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52952 - Ultra-Sensitive Minimal Residual Disease (MRD) Monitoring For Leukemia Patients Using SuperRCA Mutation Assays With Flow Cytometer Readout

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Abstract text

Background

Rare tumor-specific mutations in patient samples serve as excellent markers to monitor the course of malignant disease and responses to therapy in clinical routine, and improved assay techniques are needed for broad adoption. We describe herein - superRCA assays - which provides for rapid and highly specific detection of DNA sequence variants present at very low frequencies in DNA samples. Using a standard flow cytometer we demonstrate precise, ultra-sensitive detection of single-nucleotide mutant sequences from malignant cells against a 100,000-fold excess of DNA, to follow the course of patients treated for acute myeloid leukemia (AML) and Myelodysplastic syndrome (MDS).

Methods

Sequence of interest are first enriched by targeted PCR amplification from a patient sample and converted to DNA circles that are subjected to rolling-circle amplification (RCA). Padlock probes specific for mutant or wild-type sequences are then used to probe the repeated sequences of the RCA products with exquisite specificity, followed by RCA of the circularized probes. The large DNA clusters that result from each starting DNA circle are referred to as superRCA products.

Results

The lower detection limit and high precision of superRCA are consequences of the highly selective genotyping of the repeated target sequences in combination with the large numbers of products that may be conveniently analyzed by flow cytometry. In both AML and MDS patients, SuperRCA assay detected remaining mutations after initial treatment and clearly revealed the remaining malignant clone, subsequently leading to relapse for the patients. Even low levels of remaining leukemic markers in the post SCT-setting would prompt clinical action, mainly by reducing immunosuppressants to boost the immunological effect of the SCT in order to eradicate remaining malignant clones that risk giving rise to leukemic relapse. It is also demonstrated that, with ultra-high sensitivity, superRCA can find early sign of relapse in the blood samples than the time matched bone marrow samples analysed with ddPCR/NGS assays.

Conclusions

The superRCA assay procedure is suitable for routine use by virtue of its high sensitivity and simplicity. The 3-hr protocol contains seven solution additions to a DNA sample, separated by incubations, before reaction products are analysed using a standard flow cytometer. With ultra-high sensitivity, it’s even possible to monitoring the status of AML and MDS patient with blood samples with equal utility comparing to the bone marrow samples.

53536 - The Live Cell Imaging core facility: Advanced light microscopy at Campus Flemingsberg

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Abstract text
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53662 - CRISPR Functional Genomics facility: applying the power of CRISPR- Cas technologies to accelerate research, therapy and drug discovery

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Abstract text

The CRISPR-Cas system can be used for a variety of molecular research applications, including gene knockout, introducing small alterations, and transcriptional modulation. At CRISPR Functional Genomics (CFG), we are specialists in CRISPR technology. CFG covers the entire range of CRISPR applications, from the creation of knock-out/knock-in cell models to massively parallel, genome-wide perturbation screens. We strive to make the latest technological innovations available to the Swedish research community as quickly as possible. Examples of our toolbox include base-editing, prime-editing, pooled CRISPR loss- and gain-of-function screens, and coupling pooled screens with single cell transcriptomics. One emerging focus area is target identification and mode-of-action elucidation, which we pursue in collaboration with the Chemical Biology Consortium Sweden (CBCS) and the Chemical Proteomics unit at SciLifeLab.

53703 - Therapy with PI3K, PARP, and WEE1 Inhibitors and Radiotherapy in HPV Positive/ Negative Tonsillar Squamous Cell Carcinoma Cell Lines Reveals Synergy

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Abstract text
Human papillomavirus positive (HPV+) tonsillar and base of tongue cancer (TSCC/BOTSCC) is rising in incidence, but chemoradiotherapy is not curative for all. Therefore, targeted therapy with PI3K (BYL719), PARP (BMN-673), and WEE1 (MK-1775) inhibitors alone or combined was pursued with or without 10 Gy and their effects were analyzed by viability, proliferation, and cytotoxicity assays on the TSCC/BOTSCC cell lines HPV+ UPCI-SCC-154 and HPV- UT-SCC-60A. Effective single drug/10 Gy combinations were validated on additional TSCC lines. Finally, APR-246 was assessed on several TSCC/BOTSCC cell lines. BYL719, BMN-673, and MK-1775 treatments induced dose dependent responses in HPV+ UPCI-SCC-154 and HPV- UT-SCC-60A and when combined with 10 Gy, synergistic effects were disclosed, as was also the case upon validation. Using 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. Low APR-246 concentrations had limited usefulness. To conclude, synergistic effects were disclosed when complementing single BYL719 BMN-673 and MK-1775 administrations with 10 Gy or when combining the inhibitors, while adding 10 Gy to the latter did not further enhance their already additive/synergistic effects. APR-246 was suboptimal in the present context.

