofluorescence studies revealed that MTHFD1 and MTHFD2 are upregulated primarily in T cell populations of RA patients compared to healthy controls. Nonresponsive RA patients show dysregulated MTHFD1/2 expression. Exposure of patient-derived T cells to MTHFD1/2i constrains T cell viability, proliferation and activation. RA diseased mice treated with MTHFD1/2i exhibit increased levels of T regulatory cells and reduced levels of joint inflammation.
Conclusions: Targeting MTHFD1/2 shows promise for RA treatment. Future investigations will delve into their mechanisms of action in human tissues, with potential implications for clinical trials in RA patients.

Keywords: MTHFD1/2 Inhibitors, Autoimmunity

Understanding of the cell cycle phase differences and its associated oncogenic signaling between breast cancer subgroups

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Breast cancer tumors have traditionally been categorized into four widely recognized subtypes. However, recent single-cell advancements have shown that breast cancer tumors contain more than one subtype, highlighting their intrinsic heterogeneity. Concurrently, the crucial role of cell cycle regulation in cancer development is widely acknowledged. Yet, the specific impact of cell cycle phases on each of these subtypes remains unresolved.
In this study, our aim is to elucidate the disparities in oncogenic signaling across cell cycle phases within breast cancer subtypes, aiming to identify potential drug candidates and targets for these specific subtype-phase cases. We analyzed two publicly available breast cancer atlases, the discovery dataset with 100 064 cells and the validation dataset with 208 788 cells. After filtering based on cell type, cell malignancy, and availability of single-cell breast cancer subtype annotation we ended up with 24 489 and 96 128 cancerous epithelial cells, respectively. Differential expression analysis between cell cycle phase (G1, S, G2M) per breast cancer subtype was carried out using Limmatrend. Additionally, Gene Regulatory Networks (GRNs) were inferred using SCENIC, identifying regulons comprising transcription factors and their regulated genes. FGSEA and GEA were used to gain biological insights into the hallmarks of cancer involved. A comprehensive study of potential drug candidates was carried out by analyzing all The Comparative Toxicogenomics Database (CTD), focusing on curated drug-targets relevant to Homo sapiens and specific to breast cancer.
Initial DE analysis revealed that incorporating cell cycle phase distinctions significantly increased the number of identified hallmark pathways. Additionally, we observe a different profile of hallmarks of cancer involved in each phase within each subtype. Subtype-phase comparisons showed overall a higher pathway activity in Basal-like and Her2-enriched cells. Gene Regulatory Network (GRN) analysis further underscored the biological relevance of phase-specific stratification, identifying numerous pathways significant only when considering cell cycle phases. Notably, we identified potential drug candidates and targets specific to breast cancer subtypes and cell cycle phases, with significant pathways including p53, apoptosis, hypoxia and TNFα signaling via NFκB.
This comprehensive study emphasizes the critical role of cell cycle phases in understanding breast cancer biology and highlights critical pathways and potential drug candidates and targets.

Modelling neuroblastoma using germline ALK-R1275Q mutant patient-derived induced pluripotent stem cells

Name of Presenter: Mingzhi Liu

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Background
Amplification of Anaplastic Lymphoma Kinase (ALK) or activating mutations in the tyrosine kinase domain of the ALK gene are common somatic alterations in neuroblastoma (NB) and correlate with poor prognosis in intermediate and high-risk patients. Although hereditary NB is rare, germline gain-of-function mutations have been identified in ALK, mirroring common sporadic ALK mutations in NB. Our aim is to investigate the role of ALK mutations in the initiation of NB.

Method
We reprogrammed fibroblasts from NB patients with a germline ALK-R1275Q mutation and healthy individuals into induced pluripotent stem (iPS) cells. Using a robust differentiation protocol, we derived trunk neural crest cells (NCC) and sympathoadrenal (SA) cells from these iPS cells. Both bulk RNAseq and scRNAseq were performed all lines at multiple timepoints. Additionally, we induced MYCN overexpression in both control and patient-derived lines and injected these cells into the adrenal gland of mice.

Results and Conclusion
No differences in reprogramming capacity, expression of pluripotency markers, or ability to differentiate into migratory trunk NCC were observed, indicating the ALK-R1275Q mutation does not affect early embryonic development. Transcriptomic analysis revealed trunk NCC markers (SOX10, TFAP2A, NGFR, HOXC9) were expressed in the NCC stage and downregulated in SA cells, while SA markers (PHOX2B, ISL1, CHGA) were upregulated. ALK expression increased after SA commitment, rapidly decreasing in healthy cells but remaining high in patient cells. Gene set enrichment analysis significant downregulation of the p53 and neuronal differentiation pathways, alongside upregulation of DNA replication, protein translation, and Fanconi pathways in patient cells, suggesting a delay in differentiation and prolonged proliferative state. Single-cell data integration using control cells as a reference showed that patient cells mapped to the same stages as control cells in the NCC and early SAP stages, but in later differentiation stages, patient cells were more likely to map to the SAP stage of control cells, indicating they remain in more immature, undifferentiated stages. Orthotopic injections demonstrated that ALK mutation alone is insufficient for tumorigenesis, however it accelerated MYCN-driven tumor development. Overall, ALK maintains high expression in patient cells, leading to proliferation and an undifferentiated state, while affecting p53 pathway function. This interaction facilitates cell transformation when oncogenes like MYCN are active, highlighting the combined effect of ALK and MYCN in accelerating tumor formation and severity.

Keywords: Neuroblastoma, neural crest cell, sympathoadrenal lineage, stem cell, in vitro model

Epigenetic Modulation drives inflammatory responses in Sarcoma

Name of Presenter: Mireia Cruz De los Santos

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Compared with adult tumors, childhood malignancies, are usually driven by a relatively small number of mutations, frequently in genes encoding epigenetic regulators. This is the case for high grade osteosarcoma (OS), an aggressive bone malignancy characterized by massive genomic rearrangements and one of the most prevalent cancers in young adults, where the role of epigenetic reprogramming remains unclear. Considering the limited number of treatment options, particularly for metastatic OS, patient survival has not improved in the last 40 years. Therefore, more therapeutic options are needed for OS management, along with effective prognostic biomarkers for improved patient stratification in terms of treatment plan.
Since the role of epigenetic dysregulation in OS has been poorly studied and it could potentially identify novel treatment therapies, especially for patients with metastatic and therapy resistant disease, we aimed to study the potential of histone modifications as prognostic and therapeutic biomarkers in OS. For this purpose, an in silico predictive algorithm of histone modification landscape from RNA sequencing data was developed. Moreover, sarcoma ex vivo patient-derived spheroid models have been generated to study infiltration of autologous tumor infiltrating lymphocytes (TILs) by flow cytometry and confocal microscopy.
Our predictive algorithm allowed to cluster patients in an unbiased manner in three distinct groups. We identified that among all histone modifications, genome-wide levels of acetylation of the lysin 27 in histone 3 (H3K27ac) were inversely correlated with survival, suggesting a prognostic potential of this histone mark. Moreover, H3K27ac levels positively correlated with immune-related signatures indicating an inflamed tumor microenvironment. To confirm these results, we used sub-therapeutic doses of the histone deacetylase 1-3 (HDAC) inhibitor Entinostat to induce H3K27ac expression which resulted in higher infiltration of CD3+CD8+CD103+ tissue resident T cells in ex vivo patient-derived sarcoma spheroids. In addition, a higher frequency of tissue resident T cells was observed in Entinostat treated osteosarcoma bearing mice. Mechanistically, our preliminary results based on gene expression and ChIP analysis and ex vivo cell culture models highlight the involvement of the Hippo pathway mediators YAP1 and VGLL3 in Entinostat-mediated tumor infiltration of T cells.
Together, these findings show that increased H3K27ac levels by treatment with Entinostat resulted in enhanced infiltration into tumor spheroids. Furthermore, genome wide H3K27ac levels may be used as a biomarker for predicting survival in osteosarcoma. In addition, H3K27ac levels are associated with immune-related signatures and could be explored for novel treatment strategies.

