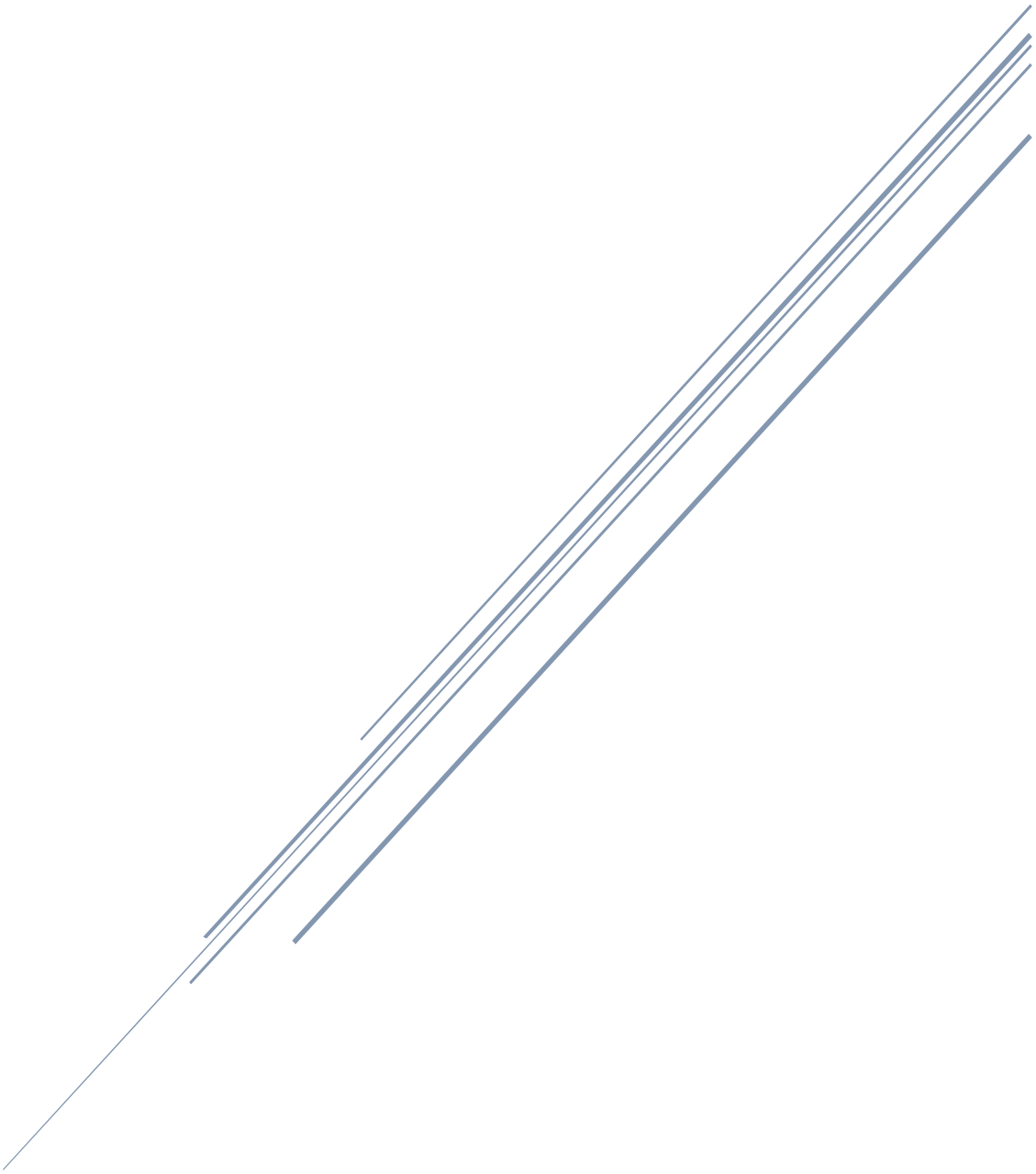
POSTER ABSTRACTS



Dual ROCK inhibitor RKI-1447 promotes cell death and emerges as a synergistic partner for BET inhibitors in neuroblastoma

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Background: Despite intensive treatment, high-risk neuroblastoma has a poor prognosis, demonstrating the need for new therapeutic strategies. Genes in the Rho/Rac pathway are frequently genetically altered in neuroblastoma, leading to active Rho/ROCK signaling. BET inhibitors target MYCN transcription and have demonstrated therapeutic efficacy against neuroblastoma, however clinical trials have suggested that resistance limits their therapeutic benefit. Notably, cytoskeletal remodeling and changes in ROCK activity have been proposed to play a role in therapy resistance.

Aims: To investigate the therapeutic potential of dual ROCK-BET inhibition in neuroblastoma.

Material and Methods: RKI-1447 efficacy was studied in vitro in cell lines through viability and clonogenic assays, and in vivo using the transgenic TH-MYCN mouse model. Transcriptomic profiling was performed with RNA-sequencing and gene signaling enrichment analysis. A drug combination screening with RKI-1447 and the FIMM oncology drug library (n=528) was performed. Combinations were validated using cell viability assays, western blots, and multicellular tumor spheroids (MCTS). Synergy was quantified using several models, including ZIP and MuSyC.

Results: Treatment with pan-ROCK inhibitor RKI-1447 in a panel of neuroblastoma cell lines, resulted in decreased cell viability, reduced clonogenic ability and increased apoptotic cell death. In accordance, treatment of homozygous TH-MYCN mice with RKI-1447 repressed tumor growth and was shown to reduce expression of MYC targets. The combination screening revealed synergistic effects between RKI-1447 and several BET inhibitors. BET inhibitors ABBV075/Mivebresib or JQ1 with RKI-1447 demonstrated synergy in both MYCN-amplified and non-MYCN-amplified neuroblastoma cell lines, while MuSyC analyses of combinations indicated an RKI-1447 induced synergistic effect on potency. Treatment with ABBV075 increased ROCK activity, measured as phosphorylated myosin light chain 2 (MLC2), an effect that was blocked when combining RKI-1447 and ABBV075. Additionally, combination treatment decreased N-MYC or C-MYC protein expression. Finally, studies in MCTS validated combinational effects, showing decreased spheroid growth and increased cell death compared to single treatment.

Conclusion: ROCK and BET inhibitors have previously demonstrated potential for the treatment of neuroblastoma; here we reveal that the combination of ROCK and BET inhibitors offer a promising treatment approach that can potentially mitigate resistance to BET inhibitors and reduce toxicity.

Harnessing p53's conformational flexibility to target and prevent leukemia

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Although the tumor suppressor *TP53* is the most commonly mutated gene in cancer, only 13% of acute myeloid leukemia (AML) patients carry mutant *TP53*. Nevertheless, p53, the protein product of *TP53* can become dysfunctional through various mechanisms even when its gene is intact. One such mechanism is acquisition of a misfolded conformation, resulting in dysfunctional activity, so-called “pseudo-mutant p53” (PMp53). PMp53 can be detected with conformation-specific monoclonal antibodies and was described in growth factor-stimulated leukemic blasts and in pre-leukemic hematopoietic stem and progenitor cells (HSPCs). This dynamic equilibrium between p53’s wild-type and the pseudo-mutant conformations, which can be influenced by several factors, can potentially be harnessed to target leukemic and pre-leukemic cells. APR-246 is the first-in-class compound that targets mutant p53. Its active metabolite MQ binds to mutant p53, thereby promoting normal p53 conformation and function. However, its effect on PMp53 has not been tested so far. The aims of this project are to test the hypothesis that APR-246 can switch PMp53 back to its wild type conformation and to explore the contribution of PMp53 to the malignant potential of HSPCs in individuals that have high risk to develop leukemia. To address the first aim, we established a sandwich-ELISA (Enzyme-Linked Immunosorbent Assay) that quantifies the dynamic equilibrium between the different p53 conformations. Next, we treated four *TP53* wild type AML cell lines with five different cytokines at various concentrations to induce PMp53 and documented the effect of APR-246 on p53 conformations and cell viability *in vitro*. To address the second aim, we conjugated p53 conformation-specific antibodies to polyadenylated oligonucleotides and optimized a p53 intra-nuclear staining protocol to enable concomitant assessment of p53’s conformations and transcriptional activity at the single cell level. This methodology will be used to study PMp53 in a cohort of individuals with various pre-leukemic conditions (e.g., clonal hematopoiesis and clonal cytopenias). The generated data can potentially open new diagnostic and therapeutic possibilities, whereby APR-246 could be used to eliminate pre-leukemic and leukemic clones that harbor PMp53.

Keywords: PMp53, AML

Characterization of heritable TP53-related cancer syndromes in Sweden - a retrospective nationwide study of genotype-phenotype correlations

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Background: Heritable TP53-related cancer syndrome (hTP53rc) is caused by germline alterations of TP53 and has a heterogenous clinical presentation. Increased understanding of genotype-phenotype relationships may improve clinical handling, diagnosis, and prognosis. Here we present the first nationwide delineation of the Swedish hTP53rc-cohort.

Methods: Genotype and phenotype data, including pedigrees, were retrieved for all TP53-variant carriers in Sweden up to March 2022 (91 families, 188 individuals). Families were classified according to classic Li-Fraumeni syndrome (classic LFS), Chompret or hereditary breast cancer (HBC) criteria and variants were reclassified using the newest ACMG guidelines.

Results: After reclassification a total of 83 families and 176 individuals were included. Among 176 variant carriers, 113 (64%) developed tumors among which 35 (31%) developed more than one primary tumor. Age at first tumor onset was higher in HBC families (42 years), compared to classic LFS (28 years, p=0.004) and Chompret families (34 years, p=0.02). The most prevalent tumor was breast cancer. Mean time between first and second primary tumor was 9 years. Patients with dominant-negative (DNE) missense variants had a lower age at first tumor onset (30 years) compared to all other variants (38 years, p=0.01).

Conclusion: Patients with DNE missense variants and patients classified as classic LFS had an earlier age at first tumor onset. This study adds granularity to personalized risk modeling with potential implications for tailored screening regimes.

Funding: The King Gustaf V Jubilee Fund (201052).

Keywords: Li-Fraumeni Syndrome, TP53

FBXL12 degrades FANCD2 to regulate replication recovery and promote cancer cell survival under conditions of replication stress

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Oncogene-induced replication stress constitutes an early obstacle pre-cancerous cells need to overcome to progress towards malignancy. Fanconi anaemia signalling represents a major genomic maintenance pathway that is activated in response to replication stress, impinging on stalled replication fork stability and recovery. We report that upon replication stress, phosphorylation of the FANCD2 N-terminus by CHK1 triggers FBXL12-dependent proteasomal degradation of FANCD2, facilitating clearance of FANCD2 at stalled replication forks. This mechanism is required to promote efficient and faithful DNA replication under conditions of CYCLIN E- and drug-induced replication stress. Notably, reconstitution of FANCD2 with mutations in the N-terminal phosphodegron fail to re-establish fork progression in FANCD2-deficient human fibroblasts in response to replication stress. In the absence of FBXL12, FANCD2 becomes trapped on chromatin leading to replication stress, excessive DNA damage, and cell death or senescence. In human cancers, FBXL12, CYCLIN E, and Fanconi anaemia signalling are positively correlated and upregulation or amplification of FBXL12 is linked to reduced survival in patients with high CYCLIN E expressing breast tumours. Finally, depletion of FBXL12 exacerbated oncogene-induced replication stress and sensitised breast cancer cells to drug-induced replication stress by WEE1 inhibition. Collectively, our results indicate that FBXL12 constitutes a vulnerability of CYCLIN E-overexpressing cancer cells and may represent a novel target for cancer therapy.

Keywords: Replication stress, Fanconi anaemia

Perturbed epigenetic transcriptional regulation in AML with IDH mutations causes increased susceptibility to NK cells

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Isocitrate dehydrogenase (IDH) mutations are found in 20% of acute myeloid leukemia (AML) patients. However, only 30-40% of the patients respond to IDH inhibitors (IDHi). We aimed to identify a molecular vulnerability to tailor novel therapies for AML patients with IDH mutations. We characterized the transcriptional and epigenetic landscape with the IDH2i AG-221, using an IDH2 mutated AML cell line model and AML patient cohorts, and discovered a perturbed transcriptional regulatory network involving myeloid transcription factors that was partly restored after AG-221 treatment. In addition, hypermethylation of the HLA cluster caused a down-regulation of HLA class I genes, triggering an enhanced natural killer (NK) cell activation and an increased susceptibility to NK cell-mediated responses. Finally, analyses of DNA methylation data from IDHi-treated patients showed that non-responders still harbored hypermethylation in HLA class I genes. In conclusion, this study provides new insights suggesting that IDH mutated AML is particularly sensitive to NK cell-based personalized immunotherapy.

Keywords: AML, NK cells

Cinical relevance of PD-1 Expression on TILs in Early-Stage NSCLC

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Introduction: Extensive research has been conducted on several biomarkers, particularly PD-L1 expression, as a crucial predictive and prognostic factor in non-small cell lung cancer (NSCLC). However, even the expression of PD-1 on tumor infiltrating lymphocytes (TILs) may have significant implications, since it plays a key role in the same signaling pathway.
This study aims to investigate the expression pattern of PD-1 on TILs in early-stage NSCLC, and its potential role as prognostic biomarker.

Materials & Methods: PD-1 was evaluated in 474 surgical resected early-stage NSCLC specimens, using Tissue microarray and immunohistochemical staining. Expression was scored as negative (<1%) or positive. Positive PD-1 expression was divided into low (1%<10%) and high (≥10%).

Results: PD-1 positivity was observed in 83.5% of cases and was statistically significant associated with pT stage (p=0.02), high pathological grading (p=0.004), and adenocarcinoma subtype (p=0.05).
High PD-1,49.8% of cases, was associated with high tumor grade(p<0.001) and adenocarcinoma subtype (p=0.03).
High PD-1 expression was found to be an unfavorable prognostic factor in the entire cohort using the Wilcoxon test (p=0.02) and in the non-adenocarcinoma subgroup using both the Log-Rank test (p=0.02) and the Wilcoxon test (p=0.005).
Furthermore, multivariate Cox regression analysis demonstrated high PD-1 expression to be an independent unfavorable prognostic factor in the non-adenocarcinoma subgroup (RR=0.85, 95% CI: 0.72-0.994, p=0.043).

Conclusions: Patients with early stage NCLC who exhibited PD-1 expression of ≥10% on TILs had an unfavorable 10-year OS rate. These findings indicate that elevated PD-1 expression may impact the selection of perioperative treatment and post- operative follow-up procedures. Further investigations on the prognostic impact of the expression of PD-L1 on tumor cells in relation to PD1 positivity on TILs are ongoing. This might provide additional information on the clinical impact of immunophenotype in lung tumors.

Keywords: Lung-cancer, PD-1.

Role of arginase 1+ microglia in brain tumour progression and resistance to treatment

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Microglia perform multiple functions to maintain brain homeostasis. Excessive or insufficient microglial response can be harmful, promoting neurodegeneration or tumorigenesis. Recent evidence suggests that microglia are heterogenous, having subtype-specific roles. One of these subsets is Arginase-1+ (Arg1) microglia with a distinct transcriptomic profile, function and spatiotemporal distribution. Arg1+ microglia are predominantly found in the cholinergic neuron-rich forebrain region during early postnatal development, where Arg1-deficient microglia result in cognitive behavioural deficiencies in female mice. Arg1 is an enzyme converting arginine into ornithine and urea, needed to produce polyamines, promoting cell growth and proliferation. Arg1 is upregulated in pro-tumoral macrophages, but its function in microglia is unknown.
To elucidate the role of Arg1 in microglia, we overexpressed (Arg1-OE) or knocked-down (Arg1-KD) the Arginase-1 in BV2 murine microglia cell line. First, we tested the advantages and limitations of our model by validating the expression of Arg1, its downstream affected and pro-inflammatory proteins (i.e., iNOS) as we hypothesize that Arg1 might be involved in maintaining sufficient response to external stimulus. We also assessed cell proliferation as Arg1 is known to have effects on cell growth. Then we tested if the presence of Arg1 in BV2 cells could regulate the expression of the signature genes (Apoe, Igf1, Lpl, Dtx3, Sap25, Leng8, Snx29, Colec12, Spp1 and Clec7a) observed in vivo in the Arg1+ microglia subpopulation. Moreover, we took advantage of a migration assay approach to test if Arg1 presence in microglia attracts C6 rat glioma cells.
Arg1 is one of the markers for pro-tumoral peripheral macrophages. However, it might not be straightforward if Arg1 has similar functions in microglia. Thus, further studies on the role of Arg1 in microglia could shed light on the presence of the subtype and a link to cognitive decline after irradiation, especially when this subtype is predominant in the developing brain.

Keywords: Microglia subtypes, glioblastoma, Arginase 1

Fine needle aspiration-based immune profiling of tumor microenvironments

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Background: Diagnostic tissue biopsies are required to select therapy for patients with solid tumors. However, core needle biopsies can cause complications and may be difficult to repeat longitudinally [1]. Sampling via fine needle aspiration biopsy (FNA) is globally established, minimally traumatic and can be repeated during treatment. FNA-samples can be used for ultra-sensitive multiplex molecular profiling, allowing for early diagnosis and for monitoring during treatment. FNA-based immune-profiling of tumor microenvironments represents an emerging opportunity and there is increasing interest in molecular cytology.

Methods: We have developed a standard operating procedure for sample preparation of minimal FNA material, compatible with clinical routines and targeted analysis of mutations, as well as gene and protein expression [2, 3]. Expression levels of 150-170 proteins per sample (leftover material only) were profiled by proximity extension assays (PEA, olink.com), a method with high sensitivity and specificity. Data were analyzed with statistical tools provided by *e.g.* Qlucore Omics Explorer (qlucore.com). We also applied machine learning strategies to identify tentative predictive biomarker signatures [5].

Results: Key results were: (1) Identification of a tentative signature (benign vs cancer) for early diagnosis of breast cancer and good correlation with established key biomarkers [2]. (2) Profiling of immune markers such as PD-L1 and many other immune-related proteins (including tentative markers for resistance to immunotherapy) in breast and lung cancer FNA-samples [3, 4]. (3) Identification of a tentative signature related to tumor stage of primary lung adenocarcinomas [4]. (5) Identification of a tentative signature related to tumor grade in prostate FNA samples, as well as analysis of immune-related proteins that may guide treatment in advanced prostate cancer [5].

Conclusions: We describe here the development of FNA-based atraumatic molecular cytology for precision cancer medicine. We have identified tentative biomarker signatures, and we demonstrated profiling of proteins related to the immune microenvironment and to resistance to immunotherapy. The methodology is highly sensitive and reproducible and permits extensive protein, RNA and mutation profiling with assessment of biomarkers for diagnosis, therapy selection and monitoring of therapy.

A novel *FLCN*-related syndrome leading to intellectual disability, immunodeficiency, and leukemia predisposition

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Background: *FLCN*, the disease-causing gene for autosomal dominant Birt-Hogg-Dubé syndrome, encodes a protein with multiple cellular roles, including the regulation of early embryonic development and metabolic modulation through the mTORC1 pathway and transcription factors TFE3/TFEB. Recently, homozygous variants in FLCN's interaction partner FNIP1, have been associated with an immunodeficiency and cardiomyopathy syndrome, while mosaic variants in *TFE3* lead to a disorder with intellectual disability and growth retardation. Here we present a 14-year-old boy with intellectual disability, short stature, coarse facial features, and immunodeficiency, who developed acute lymphoblastic leukemia at 1 year of age. In the patient, we detected a germline homozygous *FLCN* variant and aimed to characterize the genetic and molecular mechanisms leading to this novel syndrome.

Methods: We carried out 30X Whole Genome Sequencing (WGS), Sanger sequencing, and flow cytometry from blood samples to confirm variant's inheritance and evaluate patient's immunological parameters. Additionally, to investigate the effect of the variant in FLCN-mediated metabolic modulation, we used droplet-digital PCR (ddPCR), western blot (WB) and immunofluorescence staining (IF) on skin fibroblasts.