53855 - Genomic and epigenomic drivers of aggressive double-negative metastatic prostate cancer

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Abstract text

Background:
Systemic targeted therapy in prostate cancer is primarily focused on ablating androgen receptor (AR) signaling. Androgen deprivation therapy and second-generation AR-targeted therapy selectively favor the development of treatment-resistant subtypes of metastatic castration-resistant prostate cancer (mCRPC), defined by whether the tumor expresses either AR or neuroendocrine (NE) markers. Among the subtypes of mCRPC, the molecular drivers of double-negative (AR-/NE-) mCRPC are poorly defined.

Methods:
In this study, we comprehensively characterized and integrated genomic and epigenomic features of 210 treatment-emergent mCRPC subtypes by expanding the largest dataset of clinical mCRPC tumors in the US, from the West Coast SU2C-PCF Dream Team cohort with matched whole-transcriptome RNA sequencing, whole-genome sequencing, and whole-genome bisulfite sequencing and available clinical follow-up information.

Results:
We show that AR-/NE- tumors exhibit a clinically and molecularly distinct phenotype. Patients with AR-/NE- mCRPC tumors have the shortest survival, and these tumors preferentially harbor amplification of the chromatin remodeler CHD7 and biallelic-loss of PTEN. We demonstrate that methylation changes in CHD7 candidate enhancers are linked to elevated CHD7 expression in AR-/NE+ tumors. Moreover, we use genome-wide methylation analysis to nominate the Krüppel-like factor gene KLF5 transcription factor as a

driver of the AR-/NE- phenotype and link its activity to loss of the tumor suppressor RB1.

Conclusions:
These observations reveal the aggressiveness of the AR-/NE- tumors and elucidate genomic and epigenomic drivers of mCRPC subtypes, which may facilitate the identification of novel therapeutic targets in this highly aggressive disease.

53884 - Sipa1-deficiency in bone marrow microenvironment inhibits acute myeloid leukemia progression

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Abstract text
Signal-induced proliferation-associated gene 1 (Sipa1), a RAP1 GTPase-activating protein, involves in signaling of integrins, growth factors, and cytokines by inactivating RAP1. We have reported that Sipa1 deletion in mice causes phenotypic and functional alterations of bone marrow (BM) mesenchymal stem and progenitor cells (MSPCs) prior to the development of the age-dependent myeloproliferative neoplasm (MPN), and these microenvironmental alterations could lead to malignant transformation of normal hematopoietic cells, resulting in the initiation of MPN. Furthermore, in a transplantation-induced acute myeloid leukemia (AML) mouse model, Sipa1 was reduced in the BM MSPCs. However, the impact of Sipa1 expression in BM microenvironment on AML progression is unknown. Here, by using *Sipa1*$^{-/-}$ AML mouse model, we demonstrated that Sipa1 deficient recipient mice displayed a delayed AML onset with 30% of the *Sipa1*$^{-/-}$ mice free from AML while all the wild type mice developed AML within a month post-AML injection. Such a survival difference was not observed in the recipient mice where the immune cells were eliminated by lethal irradiation, indicating the involvement of Sipa1-/- immune cells in the AML progression. Further work with specific lineage depletion by neutralizing antibodies revealed that in vivo depletion of NK cells, but not T cells, could reverse the AML development in the *Sipa1*$^{-/-}$ recipient mice, suggesting that Sipa1 deficiency in NK cells might lead to the delayed AML onset in *Sipa1*$^{-/-}$ mice. In addition, phenotypic analysis of *Sipa1*$^{-/-}$ BM niche after AML onset showed the resistance of *Sipa1*$^{-/-}$ BM niche to AML remodeling, which has been considered as one of the mechanisms underlying progressive development of AML. Altogether, our study suggests that Sipa1 may serve as a new target for modulating NK cell function. However, more work is required to explore the underlying mechanisms and the therapeutic effect of Sipa1 deficient NK cells in AML.