Keywords: HDAC inhibitors, Immunotherapy

EZH2 inhibition sensitizes retinoic acid-driven senescence in synovial sarcoma

Name of Presenter: Mohammad Alzrigat

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Abstract

Synovial sarcoma (SS) is driven by a unique chromosomal translocation t(18;X) leading to expression of the SS18-SSX fusion oncoprotein, a transcriptional regulator with both activating and repressing functions. How SS18-SSX contributes to the development of SS is not completely understood. Herein, we show that SS18-SSX drives the expression of PRAME (Preferentially Expressed Antigen in Melanoma), a protein highly expressed in SS but with a poorly known function. The SS18-SSX fusion protein directly targets the *PRAME* promoter and expression of SS18-SSX and PRAME are positively correlated. We provide evidence that PRAME alters retinoic acid (RA) signaling in SS, forming a complex with RA-receptor α (RARα) and Enhancer of Zeste Homolog 2 (EZH2). As there are no pharmacological inhibitors against PRAME, we used GSK343 for inhibition of EZH2 in combination with all-*trans* retinoic acid (ATRA) to reconstitute RA signaling. GSK343 had a pronounced effect in reducing cell proliferation and triggering senescence in SS cell lines. In addition, knockdown of PRAME suppressed the response to ATRA, revealing the specificity of our approach for SS. Our data connect SS18-SSX with RA signaling and the EZH2 complex, providing insights into how this fusion oncoprotein alters normal cellular homeostasis and showing the therapeutic potential of disrupting the RARα-PRAME-EZH2 ternary complex for SS treatment.

Keywords: Synovial sarcoma, EZH2

Progesterone Receptor Modulator: Novel Avenues in Breast Cancer Prevention

Name of Presenter: Mohammed Rasul

Presenter: Mohammed Rasul (Department of Women's and Children's Health, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden).
Co-authors: Angelique Flöter-Rådestad, Alexander Zulliger, Johan Hartman, Martin Widschwendter, Twana Alkasalias and, Kristina Gemzell Danielsson

Abstract:
Women with BRCA1 or BRCA2 gene mutation have an increased risk of developing breast and ovarian cancers. Apart from the direct effect on DNA repair mechanisms, BRCA mutations via non-cell autonomous factors, including progesterone, drive cancer initiation. Our multidisciplinary combined clinical and basic research project aims at developing cancer-preventative strategies via evaluating the potential of using progesterone receptor modulators (PRM) like mifepristone. Two groups of premenopausal women are recruited for this study; The first comprises women undergoing surgery for benign breast reduction mammoplasty. The second consists of women carrying BRCA1 or BRCA2 mutations who are undergoing risk-reducing mastectomy. To investigate and validate our hypothesis, we've developed an advanced high-throughput 3D-organoid culture model using freshly isolated breast tissues. Our findings reveal that PRM effectively reduces the proliferation and growth of cancer precursor cells, encompassing luminal progenitor and basal cells, among both individuals with BRCA mutations and those without. Concurrently, it encourages the differentiation and enrichment of mature luminal cells. Intriguingly, the impact of PRM diminishes as breast cells replicate and age over time. Moreover, we've observed that PRM induces apoptosis in breast cells in a dose-dependent manner. These insights underscore the substantial role of PRM in mitigating the risk of cancer initiation and progression, demonstrating its significance for both normal and BRCA mutation carrier women.

Keywords: Breast Cancer Prevention and Progesterone Receptor Modulator

Enhancing targeted therapy by combining PI3K and AKT inhibitors with or without cisplatin or vincristine in a medulloblastoma cell culture model

Name of Presenter: Monika Lukoseviciute

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Aim
Medulloblastoma (MB) is in spite of current intensive therapy still not cured so new therapies are needed. Recently, we showed that combining phosphoinositide 3-kinase (PI3K), fibroblast growth factor receptor and cyclin-dependent-kinase-4/6 inhibitors (BYL719, JNJ-42756493 and PD-0332991 respectively) or poly (ADP-ribose) (PAR) polymerase (PARP) and WEE-1 inhibitors (BMN673 and MK1775 respectively) had synergistic effects on MB. In continuation, here we administered combinations of PI3K and AKT inhibitors with/without cisplatin or vincristine on monolayer or suspension cell cultures from different MB subgroups as well as in one MB spheroid culture and investigated the effects of the single and combined administrations.

Material and methods
MB cell lines DAOY, UW228-3, D283, Med8a and D425 grown as monolayers or suspension were treated with single and combined administrations of BYL719, AZD5363, cisplatin or vincristine. The effects were assessed using viability, proliferation, cytotoxicity and cell migration assays. Additionally, DAOY was tested as a spheroid culture model.

Key findings
While single BYL719, AZD5363, cisplatin or vincristine administrations inhibited viability in a dose dependent manner, combinations of AZD5363/BYL719, AZD5363/cisplatin or AZD5363/vincristine resulted in synergistic effects. In addition, combining drug therapy suppressed MB cell capability to migrate. Upon applying these drugs and their combinations to DAOY grown as 3D spheroid cell cultures the responses were mainly analogous.

Significance
This study provides pre-clinical evidence that single PI3K and AKT inhibitors, as well as cytostatics cisplatin and vincristine exhibit promising anti-MB activity. Moreover, the use of combining drugs allows lower doses potentially reducing side effects of the treatment. That was also confirmed in 3D spheroid model of DAOY cell line, further supporting their future potential clinical use.

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NEAR-INSTANT DETECTION OF GLIOMA STEM-LIKE CELLS IN LIVE HUMAN GLIOBLASTOMA TISSUE
Name of Presenter: Ola Hermanson

Ola Hermanson, Department of Neuroscience, Karolinska Institutet
Co-authors: Christina Neofytou, Oscar Persson, Lily Keane, Margret Jensdottir, Lisa Arvidsson, Bertrand Joseph, Shirin Ilkhanizadeh

Abstract:
Glioblastoma is classified as a grade IV glioma, a devastating brain tumor with poor outcome. Emerging research suggests that failure to target glioma stem-like and progenitor cells (GSCs) could explain the poor survival of glioblastoma patients. Glioblastoma tumors often show an infiltrative growth pattern with protrusions into the surrounding brain tissue. In the apex of the tumor, tumor cells co-exist with normal cells, immune cells, GSCs, etc., which makes GSC detection and removal particularly difficult.

Here we demonstrate the use of an oligothiophene named p-HTMI or GlioStem (GS), for selective real-time detection of GSCs ex vivo in live human glioblastoma-tissue parallel to surgery. More than 110 brain tumors including >60 glioblastoma patient biopsy samples were stained and analyzed for the presence of GS+ cells. A subset (n=21) of glioblastoma patient samples was stained with a panel of cancer stem and progenitor cell markers and GS-positive cell populations sorted. Bulk RNA-Seq for 7 glioblastoma patient samples with two paired populations of sorted cells, GS+ and GS-, revealed that the transcriptomic signatures of GS+ samples from different patients clustered together, whereas the GS- populations did not cluster close to one another or the GS+ populations pointing to a certain level of homogeneity regarding the GS+ populations, independent of intra-patient or patient-to-patient heterogeneity. Notably GS+ samples exhibited significantly higher expression of >35 genes associated with stem and progenitor cells compared to the GS- samples, including markers being associated with the pre-oligodendrocyte precursor cell (pre-OPC) and pro-neural subtypes but also cancer cells.