Results: WGS analysis in the patient identified a homozygous ultra-rare, and likely damaging missense variant in *FLCN* (p.G15S) with parental inheritance. Flow cytometry results confirmed hypogammaglobinemia, and low B- and NK-cells' counts. We did not observe changes in the activation of the mTORC1 pathway in patient's fibroblasts. However, IF staining revealed a significant increase in TFE3 nuclear translocation in the patient, resulting in the transcriptional activation of genes related with mitochondrial and lysosomal biogenesis, glycolysis, and nucleic acid metabolism, as previously seen in *FLCN*-deficient animal models.

Conclusion: Our results suggest that homozygous *FLCN* p.G15S affects TFE3-mediated transcriptional activation of genes involved in cell metabolism. We hypothesize that the variant results in a hypomorphic protein function, leading to a novel autosomal recessive *FLCN*-related syndrome, characterized by leukemia predisposition, immunodeficiency, and intellectual disability. Interestingly, this syndrome overlaps with *FLCN*-deficient animal models, mosaic *TFE3* intellectual disability syndrome, and autosomal recessive *FNIP1* immunodeficiency.

Keywords: FLCN, cancer predisposition.

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

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The *TP53* tumor suppressor gene is mutated in approximately 50% of all tumors. According to the COSMIC database, close to 11% 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 7th most common of all cancer-associated *TP53* mutations. To establish a model for assessing the impact of *TP53* nonsense mutations *in vivo*, and develop therapeutic strategies for this mutation type, we used CRISPR/Cas9 genome editing to generate 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, with development of lymphomas and sarcomas from 3-6 months of age in homozygous mice and from 10-12 months in heterozygotes. Our results so far show that *Trp53*^{R210X} mice are initially phenotypically normal. However, the proportion of female *Trp53*$^{R210X/R210X}$ mice is dramatically reduced. Based on our current numbers, 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. The most common tumor type in homozygous mice thus far is lymphoma, whereas heterozygous mice most frequently display sarcomas. The tumor phenotype of our *Trp53*^{R210X} mouse model is up until now comparable to those previously described for *Trp53*-null and *Trp53*^{R172H} homozygous mice. This new 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 compounds targeting nonsense mutations. Our long-term goal is to develop 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

Elucidating mechanisms to inactivate the chemoresistance factor SAMHD1 - targeting allosteric activation

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SAMHD1 is a deoxynucleotide hydrolase involved in the regulation of cellular dNTP levels that has recently been implicated as a resistance factor to nucleoside analogue (NA) antimetabolite chemotherapy (1). Antimetabolites were among the first chemotherapeutic agents to be used in cancer therapy, and they still remain important in the treatment of many cancer types today, though pharmacokinetic and tumour-specific factors can alter therapy outcome between patients (2). NAs enter the cell as prodrugs and are subsequently converted into their active form by the cell's nucleotide salvage pathways (3). Once activated, NAs disrupt the cellular dNTP pool or cause direct damage to the genome by mimicking the cell's own deoxynucleotides (2). SAMHD1 is therefore an attractive target for the development of inhibitors to improve the effectiveness of NA therapy which can also serve as experimental tools to further understand the enzyme's biological roles.

Catalytically competent SAMHD1 requires (d)NTP binding at two distinct allosteric sites (AS1 and AS2) to induce tetramerization, with AS1 being specific for guanine nucleotides (4). Guanine nucleotide analogues, such as the triphosphate metabolite of the antiviral acyclovir, can also activate SAMHD1 via AS1 (5). Interestingly, in the absence of their triphosphate moiety, both deoxyguanosine and acyclovir inhibit the dNTPase activity of SAMHD1 in vitro (6). However, the mode of inhibition was not described. In the present study, we are investigating this as yet unknown mode of SAMHD1 inhibition by deoxyguanosine analogues. Using a combination of biochemical and biophysical studies, we are testing the hypothesis that deoxyguanosine analogues can occupy AS1, but the lack of a triphosphate moiety prevents the formation of the SAMHD1 dimer, which is a prerequisite for forming the catalytically competent tetramer. Altogether, this study could potentially diversify the modes of inhibition towards this chemoresistance factor and provide a starting point to rationally develop AS1 targeting molecules.

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Keywords: SAMHD1, nucleoside analogues

Targeting Polo-Like Kinase 1 against childhood cancer

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Background
RNA interference (RNAi) is known for its catalytic activity and target selectivity, and it is highly suitable for precision medicine. The breakthrough for RNAi therapeutics came in 2018 when the FDA approved the first RNAi-based drug, patisiran. Since then, four more short interfering RNA (siRNA)-based drugs have been approved by the FDA/EMA for children and adults. Thus, RNAi therapeutics has become reality. However, they all target the liver.

Methods
My group is utilizing our unique RNAi prodrug technology to knockdown cancer therapy targets, selectively. RNAi prodrugs enter cells without a transfection reagent and knockdown endogenous mRNA targets such as cell cycle regulator Polo-like kinase 1 (PLK1), resulting in depletion of the PLK1 protein followed by cell cycle arrest and apoptosis. We have used methods such as qPCR, western blot, flow cytometry on both cell lines and ex vivo-cultured primary cells from pediatric leukemia patients to study the effects.

Results
RNAi prodrugs enter primary peripheral blood and bone marrow mononuclear cells collected from pediatric T- and B-cell acute lymphoblastic leukemia and acute myeloid leukemia patients and induce mRNA knockdown of an endogenous targets, PLK1, without the use of a transfection reagent. The mRNA knockdown and resulting depletion of the protein, induced cell cycle arrest and apoptosis. We also found that PLK1 knockdown sensitized pediatric leukemia cells to chemotherapeutics such as cytarabine, as a combination of RNAi prodrugs and a nontoxic dose of cytarabine increased the number apoptotic cells. Preliminary data also show that knockdown of PLK1 mRNA by RNAi prodrugs is possible in pediatric osteosarcoma cells.

Discussion/Conclusions
We have found PLK1 to be upregulated in several pediatric cancers and that its knockdown results in tumor cell death. Our hope is that PLK1-targeted RNAi prodrugs can be used for treatment of both adult and pediatric cancers and that a combination treatment may lead to a decrease in the concentration of chemotherapeutics. Moreover, as PLK1 is upregulated in cancer it could potentially serve as a biomarker. Our goal is to develop a more selective and less toxic therapy against cancer and to identify new, potential biomarkers.

Key-words:RNAi therapeutics, PLK1, childhood cancer

Unraveling novel anti-cancer properties of the CuET

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Drug repurposing has facilitated cancer treatment in recent years by minimizing both the time and the resources needed for the development of novel therapeutic compounds. We have previously shown that Disulfiram, a drug used primarily for the treatment of alcoholism, shows anticancer properties mainly through its metabolite diethyldithiocarbamate, whose activity is further enhanced by its complex with copper (CuET). CuET kills cancer cells via excessive proteotoxicity following its binding to NPL4 and the subsequent inhibition of p97-mediated protein degradation. CuET's anticancer role is distinct and separated from Disulfiram's anti-alcoholic activity which is achieved through ALDH inhibition. CuET has also been found to silence the ATR-CHK1 pathway, induce replication stress, and evoke DNA damage. We show here that one of the earliest effects of CuET treatment is translation arrest through activation of the integrated stress response. Translation repression is followed by ribosome stress and the formation of NPL4-rich aggregates that entrap p53 rendering the latter non-functional. Transcriptomic analysis of CuET-treated cells showed a time-dependent induction of cell death regulators alongside the upregulation of ribosome biogenesis and autophagic genes. Concomitant treatment with CuET and autophagic inhibition of ribosome biogenesis and/or autophagy potentiated the cytotoxic effect of CuET and holds promising therapeutic potential.

Keywords: CuET, cancer

BET inhibitors as precision medicine for treatment of cervical carcinoma

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Our group has previously shown that inhibition of MYC or MYCN causes metabolic reprogramming manifested by lipid droplet accumulation in neuroblastoma and clear cell renal carcinoma. To address whether this is a specific effect of MYCN downregulation in neuroblastoma or a general effect, we performed a screen of more than 60 human cancer cell lines of different types treated with the first-generation BET (bromodomain and extra-terminal) domain inhibitor JQ1, which among other genes inhibits c-MYC. Our data showed accumulation of cytoplasmic neutral lipids and morphological changes in five cervical carcinoma (CC) cell lines out of six analysed and we further investigated these cell lines.
Cervical carcinoma is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women. Infection by the human papillomaviruses (HPVs), specifically HPV 18 and 16, are 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 c-MYC oncogene.
We explored the initial observations in two CC cell lines carrying HPV18, one which carry HPV16, and one without HPV but with mutations in the p53 and pRB genes following treatment with JQ1 and the second generation BET inhibitor IBET762. We found that MYC levels were downregulated upon BET inhibitors treatment, together with a reduction in cell proliferation. In addition, we observed morphological changes only in the cell lines with HPV, indicating that BET inhibitors could impact cytoskeletal organization in association with virus carrying tumors.
To further unravel the effects of BET inhibitors in CC, we selected one of the cell lines and performed proteomics following treatment for 24 hours or seven days with IBET762. We discovered that the most significantly upregulated as well as downregulated proteins were involved in lipid metabolism and cytoskeleton organization, in accordance with our initial results. We are now analysing both significantly upregulated as well as downregulated proteins in these processes. Two of these, NGFR or p75NTR and RhoA play crucial roles in cellular processes related to survival as well as general cellular dynamics including cytoskeleton organization, migration, and cell division. Importantly, the NGF receptor p75 has been described to modulate RhoA which could explain the morphological changes observed. Further studies are aimed to analyse the effect on cytoskeleton as well as in lipid metabolism. Based on our data, BET inhibitors could potentially serve as an effective and innovative approach for treating CC. Third generation BET inhibitors including OTX015 are currently optimized for clinical use, aiming to minimize adverse effects and may be an attractive advance for CC.

Key words: Cervical carcinoma, precision medicine

Genetic wiring maps of co-acting transcriptional factors in single cells

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Gene expression is a fundamental process whereby genetic information is expressed to control cellular identity and plasticity. Remarkably, in isogenic cell populations heterogeneity in gene expression is often found. This phenomenon is thought to be of importance for cell survival. In the context of cancer, these fluctuations may lead to the appearance of rare cells in the population capable to survive treatment. Since the emergence of drug resistance is a major challenge in disease treatment it is important to understand how heterogeneous gene expression occurs and is regulated in at a single cell level. Transcription is driven by transcription factors (TFs), which in turn recruit cofactors (CoFs), thereby modulating gene expression. However, currently it is not possible to study the effect of the various possible TF/CoFs pairs in single cells directly. This project will contribute to solve this problem and aims to develop a method that can simultaneously quantify (unknown) co-occurring TF/CoF pairs and the transcriptome at single cell resolution. Linking TFs/CoFs interactions with the phenotypic appearance of the cell may provide new insights in the regulation of gene expression in eukaryotes. Moreover, our approach may reveal interactions that only arise in a subset of the cell population, that remain hidden in bulk studies. Our method can be applied to any other set of protein-protein interactions, opening avenues for future research.

Single cell sequencing, Heterogeneity

Implementation and validation of RNA-fusion detection in routine cancer diagnostics

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Gene fusions play an important role in the oncogenesis and progression of many tumors. Being able to accurately detect fusions can inform diagnostic, prognostic and therapy selection. Unlike traditional methods, whole transcriptome sequencing (WTS) allows for comprehensive detection of both known and novel fusions. However, the nature of the data makes fusion detection challenging.

With healthcare moving towards large-scale sequencing and precision oncology, there is a need for robust, scalable and portable pipelines that can operate in clinical settings.
Here we describe the implementation and validation of the nf-core/rnafusion pipeline (https://nf-co.re/rnafusion) for gene fusion detection using WTS data. The pipeline is built within nf-core, a community-based framework to build and maintain bioinformatics analysis pipelines. The nf-core/rnafusion pipeline uses multiple callers (STAR-Fusion, FusionCatcher, Arriba, SQUID, and pizzly) to generate a combined comprehensive report of all fusion events, which increases the confidence of the calls and aid in their interpretation.

nf-core/rnafusion has an easily maintainable codebase assembled into a continuous integration environment. Containerisation ensures reproducibility and portability of the analyses. Combining results grants confidence in fusion events repeatedly identified by different tools and increases the chances of identifying novel fusions.

To validate the pipeline we used both commercially available RNA samples known to contain a wide range of gene fusions, as well as clinical samples for which fusions have previously been detected with alternative methods. We evaluate the robustness of sample handling and library preparation as well as the sensitivity and performance of the pipeline. The validation covers the detection of known fusion events between two different genes when transcripts are highly expressed (at gene and transcript level) and the correct identification of breakpoints. Other categories of RNA fusions, such as exon-skipping events or lowly expressed gene fusions, were outside the scope of this validation and will need to be addressed separately.

Our results show that we can consistently detect all gene fusions (19/19) present in commercial samples, at both gene and transcript level when multiple fusion transcripts were present, as well as, the exact breakpoints. Our analyses show that integrating results from multiple fusion callers can considerably reduce the number of false positives. Additionally, we consistently observed high recall in highly-expressed fusion transcripts from clinical samples, while improvements are still needed in order to consistently detect lowly-expressed fusions.

Keywords: RNA-fusion, Bioinformatics

Functional fibroblast heterogeneity in the oesophageal stem cell niche

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Fibroblasts are a key component of the healthy stem and tumour cell niche. Here, we identify and functionally characterize fibroblast heterogeneity in the oesophagus during homeostasis and tumour development. We find that TROYPOS fibroblasts are differentially distributed from proximal to distal, are located close to the basement membrane and in direct contact with the oesophageal epithelium. Transcriptional profiling suggests that TROYPOS fibroblasts are different from TROYNEG fibroblast regarding extracellular matrix organization, WNT and IGF signalling factors. Newly developed oesophageal organoid-fibroblast cocultures show that both populations support organoid formation. However, TROYPOS fibroblasts show a lesser capacity to support organoid growth compared to TROYNEG fibroblasts showing functional fibroblast heterogeneity in the oesophagus.
We identify Col1a1 to be enriched in TROYPOS compared to TROYNEG fibroblasts. Ablating COL1A1POS fibroblasts, using the Col1a1iDTR mouse model, results in reduced epithelial proliferation and increase in organoid growth, establishing an important role for TROYPOS/COL1A1POS fibroblast in maintaining epithelial homeostasis in the oesophagus.
To understand the dynamics of TROYPOS fibroblasts in cancer initiation, we treated mice with the carcinogen 4NQO. At early stages of epithelial transformation, thickening of the epithelium and increased epithelial proliferation is observed. At the same time, we find a spatial reorganization of the stromal niche, where TROYPOS fibroblasts loose contact with the epithelium and are replaced with TROYNEG fibroblasts and immune cells. Organoid co-cultures reveal that TROYPOS fibroblasts remain their reduced support in organoid growth during epithelial transformation. Together these results suggest that for oesophageal cancer to progress, TROYPOS fibroblasts need to be replaced and removed from the basement membrane during the early stages of epithelial transformation. In this work, we identify an oesophageal fibroblast niche cell dynamically regulated during tumour initiation.

Keywords: Oesophagus, Fibroblasts

Harnessing immunogenic properties of senescent cells in cancer therapy

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Cellular senescence is a stress response that activates innate immune cells, but little is known about its interplay with the adaptive immune system. Here, we show that senescent cells combine several features that render them highly efficient in activating dendritic cells (DC) and antigen-specific CD8 T cells. This includes the release of alarmins, activation of IFN signaling, enhanced MHC class I machinery, and presentation of senescence-associated selfpeptides that can activate CD8 T cells. In the context of cancer, immunization with senescent cancer cells elicits strong antitumor protection mediated by DCs and CD8 T cells. Interestingly, this protection is superior to immunization with cancer cells undergoing immunogenic cell death. Finally, the induction of senescence in human primary cancer cells also augments their ability to activate autologous antigen-specific tumor-infiltrating CD8 lymphocytes. Our study indicates that senescent cancer cells can be exploited to develop efficient and protective CD8- dependent antitumor immune responses.

Keywords: antigen presentation, anticancer vaccines

Deciphering the molecular mechanisms of purine analogue nelarabine in the treatment of childhood leukemia

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Objectives: Nelarabine, in vivo quickly converted into ara-G, is increasingly used in the treatment of pediatric and young adult T-cell acute lymphoblastic leukemia (T-ALL). Despite nearly two decades of clinical use, the molecular mechanisms of how this drug kills leukemic cells remain ill-defined, limiting optimal use of this therapy in the clinic. In this study, we aim to define the molecular mechanism-of-action of nelarabine and thereby provide the theoretical basis for rational improvement of the use of this drug in clinical practice.

Methods: In this study, we use a multidisciplinary research approach with established cell line models exposed to ara-G, coupled with various functional and phenotypic read-outs, and hypothesis-free functional genomic and proteomic approaches.

Results: Given the complexity of nucleoside analogue metabolism together with cancer biology, we are using hypothesis-free functional genomic and proteomic approaches to gain insight into nelarabine metabolism and mode-of-action. To determine genes involved in resistance and sensitization, we employed genome-wide CRISPR knock-out screens in two T-ALL cell models. To identify proteins that interact with ara-G by direct binding, downstream protein-protein interactions, or post-translational protein modifications induced by ara-G, in addition to proteome expression changes, we used a thermal proteome profiling method PISA (proteome integral solubility alteration) in the same cell lines. PISA evaluation of over 9700 unique proteins following ara-G exposure at different time-points identified involvement of pathways not previously associated with this therapy. Data analysis and integration is ongoing and will be followed by functional validation of identified hits.

Conclusions: Coupling genome and proteome-wide approaches we can identify relevant biological mechanisms and obtain insights into the intracellular activation, mechanism-of-action, and resistance mechanisms of ara-G.

KEYWORDS: Nelarabine; T-cell acute lymphoblastic leukemia

Modelling neuroblastoma using human induced pluripotent stem cells

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Background:
Neuroblastoma (NB) is known to arise from the neural crest cells of the trunk (tNCCs) during development of the peripheric nervous system, but the exact cell of origin is still under debate. Mutations of a few genes have been associated with development of NB, where *ALK* and *PHOX2B* have been shown to be mutated in familial forms. Human induced pluripotent stem cells (hiPSCs) are somatic human cells that have been reprogrammed into pluri-potent stem cells, with the potential to generate cells from all the three germ layers. By controlling the composition of the culturing medium, it is possible to control hiPSC differentiation into a desired cell type, including tNCCs and cells from the sympathoadrenal lineage. Due to these properties, hiPSCs represent a reliable model to study and characterize the development of different organs and tissues, and to understand the role of gene mutations in disease development.
Aim:
We will generate a novel model of NB development based on hiPSCs. While most of the present models are focused on studying events associated with the late stages of disease, we aim to characterize initiation of NB development.
Methods and results:
Differentiation of wild type as well as mutated hiPSCs to sympathoadrenal progenitors (SAPs) and then further to sympathetic-adreno-medullar cells (SAMs). Differentiation is assessed by expression of neural crest markers by immunofluorescence, RT-qPCR and FACS. Cells will later be transplanted in the adrenal gland of new-born mice and analyzed for tumorigenic potential. We will perform single cell RNA sequencing on wild type and mutated hiPSC-derived transplant to characterize the early steps of tumor initiation.
We optimized the protocol to differentiate wild type hiPSC lines into tNCCs and further to sympathoadrenal progenitors (SAPs). This has been verified by analysis of markers expression. While undifferentiated hiPSCs are positive for pluripotency markers NANOG, OCT4 and SSEA4, the tNCCs express the trunk identity marker HOXC9 and the neural crest marker SOX10. Preliminary results from three independent hiPSCs lines show different ability of the cells to further mature into SAPs.
Conclusions:
Our approach has the advantage of identifying key steps and processes involved in NB formation. By further characterising the fate between wild type hiPSCs and cells carrying genetic mutations during differentiation into sympathoadrenal cells, we intend to elucidate how deregulation of normal developmental pathways may lead to disease development. This new NB model will be used to test therapeutical targets to induce regression of NB cells or promote their differentiation.