53889 - Small Molecule-mediated Disruption of Ribosome Biogenesis Synergizes with FGFR Inhibitors to Suppress Glioma Cell Growth

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Abstract text

High-grade gliomas (HGGs) are characterized by aggressiveness and resistance to chemotherapy. Prognosis remains dismal, highlighting the need to identify novel molecular dependencies and targets. Ribosome biogenesis (RiBi), taking place in the nucleolus, represents an interesting target as several cancer types rely on high RiBi rates to sustain proliferation. Our analysis of transcriptomics data revealed a positive correlation between RiBi rates and glioma grade, and we therefore hypothesized that HGGs could be susceptible to RiBi inhibition. BMH-21, a small DNA-intercalating acridine-like molecule preferentially blocking RNA pol I transcription was tested in adult and pediatric HGG cell lines and a zebrafish transplant model. BMH-21 reduced glioma cell viability, triggered p53-independent cell death, and impaired the growth of transplanted glioma cells in zebrafish. Combining BMH-21 with temozolomide (TMZ) potentiated cytotoxic effects. BMH-21 synergized with Fibroblast Growth Factor Receptor (FGFR) inhibitors, e g Erdafitinib (JNJ-42756493), a top hit in a combinatorial chemical synergy screen. RiBi inhibition using BMH-21, depletion of POLR1A (RNA Pol I), or Actinomycin D, revealed engagement of the FGFR-FGF2 pathway. BMH-21 downregulated FGFR1, SOX2 and c-MYC whereas fibroblast growth factor 2 (FGF2) was induced, released from the nucleolus, and secreted. Subsets of HGG lines are highly dependent on FGFR1. Pathway enrichment analysis of differentially correlated genes with FGFR1, comparing glioma and hepatocellular carcinomas, indicated a signature related to protein synthesis present in glioma. In sum, our study conceptualize the implementation of RiBi inhibition as a viable therapeutic strategy and reveals an FGFR connection to the cellular response upon RiBi inhibition.

53901 - Characterization of Bone Marrow Niche in Chronic Myeloid Leukemia Patients Identifies CXCL14 as a New Therapeutic Option

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Abstract text

Although tyrosine kinase inhibitors are effective for treating chronic myeloid leukemia (CML), they often fail to eradicate the leukemia-initiating stem cells (LSCs), causing disease persistence and relapse. Evidence indicates that LSC persistence may be due to bone marrow (BM) niche protection. However, little is known about the underlying mechanisms. We here molecularly and functionally characterized BM niches in CML patients at diagnosis and revealed the altered niche composition and function in the CML patients. Long-term culture initiating cell (LTC-IC) assay showed that the mesenchymal stem cells from CML patients displayed an enhanced supporting capacity for normal and CML BM $CD34^{+}CD38^{-}$ cells. Molecularly, RNA sequencing detected dysregulated cytokine and growth factor expression in CML patient BM cellular niches. Among them, CXCL14 was lost in the BM cellular niches in contrast to its expression in healthy BM. Restoring CXCL14 significantly inhibited CML LSC maintenance and enhanced their response to imatinib in vitro, and CML engraftment in vivo in NSG-SGM3 mice. Importantly, CXCL14 treatment dramatically inhibited CML engraftment in xenografted NSG-SGM3 mice, even to a greater degree than imatinib, and this inhibition persisted in patients with suboptimal TKI response. Mechanistically, CXCL14 upregulated inflammatory cytokine signaling but downregulated mTOR signaling and oxidative phosphorylation in CML LSCs. Together, we have discovered a suppressive role of CXCL14 in CML LSC growth. CXCL14 might offer a treatment option targeting CML LSCs.

53945 - Identifying prediagnostic colorectal cancer biomarkers using a targeted proteomics platform with extensive coverage

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Abstract text

Despite advances in treatment, colorectal cancer remains one of the leading causes of cancer-specific death, and better and more cost-efficient tools for risk prediction, prevention and early detection are needed. The primary aim of this study was to identify novel circulating protein biomarkers associated with colorectal cancer risk. A secondary aim was to validate candidate proteins identified in previous studies. We included 195 incident colorectal cancer cases and 195 matched control participants from the Northern Sweden Health and Disease Study (NSHDS). Colorectal cancer diagnosis ranged from 6 years to three months after blood sampling. Plasma samples were analyzed by proximity extension assay using the 1536-protein Olink Proteomics Explore platform. Multivariable conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (95% CI) for the association between protein concentrations and colorectal cancer risk. We identified 20 proteins that met a significant threshold of P<0.005, of which one met a strict Bonferroni-corrected threshold of P<3.9e-5. The top hit, TFF3 (Trefoil Factor 3), was strongly positively associated with colorectal cancer (OR per standard deviation increase: 1.84, 95% CI 1.38-2.45). In sub-analyses stratified by sex, TFF3 retained significance in men (FDR<0.05), but not in women ($p_{heterogeneity} < 0.05$). Other proteins that we validated in the study included AREG, CEA, and LGALS4. To conclude, we identified one novel protein, TFF3, strongly associated with subsequent colorectal cancer risk and validated previously reported associations between circulating protein levels and colorectal cancer.