Single cell annotation proved that GlioStem detected GSCs of different identities (e.g., pro-neural-, OPC-, radial glia-like cells) at different stages of maturity, again with a mix of cancer cell-signatures. In line with the clinical trial CeNo2 (https://www.clinicaltrials.gov/study/NCT05556486; K2022-4039) – lead by the neurosurgeon Dr. Oscar Persson at K – we are now analyzing whether GS can assist in identifying GSCs outside the core tumor body. Pilot data from a double-blind study of small tissue samples taken just outside of the core tumor revealed that GS indeed specifically detects GSCs also outside of the core tumor body with a significant correlation depending on distance from the core tumor body based on the proportion of normal vs tumor tissue. We propose that GlioStem is a novel pan-GSC-marker in fresh glioblastoma tumor tissue with potential for immediate use in clinical settings.

Keywords: Cancer cell progenitor, fluorescence-guided surgery

Spatially dependent heterogeneity in pancreatic cancer

Name of Presenter: Sara Söderqvist

Sara Söderqvist (Department of Clinical Science, Intervention and Technology –CLINTEC), Annika Viljamaa, Natalie Geyer, Carina Strell, Neda Hekmati, Jennie Engstrand, Ernesto Sparrelid, Caroline Salmén, Rainer L. Heuchel, Kseniya Ruksha, Argyro Zacharouli, Poya Ghorbani, Sara Harrizi, Yousra Hamidi, Olga Khorosjutina, Stefina Milanova, Bernhard Schmierer, Béla Bozóky, Carlos Fernández Moro, Marco Gerling

Pancreatic ductal adenocarcinoma (PDAC), the most common form of panreatic cancer, is one of the most lethal cancer forms. The 5-year overall survival of PDAC is curently at 11%. Yet, we lack deep knowledge of how PDAC invades then local tissue, and how it interacts with the tumor microenvironment, which often is characterized by a fibrotic stroma and a state of chronic inflammation. Recently, two main subtypes of PDAC were unraveled by bulk – and single cell RNA sequencing. Tumors of the basal-like subtype more frequently has an allelic imbalance of mutated and wild-type KRAS, undergo epithelial-to mesenchymal transition and come with the shortest survival. In comparison, the classical subtype usually come with expression of pancreas lineage markers, such as GATA6, and come with a better prognosis. However, classical and basal-like tumor cells can co-exist in one individual tumor at varying proportions.
In the current study, we spatially mapped the individual tumor cells, and quantified their classical – and basal-like related protein expression in a digitalized immunohistochemistry based QuPath pipeline. Regions of interest, containing only tumor cells, were stratified to tumor in pancreatic lobule, or tumor in desmoplastic stroma. We found that PDAC expression, or subtype state, seem to depend local microenvironment properties at the invasion front. Notably, basal-like expression (positive for Keratin 17) was upregulated at stromal invasion, while the classical expressing tumor cells (positive for Mucin 5AC) were seen at the parenchymal, lobular invasion front. Both expression patterns were often identified within the same tumor. Hence, we can for the first time elucidate what drives the classical - basal-like expression state dichotomy in PDAC and bring the previously largely unrecognized acinar invasion into the light.

Keywords: Pancreatic cancer, digital pathology

Long-term tamoxifen benefit in pre- and postmenopausal patients of high and low risk with luminal A and B breast cancer
Secondary analysis: STO-2, 3 and 5 randomized control trials

Name of Presenter: Oscar Danielsson

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Background
Breast cancer is a heterogeneous disease with some patients developing distant recurrence early and others decades later, or never. Patients with ER-positive tumors have a considerable long-term risk and can be subdivided into two main groups: luminal A and luminal B subtype. Although tamoxifen therapy is an essential treatment, the long-term benefit for patients with luminal A and B subtype is not well understood.

Methods
Secondary analysis of 952 ER-positive/HER2-negative patients with luminal A or B molecular subtype from the Stockholm Tamoxifen 2, 3 and 5 trials randomized to receive tamoxifen therapy (tamoxifen alone or with goserelin) or control. Agilent microarray gene expression data was used to classify tumors into PAM50 molecular subtypes. The primary endpoint, distant recurrence free interval (DRFI), was assessed using Kaplan Meier analysis, and multivariable Cox proportional hazards regression.

Results
Multivariable analyses showed a significant benefit from tamoxifen in both luminal A (HR=0.58, 95% CI 0.43-0.79) and luminal B patients (HR=0.67, 95% CI 0.46-0.99). Stratifying by tumor and patient characteristics revealed that most favorable prognostic markers were associated with benefit from tamoxifen in both subtypes. However, for premenopausal women a significant benefit from tamoxifen was seen for luminal B patients (HR=0.46, 95% CI 0.22-0.95) but not for luminal A (HR=0.66, 95% CI 0.34-1.29). For postmenopausal patients the reverse was noted (luminal A: HR=0.53, 95% CI 0.37-0.75, luminal B: HR=0.77, 95% CI 0.49-1.21).
When comparing the subtypes, patients with luminal B subtype had increased risk compared to luminal A patients for PR positive (HR=1.61, 95% CI 1.16-2.23), lymph node-negative (HR=1.99, 95% CI, 1.23-3.24) and low genomic risk (HR=1.67, 95% CI, 1.15-2.43) tumors.

Conclusion
Our findings suggest a long-term benefit from tamoxifen for most patients with less aggressive tumor characteristics irrespective of molecular subtype, but the benefit differed by menopausal status. Luminal B patients with PR-positive, lymph node-negative or low genomic risk tumors had a higher risk as compared to patients with luminal A tumors.

Combined targeted therapy with PI3K and CDK4/6, or FGFR inhibitors show synergistic effects in a Neuroblastoma spheroid culture model

Name of Presenter: Ourania N Kostopoulou

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Aim
Neuroblastoma (NB) is, in spite of current intensive therapy with severe side effects, still not cured so new therapies are needed. Recently, we showed that combining phosphoinositide 3-kinase (PI3K), fibroblast growth factor receptor (FGFR) and cyclin-dependent-kinase-4/6 (CDK4/6) inhibitors (BYL719, JNJ-42756493 and PD-0332991 respectively) *in vitro* in NB cell lines grown as monolayers (2D) had synergistic effects. However, there were variations depending on the combinations used and the targeted NB cell lines. To obtain further information and to mimic more natural circumstances, we investigated the effects of single and combined administrations of the above inhibitors in spheroid (3D) NB-cultures.

Material and methods
Spheroid cultures of NB cell lines SK-N-AS, SK-N-BE(2)-C, SK-N-FI and SK-N-SH were established and treated with single and combined administrations of BYL719, JNJ-42756493, and PD-0332991 and then followed for growth, viability, proliferation, cytotoxicity and cell migration.

Key findings
Single inhibitor administrations gave dose dependent responses with regard to growth and viability and their combinations were efficient and resulted in a range of additive and synergistic effects. The responses to the specific drugs alone and their various combinations were mainly analogous independent of if the cells were grown as 2D or 3D NB-cultures, however in general slightly higher drug concentrations were needed in the latter.

Significance
This study provides pre-clinical evidence that single PI3K, FGFR, and CDK4/6, inhibitors exhibit promising anti-NB activity and when combined lower doses of the drugs could be used also in 3D NB-cultures, further supporting their future potential clinical use.