Keywords: Neuroblastoma, hiPSCs

Deciphering the microglia transcriptional regulation on an early response to glioblastoma

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Glioblastoma is a highly aggressive brain tumor that creates an immunosuppressive microenvironment. Microglia, the brain's resident immune cells, play a crucial role in this environment. Recent studies suggest that glioblastoma cells can reprogram microglia to create a supportive niche that promotes tumor growth. However, the mechanisms of microglia transformation are not fully understood. In this study, we used a segregated coculture system to investigate changes in the transcriptional profile of BV2 microglia in response to C6 glioblastoma cells. RNA sequencing analysis revealed a significant upregulation of microglial Inhibitor of DNA binding 1 (ID1) and ID2, two known negative transcriptional regulators of basic HLH family of transcription factors. The observed concomitant regulation of expression of Ets proto-oncogene2, transcription factor (ETS2)-regulated target genes, *Stab1*, *Dusp6*, *Hmox1*, and *Fli1* led us to hypothesize that ETS2 may be regulated by ID proteins. ETS2 is a transcription factor involved in several biological processes, including inflammation and immune responses. Our findings suggest that the regulation of ETS2 by ID proteins could play a role in the transcriptional regulation of microglia in response to glioblastoma cells. In addition, we observed the upregulation of several microglial pro-inflammatory cytokines, including IL-1β and IL-6, that have been shown to be regulated by ETS2. Hence, our data indicates that ETS2 also plays a role in their regulation in microglia. Together, our findings provide new insights into the molecular mechanisms of microglia activation in response to glioblastoma and suggest a role for ETS2 and ID proteins in this process. Our findings also suggest a link between ETS2 and the regulation of pro-inflammatory cytokines, further supporting the idea that microglial ETS2 and ID proteins could play important roles in shaping the glioblastoma microenvironment.

Assessing the relationship of functional and phenotypic properties of tumour-reactive CD8 T cells and their expression levels of CX3CR1

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The functional properties instilled in exhausted CD8 T cells dictates their anti-tumor capacity, thereby, sparking an interest in the subtle delineation of functionally-distinct T cell populations in tumors. CX3CR1, a chemokine receptor, marks a subset with elevated cytotoxicity within the exhausted CD8 T cell pool, and its expression levels delineate functionally-distinct CD8 T cell subsets in acute infections. Thus, we asked whether the levels of CX3CR1 on CD8 T cells provide insights into their functionalities in tumors.

To study the diversity of tumor-reactive CD8 T cells, we used the OT-I system. OT-I T cells are T cell receptor transgenic CD8 T cells that recognize the model antigen ovalbumin (OVA). We transferred physiological numbers of naïve OT-I T cells into C56BL/6 mice and subcutaneously inoculated them with a melanoma cell line, B16F10, that we engineered to express a cytoplasmic variant of ovalbumin.

We first tested whether B16F10-cOVA cells elicited an *in vivo* OT-I T cell response. We detected OT-I T cells in tumors and spleens, demonstrating that the tumor-expressed model neoantigen was able to induce OT-I T cell responses. We then investigated the phenotype of the tumor-reactive OT-I T cells. Tumor-reactive CD8 T cells may exist in a continuum of exhausted states. At the end of the spectrum, CD8 T cells express higher levels of the inhibitory receptors PD1 and Tim3, and lower levels of Ly108. As expected, we found that splenic OT-I T cell phenotypes differed considerably from their tumor-infiltrated counterparts. Whereas splenic OT-I cells were $PD1^{-}$, tumor-infiltrated OT-I cells were $PD1^{hi}$ $Tim3^{hi}$, which is indicative of an exhausted state. CX3CR1 expression levels were variable on OT-I T cells in both tissues. Whereas the frequencies of $CX3CR1^{+}$ OT-I T cells were higher in tumors, CX3CR1 expression reached higher levels on OT-I T cells in the spleen. In both tissues, CX3CR1 expression was enriched on $Ly108^{lo}$ CD8 T cells, in accord with published data. In tumors, however, CX3CR1 expression was elevated in $PD1^{hi}$ $Tim3^{hi}$ T cells.

In conclusion, we established a system that allows studying the phenotypes and functionalities of CD8 T cells reactive to a non-secreted neoantigen. We found CX3CR1 to be expressed at various levels among both exhausted (PD1+) and non-exhausted (PD1-) CD8 T cells in tumor-bearing animals. We next aim to explore the functionalities of intratumoural CD8 T cell populations in relation to their CX3CR1 expression levels.

Mapping distinct tumor subpopulations in human neuroblastoma using scRNA-seq and novel spatial omics

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Neuroblastoma (NB) is the most common and deadly infant malignancy, accounting for approximately 15% of pediatric cancer-related deaths. Despite intensive multimodal therapy, survival in the high-risk group is still less than 50%. Hence, the urgent need to identify heterogeneity in NB emergence as well as the subpopulations of tumour cells displaying therapy resistance.

In our study, we have used single-cell RNA sequencing to provide a detailed description of the cellular and genetic diversity within human NB. We have profiled 17 NB samples from 15 patients representing a broad spectrum of disease characteristics and have generated a database of ~70,000 single cells. This is followed by spatial multi-omics using a combined multiplex single-molecule DNA and RNA-FISH approach on NB patient biopsies.

Unbiased clustering revealed thirteen main cell types spanning tumor cells, stromal cells, and immune cells. Among the tumor population, we found two clusters expressing known mesenchymal (PRRX1, LEPR, PDGFRA, DCN) and adrenergic (TH, DBH) genes, indicative of mesenchymal and adrenergic tumor origin, respectively. These two putative tumor populations were connected by a “bridge” population that expressed key neural crest and Schwann lineage markers (SOX10, S100B) and was annotated as Schwann Cell Precursors –like (SCPs). Transcriptional profiling of the three distinct tumor populations revealed a continuous transition to both the MES and ADR lineages via the SCP-like population which harboured malignant aberrations. To further define the three tumor cell-states and investigate a possible transition from malignant SCPs to malignant MES and/or ADR cells, we are in the proses of implementing a single-molecule DNA and RNA-FISH approach. This method allows us to simultaneously, detect and validate abnormal DNA allelic expression and study RNA signatures through which we distinguish tumor subpopulations from normal stroma cells.

We conclude that there are three subpopulations of malignant NB tumour cells and are in the proses of investigating possible links between them. Increasing our understanding of the interactions between SCPs and their downstream tumor subpopulations may provide novel information aiding in the strategy for therapeutic targeting of resistance tumor cell types in high-risk NB.

Keywords: Spatial Multi-omics, Single-Cell Transciptomics

The role of an atypical chain-specific deubiquitinase in colorectal cancer

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Background: Colorectal cancer (CRC) ranks as the third most common cancer and a leading cause of cancer-related deaths. Furthermore, metastatic CRC presents poor overall survival rates and challenges in treatment due to drug resistance. Consequently, there is a pressing need for novel therapeutic targets. Ubiquitin-related enzymes, critical for regulating stemness signaling in CRC stem cells, are potential treatment targets. Yet, their molecular mechanisms are largely unexplored. Trabid, a K29 and K33-specific deubiquitinase, whose function and role are less studied, presents an area warranting further investigation.

Methods: Utilizing online database, we examined the dependency on Trabid of tumor cell lines, the changes in Trabid expression in the intestinal mucosa of CRC patients, and the impact of Trabid expression on survival. To evaluate the growth capability of CRC cell lines following Trabid loss *in vivo*, we inoculated cells into C57BL/6 mice and monitored tumor size after Trabid knockdown. Also, we used *Apc*, *Kras*, and *Tp53* gene-edited mice to cross with intestinal Trabid knockout mice to establish a spontaneous colorectal carcinogenic and metastatic model, to evaluate survival, tumor size, and distant tumor metastasis. Finally, to investigate the effect of Trabid on cells within the tumor development critical zone—the intestinal stem cell region—we isolated intestinal crypts of systemically induced knockout, intestinal knockout, and intestinal stem cell-specific Trabid knockout mice to grow organoids, record organoid size and assess cell lineages *via* qPCR.

Results: We found that Trabid knockout has negative survival effects on most tumor cell lines. Early-stage CRC patients exhibited increased Trabid expression compared to normal tissues, correlating with poor survival rates. Trabid knockdown in MC38 xenograft models resulted in smaller tumors. In the Apc^{min} mice model, intestinal Trabid knockout mice exhibited significantly longer survival times. In spontaneous colorectal cancer mice models, intestinal Trabid knockout mice tended to have smaller tumors and fewer distant metastasis. In organoids from systemic, intestinal and intestinal stem cell-specific Trabid knockout mice, Trabid knockout organoids were smaller in size than wild type. In tamoxifen-induced knockout organoid, we observed a reduced stem cell population and an increased proportion of enterocytes and Paneth cells.

Conclusion: Trabid, acting as an oncogene, fuels tumorigenesis and metastasis. Trabid's absence leads to a reduction in intestinal stem cells but a rise in enterocyte and Paneth cell populations.

Keywords: Colorectal cancer; deubiquitinase

BET bromodomain inhibitors as precision medicine in childhood medulloblastoma

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Medulloblastoma (MB) is the most common malignant brain tumor during childhood. In these tumors *MYC* amplification and/or overexpression correlate with poor outcome. Bromodomain and Extra-Terminal domain inhibitors (BETi), a class of epigenetic modifiers that directly affect *MYC* transcription, represent a promising therapeutic approach for *MYC*-amplified tumors. Nevertheless, despite the vast number of studies conducted, the detailed mechanisms for their action have not been fully defined.

Here, we aimed to analyze the effect of BETi on MB cell viability and metabolism in order to identify novel therapeutic targets that could be exploited for combinatory treatments of *MYC*-amplified MB tumors.

We evaluated the effect of three BETi (JQ1, IBET-762, and OTX-015) on *MYC*-amplified (Group 3) and non-*MYC*-amplified (SHH) MB cell lines. Notably, proliferation and viability assays revealed BETi treatment affects growth in all cell lines tested, independently of *MYC* status. Our results further showed an increase in expression of apoptotic markers and the cell cycle inhibitor p21 in some of the cell lines. Interestingly, we found a robust increase in the levels of the redox and metabolic regulator TXNIP which correlated with decreased glycolysis and increased apoptosis in *MYC*-amplified cells. This effect was not observed when cells were incubated with small molecule MYC/MAX heterodimerization inhibitors or the MYC dominant-negative Omomyc peptide.

Together, our results show that BETi treatment inhibits the glycolytic capacity of *MYC*-amplified MB cells and that effect could be attributed to increased TXNIP expression, leading to metabolic stress, and decreased cell viability. The effect of TXNIP on glucose metabolism together with the inhibition of the TXN system might play an important role in the mechanism of action of these compounds that needs further investigation.

Key words: Medulloblastoma, BETi

A key role for HIF2α in determining differentiation potential and a noradrenergic cellular state in neuroblastoma

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Neuroblastoma arises within the sympathetic nervous system and is the most frequent extra cranial solid childhood cancer. The disease presents itself with considerable intratumor heterogeneity of which sympathetic noradrenergic (NORAD) and mesenchymal (MES) cells are two identified entities. The hypoxia inducible factor, EPAS1/HIF2a, has been proposed to be NB oncogene. However, several studies challenge this concept and high expression levels of EPAS1 is associated with increased survival of neuroblastoma patients. We have performed analysis of single cell sequenced neuroblastoma and initiated gain-and loss-of function experiments in neuroblastoma cells. Our analysis of single cell sequenced human neuroblastoma tumors revealed that EPAS1 is significantly enriched in cells from low-risk tumors characterized by high expression of sympathoadrenal markers such as PHOX2B and TH, but lower in neuroblastoma cells expressing markers defining mesenchymal cellular state, e.g. PRRX1and YAP1. This suggests that EPAS1/HIF2a could play a role in promoting a differentiated sympathetic noradrenergic cellular state. Crispr/Cas9 depletion of EPAS1 in SK-N-SH cells which harbor both MES and NORAD populations, resulted in rapid loss of PHOX2B and PHOX2A. Besides, Crispr/Cas9 depletion of EPAS1 in SH-SY5Y cells, which is NORAD population of SK-N-SH cells, leaded to a significant loss of key NORAD transcription factors such as PHOX2B, NEFL, but an enrichment of MES factors such as VIM, DESMIN and DACH1 upon RA treatment. Conversely, overexpression of EPAS1 in SH-EP2 neuroblastoma cells that only harbor the MES population, resulted in reduced YAP1 Expression and altered morphology. More interesting, overexpression of EPAS1 in NORAD state of LAN-1 cells resulted in the induction of both TH and DBH, a significant reduced in vivo xenograft tumor sizes and weights. In both SH-EP2 and LAN-1 cells, overexpression of EPAS1/HIF2a caused reduction of proliferation, a down regulation of MYCN, and a less migration and invasion capacity in vitro. This implies that high EPAS1/HIF2a levels are required to maintain the NORAD cellular state and that transition to a MES cellular states is associated with reduced EPAS1/HIF2a levels. Our preliminary data suggest a novel role for EPAS1/HIF2a which is not associated with the response to hypoxia but rather as a determining factor of neuroblastoma differentiation potential and heterogeneity.

Keywords: EPAS1/HIF2a, neuroblastoma

Peroxiredoxin 6 Disruption: Strategies to Induce Cell Death and Differentiation in High-risk Neuroblastoma

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Neuroblastoma (NB) is an embryonal tumor of the sympathetic nervous system that accounts for 7% of all childhood cancers. Heterogeneity is high, and NBs can range from spontaneous regression to aggressive and metastatic tumors. In the high-risk group, patients present poor prognosis and relapse in 50% of the cases. The *MYCN* oncogene is amplified in 40% of the high-risk group and is correlated with an undifferentiated phenotype and poor outcome. We previously described that MYCN induces a metabolic reprogramming in NB, with high rates of oxidative phosphorylation coupled with increased expression of antioxidant enzymes (Oliynyk *et al*., 2019). In this study, we explored the potential of using enzymes from the antioxidant systems as targets for NB treatment. High expression of genes from the thioredoxin, glutathione, and peroxiredoxin pathways correlates with poor prognosis in NB patients. We targeted these enzymes with different inhibitors and observed decreased cell viability, enhanced neurite outgrowth, and upregulation of markers associated with neural differentiation. The effects were very robust when inhibiting peroxiredoxin 6 (PRDX6), a unique member of the peroxiredoxin family involved in reactive oxygen species (ROS) scavenging, lipid metabolism, and ferroptosis. Inhibition of PRDX6 led to an arrest in cell proliferation, apoptosis, and neural differentiation, especially in *MYCN*-amplified NB cell lines. These changes were accompanied by decreased levels of MYC/MYCN proteins, abolishment of oxidative phosphorylation, and accumulation of lipid droplets. We validated our findings by using two *PRDX6* knockdown stable cell lines. As PRDX6 is dependent on Glutathione S-transferase P (GSTP1) for reduction and re-activation of its peroxidase function, we investigated whether simultaneous inhibition would enhance the effects. Our results showed that combined treatment potentiated cell differentiation, induced ROS and cell death (apoptosis and ferroptosis), and reduced tumor burden *in vivo*. In addition, we identified that both *PRDX6* and *GSTP1* are up-regulated during murine adrenal development (Furlan *et al*. 2017) at E12.5 in Schwann cell precursors (one-tailed Welch's *t*-test, FDR<0.05). At this developmental point, *PRDX6* is also highly expressed in bridge and sympathoblast cells. PRDX6 is characteristic of mesenchymal NB cells (van Groningen *et al.* 2017), with higher expression in high-risk NB patients (SEQC cohort, GSE62564). On a single-cell basis, *PRDX6* is observed in tumor cells with a higher expression in stroma. While *GSTP1* is also up-regulated in high-risk patients, its expression is instead higher in more differentiated noradrenergic cells (Bedoya-Reina *et al.* 2021). Patients with combined high expression of *PRDX6* and *GSTP1* have worse prognosis, high *MYCN* and lower expression of neural differentiation markers (*TH*, *SCG2*). Interestingly, there are no patients with *MYCN*-amplification and low levels of *PRDX6*+*GSTP1* (SEQC, GSE62564; Kocak, GSE45547). In conclusion, our results show the potential of using the antioxidant PRDX6 and GSTP1 enzymes as targets for differentiation-inducing therapy in NB, providing a new approach for development of less toxic and more specific strategies for cancer treatment.
Keywords: Childhood cancer; Differentiation-inducing therapy.

Title- Macrophage-derived VEGF-C decreases hematogenous metastatic dissemination and normalizes the tumor vasculature

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Expression of pro-lymphangiogenic vascular endothelial growth factor C (VEGF-C) in primary tumors correlates with the occurrence of proximal lymph node metastasis in most solid cancer types. However, the role of VEGF-C in regulating tumor cell dissemination to distant organs is currently unclear. Perivascular tumor-associated macrophages (TAMs) are key regulators of hematogenous cancer cell spreading, forming tumor microenvironment of metastasis (TMEM) doorways for breast cancer cells to intravasate tumor blood vessels and fuel distant metastases. Using an experimental breast cancer (BC) model, we show here that TAMs expressing VEGF-C decrease cancer cell dissemination to the lung while enhancing lymph node metastasis. These TAMs express podoplanin and LYVE1 and associate with normalized tumor blood vessels expressing VEGFR3. Further clinical data reveal that VEGF-C$^+$ TAMs correlate inversely with malignant grade and with the occurrence of TMEM complexes in a cohort of BC patients. Thus, our study displays an apparently paradoxical role of TAM-derived VEGF-C in redirecting cancer cells to preferentially disseminate to the lymph nodes, at least in part, by normalizing tumor blood vessels.

Keywords: Vascular Endothelial Growth Factor-C, Tumor-Associated Macrophages.

Are CD39 CD103 CD8 T cells the key to emerging immunotherapies in pancreatic cancer

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Background and Aims: Infiltration of $CD8^+$ T cells in the tumor microenvironment is a predictor of a favorable prognosis in pancreatic ductal adenocarcinomas (PDAC), but not all tumor-infiltrating T cells display tumor reactivity, and a large proportion of the T cells are entrapped in the desmoplastic stroma. Here, we aimed to look for $CD8^+$ T cells double-positive (DP) for CD39 and CD103 in resectable PDAC tumors, which recently has been described to display tumor reactivity in other types of cancer.

Methods: Tumor pieces ($5mm^3$) from the central, peripheral and non-tumor areas were cut by pathologies from freshly resected pancreatic tumors from patients undergoing surgery. A cell suspension of each tissue was obtained by mechanical disaggregation and analyzed by flow cytometry with the paired peripheral blood.

Results: DP $CD8^+$ T cells accumulated in central tumor tissues compared to paired peripheral tumor tissues and adjacent non-tumor tissues. Consistent with an antigen encounter, DP $CD8^+$ T cells were more proliferative and displayed an exhausted phenotype with higher expression of PD-1 and TIM-3 and lower levels of granzyme B compared to $CD39^-$ $CD103^-$ $CD8^+$ T cells. DP $CD8^+$ T cells also expressed higher levels of the tissue trafficking receptors CCR5 and CXCR6, but lower levels of CXCR3 and CXCR4. However, a high proportion of DP $CD8^+$ T cells was not associated to an increased overall survival in PDAC patients.

Significance: These data suggest that DP $CD8^+$ T cells with a phenotype reminiscent of tumor-specific T cells are present in PDAC tumors. Therefore, considering the presence of DP $CD8^+$ T cells to select appropriate patients for immunotherapy trials in PDAC could have a benefit impact.