53946 - The zebrafish core facility at KI: Providing zebrafish xenograft models for your research

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Abstract text
Bräutigam, L.

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The zebrafish is a powerful model for basic and pre-clinical science, especially for cancer research. It provides a clinically relevant *in vivo* platform which allows for transplantation of cancer cell lines or primary cancer material. The growth of the cancer, metastasis or response to small molecules can be followed in a high-throughput format. Thousands of transgenic lines are available allowing for *in vivo* and real time visualization of cancer progression, metastasis, or interaction with immune cells. Oncogene-driven transgenic lines are available, faithfully recapitulating disease initiation and progression in humans, and the zebrafish genome is easily accessible to investigate the oncogenic properties of candidate genes.

The zebrafish core facility at Karolinska Institutet is the largest zebrafish core facility in the Nordic countries. The facility holds ca. 20.000 animals of around 250 different genetic lines in different barriers including a BSL-2 unit. The seven staff members and our designated veterinarian provide everything from the provision of animals to running of complex research projects to internal and external academic and corporate users. The facility has established standardized pipelines for cancer cell transplantations including orthotopic implantations into the cardiovascular system or the brain. The transplanted embryos can be exposed to small molecules and imaged in-house using an automated high-throughput microscope specifically designed for zebrafish applications. A typical transplantation experiment from implantation to read-out after exposure requires only 3 days and is performed in zebrafish embryos for which no ethical license is required.

For more information, please see our poster or our webpage:
https://ki.se/en/research/zebrafish-core-facility

53967 - Spatially dependent heterogeneity in pancreatic ductal adenocarcinoma

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Abstract text

Pancreatic cancer is predicted to top cancer-related mortality in the coming years. Pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, currently has a dismal prognosis with 5-year survival of around 11%. During the recent years, evidence supporting heterogeneity in PDAC has accumulated. Both by bulk – and single cell sequencing, two main PDAC subtypes have emerged: (1) the classical subtype and (2) the basal-like subtype. Basal-like tumors are characterized by epithelial to mesenchymal transition and an overall worse prognosis.

In the current study, we spatially mapped the subtypes of individual tumor cells, representing the spectra from classical – to basal-like in an immunohistochemistry-based digital quantification pipeline. Digitalized whole-slide images were quantified in QuPath, stratifying between tumor regions invading the stroma or the healthy pancreatic lobules. We found that PDAC or subtype state is strongly driven by anatomical location, such that stromal invasion is coupled to basal-like expression (positive for Keratin 17 and Keratin 5), while classical tumor cells (positive for Mucin 5) invade the lobuli, resulting in coexisting expression patterns within the same tumor. Hence, we can for the first time elucidate anatomical drivers of the classical vs basal-like expression state dichotomy in PDAC, and bring the previously largely unrecognized acinar invasion into the light. We will now evaluate this spatially dependent heterogeneity model in an in vivo system, and explore the possibilities for clinical implementation.

54031 - Assays to lead the way1: the tricky task of specifically inhibiting glutathione peroxidase 4 (GPX4)

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Abstract text

Ferroptosis, a non-apoptotic cell death induced by iron-dependent lipid peroxidation, was first characterized in cells treated with Ras-selective lethal small molecule 3 (RSL3)[2]. RSL3, and additional ferroptosis inducing (FIN) compounds discovered in the same synthetic lethal screen, ML162 and ML210, were subsequently found to trigger ferroptosis through the inactivation of glutathione peroxidase 4 (GPX4)[3], a selenoprotein that uses glutathione to reduce lipid hydroperoxides to their alcohol forms. GPX4 thus acts as an important manager of intracellular oxidative stress broadly, and a key regulator of ferroptosis specifically. In fact, several cancer types and chemotherapy-resistant cancer lines show an up-regulation of GPX4 to compensate for the abnormally high reactive oxygen species produced during rapid proliferation, making GPX4 an attractive target for anti-cancer drug development campaigns[4]. Using a novel method of recombinant selenoprotein expression and purification[5-7] we have produced active, selenocysteine-containing GPx4 enzyme and have developed and miniaturized a glutathione reductase (GR)-coupled enzymatic assay amenable to high-throughput screening. We have screened >100,000 compounds (including approved and investigational drugs, natural products, and novel drug-like scaffolds) in dose-response using this assay to assess direct biochemical inhibitory activity of these small molecules against GPX4. Using a suite of assays to ensure specificity, namely against GR, additional isoforms of GPX, and another selenoprotein thioredoxin reductase 1 (TXNRD1), we set to identify novel inhibitors of GPX4.