Keywords: Neuroblastoma, targeted therapy

The tumour microenvironment influences long-term tamoxifen treatment response in breast cancer patients

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Background: The tumour microenvironment (TME) plays a crucial role in the response to treatment of breast cancer patients. Our study investigated whether the immune and stromal cell composition of a tumour can be prognostic for long-term survival.
Methods: The Stockholm Tamoxifen (STO-3) trial, conducted from 1976 to 1990, randomized postmenopausal patients with lymph node–negative breast cancer to receive adjuvant tamoxifen or no endocrine therapy. This study is a subset of 538 patients from STO-3 for which array-based gene expression and more than 20 years of complete patient follow-up data were analysed. TME levels of 18 cell types were assessed by applying the ConsensusTME deconvolution algorithm to the data and dividing the expression levels of each cell type into tertiles. Tertile influence on breast cancer-specific survival (BCSS, cause-specific death from breast cancer) was assessed using univariable Kaplan-Meier and multivariable Cox proportional hazard modelling, adjusting the latter for standard clinical patient and tumour characteristics.
Results: Only fibroblasts, endothelial and mast cells showed statistically significant BCSS differences in univariable analysis of all patients ($p < 0.05$, log-rank). In multivariable analysis comparing clinical trial arms, low levels of B cells, dendritic cells, NK cells, T-cells (CD4+, CD8+, gamma delta), macrophages M1/M2 and eosinophils were significantly associated with improved BCSS in the tamoxifen-treated patient arm (HRs range from 0.11-0.38 CI 95% [0.04-0.76]). Similarly, low and intermediate levels of cytotoxic cells, macrophages, neutrophils, T reg cells, fibroblasts, monocytes (HRs from 0.24-0.45 CI 95% [0.1-0.99]), or intermediate levels for endothelial cells (HR = 0.18 CI 95% [0.07-0.45]), and low or high levels of mast and plasma cells were associated with improved BCSS in the tamoxifen-treated arm (HRs from 0.20-0.49 CI 95% [0.08-0.92]).
Conclusions: In general, lower levels of TME cells were associated with improved survival in tamoxifen-treated postmenopausal breast cancer patients relative to those who were untreated, suggesting better long-term survival for lower initial TME levels in the tamoxifen-treated patients.

Keywords: breast cancer, tumour microenvironment

Pharmacological activation of p53 in endothelial cells impairs sprouting angiogenesis

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Therapies that inhibit angiogenic signaling, such as those that target vascular endothelial growth factor (VEGF), reduce tumor growth. However, since acquired resistance to these therapies can limit the duration of their effect, alternative therapies that inhibit angiogenesis through different mechanisms are needed to counter this resistance. Reduced angiogenesis has been observed in tumors treated with pharmacological activators of p53, such as those that inhibit the p53-MDM2/MDMX interaction. Yet, the molecular, structural, and functional consequences of activating p53 directly in vessels remain unclear. Here, we show that small molecule and stapled peptide inhibitors of MDM2/MDMX reduce proliferation of endothelial cells *in vitro* and *in vivo*. In our study, the most potent inhibitor *in vitro* was the small molecule navtemadlin, which induced cell cycle arrest at nanomolar concentrations and apoptosis at micromolar concentrations. On a molecular level, these effects were associated with global changes in proteins involved in DNA replication, cell cycle, and stress response. On structural and functional levels, pharmacological activation of p53 in endothelial cells reduced sprouting angiogenesis and vessel connectivity *in vitro* and *in vivo*. However, endothelial cell migration was not affected, suggesting that treatment likely impacts the specific phenotypes of endothelial cells during angiogenesis. Together, our results suggest that pharmacological activation of p53 using MDM2/MDMX inhibitors reduce sprouting angiogenesis primarily by inhibiting endothelial cell proliferation.

Keywords: p53 activation, angiogenesis

Mapping Neuroblastoma Development, Heterogeneity, and Metastasis with an Advanced Mouse Model

Name of Presenter: Peng Cui

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Neuroblastoma (NB) originates from neural crest-derived tissues, predominantly found in the adrenal glands. Nonetheless, neuroblastoma can also manifest in alternate locations, such as the abdominal region. Currently, there is a paucity of sufficient studies exploring the dynamic properties and phenotypic plasticity of neuroblastoma occurring in the adrenal and abdominal regions. Our aim is to elucidate the origins and heterogeneity of neuroblastoma in both the adrenal gland and abdominal region. To accomplish this, we intend to employ an innovative approach combining a novel GEM mouse model, single-cell and spatial transcriptomics and 3D cell culture as part of our analytical and experimental framework.
The gain-of-function point mutations of ALK (anaplastic lymphoma kinase receptor) gene has been found in around 14% of high-risk neuroblastoma. F1174 as a hotspot (30%–35%) of ALK mutations is believed to contribute to a more aggressive phenotype, increased embryonic lethality and ALK inhibitor resistance. Additionally, our previous studies have revealed NF1 (encoding neurofibromin 1) as a robust tumor suppressor, and NF1 loss is enriched in high-risk neuroblastoma tumors. By combining and Alk germline mutation (F1178S ≡ human F1178S) with a dopamine-beta-hydroxylas (Dbh)-driven Nf1 knockout, we have successfully developed an inventive mouse tumor model. This novel model exhibits the ability to generate multiple tumor types in different locations. This comprehensive model provides a valuable and unique tool for investigating the intricate interplay between these tumor types and the transition potential among different cell types.
In conjunction with H&E staining, immunofluorescence staining, and in situ hybridization (RNAscope) of mouse adrenal and abdominal tumors and healthy postnatal adrenal glands and Zuckerkandl organ (extra-adrenal chromaffin cells), we found a white mass, invading the adrenal glands with SOX10 and PHOX2B positive cells, might originate from the organ of Zuckerkandl and mesenteric ganglion. Furthermore, as the mice aged, we observed a potential transition of the white mass to neuroblastoma, which occurs in a similar anatomical location. We hypothesize the presence of a potential lineage relationship and dynamic progression from the Zuckerkandl organ/mesenteric ganglion-derived white mass to neuroblastoma during the mouse embryonic and postnatal states. Simultaneously, we are conducting single-cell and spatial transcriptomic sequencing and 3D cell culture to gain a deeper understanding of the molecular profiles and heterogeneity within the organs and tumors. By implementing these complementary approaches, we anticipate obtaining significant insights into the intricate cellular dynamics, genetic alterations, and potential therapeutic targets related to the progression and development of the adrenal and abdominal neuroblastoma.

Keywords: adrenal and abdominal neuroblastoma, tumor cells transition

Predicting brain metastasis in non-small cell lung cancer by extracellular vesicle protein profiling

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Background: Metastatic spread to the brain is major clinical dilemma for about one third of patients with non-small cell lung cancer (NSCLC) and the treatment options for these patients are limited. Thus, it would be of great benefit to, already at the time of surgery, be able to predict which patients are at risk of developing brain metastasis. This would enable more frequent monitoring and thereby detection of brain metastasis at an earlier stage.
Analysis of extracellular vesicles (EVs) for non-invasive assessment of biomarkers is of high interest for several diseases. It might be especially useful for different solid cancers where tissue biopsies are difficult to obtain or where the tumor lesions are highly heterogenous. As EVs contain proteins, RNA and miRNA from their cell of origin, they may be used as a tumor proxy biomarker source. In this work groups at KI, Sheba Medical Center and Tel Aviv University have a joint project aiming at identifying biomarkers that may predict the risk of brain metastasis development using EVs in plasma.