Keywords: Pancreatic Cancer, T cells

MYC determines the outcome of pro-senescence cancer therapy by regulating immune surveillance

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Cellular senescence, defined as permanent cell cycle arrest, is one of the main anti-tumor programs in cells, and pro-senescence therapy has therefore been proposed as a new strategy to treat cancer. However, this concept is controversial since the senescence-associated secretory phenotype (SASP) can possess both anti- and pro-tumorigenic properties. What determines the outcome of senescence induction in tumor settings is largely unknown. Here we utilized an immunocompetent, conditional $BRAF^{V600E}$/MYC-ER-driven mouse lung tumor model to study the consequences of CDK2 depletion/inhibition, which previously was shown to trigger senescence in tumor cells. After generation of $BRAF^{V600E}$/MYC-ER/$CDK2^{FLOX/FLOX}$ mice, inhalation of Ad-CRE virus led to activation of $BRAF^{V600E}$ and MYC-ER, and deletion of CDK2 in the mouse lung epithelium. In addition, MYC-ER activity can be regulated by administration of tamoxifen (TAM). While activation of MYC by TAM accelerated lung tumor development, CDK2 deletion or pharmacological inhibition resulted in delayed onset of disease, reduced tumor burden and significant prolongation in survival also in the presence of TAM. This was accompanied by induction of senescence. RNAseq analysis of whole lung tissue further demonstrated that CDK2 depletion induced expression signatures related to senescence, SASP and enhancement of immune response signaling. Additionally, immunohistochemistry and flow cytometry showed that CDK2-depleted lung tumor tissues exhibited an increased infiltration of CD8 T cells and macrophages. Based on bulk RNA-seq and scRNAseq-data, 150 genes representative of tumor and senescence markers, SASP and immune and other cells in the microenvironment were selected for spatial transcriptomics by HybISS. The analysis showed that MYC activation by TAM caused a proliferative and immune suppressive milieu, while CDK2 depletion in addition to senescence and SASP resulted in infiltration and activation of a different types of immune cells and a shift towards a more immune-reactive environment. However, some immune suppressive characteristics remained, which may explain why small, essentially non-proliferative tumors persisted also after CDK2 depletion. Interestingly, MYC inactivation by tamoxifen withdrawal resulted in a drastic regression of CDK2-deleted tumors, and increased activity tumor-infiltrating cytotoxic T cells. Finally, immune depletion of T-cells, and to a lesser extent NK cells, concurrently with MYC inactivation abolished the regression of CDK2-depleted tumors. In conclusion, our results suggest that senescence and SASP induction by depletion/inhibition of CDK2 leads to partial tumor inhibition and increased infiltration of immune cells into the tumor microenvironment. However, optimal immune surveillance and tumor eradication requires elimination of immunosuppressive MYC activity. These findings have relevance for the design of future pro-senescence and immune cancer therapies.

Keywords: Pro-senescence therapy, Immune surveillance

High-throughput neural stem cell-based drug screening identifies S6K1 inhibition as a selective vulnerability in SHH-medulloblastoma

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Co-authors: Niek van Bree, Lola Boutin, Simon Moussaud, Margareta Wilhelm

Medulloblastoma (MB) is one of most common malignant brain tumors in children. Current treatment include surgery, chemotherapy and craniospinal irradiation. Though the combined treatment has increased overall survival, it can lead to devastating side effects in survivors. This shows the urgent need for more effective and targeted therapy which minimize the harmful side effects for MB treatment.
We have previously established a human neuroepithelial stem (NES) cell model by reprogramming of noncancerous somatic cells carrying a germline PTCH1 mutation. Orthotopic transplantation of patient-derived NES cells in mouse cerebellum result in tumors mimicking human SHH-MB. Re-injection of isolated tumor NES (tNES) cells showed accelerated tumor growth with increased malignancy. We used our NES model (both naïve and after tumor formation) for high-throughput screening of a 172 oncodrug-library and identified PF-4708671 (p70S6K1 inhibitor) to specifically target tumor NES cells compared to normal NES cells with same genetic background. Inhibition of p70S6K1 impaired tumor cell growth and acted in synergy with conventional chemotherapy. Importantly, inhibition p70S6K1 had only mild effects on normal human neural stem cells and neurons. Knock down of RPS6KB1 gene in tumor NES and ONS76 cells significantly decreased cell proliferation. Our data demonstrate that the patient-derived NES cell platform can be used for identifying new effective therapies or targets for SHH-MB. Targeting S6K1 could potentially benefit MB treatment.

Keywords: Medulloblastoma, precision cancer medicine

Ensemble-based deep learning improves detection of invasive breast cancer in routine histopathology images

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Accurate detection of invasive breast cancer (IC) can provide decision support to pathologists as well as improve downstream computational analyses, where detection of IC is a first step. Tissue containing IC is characterized by the presence of specific morphological features, which can be learned by convolutional neural networks (CNN). Here, we compare the use of a single CNN model versus an ensemble of several base models with the same CNN architecture, and we evaluate prediction performance as well as variability across ensemble based model predictions.

Two in-house datasets comprising 587 WSI are used to train an ensemble of ten InceptionV3 models whose consensus is used to determine the presence of IC. A novel visualization strategy was developed to communicate ensemble agreement spatially. Performance was evaluated in an internal test set with 118 WSIs, and in an additional external dataset (TCGA breast cancer) with 157 WSI.

We observed that the ensemble-based strategy outperformed the single CNN-model alternative with respect to accuracy on tile level in 89% of all WSIs in the test set. The overall accuracy was 0.92 (DICE coefficient, 0.90) for the ensemble model, and 0.85 (DICE coefficient, 0.83) for the single CNN alternative in the internal test set. For TCGA the ensemble outperformed the single CNN in 96.8% of the WSI, with an accuracy of 0.87 (DICE coefficient 0.89), the single model provides an accuracy of 0.75 (DICE coefficient 0.78)

The results suggest that an ensemble-based modeling strategy for breast cancer invasive cancer detection consistently outperforms the conventional single model alternative. Furthermore, visualization of the ensemble agreement and confusion areas provide direct visual interpretation of the results. High performing cancer detection can provide decision support in the routine pathology setting as well as facilitate downstream computational analyses.

Keywords: Breast Cancer, Deep learning ensemble

KI Biobank for Researchers - Research core facility at KI

Name of presenter: Li-Sophie Rathje, PhD
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Co-authors: KI Biobank

KI Biobank at Karolinska Institutet in Solna is a modern and high-tech infrastructure for pre-analytical handling, storage and distribution of human biological samples. We provide services for collection, handling, storage and traceability of biobank samples and support scientists both within and outside of Karolinska Institutet. KI Biobank assists researchers with advice on how to start a sample collection, how the specimen should be collected and labeled, and what legal requirements that must be met in order to be able to collect human biological samples. On July 1, 2023, a new Swedish Biobank Act (2023:38) will come into effect, representing a modernization of the current law. We help to establish the necessary agreements under the Biobank Act, such as biobank agreements, extradition treaty and Material Transfer Agreement (MTA). We offer design of study specific paper referrals and automated sample handling, such as e.g. plasma- and serum aliquoting, DNA extraction, preparation of cell-free plasma DNA from STRECK tubes, formatting of samples before analysis, and assistance with various forms of transportation. We also provide robust IT systems, ensuring full traceability of each sample for customers with samples stored at the KI Biobank as well as other departments at KI.

Keywords: Biobanking, Services

Microglia secrete extracellular matrix modulators that are essential for the invasive nature of diffuse midline gliomas (DMG)

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Paediatric diffuse midline glioma (DMG), with H3K27M mutation, has proven to be one of the most challenging paediatric cancers to date with a median survival rate of less than one year from diagnosis. Despite significant efforts over the past four decades, the treatment options for these patients remains isolated to fractionated radiation therapy that only mitigates neurological symptoms for a short period of time and extends overall survival by a mean of 3 months. The diffuse nature of DMG allows extensive infiltration into neighbouring normal tissue contributing to the aggressive nature of the disease, however the factors responsible for promoting DMG invasion are unclear. Recently, we showed that microglia, the immune cells of the brain, become pro-tumoural in DMG and therefore aimed to further characterise the activation state of DMG-associated microglia. We found that there is a striking increase in the number of microglia in human DMG patient tissue compared to controls as well as increased microglial activation measured by IBA1 intensity. RNA sequencing of microglia exposed to patient derived DMG cells, SF8628, acquired a unique activation state characterised by increased expression of extracellular modulator genes such as Fibronectin (Fn1), Collagen1 (Col1a1, Col1a2), and Metalloproteinases (Mmp2, Mmp3, Mmp14, Mmp15). Interestingly increases in ECM modulator gene expression were unique to microglia exposed to DMG cells and were not present when microglia were exposed to another type of paediatric high-grade glioma, SF188. This unique transcriptional upregulation of ECM modulators was also found in human microglia isolated from DMG patient tissue. Using immunofluorescence, we further validated these results in human DMG tumours, whereby we found large deposits of both Collagen1 and Fibronectin that were absent from control tissue. These deposits were found in close proximity to activated microglia. Finally, we carried out invasion assays whereby microglia treated with inhibitors of fibronectin or the metalloproteinase responsible for cleaving Fibronectin, MMP2, lead to a dramatic decrease in the invasion of DMG cells. In conclusion this work suggests that microglia are essential for remodelling the extracellular matrix and are indispensable for DMG invasion into neighbouring tissue. Targeting microglial secretion of important ECM modulators in DMG may represent a novel therapeutic strategy for DMG patients.

Keywords: Microglia, diffuse midline glioma (DMG)

Sialic acid induce vascular endothelial dysfunction by triggering ferroptosis pathway

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Vascular complications rank among the most life-threatening adverse effects following cancer treatment. Moreover, metastasis remains to be the leading cause of cancer-related death. The interactions between circulating tumor cells and endothelial adhesion molecules was found to be the key step in hematogenous metastasis. Several investigations have reported that endothelial inflammatory dysfunction acts as an essential precursor for tumor metastasis. However, the underlying mechanisms are not fully understood. Sialic acid (SIA) is a metabolite produced by hexosamine-sialic acid pathway during glucose metabolism. Researchers have found that SIA showed positive correlation with endothelial cell (EC) function and was introduced as a biomarker for several tumors. While few studies showed that the accumulated SIA is responsible for ECs dysfunction which is associated with the metastasis pathway, the underlying mechanisms remain unknown. In the present investigation, we showed that accumulation of SIA in ECs could induce inflammatory response with an increasing expression of IL-1β, ICAM-1 as well as VCAM-1, and promote monocyte adhesion to ECs. Mechanistic studies showed that SIA triggered SLC3A2 ubiquitination degradation and hence accumulation of lipid peroxidation in ECs. Fer-1 could inhibit the ferroptosis pathway and further reverse ECs injury. Interestingly, mitochondrial dysfunction was also partly involved in ECs injury after SIA treatment and been reversed by Fer-1. Together, our study reveals a new mechanism showing that SIA could promote endothelial ferroptosis that activate ECs inflammatory response. This knowledge might give new insight into the pathogenic mechanisms underlying endothelial injury and provide new therapeutic approaches to prevent or reverse tumor metastasis.

Key words: Endothelial dysfunction, SIA.

In situ identification of prognosis-associated human colon cancer fibroblast subsets with distinct and differential associations to T-cells and cancer cells

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Introduction: Better characterization of mesenchymal cell subsets in colon cancer are needed to support development of biomarkers and drug targets.
Results: scRNAseq from three human colon cancers, following negative selection of malignant cells, endothelial cells and immune cells, identified two main cell types with similarities to fibroblasts (A-cells) and perivascular cells (B-cells). Multiplex staining confirmed the existence of two main subsets (PDGFRA+/M-CAM-; A-cells) and (PDGFRA-/M-CAM+; B-cells) and demonstrated strong perivascular enrichment of the B-cells. Subsequent analyses focused on the fibroblast-like A-cells. Three A-cells subclusters were suggested by gene expression profiles. Bioinformatics analyses suggested similarities between A1 cells and inflammatory CAFs, and between A3 cells and telocytes, a previously described intestinal fibroblast-like cell subset. For in situ validation multiplex staing was performed on 6 stage II/III colon cancer tumors where 5-10 high-power-fields analyzed from each case. Based on differentially expressed genes, PDGFRA, Tissue Factor (TF) and FAP was selected for multiplex profiling. Digital image analyses confirmed the existence of A1 cells (PDGFRA+/FAP-/TF-), A3 cells (PDGFRA/TF+/FAP-) and two subgroups of FAP+/TF- A2 cells; ($PDGFRA^{high}$ and $PDGFRAl^{ow}$). Case-based analyses demonstrated large variations between tumors regarding overall composition of fibroblast subsets. A1 (PDGFRA+/FAP-/TF-) and A3 cells (PDGFRA+/FAP-/TF+) were spatially enriched in stroma areas surrounding tumor cell islands. Furthermore, high fraction of peri-epithelial A1 and A3 cells was associated with reduced cancer cell proliferation. Both cell subsets showed positive associations with T-helper-cell proliferation, and with a high epithelial/stroma localization ratio of cytotoxic T-cells. For these T-cells features, A1 showed stronger associations than A3. $PDGFRA^{high}$ and $PDGFRA^{low}$ A2 cells (FAP+/TF-) showed a series of properties contrasting with A1 and A3. $PDGFRA^{high}$ A2 peri-epithelial was associated with high cancer cell proliferation. $PDGFRA^{low}$ A2 was negatively associated with T-cell abundance. Also, these cells were associated with reduced T-helper cell proliferation and with a low epithelial/stroma localization ratio of cyto-toxic T-cells. Based on these findings, preliminary analyses were performed to explore clinical associations of the A-cell subsets based on quantitative spatial analyses of the well-annotated U-CAN cohort of approximately 200 stage I-IV cases. Regarding stage-associations, both A2 subsets increased from stage 1-3, whereas A1 fraction was reduced from stage 1-3. Total relative density of fibroblasts subsets was not associated with survival. Notably, high relative abundance of A1 and A3 in peri-epithelial regions was associated with better survival (10-50µm, $P<0.05$).
Conclusion: Four novel multi-marker-defined, and spatially distinct, human colon cancer fibroblasts subsets were identified. Tumor-restraining functions were associated with A1 and A3 subsets, with A1 predominantly linked to T-cell features and A3 to cancer cell features. In contrast, PDGFRAhighA2 was associated high cancer cell proliferation and PDGFRAlowA2 with inhibitory effects on T-cells. Good prognosis associations was detected for peri-epithelial A1 and A3 cells. In summary, novel human CRC CAF subsets was identified for further exploration as biomarkers and drug targets.

Gamma Delta T cell recognition and activation potential in Medulloblastoma

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Medulloblastoma (MB) is a heterogeneous group of tumors developing in the cerebellum and is one of the most common malignant brain tumors in children. Fatal left untreated, the standard therapies for MB involve surgery, chemotherapy, and irradiation (only for >5 years old). Despite an overall good 5-year survival rate around 70%, first line treatment often results in severe neurological and endocrine deficits in the developing brain. Thus, there is a strong need to identify less toxic and more efficient therapeutic strategies. The emergence of cancer immunotherapy has revolutionized cancer treatment, including immune checkpoint blockade, CAR-T cell therapy, and infusion of T cells or NK cells. Gamma Delta (γδ) T cells, a non-conventional T cell population, are in the spotlight as a novel cancer immunotherapy strategy due to their advantageous combination of non-alloreactivity, a strong tumor cell lysis potential and a broad antigen recognition. However, their ability to target and eliminate MB cells is poorly understood. In humans, γδ T cells are classified according to their Vδ chain (Vδ1, Vδ2, Vδ3 and Vδ5) where each subpopulation has different functionality and tissue distribution.
To explore the possibility of using γδ T cells to recognize and target MB we have ex-vivo expanded different human γδ T cell subpopulations and tested their ability to target a panel of MB cells. In addition, we have characterized the expression of known γδ T cells ligands on both MB cells and in MB patient datasets. We identified Ephrin-A2 receptor and the phosphoantigen/Butyrophilin complex as ligands of interest in triggering respectively Vγ9Vδ1 and Vγ9Vδ2 T cell activation leading to MB cell lysis. Preliminary results have shown that differentiated neurons and neuroepithelial stem cells, generated from IPS cells, are not targeted by Vγ9Vδ2 T cells, demonstrating the safety of this approach. Furthermore, we are exploring how targeted therapy may influence the expression profile of both known and unidentified γδ T cell ligands. The optimization of MB cell killing by γδ T cells aim to propose a novel therapeutic strategy for MB patients.

Keywords: Medulloblastoma – Immunotherapy

Reprograming of glutamine metabolism and enhanced HILPDA expression drives MYC-mediated lipid droplet accumulation in clear cell renal cell carcinoma

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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 specialized organelles composed of a core rich in triglycerides and sterol esters, surrounded by a phospholipid monolayer. For long, they have been considered as inert vesicles for fat deposit, product of altered metabolism. However, in recent years, they have gained recognition as emerging regulators of tumorigenesis. Yet, the mechanisms and factors regulating their biogenesis are still 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, resulting in accumulation of triglycerides, the main component of LDs. In contrast, MYC inhibition upon VHL expression led to an increase in inositol-related lipid species, and thus LD formation was not observed. Importantly, concomitant inhibition of both MYC and glutamine metabolism reduced tumor burden and impaired LD accumulation *in vivo*. Moreover, using RNAseq analysis, we identified the hypoxia inducible lipid droplet associated protein (HILPDA) as the key driver for MYC inhibition-derived LD accumulation, and demonstrated that it impairs proliferation and LD formation upon downregulation. Finally, analysis of ccRCC and healthy renal control samples, postulated HILPDA as a specific biomarker for ccRCC.
Taken together, our study characterizes the molecular interplay between hypoxia and MYC signaling resulting in LD accumulation. These discoveries provide an attractive approach for development of novel therapeutic interventions for treatment of ccRCC.

Keywords: clear cell renal cell carcinoma, HILPDA

Expansion of tumor-reactive T cells using BETi-treated melanoma cells leads to an improved phenotype and superior in vitro performance.

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While adoptive cell therapy (ACT) using ex vivo expanded tumor-infiltrating lymphocytes (TIL) has led to greatly improved survival in metastatic melanoma, many patients still respond poorly to it. To increase the clinical efficacy of ACT in melanoma, improved methods that more selectively expand tumor-reactive T cells are thought to be crucial. One way to achieve this is by a so-called Mixed Lymphocyte Tumor-cell Culture (MLTC), in which patient-derived T cells are stimulated ex vivo with autologous tumor cells to selectively expand polyclonal tumor-reactive T cells. Though this was indeed applied to treat metastatic melanoma with some degree of success, the approach is clearly limited by low immunogenicity of the autologous tumor cells used in the MLTC. Our lab found that JQ1, a small-molecular BET inhibitor (BETi), strongly enhances tumor immunogenicity through multiple mechanisms (manuscript in preparation). Therefore, we studied whether JQ1-treated melanoma cells in an MLTC would lead to improved expansion of tumor-reactive T cells. Indeed, our MLTC approach led to clearly enhanced TIL proliferation and overall yield, which coincided with a favorable increase in the CD8/CD4 T cell ratio and a more beneficial differentiation status, as indicated by higher frequencies of less differentiated CD8+ CCR7+ CD62L+ T-cells. Importantly, TIL expanded in our MLTC approach displayed markedly improved capacities for recognition and killing of matched tumor cells. Overall, our data suggests that T-cell expansion using BETi-treated cancer cells may be exploited to improve ACT strategies in metastatic melanoma.

Keywords: ACT, MLTC

THE IDENTIFICATION OF GAMMA DELTA T CELLS AS CELLS WITH 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. In addition, γδ T cells have been and are being tested in several clinical trials focused on different cancer types. Unfortunately, so far without clear clinical benefit. This may be due to main usage of Vδ2 cells whereas Vδ1 may be more cytotoxic and the general lack of knowledge on γδ T cell clonal expansion and ligand recognition.

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

Methods
We ran flow cytometry identifying γδ T cell subtypes in four human neuroblastoma patient samples. In addition, we performed a pilot scRNA/VDJ-seq experiment to characterize clonal composition and functional states of ab and γδ T cells infiltrating neuroblastoma.