Surprisingly, the previously described FINs showed no biochemical inhibition of GPX4. However, both RSL3 and ML162 demonstrate potent biochemical inhibition of TXNRD1. The potential importance of this off-target effect has yet to be understood but was further confirmed both in cells, using a TXNRD1-specific activity probe, RX1, and by assessing biophysical binding of the molecules to TXNRD1, but not GPX4, using nanoscale differential screening fluorimetry. Finally, we present a validated pipeline for developing specific GPX4 inhibitors, as well as nine novel small molecules showing biochemical inhibitory activity against GPX4 but not other GPX isoforms nor other selenoproteins.

1) Othello 3.3.1352; 2) 18355723; 3) 24439385; 4) 31105042; 5) 28193838; 6) 28917049; 7) 34304108;

54034 - Revealing Altered Lipid Metabolism as a Key Vulnerability of Germinal Centre Derived B-cell Lymphoma

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Abstract text
Germinal centre (GC) derived B cell lymphomas are amongst the most aggressive lymphoma subtypes. These include Burkitt's Lymphoma (BL) and GC B cell subtype Diffuse Large B Cell Lymphoma (GCB-DLBCL). Investigation of their aggressiveness is mostly based on gene expression and phenotype, but the link between gene function and behaviour is less explored.

We conducted a bias-free functional bioinformatic search using the Cancer Dependency Map (DepMap) that contains transcriptome and genome-wide CRISPR screening on cancer cell lines. Using principal component analysis (PCA) and unsupervised clustering, we identified a distinctive cluster composed of GC-derived malignancies that we called intra-GC cluster. To identify the unique genes for survival of intra-GC lymphoma, we combined differential expressed genes (DEGs) analysis, gene set enrichment analysis (GSEA), and other hypothesis tests in comparison with all other types of haematological cancer.

We identified altered lipid metabolism as a main characteristic for the intra-GC cluster. This was signified by top DEGs that related to lipid metabolism, as well as significantly increased dependency for *RHOA, FDFT1*, and *COX10* that all require the usage of farnesyl pyrophosphate (FPP), a mevalonate pathway product for lipogenesis. Genes that mediate FPP production did not show the same dependency pattern, suggesting that repurposing of FPP was a unique feature. Among all genes, *RHOA* was identified as the most required gene, specifically for the intra-GC cluster and deserves further investigation.

Overall, we identified that GC-derived malignancies were transcriptomically distinct among haematological cancers and displayed a unique dependency on lipid metabolism.

54037 - Neural-network learning-based deconstruction of the developmental genetic programs in glioblastoma and neuroblastoma

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Abstract text

The cellular plasticity and the resulting intratumoral heterogeneity of cancer cells inevitably contribute to tumor malignancy. Therefore, it is critical to understand the fundamental mechanisms that govern the re-initiation of the developmental genetic programs for cellular plasticity in the tumor cells. Glioblastoma is the most malignant central nervous system cancer, and neuroblastoma is the most common extracranial pediatric solid tumor in the peripheral nervous system. Here, we systematically investigated the developmental gene programs in glioblastoma and neuroblastoma.

Using our novel deep-learning software, we recently projected 73495 single tumor cells of 100 glioma patients into cell-type differentiation trajectories of normal brain development. We computationally and experimentally revealed two principal cell lineage patterns in glioblastoma: the brain-neural and neural-crest-brain-perivascular lineage (Ref: 1).

In our latest study, combining single-cell MultiOmics with Spatial Transcriptomic profiling, we simultaneously explored the single-cell transcriptional states, tumor states-associated ecosystem, and open-chromatin landscapes of neuroblastoma along the developmental neural crest cell differentiation trajectory. We identified the neural crest lineage cell states that exist predominantly only in high-risk neuroblastoma and uncovered this lineage states' transcriptional regulatory logic. We experimentally observed that the epigenetic priming of cellular plasticity exists broadly in cancer cells, but it usually remains latent until triggered by either endogenous or exogenous stimuli. These findings underscore the crucial role of developmental genetic programs in driving the emergence of phenotypic plasticity in central and peripheral nervous system tumor types.