Material and Methods: A patient cohort of early-stage (IA-IIIA) NSCLC patients (n=40) who underwent surgery and later did or did not develop brain metastasis (n=20/group) was used. EDTA-plasma samples were collected prior to surgery and EVs were isolated from 0.3-1ml plasma using Izon's qEV1 70nm columns. Size and concentration of the EVs were analysed using Nanoparticle Tracking Analysis (NTA) and the total EVs proteome studied by mass spectrometry (MS) analysis. Qlucore® Omics Explorer was used for data analysis in relation to clinical parameters. Western blotting was used to characterize EV markers and for validation of putative biomarkers.

Results: The samples contained EVs with median sizes between 100 and 200 nm. The MS analysis identified over a thousand proteins and after removing abundant plasma proteins and adjusting for proteins expressed in at least half of the patient samples the proteins taken for further analysis was around 50% of those initially identified. Both cohorts expressed over 60 of the top-100 proteins reported to be associated to EVs in Vesiclepedia. Preliminary analyses of the MS-data identified 42 proteins that correlated to brain metastasis development with p-value of 0.05, and seven proteins with p=0.001. These are further explored in context of tumor signaling.

Conclusion: EVs can be used to identify protein signatures that differ between early-stage NSCLC patients that will or will not develop brain metastasis. If these markers are robust enough to be used as biomarkers need further analysis which must also involve a larger patient cohort.

Keywords: Extracellular vesicles, brain metastasis prediction

Computational Analysis for Investigating Intra-tumoral Heterogeneity in Breast Cancer

Name of Presenter: Qiao Yang, Ph.D. student

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Intra-tumoral heterogeneity occurs at various levels, such as genomic and transcriptomic, contributing to the high recurrence and metastasis rates in breast cancer (BC). To examine how this variability impacts treatment and prognosis in BC, we analyzed 32 topographic tumor regions from freshly resected large breast tumors and two regions from lymph node metastases of seven patients. Tissues from these regions underwent homogenization, followed by drug screening and flow cytometry analysis. Additionally, the homogenized tissue samples were subjected to high-throughput sequencing, including whole genome sequencing, whole genome bisulfite sequencing, and bulk RNA sequencing. Genomic data were used to estimate somatic mutations. Dimensionality reduction techniques clustered samples by patient and tissue origin based on gene expression data. Preliminary results indicated varying levels of intra-tumoral heterogeneity in terms of somatic mutations, immune cell distribution, and biological signaling pathway enrichment across patients, BC subtypes, and regions. Moreover, regions displayed differing responses to drugs. By integrating results from ex vivo experiments and bioinformatic analysis, we aim to move beyond single-level analysis and interpret the multi-omics data using machine learning. This study seeks to better understand the intra-tumoral heterogeneity of BC and provide advanced insights into the mechanisms underlying tumor progression, drug resistance, and metastatic spread in BC.

Keywords: breast cancer, intra-tumoral heterogeneity

The anticancer compounds auranofin and TRi-1 have distinct cytotoxicity profiles with regards to thioredoxin reductase inhibition

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Cancer cells' reliance on the selenoprotein thioredoxin reductase 1 (TrxR1, TXNRD1)
for sustained proliferation has led to the development, aided by high throughput screening, of TrxR1 inhibitors such as auranofin and TRi-1, with pronounced anticancer effects and varying target specificity (1). TRi-1 and auranofin additionally create prooxidant forms of otherwise inhibited TrxR1 known as SecTRAPs, potentiating their effects. The cellular signaling
programs triggered by these inhibitors, however, are still poorly understood despite recent proteomics investigations (2). We here report observations regarding the kinetics of intracellular TrxR1 inhibition relative to the compounds' cytotoxicity as well as their broad effects on cell cycle regulation and proteostasis.
Whereas cell death following auranofin treatment can be rather swift, TRi-1 treatment produces distinctly delayed cytotoxicity being significant not until a few days after compound
exposure. Nevertheless, TRi-1 produces the fastest and most pronounced inhibition of TrxR1
activity which returns to pretreatment levels well before the onset of cell death. Both compounds deplete TrxR1 activity but not protein abundance, creating a potential window for
additional cell damage via formation of SecTRAPs.
The molecular mechanisms governing the inhibitors' cellular uptake are not known, but we have found dose-dependent delayed cytotoxicity seen even with a short pulse of 10 min treatment and subsequent compound removal. This suggests rapid uptake before cytotoxicity can be detected. In contrast to auranofin's well documented activity as a proteasomal inhibitor, TRi-1 displays no overt effects on proteostasis, which further highlights the importance of distinct uptake and signaling pathways for each of these inhibitors' activity.
Outlining the differences in the specificity, speed, and uptake of different TrxR1 inhibitors is crucial for the full understanding of the effects of antioxidant system inhibition and furthering its therapeutic potential.

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2. Sabatier P, Beusch CM, Gencheva R, Cheng Q, Zubarev R, Arnér ESJ. Redox Biol. 2021.
doi.org/gnprb5.

Keywords: Thioredoxin reductase, Anticancer inhibitors

Identifying targetable features of senescence escape following cancer therapy
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Abstract
Globally, there are 10 million annual cancer deaths. A greater understanding of therapy resistance and relapse is crucial. Senescence, an anti-tumour mechanism also associated with development and wound repair, has recently been causally linked to therapy resistance and cancer relapse. This project employs a novel transgenic model with dual purpose. First, p21:CreERT2 and lsl-tdTomato can lineage trace p21 positive cells, a marker of senescence. Second, clonal dynamics can be tracked *in vivo* with TREX:eGFP, a lentiviral barcode library. Following Doxorubicin-induced senescence, p21:CreERT2; lsl-tdTomato; TREX:eGFP cancer cells are transplanted *in vivo,* and analysed for senescence escape over time. Smart-seq3 scRNAseq will identify and distinguish stably senescent cells (with a unique cloneID), and relapse-causing cancer clones (with common cloneIDs). We hope that the advanced assessment of these clonal varients will delineate features of therapy resistant cells that drive cancer relapse, informing the development of targeted treatments and refining cancer therapy strategies.

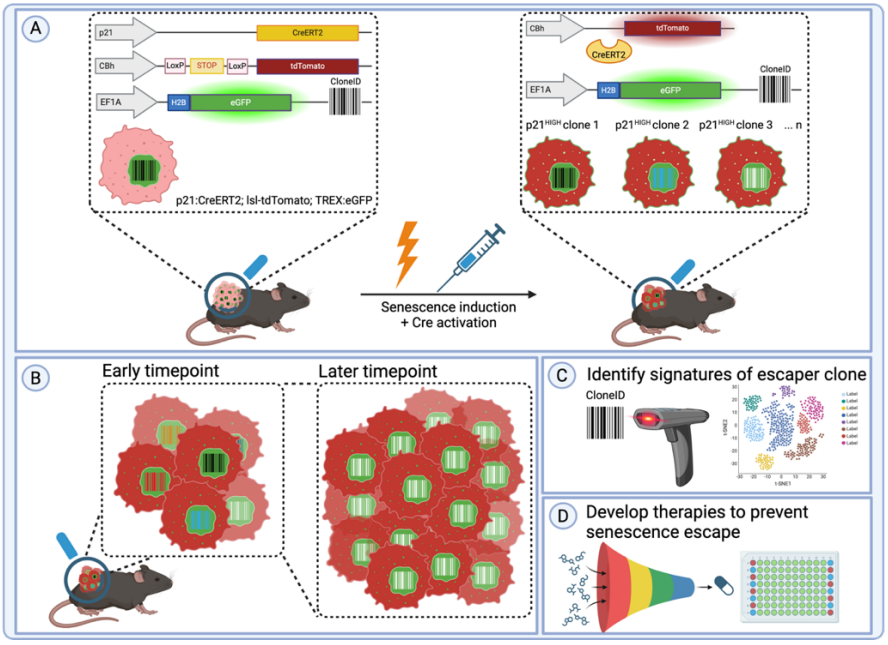

Figure: depiction of senescent cancer cell escape model and downstream applications.