Results
The γδ T cell compartment in human neuroblastoma consists of the same subtypes and varies in proportion between individual patients. A large proportion of the detected infiltrating cells were Vδ1+ cells. The pilot scRNA/VDJ-seq experiment revealed prominent clonal expansion of both ab and γδ T cells in the tumor. In both cases expanded clones exhibited a clear cytotoxic signature. Clonal expansion was particularly prominent for γδ T cells where nearly half of the cells represented one of the two top expanded clones with private Vδ1 and Vδ3 TCRs.

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

Keywords: neuroblastoma, γδ T cells, clonal expansion, immunotherapy

Determining the contribution of mitochondrial alterations to lung cancer in vivo

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Mitochondria are the metabolic centres of the cell and besides energy conversion, they provide many of the building blocks necessary for cell proliferation and play a central role as signalling hubs in apoptosis, chemoresistance and tumour microenvironment remodelling. Recently, a growing body of evidence highlighted the importance of mitochondrial function in cellular transformation to malignancy and cancer progression1. However, the role of mitochondrial function and dysfunction in cancer progression is much debated and it seems to be dependent on both cancer type and disease stage[2–6].
Mitochondria contain their own genome (mtDNA), whose expression is necessary for the oxidative phosphorylation (OXPHOS) system to function. mtDNA is present in thousands of copies within the cell and the amount of mtDNA is known to vary greatly among cancer types and stages. The importance of mtDNA content and mtDNA mutations to tumour progression is still a matter of debate, and most of the data collected in human patients are divergent even among the same cancer type[7].
Lung cancer is the deadliest and the second most common cancer among men in Europe[8]. Recent studies suggested that lung adenocarcinomas are highly dependent on mitochondrial function, as they rely more on mtDNA expression than other cancers[4,9,10]. Lung cancer was reported to correlate with both increased and decreased mtDNA levels in different patients cohort-based studies[7]. Therefore, to date, the contribution of mtDNA copy number to tumour development is still unclear.
We have employed a well-established genetically-driven cancer model[11,12] to characterise the contribution of mitochondria to tumour onset. We found that tumour tissue isolated from a Kras-inducible mouse model contained higher levels of mtDNA when compared with surrounding non-affected tissue. We now aim to continue to understand if and how mtDNA contributes to lung adenocarcinoma development, what are the physiological mechanisms involved and to investigate whether mtDNA expression can be exploited for blocking tumour progression in vivo.

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Adenoid Cystic Carcinoma (AdCC): A Clinical Survey of a Large Patient Cohort

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Adenoid cystic carcinoma (AdCC), a rare heterogenous disease, presents diagnostic, prognostic, and therapeutic challenges. To obtain more knowledge, we conducted a retrospective study on a cohort of 155 patients diagnosed in 2000-2022 with AdCC of the head and neck in Stockholm and investigated several clinical parameters in correlation to treatment and prognosis in the 142/155 patients treated with curative intent. The strongest favourable prognostic factors were early disease stage (stage I and II) as compared to late disease (stage III and IV) and major salivary gland subsite as compared to other subsites, with the best prognosis in the parotid gland, irrespective of the stage of the disease. Notably, in contrast to some studies, a significant correlation to survival was not found for perineural invasion or radical surgery. However, like others, we confirmed that other common prognostic factors, e.g., smoking, age, and gender, did not correlate to survival and should not be used for prognostication of AdCC of the head and neck. To conclude, in AdCC early disease stage, major salivary gland subsite and multimodal treatment were the strongest favourable prognostic factors, while this was not the case for age, gender and smoking nor perineural invasion and radical surgery.
Most AdCC studies include limited amount of study individuals, we have a uniquely large cohort and plan to continue with further studies. We have gathered tumour material from patients diagnosed between 2000-2014 out of the cohort described above (n = 101). These will be analysed by immunohistochemistry for possible immunological and stem cell markers (CD8, CD4, FoxP3, HLA, Myb, and Notch1). The data will be paired to the clinical outcome of the corresponding patients.

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Keywords: Head- and neck cancer, Adenoid cystic cancer

Long- and short-term effects of a high-intensity exercise intervention on biomarkers and imaging of cardiac function in breast cancer survivors

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BACKGROUND
Chemotherapy-associated cardiotoxicity is a major adverse effect in breast cancer patients increasing the risk of cardiovascular diseases such as heart failure even after successful cancer treatment. Adjuvant exercise has been shown to reduce fatigue and improve cardio-respiratory fitness in individuals undergoing chemotherapy, and exercise may reduce the cardiotoxic effects of chemotherapeutic agents. In the Optitrain randomised controlled trial (RCT), the effects of adjuvant aerobic and resistance high-intensity interval training (HIIT) for breast cancer patients were examined.

AIM
To investigate whether high-intensity exercise was effective in reducing biomarkers of cardiotoxicity and echocardiography-assessed clinical cardiac dysfunction.

METHODS
A total of 240 women were initially randomised to either aerobic HIIT (AT-HIIT), resistance HIIT (RT-HIIT) intervention groups, or the control group receiving usual care (UC). Biomarkers for acute cardiotoxicity (plasma troponin-T, cTnT) and long-term myocardial remodelling (Nt-pro-BNP) in addition to cardiorespiratory fitness ($VO2_{peak}$) were recorded at baseline, post-intervention, and the 1- and 2- year follow-up. At the five-year follow-up, all participants were invited to undergo an echocardiogram in combination with another $VO2_{peak}$ assessment.

RESULTS
Overall, 88 women were included in the analysis of the biomarker for cardiotoxicity. Additionally, 55 and 94 women returned for an echocardiogram and a VO2 peak assessment at the 5-year follow-up investigation, respectively. An increase in plasma cTnT was recorded in all groups post-intervention. Moreover, exercise groups presented lower Nt-pro-BNP compared to UC at the 1-year follow-up. No significant differences across groups were determined for $VO2_{peak}$ or echocardiogram-assessed cardiac function at the five-year follow-up.

CONCLUSION
While biomarkers of acute cardiotoxicity differed across intervention groups, findings remain unclear regarding the effect of high-intensity exercise on long-term myocardial remodelling and cardiac function. Future RCTs should examine the effects of exercise interventions on cardiac function and cardiotoxicity in cancer patients undergoing cytotoxic treatment.

Keywords: Exercise Oncology, Cardiotoxicity

From Protectors to Promoters: The Role of Microglial Aging in Glioblastoma Progression

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Microglia, the resident immune cells in the brain, play a versatile role throughout the lifespan, acting as supportive cells or contributing to the spread of neuroinflammation, depending on their phenotype. Aging is a known risk factor for neoplasm development and is also associated with the deterioration of the immune and neural systems. Consequently, the occurrence of glioblastoma, the most common and lethal brain tumor among adults, is correlated with increasing age. Microglia constitute a significant proportion of the tumor mass and can contribute to its progression and invasion into surrounding tissue. Previously, we have developed a unique microglial aging model based on long-term cultivation of BV-2 microglia, enabling us to study the effects of cellular aging and microglial phenotypic alterations.
In this study, we report that age-related changes in microglia lead to a more tumor-supportive phenotype. Glioblastoma cells exhibit a higher migration to aged microglia, and *vice versa*, compared to their younger counterparts. Furthermore, we observed differential protein levels of chemotactic cytokines, specifically the CCL-family chemokines, in microglial aging or mutual co-culture with glioblastoma cells compared to the younger microglia. Further investigation of the microglia-glioblastoma microenvironment in aging revealed alterations in the expression of certain matrix metalloproteinases, which could potentially contribute to tumor expansion. Indeed, utilizing a cell migration assay with the addition of Matrigel to simulate a more complex extracellular environment, increased invasion was observed after exposure of glioblastoma cells to aged microglia compared to young ones.
Having evaluated the more tumor-supportive properties of aged microglia compared to young microglia, we aimed to examine whether previously identified drivers of microglial aging play a role in this phenomenon. Microglial aging is associated with the dysregulation of purinergic receptors, specifically P2RY12 and P2RY13, which are essential for microglial immune functions. These receptors are involved in ATP and glioblastoma sensing and microglial process branching. We found that these receptors were downregulated in microglia with aging and in the glioblastoma. Our findings highlight the important role of microglial purinergic signaling in glioblastoma, as decreased levels of *P2RY12* or *P2RY13* were associated with worse survival in patients from the TCGA cohort of low-grade gliomas and glioblastoma, as well as data from the Protein Atlas. Notably, genetic attenuation of P2RY13 in young microglia demonstrated glioblastoma attraction akin to that of aged microglia, while attenuation in aged microglia further increased tumor cell migration compared to the levels of aged microglia.
The presented data provide new insights into the molecular and cellular mechanisms underlying microglial function and how they change with age and in the context of disease.

Keywords: microglia, glioblastoma, aging

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

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Tumor associated brain resident microglia (TAM microglia), as well as peripherally recruited bone-marrow derived macrophage (TAM macrophage), can 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 show less TAM macrophage infiltration. TAM macrophage and TAM microglia have distinct transcriptomes and are likely to exert different functions in the tumor microenvironment. However, TAMs have been mostly studied as a single entity. Here, we study them as two separate populations, assessing their communication with one another, as well as their unique functions. We hypothesized that TAM microglia acquire specific activation states 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 worse prognosis. In addition, migration and proliferation assays confirmed a distinct microglial activation state depending on the glioma *IDH1* mutational status. RNA sequencing of microglia exposed to *IDH1* WT, or *IDH1* MT tumor cell revealed distinct gene expression profile. To assess whether this microglial change of phenotype could drive macrophages recruitment, we performed cocultures between microglia and *IDH1* WT or *IDH1* MT glioma cells followed by macrophage migration assays. These results showed that microglia presence drives macrophages recruitment in *IDH1* WT glioma but not in *IDH1* MT glioma. Currently, we are examining the factors, secreted by *IDH1* WT glioma activated-microglia, that may be responsible for macrophage recruitment. Blocking these essential macrophage recruitment factors may represent a novel treatment strategy for *IDH1* WT gliomas.

Keywords: glioma, microglial activation.

Bridging the divide – a review on the implementation of Personalised Cancer Medicine

Michele Masucci

Background: The introduction of Personalised Cancer Medicine entails a paradigmatic shift in the medical profession and healthcare. At the forefront of this endeavor are research groups and organisations that are striving to create environments that support the implementation of PCM.

Methods: This review provide insights into some of the determining factors for the success of Personalised Cancer Medicine (PCM) programs supported by recent literature.

Results: The emergence of PCM stems from the understanding how cancer develops as a disease starting in single cells due to genetic errors and reprogramming, and the ensuing rapidly increased access to complex biological information on individual patients and the cause of disease. Healthcare providers and organisations at the forefront of PCM have met three fundamental challenges: the fostering of leadership with the capacity to support innovation and facilitate translational processes, the harmonization of organizational differences between different disciplines and professions, and the coordination of multiple sources for funding and regulatory bodies. Research funding is often oriented towards specific investigators or research groups. Investment in large common infrastructures requires coordinated efforts of complex governmental and multi-stakeholder organizations and time-consuming collaborative efforts. Fragmented and poorly informed leadership is a major obstacle to implementing a multidisciplinary approach to personalised treatment.

Conclusions: The implementation of PCM is dependent on the establishment of collaborative multidisciplinary environments. With PCM, new professions and expertise have emerged, which requires dedicated training programs and the fostering of team science. The organisational and cultural transformation needed for implementing PCM in institutions built on older medical paradigms requires strong leadership with the ability to bring together a multitude of actors towards a shared vision.

A Clinical Risk Model for Personalized Screening and Prevention of Breast Cancer

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Purpose: Image-derived artificial intelligence based short-term risk models for breast cancer have shown high discriminatory performance compared with traditional lifestyle/familial-based risk models. The long-term performance of image-derived risk models have not been investigated.
Methods: We performed a case-cohort study of 8,604 randomly selected women within a mammography screening cohort initiated in 2010 in Sweden for women aged 40-74. Mammograms, age, lifestyle and familial risk factors were collected at study-entry. In all, 2,028 incident breast cancers were identified through register matching in May, 2022 (206 incident breast cancers were found in the subcohort). The image-based model extracted mammographic features (density, microcalcifications, masses, left-right breast asymmetries of these features) and age from study-entry mammograms. The Tyrer-Cuzick v8 risk model incorporates self-reported lifestyle and familial risk factors and mammographic density to estimate risk. Absolute risks were estimated, and age-adjusted model performances (aAUC) were compared across the 10-year period.
Results: The aAUCs of the image-based risk model ranged from 0.74 (95%CI 0.70-0.78) to 0.65 (95%CI 0.63-0.66) for breast cancers developed 1-10 years after study-entry; the corresponding Tyrer-Cuzick aAUCs were 0.62 (95%CI 0.56-0.67) to 0.60 (95%CI 0.58-0.61). For symptomatic cancers, the aAUCs for the image-based model were ≥0.75 during the first 3 years. Women with high and low mammographic density showed similar aAUCs. Throughout the 10-years of follow-up, 20% of all women with breast cancers were deemed high-risk at study-entry by the image-based risk model compared with 7.1% using the lifestyle familial-based model ($p<0.01$).
Conclusion: The image-based risk model outperformed the Tyrer-Cuzick v8 model for both short-term and long-term risk assessment and, could be used to identify women who may benefit from supplemental screening and risk reduction strategies.

Keywords: Breast cancer, risk assessment

Chromatin damage generated by DNA intercalators leads to degradation of RNA Polymerase II

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In cancer therapy, DNA-binding drugs are mainly known for their capacity to kill cells by inducing DNA damage. Recently several DNA-binding compounds have attracted interest given their ability to inhibit nucleolar RNA pol I transcription (BMH-21, an acridine-like quinazolinone derivative), or evicting histones (Aclarubicin, an anthracycline), or inducing chromatin trapping of FACT (CBL0137/curaxin, a carbazole derivative). Interestingly these DNA intercalators lack the capacity to induce DNA damage while still retaining cytotoxic effects and ability to activate p53. Here we show that these DNA intercalators impact chromatin biology by concomitantly interfering with the functions of RNA polymerase I, II, and III. The compounds all have the capacity to induce degradation of RNA polymerase II, a process that requires ongoing transcription initiation and/or elongation. Simultaneously, they induce trapping of Topoisomerases TOP2A and TOP2B on the chromatin. Using RADAR-assay we can show that BMH-21 behaves as a catalytic inhibitor of Topoisomerase II, just like Aclarubicin and CBL0137. Moreover, Aclarubicin, CBL0137, and BMH-21 induces chromatin trapping of FACT, emergence of Z-DNA, and histone eviction. CBL0137 and aclarubicin are as effective inhibitors of RNA pol I transcription as BMH-21. An intriguing aspect in the development of BMH-21, CBL0137, as well as the CX-5461 compound (a G4 stabilizer / TOP2 poison and inhibitor of Pol I transcription) is that, at some point, the mechanistic understanding of their respective precursor compounds moved between having or lacking the capacity to induce DNA damage. DNA-binding drugs studied herein have a cumulative impact on general transcription machinery by inducing accumulation of topological defects and impacting all chromatin, and therefore, their cytotoxic capabilities may at least in part be the result of compounding deleterious effects on chromatin homeostasis.

Keywords: chromatin damage, RNA polymerase II

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

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Background
Amplification of Anaplastic Lymphoma Kinase (ALK) or activating mutations in the tyrosine kinase domain of the ALK gene is a common somatic alteration in neuroblastoma (NB) and has been correlated with poor prognosis in intermediate and high-risk patients. Although hereditary NB is rare, germline gain-of-function mutations have been found in ALK, mimicking common sporadic ALK mutations in NB.
Aim
Our aim is to study the role of ALK mutations in NB initiation.
Method
The induced pluripotent stem (iPS) cell technology allows somatic cells to be reprogrammed into pluripotent stem cells with the ability to self-renew and differentiate to almost all cell types. To study the contribution of ALK mutations in NB development, we reprogrammed non-cancerous fibroblast from NB patients carrying a germline ALK-R1275Q mutation and healthy individuals to iPS cells. The origin of NB is thought to be neural crest cells (NCC) and its derivative sympathoadrenal (SA) cells. Importantly, we established a robust NCC and SA differentiation protocol deriving trunk NCC and SA cells from iPS cells to analyze the impact of ALK-R1275Q mutation during SA differentiation.
Results and Conclusion
No differences in reprogramming capacity, expression of pluripotency markers, or ability to differentiate to migratory trunk NCC were observed, suggesting that ALK-R1275Q mutation does not interfere with early human embryonic development. Analysis of the transcriptomic landscape during the differentiation process from NCC to SA cells shows that trunk NCC-relevant markers, like SOX10, TFAP2A, NGFR, and HOXC9, are mainly expressed in the NCC-stage and downregulated in SA cells. On SA-lineage commitment, we observe upregulation of SA-lineage markers PHOX2B, ISL1, and CHGA, suggesting differentiation to both sympathoblasts and chromaffin cells. Interestingly, we observe increased expression of ALK after SA-lineage commitment which rapidly decreases during SA differentiation in cells derived from healthy individuals but remains highly expressed in cells derived from ALK-R1275Q NB patients. Pathway analysis identifies significant downregulation of neural differentiation and p53 signaling pathway with concomitant upregulation of DNA replication and protein translation pathways in patient cells compared to control cells, suggesting a decrease or delay in differentiation and a lingering of ALK-R1275Q cells in a proliferative state.

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

The role of histone modifications in the osteosarcoma immune landscape

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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, a 3D spheroid model of osteosarcoma was generated to study infiltration 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 (TME) in H3K27ac high tumors. To confirm these results, we employed entinostat, a histone deacetylase 1-3 (HDAC) inhibitor to upregulate H3K27ac. Sub-therapeutic doses of entinostat significantly upregulated H3K27ac and primed tumors for higher infiltration of CD3+CD8+ T cells. Moreover, baseline H3K27ac levels in different cell lines correlated with improved CD3+ infiltration.

Together, these findings show that genome wide H3K27ac levels may be used as a biomarker for predicting survival in osteosarcoma. Furthermore, H3K27ac levels are associated with immune-related signatures and could be explored for novel treatment strategies. Additionally, increased H3K27ac levels by treatment with entinostat resulted in enhanced infiltration into tumor spheroids.

Keywords: Osteosarcoma, HDAC inhibitors, Immunotherapy

CDK2 loss induces neuronal differentiation in MYCN-amplified neuroblastoma

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Neuroblastoma (NB) is the most common extra-cranial solid tumor of childhood cancers. It arises from the neural crest and has been estimated to account for approximately 15% of cancer-related deaths in children. Amplification of the MYCN gene is detected in about 20% of all NB cases and is a marker of poor prognosis. Previous studies have suggested cyclin dependent kinase 2 (CDK2) to be a promising therapeutic target in NB with high MYCN expression. How CDK2 cooperates with MYCN in NB is not fully understood. In the present study, we uncovered a novel role of CDK2 as regulator of neuronal differentiation in NB. We demonstrate that genetic and pharmacological inhibition of CDK2 induces neuronal differentiation in MYCN-amplified NB cell lines. We show that CDK2 depletion reduces MYCN protein phosphorylation and MYCN protein levels and induces gene expression changes that mimic changes in response to MYCN depletion. Finally, we demonstrate that combinations of CDK2 and MYC/N inhibitors demonstrate synergistic anti-NB activity. In conclusion, this work sheds new insight on the role of CDK2 in NB and its functional interplay with MYCN and suggests that combination of inhibitors targeting CDK2 and MYCN could be a promising therapeutic approach for patients with high-risk NB with MYCN-amplification.

Keywords: CDK2, Neuroblastoma

Unraveling cellular crosstalk in liver metastases of gastrointestinal cancer: Multimodal analysis of metastatic invasion

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Background
The diagnosis of gastrointestinal cancer liver metastases (LM) characterizes an advanced disease stage with a 5-year overall survival below 10 %. Histologically, two major growth pattern of liver metastases have been described: (i) encapsulated tumors with a separation of liver and tumor by a fibrous rim and (ii) “replacement” type liver metastases. The latter is associated with a worse prognosis and characterized by direct cellular contact between tumor and liver parenchymal cells. This implies that cellular interactions in the tumor microenvironment are of uttermost importance for tumor aggressiveness.