Reference:

1. Hu Y., Jiang Y., et al., <Science Advances>, 2022 Jun. PMID: 35675414

54038 - Modeling and understanding glioblastoma edge cells

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Abstract text

Glioblastoma is a devastating disease with an overall median survival of 8 months from diagnosis. The majority of patients die of tumor relapse in close proximity to the resected primary tumor. Glioblastoma is intensely researched but most studies have been performed on tissues and cultures derived from the bulk tumor while it is the remaining edge cells that cause lethality. Investigations of glioblastoma edge cells are rare and few experimental models exist. Here, we have established and analyzed a series of matched cell cultures derived from the tumor bulk and outer edge of six glioblastoma patients to understand glioblastoma edge cell biology. Tumor samples were resected guided by 5-ALA fluorescence using neuro-navigation and stringent procedures to not contaminate edge samples with bulk tumor cells. First, two bulk tumor samples were resected from 5-ALA fluorescent tissue. One was annotated and represented the highest fluorescent part of the bulk tumor, and the other was non-annotated and represented a large part of the bulk tumor. After removing all fluorescent areas and careful irrigation of the cavity, the edge sample was taken 1-2 cm outside the fluorescent border in a non-eloquent area using clean instruments. Samples were dissociated, explanted, and cultures established. Functional analyses were performed on the primary and established cultures of six patients from which we managed to establish matched bulk and edge cultures. Across all six patients, there were significant differences between matched bulk and edge cells, where bulk cells had a higher self-renewal capacity and edge cells were more invasive. To investigate the molecular basis of these findings we performed whole exome sequencing (WES) and combined single-cell RNA- and ATAC-sequencing (10X Multiome). Analyses are ongoing but WES data does not support genetic causes of the phenotypic difference between bulk and edge cells of the same patient and across patients. The 10X Multiome data display bulk and edge-specific clusters and candidate genes and pathways are currently being investigated. In summary, we have generated a novel set of glioblastoma cell culture models of the relapse-prone outer tumor edge that display distinct functional and molecular properties compared to their matched bulk tumor cells. These will be valuable tools in search of therapeutic targets of the relapse-causing cells and should provide more relevant models for glioblastoma drug screening and development.

54039 - The association of Parvimonas micra and Fusobacterium nucleatum to the tumour immune response in colorectal cancer

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Abstract text

The gut microbial composition has a substantial impact on colorectal cancer (CRC) development and progression, partly through modulation of the tumoral immune response. We have studied the presence of two CRC associated bacteria, *Parvimonas micra* and *Fusobacterium nucleatum*, by qPCR in fresh frozen tumour tissue and in faeces of CRC patients in relation to different tumour immunological traits. In a cohort of 69 CRC patients, we found a significant association of *P. micra and F. nucleatum* in tumour tissue with tumours of the immune activated CMS1 subtype. Immunological analyses using flow cytometry revealed significant associations for *P. micra* with increased percentages of antigen-presenting HLA-DR$^+$ B lymphocytes and activated CD69$^+$ T lymphocytes. Furthermore, *P. micra* was positively associated with both M1 and M2 macrophage traits. These findings were validated using transcriptomics. We further analysed the presence of *P. micra* and *F. nucleatum* in 112 tumour tissue samples and 250 faecal samples from a larger CRC patient cohort. High levels of *P. micra* and *F. nucleatum* in tumour tissue were associated with a decreased five-year cancer specific survival. Both high levels of *P. micra* and *F. nucleatum* were associated with tumours mutated in *BRAF*^{V600E} and tumours of the MSI subtype. Preliminary results further suggest an association between high levels of *P. micra* and *F. nucleatum* to decreased levels of regulatory T-cells as quantified using the VECTRA system for multispectral imaging. A deeper understanding of the role of the gut microbiota in CRC has the potential to contribute to improved personalized cancer treatments.

54051 - Complement factor H is a novel ligand for the inducible T cell co-stimulator and promotes regulatory T cells survival in the glioma microenvironment

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Abstract text

The survival of glioma patients has not significantly increased in recent years despite aggressive treatment and advances in immunotherapy. The lack of response to the treatments is partially attributed to the notoriously immunosuppressive microenvironment, with regulatory T cells (Tregs) playing a pivotal role in immunologic tolerance. Here we investigated the impact of the complement factor H (FH) on Tregs in the glioma microenvironment. We showed that FH is a novel ligand of the inducible T cell costimulator (ICOS). The binding of FH to this immune checkpoint molecule promotes the survival of Tregs. Additionally, FH enhances Treg functions crucial for the modulation of the tumor microenvironment, including secretion of transforming growth factor beta, interleukin 10, and suppression of the effector T cells proliferation. Staining of human and mouse gliomas revealed that FH is produced directly by cancer cells, and database investigation showed that upregulation of FH expression is associated with the occurrence of Tregs and poor prognosis for glioma patients. This was confirmed using glioma mouse model, where FH knockdown showed a clear tendency to prolong survival. Since the accumulation of Tregs represents an auspicious prognostic and therapeutic target, FH expression should be scrutinized when considering the effectiveness of immunotherapies against glioma.