Keywords: Senescence, Relapse.

TARGETING TENEURIN 4 SUPPRESSES TUMOR GROWTH AND INDUCES DIFFERENTIATION IN NEUROBLASTOMA

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High-risk neuroblastoma (NB) presents significant clinical challenges, and further therapeutic options are needed. NB originates from neural crest cells, which results in impaired neuronal differentiation. Teneurins (TENM1-4) are cell adhesion molecules highly expressed during embryonal development and function in differentiation. We and others have identified somatic mutations and structural aberrations of TENM genes in NB. We aim to elucidate the role of TENM4 in NB growth, tumorigenicity, and differentiation. TENM4 immunohistostaining was performed in NB tumors. Genetic inhibition was mediated by siRNA, CRISPR-Cas9, and inducible CRISPR-Cas13d in NB cells to analyze the effects on morphology, proliferation, tumorigenicity, and molecular signaling through transcriptomics and quantification of gene expression. Elevated TENM4 protein and mRNA levels were observed in high-risk and MYCN-amplified NB tumors, correlating with poor patient outcome. siRNA-mediated knockdown of TENM4 significantly decreased proliferation in all investigated NB cell lines. Transcriptomics analyses in TENM4-inhibited NB cells identified key cell components such as induced differentiation, inhibited cell cycle progression, epithelial to mesenchymal transformation, and mTOR signaling as TENM4 targets. We also observed a trend toward more adrenergic and less mesenchymal phenotype in the TENM4-inhibited cells. CRISPR-Cas9 gene-edited SK-N-BE(2) TENM4-/- clones demonstrated neuronal differentiation-like morphology with impaired clonogenic capacity and reduced proliferation compared to wildtype cells. TENM4-/- cells did not lead to tumor formation when grafted into nude mice as opposed to wild-type SK-N-BE(2) cells that formed tumors. We generated a CRISPR-Cas13dmediated TENM4 knockdown in SK-N-BE(2), which resulted in a downregulation of TENM4 expression on mRNA and protein levels and demonstrated decreased tumorigenicity in mice bearing subcutaneous tumors compared to their controls. Our findings show that TENM4 is expressed in a subpopulation of NBs with MYCN-amplification and plays an essential role in NB growth and differentiation, suggesting it as a potential therapeutic target.

Keywords: Neuroblastoma, Teneurin

Novel therapeutic vulnerabilities in del(5q) myeloid malignancies

Name of presenter: Sergio Martinez-Høyer

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Background:
Deletion of the long arm of chromosome 5 (del(5q)), is one of the most common chromosomal alterations in myelodysplastic syndromes (MDS) and secondary Acute Myeloid Leukemias (AML)[1]. A common deleted region (CDR) has been defined to be universally deleted in all del(5q) MDS/AML patients. Several studies have shown the contribution of CDR genes, alone or in cooperation, to the pathogenesis of the disease. The role of other passenger genes within the CDR has not been yet defined. Lenalidomide is the treatment of choice in del(5q) MDS. Mechanistically, this drug triggers specific CK1a protein degradation, whose gene *CSNK1A1* is contained witin the CDR, leading to apoptosis induction and elimination of del(5q) cells[2]. Despite its initial success, high rates of relapse after treatment frequently associated to the expansion of *TP53* mutant del(5q) clones, may complicate management of patients after drug exposure[3]. Thus, novel treatments are needed to eliminate the del(5q) clone and prevent relapse.
Aims:
We sought to identify novel molecular vulnerabilities in del(5q) myeloid malignancies. We focused our approach on passenger alterations within the commonly deleted region (CDR), universally deleted in all del(5q) MDS/AML. We hypothesize that, similarly to Lenalidomide and *CSNK1A1* haploinsufficiency, one or several genes within the CDR could provide alternative vulnerabilities in del(5q) hematopoietic stem and progenitor cells (HSPCs).
Methods:Data analysis of the Depmap portal database. CRISPR/Cas9 editing of del(5q) cell lines and primary umbilical cord blood CD34+ hematopoietic stem and progenitor cells (HSPCs). The effect of genetic editing was assesed by flow cytometry (differentiation, cell cycle, apoptosis) colony forming assays and RNA-seq analyses.
Results:
We first analyzed the Depmap portal to find essential genes in blood cell lines. The RNA binding protein *RBM22* resulted as the most essential gene in functional genetic screenings in a panel of hematopoietic cell lines. *RBM22* is highly expressed in the hematopoietic system and is one of the few genes within the 5q CDR whose gene expression is consistently downregulated in del(5q) MDS CD34+ cells compared to healthy controls[4]. To validate this data in del(5q) cells, we targeted *RBM22* using CRISPR/Cas9 in del(5q) cell lines. *RBM22* KO del(5q) cells showed reduced clonogenic potential, cell cycle alterations and increased cell death. Elimination of del(5q) cells happened irrespective of *TP53* status. Of note, targeting *RBM22* in healthy CD34+ cells did not alter HSPC function. Transcriptome analysis of edited del(5q) cells unveiled dysregulated pathways in *RBM22* KO cells, such as mitochondrial metabolism. Finally, we identify *FLI1* alternative splicing to be altered following loss of *RBM22*. *FLI1* is overexpressed in del(5q) HSPCs and is involved in malignant growth advantage[5].
Summary/Conclusion:
Our results postulate *RBM22* as a promising target with a wide therapeutic window in del(5q) myeloid neoplasms. Future work will look to develop agents to mimic *RBM22* KO phenotype in del(5q) cells, while sparing normal hematopoiesis. More generally, our approach may be useful to identify novel vulnerabilities associated with other deletions in cancer.

Inhibition of RNA Pol I transcription and induction of nucleolar stress in cancer cells treated with the experimental alpha-adrenergic receptor antagonist JP1302

Name of Presenter: Sheetanshu Saproo

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Increased RNA polymerase I (Pol I) transcription and subsequent ribosome biogenesis are recognized as hallmarks of nearly all cancer types. Consequently, targeting Pol I transcription through pharmacological inhibition has emerged as a promising approach in cancer therapy. Acridine derivatives display anti-cancer properties but have primarily been used as antibacterial and anti-parasite agents. The activity of acridines is mainly attributed to the planarity of these aromatic structures, which can intercalate within the double-stranded DNA structure, and inhibition of chromatin associated biological targets including topoisomerases. JP1302 is a poorly characterized drug, based on a 9-aminoacridine structural scaffold, it is known as an experimental alpha-2 adrenergic receptor antagonist. JP1302 acts as a transcription inhibitor, blocking Pol II phosphorylation and inhibiting p21 expression at a concentration of 10 µM (Mitchell DC, et al., Nat Biotech, 2023). Given JP1302's structural similarity to other acridine derivatives such as aminacrine, ethacridine, and the experimental compound BMH-21, known for blocking Pol I transcription, inducing nucleolar stress, and activating p53, we hypothesized that JP1302 might share similar properties. Here we show that JP1302 acts as an inhibitor of Pol I transcription at concentrations exceeding 0.5 µM. Treatment with JP1302 induced nucleolar stress, evidenced by a rapid decrease in the levels of the catalytic subunit POLR1A (also known as RPA194 or RPA1), leading to a reduction in rRNA synthesis within six hours at a concentration of 0.5 µM. JP1302 stabilized p53 and increased p21 expression in osteosarcoma cell line U2OS and glioblastoma cell line A172. Downregulation of RNA Pol I activity occurred in the absence of DNA damage response, as determined by the absence of g-H2A.X staining. We found that JP1302 impaired the growth of both p53 wild type (wtp53) and p53 null HCT116 cells with GI50 values of around 0.6 uM at 48 hours, and in U2OS wtp53 at 0.8 uM. Interestingly, in the lower nanomolar concentration range JP1302 appeared to transiently stimulate RNA synthesis, a phenomenon that requires further investigation. In summary, we identify JP1302 as a novel inhibitor of rRNA synthesis, exerting its effects at concentrations that do not induce DNA damage and independently of p53. Given that JP1302, as a receptor antagonist, easily passes the blood-brain barrier in rodents at low concentrations and accumulates in different brain regions it is of future interest to test this drug in brain tumor models and to further establish its effects on chromatin and transcription.