Results
We have combined immunohistochemistry with multiplex immunofluorescence and multiplex RNA in situ hybridization to investigate the process of capsule formation in colorectal cancer LM patients from the KaroLiver cohort. We could show that the capsule is zonated with higher expression of stromal markers for benign fibrosis in the outer parts. In addition, we provided evidence that capsule formation is a reaction of failed tumor cell invasion in combination with perimetastatic liver injury.

In parallel, we used single-cell RNA sequencing (scRNAseq) and cellular interaction profiling to globally analyze the tumor microenvironment of murine LM. We could characterize liver parenchymal cell states associated with the perimetastatic injury and found novel tumor-liver interactions. The results were validated in LM patients from the KaroLiver cohort.

Discoveries & Future directions
Taken together we could show that LM induce a perimetastatic liver injury reaction which can, in areas of failed tumor cell invasion, trigger tumor encapsulation. Our data revealed potential regulatory roles of Il-6 and Jagged/Notch signaling in LM-associated liver injury and we will further investigate whether these signaling pathways can be leveraged to interfere with tumor growth.

Studies of adaptive NK cells in CMV and CMV-related tumors

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Natural Killer (NK) cells are innate immune cells able to reject hematological malignancies and correlate with better prognosis in solid tumors. However, conventional NK (cNK) cell anti-tumor activity is limited by the immune suppressive tumor microenvironment (TME). A newly discovered subset of NK cells, termed adaptive NK (aNK) cells have immunological memory and accumulate in cytomegalovirus (CMV) infected individuals. These aNK cells are able to resist immune suppression of the TME. Glioblastoma (GBM) is the most aggressive brain tumor that has very poor dismal prognosis. Our data suggest that aNK cells recognize tumor antigens. Using GBM spheroids, we demonstrate that NK cells can infiltrate and kill tumor cells, especially when loading DCs with GBM antigens. DCs were loaded with commonly mutated GBM-proteins and cocultured with NK cells to stimulate aNK cells responses. Our data show that IDH1 mutation stimulated aNK cells. Furthermore, we have discovered that aNK cells have a different metabolic profile than cNK cells, and when cNK cells are shut down in the TME when inhibiting glycolysis, aNK cells are still activated because they can use alternative metabolic pathways, such as the TCA cycle. aNK cells seem to have a great potential to use in therapy against GBM tumors, using a DC vaccine and boosting their metabolism in the TME.

Keywords: NK cells, Glioblastoma, tumor microenvironment

Galectin-3 regulates amino acid uptake and is essential for sonic hedgehog-driven medulloblastoma

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Galectins are a family of carbohydrate-binding proteins that are important for regulating cell-cell and cell-extra cellular matrix (ECM) interactions. Galectins are often found upregulated in solid tumors and have been shown to enhance cancer cell migration, invasion, immune evasion, and angiogenesis, and correlate with poor prognosis in many cancer types. We have previously developed a model for Sonic hedgehog (SHH)-driven MB using neural stem cells derived from reprogrammed PTCH1-mutant patient cells. Using our model, we identified a progressive upregulation of Galectin-1 and Galectin-3 with increasing MB malignancy. We have shown that Galectin-1 is a direct target gene of the SHH-pathway transcription factors GLI1 and GLI2, and a potential therapeutic target for SHH-MB (Susanto et al., PNAS, 2020). Here we further studied the biological role of Galectins in MB, focusing on Galectin-3.
We found that high Galectin-3 expression is specific to the SHH-MB subgroup compared to other MB subgroups, suggesting that Galectin-3 may play an important biological role in SHH-MB. We found that CRISPR/Cas9-mediated deletion of Galectin-3 in SHH-driven MB cells resulted in a significant reduction of proliferative, migratory, and neurosphere formation capacity. In addition, to further examine the potential of Galectin-3 inhibition as a novel treatment option for SHH-driven MB, we took advantage of an orthotopic zebrafish embryo MB model. Injection of the Galectin-3 KO cells showed decreased tumor viability compared to the control. This was further confirmed by orthotopic transplantations in mice. Galectin-3 KO cells were unable to form tumors *in vivo*, whereas all mice transplanted with WT cells developed tumors. RNA sequencing data revealed an extensive impact of Galectin-3 KO on transcriptomics level, associated with downregulation of numerous biological pathways involved in ECM remodeling and cell adhesion. Strikingly, many biological pathways involved in amino acid transport were also downregulated whereas starvation responses were upregulated upon Galectin-3 KO. Interestingly, we identified a global downregulation of SLC transporters important for uptake of essential metabolites. These effects seen on transcriptomics level were confirmed by LC-MS/MS metabolite profiling, which points towards a crucial role for Galectin-3 in amino acid uptake. Studies are ongoing to further decipher the molecular mechanism connecting Galectin-3 with SLC transporter expression.
Taken together, we have shown that Galectin-3 is essential for SHH-driven MB tumor formation and progression. Our results point towards a crucial role for Galectin-3 in amino acid uptake which makes Galectin-3 inhibition an attractive novel treatment option for SHH-driven MB.

Keywords: Medulloblastoma, Galectin-3

The role of IL40 in the investigation of shared B-cell activation and selection in development of lymphoma and rheumatoid arthritis

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Autoimmune diseases are associated with a greater risk of lymphoma development. For rheumatoid arthritis (RA) the risk of developing lymphoma is estimated to be 2-fold compared to the general population. However, among autoantibody positive RA patients with high disease activity, the risk of developing diffuse large B cell lymphoma (DLBCL) has been described to be up to 90-fold. While the association between lymphoma and RA is well established, the underlying biological and molecular mechanisms responsible remain to be elucidated. B cell activation and the BCR specificity are hypothesized to play an important role in lymphoma development.

The objective of our study is to investigate shared B-cell activation pathways in RA and B cell lymphoma (BCL). In total 35 RA patients with DLBCL are included from the nationwide Auto - Lymphoma" study in Sweden. PBMC, serum, plasma samples have been obtained at four different time points, at time of lymphoma diagnosis and after treatment (t=0, t = 6, t=12, t=24, in months). In addition, we also have access to cryopreserved- and FFPE DLBCL tissue from RA patients.

Previous studies have shown that the novel cytokine IL40 is significantly increased in the serum of RA patients compared to healthy controls and patients with osteoarthritis. IL40 has also been shown to be produced constitutively in DLBCL cell lines. IL40 ELISA on plasma from RA patients with and without lymphoma, demonstrated significant increase of IL40 in RA Lymphoma patients ($p < 0.01$). In addition, we found increased level of IL40 in 'Early RA' patients compared to 'Established RA', which in consensus with previous studies can likely be explained by differences in treatment. Furthermore, we found an inverse correlation between IL40 and anti-CCP2 IgG levels in 'RA Lymphoma' patients at baseline ($R = -0.43$, p-value = 0.02) and for RF IgG among RF IgG+ subset ($R= 0.65$, p-value = 0.02). Further studies are needed to determine which cell subsets that are responsible for the elevated IL40, and which role this novel cytokine may play in Rheumatoid arthritis and lymphoma.

Exploring small extracellular vesicles from EML4-ALK driven Non-small cell lung cancer for ALK-TKI response and resistance

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Background: Precision cancer medicine (PCM) targeting oncogenic drivers is an important treatment regimen for non-small cell lung cancer (NSCLC) patients with a tumor driven by an EML4-ALK fusion. In these patients the constitutively active ALK kinase are targeted by tyrosine kinases inhibitors (ALK-TKIs). Albeit impressive responses are seen, a substantial number of patients relapses calling for ways to monitor treatment response in a non-invasive way. Small extracellular vesicles (sEVs)/exosomes released from tumor to blood may be such an approach as they carry protein and RNA from their cell of origin. We studied how the ALK-TKI lorlatinib altered the protein cargo of an EML4-ALK positive cell line and their released sEVs to point out possible response biomarkers. Some of these were validated in sEVs isolated from pleural effusion (PE) fluid of NSCLC patients with an EML4-ALK positive tumor.
Material and Methods: The EML4-ALK v.1 DFCI032 cells were treated with lorlatinib and effect on cytotoxicity and apoptotic cell death examined. ALK expression was studied by immunocytochemistry (ICC) and western blot (WB). sEVs were isolated from cell culture media using size exclusion Izon's qEVoriginal gen2, 70nm columns and characterized by Nanoparticle Tracking Analysis (NTA). Proteins were extracted from cells and corresponding sEVs and were subjected to peptide-based mass spectrometry profiling (MS) at the Biomedicium protein facility, KI. Protein signalling networks were explored by STRING and Qlucore bioinformatics. sEVs from PE-fluid of ALK-positive NSCLC tumours were profiled by proximity extension assay (PEA), on Immuno-Oncology® and Oncology II® panels. Immunofluorescence single vesicle analysis was carried out to validate some of the identified proteins.
Results: Cells responded to lorlatinib treatment with reduced cell viability and induction of apoptosis. NTA profiling of their corresponding sEVs showed no alteration in particle size or amount after lorlatinib treatment while WB confirmed endosomal origin. MS-based profiling of cells and sEVs revealed over 1500 proteins in each compartment. In the sEVs around 100 proteins were in total regulated by lorlatinib. STRING analyses revealed alterations in the epithelial to mesenchymal transition (EMT) regulated proteins VIM, CDH2, some TKI bypass drivers e.g., EPHA2 and SRC as well immune signaling components. Single vesicle analyses confirmed expression of EPHA2 and PD-L1 expression. PEA profiling identified EPHA2, CD73, and PD-L1also in sEVs from PE-fluid.
Conclusion: Protein profiling of sEVs can reveal biomarkers for non-invasive profiling of NSCLC patients during a clinical ALK-TKI treatment course.

Keywords: non-small cell lung cancer, extra cellular vesicles, ALK-TKI, response biomarkers

Combination of PARP and WEE1 inhibitors *in vitro*: Potential for use in the treatment of SHH medulloblastoma

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Medulloblastoma (MB), grouped as either WNT-activated, Sonic hedgehog (SHH)-activated, or non-SHH group 3 or group 4, accounts for almost 20% of all childhood brain cancers. In spite of current intensive treatments, not all patients are cured and survivors suffer from severe side-effects.

The present study therefore examined the effects of the poly-ADP-ribose polymerase (PARP) and WEE1-like protein kinase (WEE1) inhibitors, BMN673 and MK-1775, respectively, alone or in combination on four MB cell lines. More specifically, the MB cell lines, DAOY, UW228-3, MED8A and D425, were tested for their sensitivity to BMN673 and MK-1775 alone or in combination, using cell viability, cell confluency and cytotoxicity assays. The effects on the cell cycle phases were also examined using FACS analysis. 3D cultures will be established soon in order to validate the 2D data.

Monotherapy with BMN673 and MK-1775 exerted dose-dependent inhibitory effects on the viability of almost all MB cell lines. Notably, when BMN673 and MK-1775 were used in combination, synergistic effects were noted in the SHH group cell lines (DAOY and UW228-3), but not in the already WEE1-sensitive group 3 (MED8A and D425) lines. Moreover, the combination treatment decreased the percentage of cells in the G1 phase and induced the novel distribution of both DAOY and UW228-3 cells in the S and G2/M phases, with the UW228-3 cells exhibiting a greater delay.

To conclude, MK-1775 was efficient in all and BMN673 in most cell lines, and their combined use exerted synergistic effects on the SHH, but not the group 3 cell lines. These data suggest that MK-1775 alone may be of interest for all MB cell lines, and that the combination of PARP/WEE1 inhibitors may provide possible therapeutic opportunities for the therapy of SHH MBs. Their use warrants further investigations in the future.

Keywords: medulloblastoma, BMN673, MK-1775

Trace elements Se, Cu, and Zn modulate the response of cancer cells to drugs *in vitro* by targeting thioredoxin reductase

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Selenium (Se), copper (Cu), and zinc (Zn), among other trace elements, are essential minerals for human health. They play crucial functions as parts of necessary transcription factors and enzymes, including those involved in cellular antioxidant defense systems like thioredoxin reductase (TXNRD). However, these trace elements can act as pro-oxidants and cause oxidative stress if they are present in high levels or circulating as free ions. Cellular redox equilibrium can be disturbed by the complex interactions of trace elements with essential transcription factors and enzymes, which can then affect redox-dependent signaling pathways and control the fate of individual cells. Since cancer often shows accelerated metabolism, increased cell replication, and activation of NADPH oxidases, resulting in increased levels of hydrogen peroxide and other oxidizing agents, the dual role of trace elements becomes more important in the context of this disease.

Based on this context, we decided to study the potential impact of Se, Cu, and Zn on the resistance or susceptibility of cancer cells to specific treatments, given their capacity to modify the redox status of the cell.

Due to their high endogenous TXNRD1 (also known as TrxR1) expression, human lung carcinoma cells (A549) were used. Cells were incubated with different Cu and Zn concentrations in the presence or absence of Se. Cells were treated with LD50 doses of auranofin, RSL3, ML210, ML162, erastin, heme, and curcumin for 24 h, and cell viability was then measured. The role of TRP14 –an enzyme from the thioredoxin family– was studied using the same experimental setup in TRP14-knockout cells. Furthermore, TrxR1 activity was assessed using the novel fluorescent probe RX1 in intact living cells.

Thioredoxin reductase 1 (TrxR1) was shown here for the first time to be inhibited by both Cu and Zn in a cellular context. However, pre-treatment with Se could counteract the inhibitory effects of Cu and Zn on TrxR1. Additionally, the presence of Cu, Zn, or a combination of both increased the toxicity of some drugs, while Se pre-treatment prevented cell death upon drug addition in some other cases. Notably, the absence of TRP14 conferred resistance against several drugs when compared to wild-type cells, emphasizing the complex interplay between trace elements, drug toxicity, and redox signaling in the treatment of a complex and multifactorial disease such as cancer.

Keywords: *Trace elements, thioredoxin reductase*

Characterization of gamma delta T cell subsets allows prediction of allogeneic hematopoietic stem cell transplantation outcome

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Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative therapy for most patients diagnosed with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). A major complication of this therapy is graft-versus-host-disease which leads to severe tissue damage and death. γδ T cells, a rare subpopulation of T cells with innate and adaptive properties, have been shown to participate in anti-cancer immune responses. However, studies investigating their reconstitution and role after HSCT reveal contradictory results. In addition, the specific role of γδ T cell subsets in this context is understudied.
In this study, we aim at characterizing the reconstitution of γδ T cells after HSCT, in relation to the occurrence of acute graft-versus-host-disease (aGvHD). Blood samples from AML and MDS patients undergoing HSCT were collected before and 1, 2, 3, 6 and 12 months after transplantation. Samples were analyzed in flow cytometry. NGS was performed on the γ chain of the retrieved γδ T cells to analyze clonal evolution and diversity.
We observe specific differences of γδ T cell subsets between aGVHD and non-aGvHD patients. No change in the Vδ1 frequency was observed in patients with severe aGvHD over time, while interestingly the Vδ1 frequency increased in patients with no or mild aGvHD. This indicates a possible protective role of the Vδ1 subset. Moreover, a larger effector memory Vδ2 population is observed in aGvHD patients compared to non-aGvHD patients, at the expense of the central memory Vδ2 population. Last, CX3CR1 was identified as a marker for aGvHD in all T cell populations, especially in the earlier timepoints.
The reconstitution of γδ T cell subsets after HSCT seems to influence aGvHD. These results could have meaningful impact for the prediction of HSCT outcome and for the development of better transplantation techniques.

Keywords: Gamma Delta T cells, Hematopoietic Stem Cell Transplantation.

Analysis of plasma-isolated extracellular vesicles – a way to predict treatment response in immune checkpoint inhibitor treated metastatic non-small cell lung cancer patients?

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Background: Liquid biopsies for non-invasive analysis of biomarkers (BMs) is highly needed for metastatic non-small cell lung cancer (NSCLC) patient treatment monitoring since tissue biopsies are difficult to obtain and different metastatic lesions show great heterogeneity in signaling. One way is to analyse small extra cellular vesicles (sEVs) isolated from plasma prior and post therapy. sEVs are important communicators for tumors with their adjacent- and distant microenvironment including the immune system and contain RNA, miRNA, DNA and proteins partly reflecting their cell of origin. We have isolated sEVs from plasma of NSCLC patients at Karolinska prior start of immune checkpoint inhibitor (ICI) pembrolizumab treatment. Our aims are to to identify protein biomarker (BM) signature related to ICI treatment response as well as to PD1 and PD-L1 expression in sEVs.

Material and Methods: sEVs were isolated from 0.5 ml EDTA plasma of NSCLC patients (n=15) prior ICI therapy with the PD1 inhibitor pembrolizumab, alone or in combination with chemotherapy at Karolinska University Hospital (PI: Dr L. De Petris). The Izon's qEVoriginal gen 2, 70nm columns were used for the isolation and sEVs concentration and size were examined using Nanoparticle Tracking Analysis (NTA). Proteins were extracted from sEVs and profiled using proximity extension assay (PEA), on Immuno-Oncology® and Oncology II® panels. Qlucore® Omics Explorer was used for data analysis and visualization. Western blotting and ELISA were applied to characterize sEV markers and for validation of putative BM protein profiles.

Results: The median size of the sEVs were around 90 nm with concentrations ranging 1.5x 10^{10} to 1.4 x 1011particles/ml plasma. The sEVs expressed the exosome markers CD9 and TSG101. PEA protein profiling revealed that both oncogenic and immune signaling proteins were expressed over RIPA-negative control with heterogeneity seen among individual samples. Protein signatures that correlated to genomic makeup of the tumor, PD-L1 expression level, survival- and treatment response were evaluated. We also looked on PD1 and PD-L1-associated protein cargo.

Conclusion: We found that sEVs isolated from small amount of EDTA-plasma is a possible source of BMs. Multiple protein signatures rather than individual protein profiling may allow for non-invasive BM profiling of NSCLC patients when given ICI pembrolizumab alone or combined with chemotherapy.

Keywords: non-small cell lung cancer, immune checkpoint inhibitors, extracellular vesicles, liquid biopsies, biomarkers

The ACROBAT 2022 Challenge: Automatic Registration Of Breast Cancer Tissue

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Introduction
WSI registration is an enabling technology both for research and diagnostics. The ACROBAT 2022 challenge aimed to evaluate image registration algorithms that align WSIs of differently stained histopathological slides that originate from routine diagnostics.

Materials and Methods
The data set that was published for the challenge consists of 4,212 WSIs of resection specimens from 1,153 breast cancer patients, which exceeds previous WSI registration data sets by one order of magnitude. For each patient, one H&E WSI is available. The training set consists of 750 cases (3,406 WSIs), with one to four IHC WSIs each from the routine diagnostic stains ER, PGR, HER2 and KI67. The H&E WSIs in the validation set (100 cases, 200 WSIs) and test set (303 cases, 606 WSIs) are paired with one randomly selected IHC WSI each. 13 annotators generated ca. 37,000 pairs of corresponding landmarks in the validation and test set image pairs. Within each image pair, the 90th percentile of distances between registered and annotated landmarks was computed. Participants were then ranked on the median of these 90^{th} percentiles.

Results
Median 90th percentiles for eight teams that were eligible for ranking in the test set ranged from 60.1 µm to 15938.0 µm. The best performing method therefore has a score slightly below the median 90th percentile of distances between first and second annotator of 67.0 µm.

Conclusions
The ACROBAT 2022 challenge contributed to establishing the state-of-the-art in WSI registration under realistic conditions. Top-performing methods exceeded our expectations regarding robustness and precision and will enable future avenues of research.