54057 - Novel Affibody-based tracer for radionuclide imaging of HER2: Clinical translation

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Abstract text

Introduction: A level of expression of human epidermal growth factor receptor type 2 (HER2) in breast and gastric cancers is a predictive biomarker for outcome of HER2-targeting treatments. Affibody molecules are small targeting proteins based on non-immunoglobulin scaffold, which provide highly sensitive molecular imaging at the day of injection. Clinical evaluation of ^{68}Ga-labelled Affibody molecule ABY-025 showed that this imaging probe is capable of quantitative measure of HER2 expression in the breast cancer 3-4 h after injection [1]. Recent progress in development of SPECT/CT cameras enabled reasonable accuracy in quantitative measurement of activity concentration in vivo. Implementation of a ^{99m}Tc-labelled Affibody molecule would increase availability of HER2 imaging for clinical community. The aim of this study was to evaluate biodistribution, dosimetry and safety of [^{99m}Tc]Tc-ZHER2:41071 Affibody molecule in a Phase I trial. **Methods**: Biodistribution of [^{99m}Tc]Tc-ZHER2:41071 was studied in mice bearing HER2-expressing SKOV-3 xenografts. To evaluate dosimetry in humans, uptake values in mice was evaluated and absorbed doses were estimated using OLINDA/EXM 1.0 software. Phase I trial was a prospective, open-label, non-randomized Phase I diagnostic study in patients with untreated primary breast cancer (ClinicalTrials.gov Identifier: NCT05203497). Three cohorts of patients (injected with 500, 1000, or 1500 μg ZHER2:41071) with primary breast cancer were enrolled in the study. Each cohort included at least five patients with high HER2 expression (immunohistochemistry (IHC) score 3+ or IHC score 2+ and FISH positive) and five patients with low HER2 expression in tumors (IHC score 0, 1+ or 2+ and FISH negative). The injected activity was 451±71 MBq. Planar scintigraphy was performed after 2, 4, 6 and 24 h and SPECT/CT imaging after the planar imaging 2, 4 and 6 h after injection. Vital signs were monitored before, during and after the imaging.**Results**: Biodistribution results in murine model demonstrated that the tumor-to-kidney ratio and tumor-to-liver ratio were 2.2 ± 0.5 and 52 ±11 4 h after injection, respectively. microSPECT/CT imaging demonstrated a high-contrast visualization of HER2 expression. An evaluation of absorbed doses for humans demonstrated very favourable dosimetry (effective dose of 0.00066 mSv/MBq). Phase I trial showed that there is no adverse events after injection of [^{99m}Tc]Tc-ZHER2:41071. Kidney was the normal organ with the highest accumulation. The effective dose was 0.019±0.004 mSv/MBq. The best discrimination between HER2-positive and HER2-negative primary tumors was achieved in patients injected with 1000 μg. Already 2 h after injection, the uptake in tumors with high expression (SUVmax 16.9±7.6) was significantly ($p<0.005$, Mann–Whitney U test) higher than in tumors with low expression (SUVmax 3.6±1.4). [^{99m}Tc]Tc-ZHER2:41071 uptake in HER2-positive lymph node metastases was also significantly ($p < 0.05$) higher than in HER2-negative 2 h after injection of 1000 μg.**Conclusions**: [^{99m}Tc]Tc-ZHER2:41071 was successfully tested in preclinical murine model. Imaging using [^{99m}Tc]Tc-ZHER2:41071 was safe in patients. Dosimetry data showed that multiple injections of [^{99m}Tc]Tc-ZHER2:41071 for monitoring HER2 expression over time are feasible. Injected protein dose of 1000 μg is

preferable for discrimination between tumors with high and low expression of HER2.

54060 - Transcriptional guardian of lipid metabolism in liver cancer

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Abstract text
The regulation and expression of genetic information is a fundamental process in all living organisms. How the cell controls optimal usage of the genetic information under physiological and pathological conditions remains an area of intense investigation. To study cellular economics in healthy and diseased cells, we investigate the regulatory control and dependencies of the coding and noncoding part of the genome by using multi-omics approaches.

Our work revealed that RNA-binding proteins (RBPs) play crucial roles in liver cancer. Deregulation of RBPs negatively affects patient survival. We found that RBP functionality is mediated through long non-coding RNAs (lncRNAs). One of the most noticeable RBP-lncRNA connections impacts hepatic lipid metabolism. Correction of the RBP-lncRNA regulatory network improved lipid metabolism *in cellulo* and diminished tumor growth *in vivo*[1]. Since many cancer types, including liver cancer, are linked to increased dietary lipid uptake (obesity), we investigated the molecular changes in response to high fat diet (HFD). We found transcriptional deregulation of genes affecting a wide range of cellular processes and identified that female sex hormones contribute to the reversal of the disease phenotype[2].