Keywords: transcription inhibitor, RNA polymerase I, repurposing

Role of collagen organization in cancer

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Increased deposition and stiffening of tumor extracellular matrix (ECM) contribute to the palpable, “hard lump” that is often the first clinical sign of cancer. Via mechanotransduction, this increased stiffness is translated into intracellular signaling and influences cellular behavior (Paszek et al, 2005). Collagen is a major contributor to tumor mechanics. During tumorigenesis, tissues progressively stiffen, at least in part, due to incremental increase in collagen deposition (i.e. collagen stiffness increases as a function of concentration), collagen remodeling (i.e. progressive linearization, alignment and thickening of interstitial collagen) and with LOX-mediated crosslinking (Acerbi et al, 2015).

A number of methodologies can be used to measure tissue stiffness with varying degrees of resolution and throughput. In our lab, we infer tissue stiffness from the classic picrosirius red staining of collagen combined with polarized light microscopy (cPOL). This technique is compatible with routinely fixed and processed human and mouse tissues and allows the analyses of large regions, i.e., entire tissue sections. Importantly, tissue stiffness assessed by cPOL correlates well with atomic force microscopy measurements (Stashko et al, 2023). Additionally, we have established protocols for co-detection of collagen organization and various cellular and molecular markers by immunohistochemistry and Opal-based multiplex immunofluorescence in the same tissue section. We have also established image analyses pipelines to quantitatively determine the relationship between marker expression and local ECM organization features (such as fiber density, thickness, length, alignment, type and spatial distribution).
One branch of our ongoing research applied these protocols and pipelines to characterize the temporal and spatial interplay between the immune cell compartment and ECM organization in various sets of tumor tissues from mouse models of breast, pancreatic and lung cancer, as well as in human breast cancer tissues. Specifically, we see distinct accumulation and distribution of T cell subpopulations, in function of local collagen organization, across various stages of tumorigenesis. We are also characterizing how this correlates to PD-1 and PD-L1 expression. To functionally validate the dependence of immune cell distribution on ECM stiffness, we are modulating the ECM stiffness in lung cancer mouse models driven by KrasG12D and BRAFV600E by inducing fibrosis with bleomycin (and thereby increasing stiffness) or by inhibition of LOX-mediated collagen crosslinking with BAPN (leading to tissue softning).
Stromal collagen alignment has clinical significance in a variety of cancers as well as in other diseases accompanied by fibrosis (Drifka et al., 2016). Comprehensive assessment of collagen alterations in tumor tissues conveys additional, valuable information with the potential of better predicting cancer risk and enhancing antitumor therapies.

Studies on the effects of curcumin targeting CDC27 alone or combined with other inhibitors on HPV positive and negative head and neck cancer cell lines

Name of Presenter: Tina Dalianis

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Background/aim. Human papillomavirus positive (HPV^+) tonsillar and base of tongue cancer (TSCC/BOTSCC) accounting for 40% of all head neck cancer (HNSCC) in Sweden are still rising in incidence, and are despite their better outcome compared to HPV-negative (HPV^-) HNSCC (80% vs. 50% resp. 5-year survival) treated like HNSCC with chemoradiotherapy (ChRT). ChRT has severe side effects reducing the quality of life without improving the previously obtained efficacy of 80% 5-year survival with only radiotherapy (RT) given before, so new therapeutic options are urgently needed.

Previous studies by others and us have focused on identifying prognostic or targetable markers for HPV^+ TSCC/BOTSCC. Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA) and Fibroblast Growth Factor Receptor (FGFR3) mutations have been found fairly frequently in HPV^+ TSCC/BOTSCC and in vitro targeted therapy combining phosphoinositide 3-kinase (PI3K) and FGFR inhibitors have shown synergistic effects. More recently, upon whole exome sequencing (WES) we identified a deletion variant of CDC27 in patients with a poor prognosis.

Here we therefore wanted to investigate the effects of Curcumin a drug that potentially targets CDC27, either using it alone or combination with other inhibitors on TSCC/BOTSCC cell lines grown *in vitro* as monolayers (2D) or spheroids (3D).

Methods: The effects of Curcumin as single inhibitor or combined with other inhibitors e.g. BYL719 (a PI3K inhibitor) on TSCC/BOTSCC cell lines such as e.g. HPV^+ CU-OP-2, and -20 and others grown as monolayers (2D) and spheroids (3D) are now being assessed. Effects of the various drug treatments will be analysed by viability (WST-1 assay), proliferation (IncuCyte S3 Live-cell Analysis System), and FACS assays on various TSCC/BOTSCC.

Results: Preliminary data using Curcumin as a single agent presented dose dependent responses with decreased viability and proliferation. Combination experiments are now ongoing.

Conclusion: To summarize, our preliminary data disclose that using Curcumin as a single agent reveals promising effects on HPV^+/HPV^- TSCC/BOTSCC cell lines.

Regional regeneration in the esophagus

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The esophagus is considered a homogeneous organ along the longitudinal axis. However, previous work in our lab showed that the esophagus differs axially in various aspects, remains largely understudied. To better understand the esophageal heterogeneity and regeneration, we took advantage of a regeneration mouse model and lineage tracing method. We identified the cell behavior dynamics during regeneration. Also, we found the esophageal epithelium responded differently along the axis upon regeneration, with the proximal region proliferating less but prone to forming bigger clones and the opposite at the distal region. Sc- RNA-seq confirmed the regenerative dynamics we saw in the mouse in vivo. Interestingly, we identified a cell population, that exists only in the distal region and enriched in the folded region, responds dynamically to the injury. Further analysis on sc-RNA-seq and spatial transcriptomics would be conducted to unravel the cellular and molecular mechanisms in esophagus regeneration in general and in the context of regionality. Furthermore, to understand the regional differences in esophageal cancer, we generated a genetic cancer mouse model, enabling us to induce oncogenic events evenly along the esophageal epithelium. It would be interesting to know if tumors form evenly along the esophagus or if it biased to a specific region in our model. The insights from the combination of the regeneration and cancer models would give us implications for esophageal cancer treatment.