Computational Analysis for Investigating Intra-tumoral Heterogeneity in Breast Cancer

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Intra-tumoral heterogeneity occurs on different levels, like genomic and transcriptomic levels, and causes a high level of recurrence and metastasis in breast cancer (BC). In order to investigate how this variability could influence treatment and prognosis in BC, we dissected 32 topographic tumor regions from freshly resected large breast tumors and two regions from the lymph nodes of seven patients. Tissues from these regions all went through homogenization, followed by drug screening, flow cytometry analysis, and high-throughput sequencing. Dimensionality reduction techniques clustered samples by patient and tissue of origin. Preliminarily results showed different levels of intra-tumoral heterogeneity score and immune cell distribution. Also, somatic variants varied across tumor regions within patients. We further found that regions from the same tumor presented divergence in subtype classification. Correspondingly, regions showed unequal ability to respond to drugs and pathway activation. Committed to integrating results from *ex vivo* experiments and bioinformatic analysis, we hope to go beyond pure single-level analysis and interpret the multi-omics results in a machine-learning-driven method. Based on this study, we try to decipher the intra-tumoral heterogeneity of BC better and provide advanced insights into mechanisms behind tumor progression, drug resistance, and metastatic spreading in BC.

Keywords: breast cancer, intra-tumoral heterogeneity

Targeting the replication stress response in neuroblastoma

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Replication stress (RS) is an early driver of tumorigenesis that has been associated with activation of oncogenes such as MYC and Cyclin E. Several studies provide evidence that the vulnerability of cancer cells to oncogene-induced replication stress (OIRS) can be enhanced by targeting components of DNA replication and repair pathways. We have recently demonstrated a pivotal role of the ubiquitin ligase FBXL12 as critical regulator of replication-stress tolerance in cancer cells. In response to OIRS, FBXL12 facilitates the clearance of FANCD2, a key component of Fanconi Anemia (FA)/homologous recombination (HR) DNA repair pathway, through ubiquitin-mediated proteasomal degradation of CHK1-phosphorylated FANCD2 at stalled replication forks. Consequently, in the absence of FBXL12, FANCD2 becomes trapped at replication forks, resulting in fork collapse and DNA damage, ultimately inducing senescence and cell death in cancer cells experiencing high levels of RS (Brunner et al).
In this study, we characterized FBXL12-FANCD2 signaling in Neuroblastoma (NB), a cancer distinguished by the presence of MYCN amplifications and disruptions in the normal functioning of DNA repair pathways. Our experimental and pre-clinical data support a functional link between FBXL12-FANCD2 signaling and NB tumorigenesis. First, *FBXL12* is an independent prognostic factor in multiple NB cohorts, and expression of *FBXL12/FANCD2* strongly correlates with activation of DNA replication/repair and MYC target genes. Second, *FBXL12* predicts poor patient outcome in both MYCN-amplified and 11q-deleted NB making FBXL12 a potential target in both MYCN-overexpressing and DNA repair deficient cancers. Third, *FBXL12* and *FANCD2* are induced during NB progression in TH-*MYCN* mice as compared to ganglia in control mice, arguing for a critical function of the FBXL12-FA pathway during NB progression.
Since activation of replication stress/DNA repair tolerance pathways antagonize DNA damage-induced activation of anti-tumor immune cells, and NB tumors are so called “cold” tumors largely devoid of infiltrating inflammatory cells, we hypothesize that targeting FBXL12-FANCD2 signaling has the potential to convert these immunologically "cold" tumors into "hot" tumors, potentially sensitizing NB cells to immune checkpoint inhibitors.

Keywords: FBXL12, Neuroblastoma

Small polyanions improves transduction efficiency of enveloped viruses

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Viral vectors have been extensively used in gene therapy applications. However, some challenges remain due to low viral transduction efficiency on certain tissues and cell types. To address this issue, many additives have been applied to reduce the toxicity and enhance the transduction efficiency such as polymers, lipids, peptides and polycationic compounds. It was known that polycationic additives such as protamine sulfate and polybrene enhance the transduction efficiency, whereas anionic molecules such as pyran and heparin inhibit the transduction efficiency. However, in this study, we found that when we used lower concentrations of heparin and its lower molecule analogues, we observed a significant increase in transduction efficiency in immune cells. These findings encourage us to conduct more investigations to understand the function that these compounds play in enhancing the viral uptake and the transduction efficiency.

Keywords: Transduction efficiency, Heparin

Inhibition of Teneurin-4 promotes differentiation and suppresses neuroblastoma growth

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Neuroblastoma (NB) originates from the neural crest cells resulting in impaired neural differentiation. Teneurins (TENM1-4) are cell adhesion molecules that are highly expressed during embryonal development and function in differentiation. Somatic mutations and structural aberrations of *TENM* genes have been identified in NB. High-risk NB presents significant clinical challenges, and further therapeutic options are needed. We aim to identify the significance of *TENM4* in NB growth, tumorigenicity, and differentiation. TENM4 immunohistostaining was performed on NB patient tumors. Genetic inhibition was mediated by siRNA and CRISPR-Cas9 in NB cells to analyze the effects on morphology, proliferation, tumorigenicity, and molecular signaling through transcriptomics, proteomics, and quantification of gene expression. We examined primary NBs and detected a significantly higher protein and mRNA expression level of *TENM4* in high-risk vs. non-high-risk and *MYCN*-amplified vs. non-*MYCN* amplified tumors. Moreover, tumors positive for TENM4 protein were associated with poor patient outcome. siRNA-mediated knockdown of *TENM4* significantly decreased proliferation in all investigated NB cell lines. Two *TENM4*$^{-/-}$ clones from the CRISPR-Cas9 gene-edited SK-N-BE(2) were uncovered; both clones demonstrated neuronal differentiation-like morphology with impaired clonogenic capacity and reduced proliferation compared to wild-type cells. Using RNA-Seq, qPCR, and proteomics, we characterized NB cell responses of inhibited *TENM4*, identifying key components as induced differentiation, inhibited cell cycle progression, and mTOR signaling together with metabolic changes as TENM4 targets in NB cells. More specifically, genes associated with neuronal differentiation, such as *SCG2* and *NGFR* were upregulated after the siRNA knockdown of *TENM4*. Similar changes were observed in the *TENM4*$^{-/-}$ clones. Also, protein levels of SCG2 were upregulated in all *TENM4*-inhibited cells. Furthermore, both *MYCN* and the Schwann cell precursor marker *ERBB3* were downregulated in the *TENM4*-inhibited cells compared to their controls. Finally, *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. Our data suggest that TENM4 is expressed in a subpopulation of NBs with *MYCN* amplification and plays an important role in NB growth and differentiation. TENM4 could be a potential target in NB therapy.

Keywords: Neuroblastoma, Teneurin-4

Spatially dependent heterogeneity in pancreatic ductal adenocarcinoma

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The 5-year overall survival of pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, has stagnated at 11%. Two main subtypes of PDAC has been unraveled by bulk – and single cell RNA sequencing, the basal-like and the classical. While the classical subtype is characterized by a less frequent mutated *KRAS* allelic imbalance and expression of pancreas lineage markers such as GATA6, the basal-like suptype is known for its proneness for epithelial-to-mesenchymal transition and a worse prognosis. However, the intratumor heterogeneity is high, and classical and basal-like clones frequently co-exist in the individual tumor.

However, the molecular background of this aforementioned heterogeneity in PDAC is unknown. 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 stratifying between tumor invading the stroma *versus* pancreatic lobules. We found that PDAC expression, or subtype state, can depend on local microenvironment properties at the invasion front. Notably, basal-like expression (positive for Keratin 17 and Keratin 5) was upregulated at stromal invasion, while the classical expressing tumor cells (positive for Mucin 5) 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. We now seek to evaluate this spatially dependent heterogeneity model in an in vivo system, and explore the possibilities for clinical implementation.

Keywords: Pancreatic cancer, immunohistrochemistry

Mixed response after radiopharmaceutical therapy with 177Lu-DOTATATE in a refractory high-risk neuroblastoma patient – early insights into the LuDO-N Trial

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Background
A 21 months old, previously healthy female was diagnosed with high-risk neuroblastoma with widespread skeletal and bone marrow metastasis. Induction therapy was initiated according to the SIOPEN/HR-NBL1 protocol followed by 4 courses of topotecan/vincristine/doxorubicin (TVD) and three courses of temozolomide/irinotecan (TEMIRI) due to persistent bone marrow involvement. This was followed by radioisotope (...I-mIBG) therapy with concomitant topotecan and stem-cell rescue prior to high-dose chemotherapy (busulfan-melphalan) with stem-cell rescue. Due to progression of the distal femoral metastasis, the patient was screened for inclusion in the LuDO-N Trial.

Methods
^{68}Ga-DOTATOC PET/CT imaging was performed to confirm trial inclusion criteria as well as at 1 and 2,5 months after end of treatment with .^{77}Lu-DOTATATE. Dosimetry was performed by 4 serial SPECT/CT scans during days 1-7 after each of the two treatment cycles. SNP microarray and post-hoc analysis with SSTR-2 immunohistochemistry (IHC) was performed on the primary tumor and the distal femoral metastasis that eventually progressed.

Results
Treatment with .^{77}Lu-DOTATATE was well tolerated. ^{68}Ga-DOTATOC PET/CT imaging showed uptake in the primary tumor and at multiple metastatic lesions. The uptake of .^{77}Lu-DOTATATE resulted in the delivery of 54 Gy of radiation to the primary tumor, but only 2 Gy to the distal femoral metastasis. Response evaluation at 1 month showed a stable disease with clinical improvement. At 2.5 months, however, the patient developed a pathological fracture at the metastatic site in the distal femur. IHC of the metastatic tumor tissue showed overall weak SSTR-2 expression. Sequencing revealed a KIAA1549::BRAF fusion, that was not present in the primary tumor at diagnosis. A request for compassionate use of with pan-RAF inhibitor tovorafenib was filed, however the disease progression was rapid, and the patient died of the disease before treatment could be initiated.

Conclusion
We report a patient with refractory high-risk neuroblastoma, presenting an early response to .^{77}Lu-DOTATATE at 1 month followed by a mixed response and local progression of the distal femur metastasis at 2.5 months. Further analysis of the progressed metastatic tissue revealed poor SSTR-2 expression and a novel KIAA1549::BRAF fusion acquisition, possibly contributing to the resistance to the radiopharmaceutical therapy 177Lu-DOTATATE.

Keywords: Neuroblastoma, Radiopharmaceutical Therapy

Efficacy of combined targeted therapy with PI3K and CDK4/6, or PARP and WEE1 inhibitors in neuroblastoma cell lines

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Neuroblastoma (NB), the most frequent solid extracranial tumor in children, is in spite of current aggressive therapies with heavy adverse effects not always cured, so novel treatments are necessary. Recently, when combining phosphoinositide 3-kinase (PI3K) and fibroblast growth factor receptor inhibitors we revealed synergistic effects in NB cell lines. Here this approach was extended.

Single treatments and combinations of Food and Drug Administration (FDA)-approved PI3K, Cyclin-Dependent-Kinase-4/6 (CDK4/6), Poly-ADP-ribose-polymerase (PARP) and WEE1 inhibitors (BYL719, PD-0332991, BMN673 and MK-1775 resp.), were used on NB cell lines SK-N-AS, SK-N-BE(2)-C, SK-N-DZ, SK-N-FI and SK-N-SH and viability, proliferation and cell cycle changes were followed. Furthermore, 3D cultures are under establishment and so far spheroids have been established with SK-N-BE(2)-C, SK-N-SH and SK-N-DZ NB cell lines and soon experiments with the best 2D drug combinations will be validated in 3D cultures.

Treatments with single drugs presented dose dependent responses with decreased viability and proliferation and combining BYL719 with PD-0332991 or BMN673 with MK-1775 resulted in synergistic effects in many cell lines. Moreover, combining MK-1775 and BMN673 decreased the numbers of cells in S-phase much more than using either drug alone, while the effect of PD-0332991 dominated in the PD-0332991 and BYL719 combination.

To summarize, our data disclose that combining PI3K and CDK4/6 or PARP and WEE1 exhibited synergistic anti-NB effects and further evaluation of the 2D data will be performed in 3D grown cells lines and *in vivo in mouse models*. Therefore, lower doses of the inhibitors could be utilized, thereby potentially reducing the risk for adverse side effects.

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Keywords: Neuroblastoma, targeted therapy

The inhibition of mitochondrial RNA polymerase as a novel cancer treatment

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Mitochondria exhibit profound influence over cellular energy production, metabolism and cell death. As such, it is unsurprising that there is accumulating evidence for a crucial role for mitochondria in the onset, development and treatment of most cancers. As tumors are almost always characterised by a highly-proliferative phenotype, which requires extensive cellular energy production, targeting drugs to inhibit mitochondrial processes may be a powerful and, as yet, untapped therapeutic tool. The first-in-class specific inhibitors of mitochondrial transcription (IMTs) are an example of such a therapeutic approach. By inhibiting the mitochondrial RNA polymerase (POLRMT), IMTs impede the biogenesis of the oxidative phosphorylation (OXPHOS) system and induce a cellular energy crisis. Elevated POLRMT levels have been identified in biopsies from several cancer types including acute myeloid leukaemia and non-small cell lung cancer, however, elevated levels of POLRMT is associated with improved survival in patients with endometrial cancer. To better understand POLRMT function, we used a Kras based cancer mouse model crossed a panel of POLRMT mouse models: POLRMT overexpression, POLRMT heterozygous knock-out and a *de novo* dominant POLRMT mutation in the N-terminal domain which was identified in patients. We also generated mouse embryonic fibroblasts (MEFs) from these models to better investigate the molecular mechanisms involved.

Keywords: Mitochondria, Lung Cancer

Dissecting the anticancer properties of Actinomycin D

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Actinomycin D (ActD) is the first antibiotic to have been used in cancer therapy, currently used as a first line chemotherapeutic in various cancer types such as Wilms Tumor and Ewing's Sarcoma at concentrations with ambiguous mechanisms of action. Its anticancer activity originates from its capability to intercalate into GC-rich regions and stabilize topoisomerase- I DNA complexes that prevent RNA polymerase-associated transcription elongation. Even though most DNA intercalators block preferentially polymerase I (pol I) transcription, it has been shown that ActD blocks all three RNA polymerases in a dose-dependent manner starting from pol I at a low dose and gradually blocking pol II and III when administered in higher doses. Using a low and a high concentration of ActD, we hereby characterize its multiple mechanisms of action and broaden its anti-cancer potential. We provide evidence that ActD dosage is an essential factor to consider in cancer treatment following patient profiling according to their ribosome biogenesis status. Our data pinpoint the multiple roles a single chemical inhibitor can have and support the concept of dose redirecting (optimizing, adjustment) based on the metabolic profiling of cancer patients.

Keywords: Actinomycin D, Cancer.

USP39 controls pyruvate handling and tumor growth in NSCLC

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Lung cancer remains the most common cause of cancer-related deaths worldwide despite the recent advances in the development of oncogene-targeted therapies. These treatments are highly effective, however rarely curative, and only about a quarter of lung cancer patients have identifiable targets. This highlights the need for developing new treatments to improve patient care and reduce the mortality rate.
To provide the necessary energetic and biosynthetic demands cancer cells upregulate the required metabolic pathways facilitating their rapid growth and proliferation. We have identified the deubiquitinase USP39 as a new regulator of cancer cell metabolism and tumor growth in non-small cell lung cancer (NSCLC). High USP39 expression correlated with poor prognosis in NSCLC patients, and cancer cell growth was reduced upon USP39 knockdown both *in vitro* and *in vivo*. Mechanistically, we found that USP39 directly regulates the pyruvate dehydrogen-ase (PDH) complex and thereby redirects the metabolic fate of pyruvate towards conversion to acetyl-CoA. Overall, our study provides evidence for a new role of USP39 in cancer metabolism and provides a novel mechanism behind NSCLC's rapid proliferation. Thus, USP39 may present a potentially valuable therapeutic target for cancer treatment, which remains to be fur-ther explored.

Combining specific PI3K, FGFR, CDK4/6, PARP, and WEE1 Inhibitors or Corresponding single inhibitors with Radiotherapy in HPV Positive and Negative Tonsillar Squamous Cell Carcinoma Cell Lines Reveals Synergistic Effects

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Background and aim. Human papillomavirus positive (HPV+) tonsillar and base of tongue cancer (TSCC/BOTSCC) is rising in incidence, but chemoradiotherapy is not curative for all and moreover it comes with serious acute and chronic side effects, so novel therapeutic options could be of use. Since, we have recently disclosed that HPV+ TSCC/BOTSCC frequently display PI3K and FGFR3 mutations, we wanted to investigate whether targeted therapies could be of potential use for our patients.

Design. To explore and disclose novel therapeutic options, in our lab the effects of targeted therapy with PI3K (BYL719), FGFR3 (JNJ-42756493), CDK4/6 (PD-0332991), PARP (BMN-673), and WEE1 (MK-1775) inhibitors alone or combined was investigated in TSCC/BOTSCC cell lines grown as monolayers (2D). In addition, targeted therapy with PI3K (BYL719), PARP (BMN-673), and WEE1 (MK-1775) inhibitors alone or combined was also been pursued with or without 10 Gy. The best combinations in 2D grown cell lines will then be validated in TSCC/BOTSCC cell lines grown as spheroids (3D) and *in vivo*.

Material and Methods. More specifically, the effects of the above single inhibitor, inhibitor/inhibitor combinations or inhibitor/10 Gy combinations were analyzed by viability, proliferation, and cytotoxicity assays on various TSCC/BOTSCC cell lines such as e.g. HPV+ UPCI-SCC-154, CU-OP-2, 3 and 20 and HPV- UT-SCC-60A and CU-OP17 all grown as monolayers (2D).

Results. Single inhibitors all induced dose dependent effects. Furthermore, combinations of BYL719 with JNJ-42756493 or PD-0332991 presented synergistic effects. In addition, BYL719, BMN-673, and MK-1775 treatments induced when combined with 10Gy synergistic responses in HPV+ UPCI-SCC-154 and HPV- UT-SCC-60A. Moreover BYL719/BMN-673, BYL719/MK-1775, or BMN-673/MK-1775 combinations on HPV+ UPCI-SCC-154 and HPV- UT-SCC-60A also induced synergy compared to single drug administrations but adding 10 Gy to these synergistic drug combinations had no further major effects.

Conclusion. To summarize, synergistic effects were disclosed when combining e.g. BYL719/JNJ-42756493, BYL719/PD-0332991 BYL719/BMN-673 or BYL719/MK-1775 or complementing single BYL719, BMN-673 and MK-1775 administrations with 10 Gy, while adding 10 Gy to combinations of the latter three did not further enhance their already additive/synergistic effects. The next step will now be to validate the above best combinations in 2D grown cell lines in corresponding 3D grown cells lines and *in vivo*.

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Keywords: HPV, head and neck cancer, targeted therapy

Progesterone Receptor Modulator: Novel Avenues in Breast Cancer Prevention

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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.

Real world evaluation of the Prosigna/PAM50 test in a node-negative postmenopausal Swedish population: A multicenter study

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Molecular signatures to guide decisions for adjuvant chemotherapy are recommended in early HR-positive, HER2-negative breast cancer. The objective of this study was to assess what impact genomic testing with Prosigna has had following its recommendation by Swedish national guidelines. Postmenopausal women with HR-positive, HER2-negative and node negative breast cancer at intermediate clinical risk were identified retrospectively from five Swedish hospitals. Tumor characteristics, results from Prosigna test and final treatment decision were available for all patients. Treatment recommendations were compared with the last version of regional guidelines before the introduction of routine genomic testing. Among the 360 included patients, 41% had a change in decision for adjuvant treatment based on Prosigna test result. Out of the patients with clinical indication for adjuvant chemotherapy, 52% could avoid treatment based on results from Prosigna test. Contrary, 23% of the patients with no indication were escalated to receive treatment after testing. Ki67 was not significantly different between the different risk groups, intrinsic subtypes or between groups in which treatment was changed after Prosigna testing. In conclusion, we report the first real-world data from implementation of genomic testing in a Swedish context, which may facilitate the optimization of future versions of the national guidelines.

Keywords
Adjuvant chemotherapy; breast cancer; Prosigna; PAM50, gene expression signature; genomic test; impact

MNT EFFECTS ON CELL MIGRATION AND DNA REPAIR THROUGH CCDC6 INTERACTION

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Introduction
The MXD family is composed of transcription factors that act as dimers with MAX. It includes MNT, which modulates the activity of MYC, a frequently deregulated oncogene in human cancer. We explored new biological functions of MNT by studying the effect of MNT knockout on the transcriptome. Next, through proteomic analysis, we discovered a new interaction between MNT and coiled-coil domain-containing protein 6 (CCDC6), implicated in DNA damage response and fusion genes in cancer. Given their relevance in cancer, our aim to study the effect of MNT and CCDC6 knockout, their physical interaction, and its biological consequences.
Material and Method
To analyze the biological effects of MNT knockout, we used the HAP1 cells, derived from human myeloid leukemia. We performed RNA-seq using the Illumina platform and analyzed the datasets with Cufflinks, DESeq2 and RNA eXpress. Additionally, we analyzed the effect of MNT knockout on cell proliferation and migration. MNT interactome was analyzed using Affinity Purification-Mass Spectrometry in two different rat pheochromocytoma cell lines, without and with inducible MAX. MNT-CCDC6 interaction was confirmed by co-immunoprecipitation (Co-IP) in different cell lines, and the domains involved in the interaction were studied using deletion mutants. The subcellular localization of the complex was determined using subcellular fractionation assays followed by Co-IP and confirmed through Proximity Ligation Assay using confocal fluorescence microscopy.
Results and discussion
Analysis of the RNA-seq data in wild-type and in MNT-deleted cells showed that MNT could play a role as both a transcriptional repressor and activator in HAP1 cells. The crossed analyses with three different bioinformatic tools revealed that MNT knockout changes the expression of 460 genes in HAP1 cells. These included the upregulation of *THBS1* (Thrombospondin 1), which inhibits angiogenesis and stimulates cell migration. Consistently, HAP1 cells lacking MNT also showed increased migration and proliferation. We identified an interaction between MNT and CCDC6, which mainly takes place in the cytoplasm, and found that CCDC6 knockdown increased the resistance to DNA damage. Co-IPs with deletion mutants suggest that the interaction needs, at least, the 101-223 amino acids of CCDC6, together with the N-terminal domain of MNT. Together, these findings suggest that MNT may act as a tumor suppressor via its interaction with CCDC6. Experiments are underway to elucidate the mechanisms involved.
Conclusion
We have discovered MNT-CCDC6 interaction, which opens a new path to the understanding of MNT´s functions and its effects as a modulator of MYC-mediated oncogenic activity.

Keywords: MNT, CCDC6.

The regional differences along the esophageal longitudinal axis

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The esophageal epithelium has been thought of as a homogeneous organ along the longitudinal axis. However, previous work in our lab showed that the esophagus differs in various aspects axially, such as the gene expression profile in the esophageal epithelium, organoid-forming ability, signaling between the epithelium and the fibroblasts, and so on. Furthermore, the incidence of esophageal adenocarcinoma and squamous cell carcinoma is higher in the distal region of the esophagus than that in the proximal part. This evidence suggests that, instead of a homogenous organ, the esophagus varies along the axis. To further dissect this, we focused on the esophageal epithelium, where the stem cells reside, and took advantage of a regeneration mouse model and lineage tracing method. We found that under physiological conditions, the stem cells do not differ significantly in proliferation along the axis. However, under stress conditions, they responded and behaved differently, with the proximal region prone to form bigger clones, whereas the distal region is more prone to form smaller clones. We also identified the stem cell behavior dynamics using this regeneration model. We would further exploit the organoid culture system to investigate the esophageal stem cell behavior after injury. To find out the underlying molecular mechanisms, we would do RNA and ATAC sequencing. 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: esophageal cancer, regional differences

A CX3CR1 small molecular inhibitor (KAND567) suppressed the growth supportive effect of monocytes on Chronic Lymphocytic Leukemia cells

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Background: Chronic lymphocytic leukaemia (CLL) is the most prevalent leukemia. Novel treatment strategies are needed to improve the prognosis. Non-malignant cells of the tumor microenvironment (TME) are of importance for the growth of tumor cells. Monocytes expressing CX3CR1 (the fractalkine receptor) have been shown to support the growth of tumor cells. In CLL, monocyte derived nurse-like cells (NLC) support the growth of CLL cells, which promotes leukemic cell proliferation and survival by secretion of chemokines/cytokines. In this study, we have studied the role of autologous monocytes on CLL cell survival and the effects of a small molecule inhibitor of CX3CR1 (KAND567).
Methods: Primary leukemic cells (CLL cells) or healthy B cells ($CD19^+$ cells) as well as autologous monocytes ($CD14^+$ cells) from CLL patients (n=22) and age-matched healthy controls (n=11) were cultured alone or in co-culture experiments with or without the CX3CR1 inhibitor (KAND567) for up to 120 h. Apoptosis of $CD19^+$ cells was analysed by Annexin V/PI staining. Plasma conc. of fractalkine (CX3CL1) was determined by ELISA.
Results and discussion: In plasma of CLL patients, a significant (p= 0.0001) increase in the concentration of CX3CL1 compared to healthy controls was noted. CLL monocytes expressed CX3CR1 as did normal monocytes while in CLL patients a significant proportion of pro-inflammatory intermediate monocytes ($CD14^+/CD16^+$) was found (flow cytometry). However, leukemic CLL cells and normal B cells did not express CX3CR1.
A growth supportive effect of autologous monocytes was observed on the survival of both CLL cells and healthy $CD19^+$ B cells in 120 h co-culture experiments. KAND567 reduced the number of alive CLL cells in a dose-dependent manner in cell co-cultures with autologous monocytes, while no effect was found on healthy $CD19^+$ B cells. KAND567 did not kill CLL cells when cultured alone, suggesting that KAND567 may inhibit the growth promoting effect of autologous monocytes on CLL cells. In co-culture experiments a reduced frequency with time of NLC was noted in the presence of KAND567.
Conclusion: The CX3CR1/CX3CL1 (fractalkine receptor/ligand)) axis seems to be activated in CLL and might be of importance for the NLC-driven growth of CLL cells. A small molecule inhibitor of CX3CR1 reduced the growth supportive effect of autologous monocytes on CLL cells. Disruption of growth promotion induced by monocytes may prevent leukemic cell proliferation and survival. Inhibition of autologous monocytes in the TME might represent a new therapeutic strategy in CLL, complementary to treatment with drugs targeting the leukemic cells.

Key words: CX3CR1, nurse like cells

Identification of novel PDGFRalpha-dependent sarcoma-supportive functions of fibroblasts

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Purpose
Although widely known to support cancer progression and metastasis in epithelial tumors, the role(s) of non-malignant, stromal cell in mesenchymal tumors remains less characterized. This study therefore explored possible pro-tumoral effects of fibroblasts in sarcoma.

Experimental Design
Potential pro-tumoral effects of fibroblasts was characterized in co-culture models, of fibroblasts and sarcoma cells with sarcoma cell proliferation and migration as main endpoints. Molecular mechanistic studies focused on the potential involvement of PDGFRalpha signaling. Fibroblast-mediated effects on sarcoma cell proliferation and migration were also analyzed in vivo using zebrafish.

Results
A series of co-culture studies, using two different fibroblast cultures and three sarcoma cell lines, demonstrated consistent stimulatory effects of fibroblasts on sarcoma cell proliferation and migration.
Notably, these effects were attenuated by a specific PDGFRalpha antagonist. PDGF receptor profiling of fibroblasts and sarcoma cells suggested that the inhibitory effects of the PDGFRalpha antagonist on sarcoma cell proliferation and migration in the co-culture models was exerted through inhibition of PDGFRalpha in fibroblasts.
Stimulatory effects of fibroblasts on sarcoma cells were also supported by zebrafish embryo experiments. In these studies, fibroblast-exposed sarcoma cells demonstrated enhanced migration following injection into the perivitelline space of zebrafish embryos.
Ongoing studies are characterizing the PDGFRalpha-dependent secretome of fibroblasts for identification of factors mediating the stimulatory effects of fibroblasts.

Conclusions
The study identifies a novel PDGFRalpha-dependent role of stromal cells in supporting sarcoma cells proliferation and migration in vitro. Furthermore, studies suggest continued exploration of PDGFRalpha-focused interference of stromal/malignant cell interactions as a therapeutic approach for sarcoma.

Keywords: Sarcoma, Stromal cells

Inactivation of ICMT reduces the development of $BRAF^{V600E}$-driven cancer

Xijie Yang, Martin Bergö´s group

Isoprenylcystein carboxyl methyltransferase (ICMT) is an enzyme mediating posttranslational methylation at the C-terminal end of so-called CAAX proteins, such as RAS and RHO proteins. ICMT has been implicated as a target for treatment of cancer driven by KRAS mutations. However, the impact of ICMT deficiency in non-KRAS–driven cancer has not been investigated with genetic methods. In this study, we tested the potential of ICMT inhibition as treatment of cancer with the $BRAF^{V600E}$ mutation, which is present in 7% of human cancer and in 50% of malignant melanomas. Knockout of Icmt reduced tumor growth and increased survival in mice with $BRAF^{V600E}$ mutated lung tumors or metastasizing malignant melanoma induced by $BRAF^{V600E}$ and knockout of Pten. ICMT depletion reduced proliferation and invasion capacity of human malignant melanoma cell lines driven by BRAF or NRAS mutations. Also, ICMT inactivation markedly inhibited the growth of $BRAF^{V600E}$-mutated melanoma cells resistant to a $BRAF^{V600E}$ inhibitor. Additionally, ICMT specific inhibitor C3 had the similar effect as the ICMT genetic inactivation in vitro and in vivo. ICMT deficiency did not affect RAS or MAPK signaling, suggesting that the growth-inhibitory effect is not mutation-specific. We found inositol polyphosphate-5-phosphatase E (INPP5E), which is a CAAX protein, is a potential substrate of ICMT and ICMT achieves its function partially through INPP5E. These results introduce ICMT as a potential target for treatment of BRAF induced cancer, particularly malignant melanoma.

Keywords: ICMT, $BRAF^{V600E}$-driven cancer

Gene targets of the MYC proteins with prognostic prediction in neuroblastoma exhibit different expression during sympathoadrenal development

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Deregulation of the MYC family of transcription factors c-MYC (encoded by *MYC*), MYCN, and MYCL is prevalent in the majority of human cancers with an impact on tumor initiation, progression, and response to therapy. In neuroblastoma (NB), amplification of the *MYCN* oncogene and over-expression of *MYC* characterize approximately 40% and 10% of all high-risk NB cases, respectively. Nevertheless, the mechanism and stage of neural crest development in which MYCN and MYC contribute to the onset and/or progression of neuroblastoma are not yet fully understood. In this study, we hypothesized that an additive effect of subtle differences in the expression of MYCN and/or c-MYC target genes can more accurately stratify NB patients in different risk groups than using the expression of either MYC or MYCN alone. To test this hypothesis, we utilized an integrative approach using the transcriptome from 498 NB patients and defined c-MYC and MYCN target genes to model a multigene transcriptional risk score. Several techniques were employed for the analysis, including differential expression analysis, univariate Cox regression, protein-protein network analysis, and lasso-Cox regression analysis. These methods were used to screen and identify genes with potential prognostic significance in NB. Our findings demonstrate that a defined set of c-MYC and MYCN targets, with significant prognostic value, effectively stratify NB patients into different groups with varying overall survival probabilities. Importantly, these targets differ between c-MYC and MYCN and exhibit distinct expression patterns within the developing sympathoadrenal system by using single-cell sequencing analysis. Additionally, target genes predicting poor survival were more likely to be expressed in sympathoblast than in chromaffin cells. In conclusion, our results demonstrate that the expression of a specific set of MYCN and c-MYC target genes accurately classifies NB patients in groups with significantly different survival probabilities.

Keywords: MYC, neural development

Spatial multi-omic characterization of glioblastoma

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Glioblastoma (GBM) is the most malignant primary tumors in the brain. High inter- and intratumor heterogeneity are the main causes of treatment failure. Molecular characterization based on genomic and transcriptome 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 are using a recently developed technology, deterministic barcoding in tissue for spatial omics sequencing (DBiT-seq) to analyze patient GBM tissues. DBiT-seq is a microfluidic-based platform to deliver barcodes to the surface of a tissue slide to allow for spatial omics sequencing. It has been shown as an robust tool to profile transcriptome and proteome, epigenomes such as accessible chromatin and chromatin binding proteins. Our lab recently developed nanobody based single cell CUT&Tag that enabled profiling of multi epigenetic modalities from the same cells. We found the adoption of this method in DBiT-seq platform to be an ideal tool to characterize GBM microenvironment. Currently we are working on optimizing a protocol that allows us to profile accessible chromatin or multi histone modifications with transcriptome simultaneously from GBM tissue sections. This study is expected to better our understanding of pathogenesis and clinical diagnosis of GBM.
Keywords: Multi-omics; glioblastoma

Introducing the Chemical Biology Consortium Sweden (CBCS)

Weiyingqi Cui, CBCS KI, MBB

The Chemical Biology Consortium Sweden (CBCS) (www.cbcs.se) is a national research infrastructure in Sweden funded by the Swedish Research Council, Science for Life Laboratory, and the host universities. The aim of CBCS is to provide world-leading expertise in the field of chemical biology, as well as to strengthen research in chemical biology at a national level. Along this line, CBCS is a core part of the Chemical Biology and Genome Engineering (CBGE) facility at SciLifeLab, which also includes the CRISPR Functional Genomics and Chemical Proteomics platforms.

The mission of CBCS is to provide a state-of-the-art platform and expertise for the generation of high-quality bioactive chemical tools for proof-of-concept applications within life science research in general and with the goal to explore complex biology. To do this CBCS provides support to academic researchers in assay development, screening, chemistry, compound profiling, disease profiling, access to compound libraries and analysis. Additionally, CBCS provides access to a high-quality chemical collection (~350 000 compounds) that originates from the pharmaceutical industries and has been developed and expanded with compounds from various commercial vendors. The available screening collections include chemically diverse sets to approved drugs and pharmacological tool compounds. CBCS has well-functioning logistics and routines for distribution of assay ready plates and library compounds nationally.

Collectively, CBCS has been involved in more than 300 collaborative projects. The screening projects cover a wide range of model systems and readouts, covering isolated protein targets, targeted cell-based assays, phenotypic cell-based assays, and whole organisms, such as parasites and plants. All research is conducted in close collaboration with users and project outputs range from scientific publications to commercialization of projects and technology developments. Overall, CBCS has supported >230 individual users from all major Swedish universities, resulting in 174 publications and the establishment of seven companies and several patent applications.

Keywords: High-throughput screening, Compound libraries

The Autoradiography (ARG) core facility

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The Autoradiography Core Facility is a part of the **Center for Imaging Research** (CIR) at Bioclinicum.

We provide expertise and equipment to perform in vitro autoradiography and ligand binding in histological sections and tissue or cultured cell homogenates. **Autoradiography** is a method employed to visualize the location and distribution of a radioisotope-labelled compound or antibody in a sample and assess its target-specificity. **Radioligand binding** is used to study the binding properties of a radioisotope-labelled compound or antibody to a specific target, such as a receptor or enzyme. These methods are commonly used for characterization of PET imaging tracer, drug, or antibody target specificity, affinity, and helps predict suitability of the tracer application in vivo.

Our services are commonly used in drug, theranostics or PET tracer development projects. Working closely with Radiopharmacy (RCF) and the pre-clinical and clinical imaging facilities (KERIC, BMIC), we have extensive experience with academic projects and pharmaceutical industry. We're therefore able to give you the highest quality service.

ARG CF helps you:

- **Visualize** radioligand **localization** in different anatomical regions / **biodistribution**
- Assess **target-specificity** of a candidate drug or PET tracer
- Characterize the **binding affinity** properties of a candidate drug/compound for a given target (e.g. enzyme, receptor)
- Measure the maximum **density of the target protein** (e.g. receptor) available to the radioligand in a given tissue/organ.
- **Test drug candidates**, screening for compounds that compete with high affinity for binding to a particular target

More info and service requests: https://karolinska.corefacilities.org/service_center/show_external/3680
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Chemical Proteomics

Massimiliano Gaetani, Ph.D., Head of Core facility / Unit

Correct target identification and mechanism of action (MoA) elucidation of candidate drugs or already approved molecules for drug repurposing are bottlenecks and keys in drug development. For phenotype-based approaches, our Chemical proteomics unit provides research support by proteome-wide analyses of treatments in cells and lysates, adopting and developing the most efficient MS-based methods to decipher how any small molecule, compound, drug, or treatment works in any biological systems. Our goal is to apply unbiased and deep MS-based proteomics at throughput, multiplexity, sustainability, proteome depth and sensitivity needed in drug development. We address the limitations of the affinity-based approaches using MS-based proteome profiling approaches with unmodified ligands. With our development of Proteome Integral Solubility Alteration (PISA) assay for identification of targets and early mechanistic proteins as solubility-shifted proteins in a 30-90 min treatment, we have addressed the limitations of thermal proteome profiling, which sustainability for the multiple molecules/conditions needed to deconvolute targets and MoA is limited by costs, analysis time, number of replicates, missing values, loss of proteome depth, and uncertain “protein melting” interpretation. PISA represents a profound innovation and is a well-recognized trademark used by prominent MS-based proteomics laboratories. PISA enables 10–100-fold throughput increase, up to five test molecules/conditions plus control in biological triplicates, small sample amounts (e.g., primary cells and iPSCs), deep proteome coverage (~10,000 protein IDs), and minimal missing values. Orthogonal approaches needed for MoA elucidation can be integrated into one PISA assay: PISA-Express integrates cell proteome regulation; PISA-REX adds to that the reduction-oxidation changes. PISA can also measure drug residence time on targets.

For target-based approaches, mapping the protein binding site (e.g., small molecules, antibodies) and monitoring conformational changes are provided by Hydrogen/Deuterium exchange MS (HDX-MS) on purified protein samples, by measuring the exchange of the backbone`s hydrogens with the buffer in the identified protein fragments.

Cancer Research Horizons Therapeutic Innovation

Claire Heride , Louise Slater

Therapeutic Innovation (TI) is part of Cancer Research Horizons, the innovation arm of Cancer Research UK (CRUK). TI comprises six drug discovery centres, each with complementary state-of-the-art drug discovery technology platforms, operating under one leadership team, with a shared portfolio and budget. We engage closely with CRUK-funded researchers (~4,000) and a global research community through strategic partnerships (e.g., Oncode Institute, Netherlands and Karolinska Institutet, Sweden). Our aim is to identify and progress novel ideas in close partnership with academic researchers, with the objective of discovering breakthrough medicines for cancer patients. CRUK has a long track-record of translating academic science into the clinic, having played a role in the discovery and/or development of ten cancer medicines.

Our purpose is to ensure the translation of emerging cancer biological knowledge and discoveries. We are interested in novel targets and emerging areas of cancer biology or approaches to known challenging cancer targets with promising therapeutic potential in areas of unmet need. We have the expertise to progress small molecule or biological approaches to modulate cancer targets. Our approach is centred on collaboration, working closely with individual academic researchers, academic institutes, and commercial entities to enable us to drive progression of our portfolio further, faster, together.

KI Stem cell and Organoid: Disease modelling using spheroids and organoids.

Mukesh Varshney

The main goal of KISCO is, to assist researchers to harbour the potential of pluripotent stem cells (PSCs) in studying complex human disease mechanisms. Our services include iPSC reprogramming, characterisation, consolidation, differentiation to desired lineage (e.g. ectoderm, mesoderm and endoderm) and tissue-specific organoid generation that closely mimics the in vivo organ or tissue setting. Among new applications that have been tested and implemented at our core recently, are the use of animal component free ECMs and a 12-well plate format spinning bioreactor for long-term organoid cultivation.