Keywords: Esophagus, Regional regeneration

BRAF-induced *EHF* expression affects *TERT* in aggressive papillary thyroid cancer

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BRAFV^{600E} and *TERT* promoter mutations in papillary thyroid carcinoma (PTC) have a synergistic effect on prognosis. This effect is believed to arise from MAPK activation triggered by BRAFV^{600E}, leading to the upregulation of ETS transcription factors that bind to the mutant *TERT* promoter. Here, we explored the role of ETS factors in relation to clinical features, *BRAFV*^{600E} and *TERT* promoter mutations in PTC. Transcriptomic data for 28 ETS factors were analyzed in the PTC cohort of The Cancer Genome Atlas (TCGA, n=399) and subsequently validated in a local cohort (n=93). *In vitro* experiments were performed to investigate the regulatory role in relation to *BRAFV*^{600E} and *TERT* expression. TCGA identified *ETS1*, *ERG*, *FLI1*, *GABPA*, *EHF*, *ETV6* and *SPDEF* as differentially expressed genes between stages I+II and III+IV. In both cohorts, *EHF* was consistently associated with adverse clinical features, *BRAFV*^{600E} and *TERT* promoter mutation/expression. Notably, in *BRAFV*^{600E} mutated PTC, high *EHF* expression was associated with shorter disease-free survival. Cases harboring concurrent *BRAFV*^{600E}, *TERT* promoter mutations and high *EHF* expression exhibited the shortest disease-free survival. In cells harboring concurrent *BRAFV*^{600E} and *TERT* promoter mutation, over-expression of EHF significantly increased *TERT* expression while knockdown or pharmacological inhibition of BRAF significantly decreased both *EHF* and *TERT* expression. The ETS transcription factor *EHF* is associated with poor prognosis in PTC. This is potentially mediated by BRAF-induced upregulation of *EHF* which in turn increases *TERT* expression in *TERT* promoter mutated cells.

Keywords: *TERT* promoter mutation, papillary thyroid carcinoma

Multi-omics characterization of glioblastoma with spatial tools

Name of Presenter: Yonglong Dang

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Glioblastoma (GBM) is the most malignant primary tumor in the brain. High inter- and intratumor heterogeneity are the main causes of treatment failure. Molecular characterization based on genomic and transcriptomic analysis uncovered sub- categories of this malignant tumor, yet the epigenomic regulatory networks in the tumor are much less known due to the lack of good tools to characterize the tumor microenvironment.

In this project, we use cutting edge tools such as deterministic barcoding in tissue for spatial omics sequencing (DBiT-seq), Xenium and MERFISH to molecularly characterize GBM microenvironment at cellular resolution. The microfluidic-based DBiT-seq platform has been shown to unbiasedly and robustly profile genome-wide multi-omics such as the transcriptome, accessible chromatin and chromatin binding proteins landscapes. As a proof of concept, we performed H3K27me3 modification profiling of platelet-derived growth factor receptor beta (PDGFB) driven tumor xenografts. We could recover an average of 10,000 unique fragments per spatial pixel, with high quality (fraction of reads in peaks being 12%). With this data, we could already uncover distinct cellular niches or neighborhoods in the tumors with spatial preference.

Furthermore, we recently developed nanobody-Tn5 based DBiT-seq protocol, with the potential to profile up to 3 histone modifications (e.g., H3K27ac, H3K27me3, and H3K4me3) with the transcriptome simultaneously from each pixel of the same tissues. We are currently applying this novel technology to the primary GBM tissues. This study is expected to help us explore and generate novel hypotheses for understanding GBM tumor development and identifying targetable driving mechanisms towards treatment.

Keywords: Spatial epigenomics; Glioblastoma.

CRISPR-based screening of oligodendroglia specification transcription factors on the development and maintenance of glioblastoma subpopulations.

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Glioblastoma (GBM) is a malignant brain tumour that arises *de novo* from glial progenitors or via malignant transformation of low-grade astrocytoma. Despite this, gene expression data from human GBM cell lines indicate differential regulation of oligodendroglia lineage markers such as Platelet-derived growth factor receptor A (PDGFRA), Oligodendrocyte transcription factor (OLIG2) and SRY-related HMG-box 10 (SOX10) between glioblastoma subtypes and their subpopulations. Moreover, oligodendrocyte precursor cells (OPCs) were reported to upregulate tumour survival, proliferation and chemoresistance in GBM *in vitro*, suggesting a more complex interaction between GBM and oligodendroglia development than previously thought. To investigate the role of oligodendroglia in GBM, we plan to conduct clustered regularly interspaced short palindromic repeats (CRISPR) based, gain-of-function and loss-of-function high throughput screens to identify novel effects of known oligodendroglia transcription factors on GBM subpopulations. The candidate transcription factors are collated from a series of single cell studies conducted within the lab that influence oligodendrocyte specification. Additionally, the CRISPR-based screens will be coupled with chemotherapy, proliferation modulators, and metabolic regulators to phenotypically assess the function of oligodendroglia transcriptional factors in GBM. To identify the transcriptomic and epigenomic changes due to TF gain/loss of function, we will perform multi-omic scRNA/ATAC-seq and nanoCUT&TAG targeting H3K4me3 (active mark) and H3K27me3 (repressive mark). We also plan to investigate the spatial distribution of these candidate oligodendroglia TFs within GBM tumours and their surrounding microenvironment via RNAscope and spatial transcriptomics. This data will provide a better understanding of the complex interactions between oligodendroglia and GBM and identify potential therapeutic targets for high-grade gliomas.

Keywords: Glioblastoma, Oligodendroglia

Enhanced activity of the human Peroxiredoxin 2 variant: the effect of selenocysteine substitution at the catalytic sites

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In our study, we expressed and purified a selenocysteine variant of human Peroxiredoxin 2 (hsPrx2), and compared the kinetic properties with the wild type (cysteine containing) enzyme. The wild type form of hsPrx2 is a universally expressed cytosolic protein that plays important roles in reduction of cytosolic hydrogen peroxide, organic and nitrogen peroxides. By controlling the level of reactive oxygen species (ROS), the Prx2 is involved in tumor formation and suppression.

Like any other two-cysteine Prxs, hsPrx2 contains two active site Cys: the peroxidatic (C_P) and resolving (C_R) residues. In the normal catalytic cycle, the nucleophilic C_P attacks the peroxide substrate and forms Cys-sulfenic acid (C_P-SOH). In the next, resolution step, two Prx2 monomers forms a homodimer unit by an intermolecular disulfide bond between the oxidized C_P site and C_R site (C_P-S-S-C_R). In the final step this disulfide is reduced by flavoprotein disulfide reductase-dependent enzyme systems, mainly the thioredoxin and glutathione systems.

However, if the Prx2 is exposured to higher H_2O_2 concentrations it is likely to escape from the catalytic cycle as the C_P forms a catalytically inactive sulfinic acid (C_P-SO_2H) derivate. This transition is reversible in an ATP-dependent reaction by Sulfiredoxin; however, any further oxidation by H_2O_2 is irreversible as lead to sulfonic acid (C_P-SO_3H) derivate.
Interestingly, in a few bacterial species, there are isoforms of peroxiredoxins with selenocysteine (Sec, U) replacing the C_P residue. The use of selenium instead of thiols in redox-related proteins can not only increase the redox potential of protein, but may also protect against the over-oxidation.

Thus, the generation and study of recombinant selenium-containing Prx2 may bring us better understanding the role of over-oxidation in Prx2 catalytic cycle, and shed light upon the evolutionary or functional constraints that govern the choice of Cys over Sec in nature.

By expressing, purifying and characterizing recombinant human Prx2 C51U we found that the selenoprotein was an order of magnitude more active peroxidase than the wild-type enzyme when coupled to thioredoxin as the recycling system. Further characteristics are also reported, suggesting that the mammalian Cys- rather than Sec-containing variants of Prx may have evolved to better support their roles in redox regulation rather than solely as antioxidant peroxidases.

Keywords: selenoprotein, peroxiredoxin

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