In conclusion, our work revealed disease-specific changes in the regulation of the coding and noncoding part of the genome that can be exploited to predict and revert pathological liver cell stages.

1. CCT3-LINC00326 axis regulates hepatocarcinogenic lipid metabolism. Søndergaard JN et al. *Gut*. 2022. PMID: 35022268
2. Hepatoprotective effects are mediated by systemic estrogen receptor activation and uncovers clinical biomarker and targets. Sommerauer, Gallardo Dodd et al. *manuscript*

54065 - Exploiting nucleotide metabolism to potentiate nucleoside analogue-based cancer therapies

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Abstract text

Nucleoside analogues have long been exploited for the treatment of diverse pathologies, such as cancer. However, these therapies are administered as nucleoside prodrugs that require activation through sequential phosphorylation, often resulting in an active triphosphate metabolite. Thus, the enzyme SAMHD1, a triphosphohydrolase, can constitute a major barrier to therapeutic efficacy. Consequently, its inactivation could potentiate the effectiveness of nucleoside-based treatments, as has already been demonstrated preclinically for the case of acute myeloid leukaemia standard-of-care therapy cytarabine. Unfortunately, no small molecule has been reported to date producing the direct engagement of SAMHD1 activity in cell models, and indirect inactivation has been observed with the use of ribonucleotide reductase (RNR) inhibitors, though it remains to be deciphered the exact mechanism that provokes SAMHD1 inhibition. To satisfy this necessity, we screened a collection of approved drugs (AD) targeting enzymes involved in nucleotide biosynthesis and studied SAMHD1-dependent synergy with cytarabine in the leukemic THP-1 cell line. As a result, two approved drugs (AD1 and AD2) targeting the same enzyme potentiated cytarabine in a SAMHD1-dependent manner. Subsequently, SAMHD1 indirect inactivation by AD1-2 and was thoroughly characterised using biochemical, biophysical and cell-based assays. Additionally, enhanced activation of the DNA damage response and cell cycle checkpoints upon the combination of cytarabine and AD1-2 were also explored. As a result, we provide a more detailed mechanism involved in regulation of SAMHD1 activity, which can be further utilised to repurpose already approved drugs in haematological diseases to abrogate SAMHD1 drug resistance factor and improve current chemotherapies.

54083 - Mechanisms of response and resistance to p53 reactivation in glioblastoma multiforme

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Abstract text

Glioblastoma multiforme (GBM) is an aggressive and universally fatal brain tumor with a median survival of 15 months following diagnosis. No targeted therapies have yet been proven successful in the treatment of these tumors, reason for which standard of care still consists of surgery and radiochemotherapy regimens. Over half of GBM patients retain wild-type p53, resulting in potential susceptibility to MDM2 inhibitors. These molecules disrupt the interaction between p53 and its negative regulator MDM2, allowing downstream pathway signaling and cell fate decisions such as growth inhibition and death resulting from cellular stress. We have analyzed clinical samples from 9 patients with recurrent glioblastoma multiforme (GBM) treated with the MDM2 inhibitor KRT-232, all of which relapsed despite the biological promise of this small molecule. By analyzing on-treatment biopsy samples, we detected upregulation of p53 transcriptional targets, suggesting blood brain penetrance and drug pharmacodynamics. However, in patient-derived cell lines, treatment with KRT-232 at the clinically achieved concentration was only sufficient to stall but not reduce tumor growth. Cell death via apoptosis was achieved in these cell line models when MDM2 inhibition was combined with the chemotherapeutic agent temozolomide. These drugs have non-overlapping mechanisms of action and exhibit synergy in our cell line models. Bone marrow cells exhibited a reversible growth inhibition phenotype when treated with KRT-232 and TMZ, supporting the idea that a therapeutic window exists with this combination. We additionally observed that tumor progression occurred in the absence of p53 inactivating mutations, suggesting that other mechanisms of resistance to KRT-232 may modulate drug response and resistance in GBM. Indeed, drug treatment induced an adaptive response in human tumors, characterized by the upregulation of genes involved in glial cell differentiation processes. Ongoing work is focused on understanding how tumor heterogeneity and cell state modulate response and resistance of GBMs to MDM2 inhibition and other therapeutic strategies that reactivate p53 signaling. Our study highlights the utility of tissue sampling during clinical trials and represents the first clinical effort to detect adaptive changes in human tumor tissue associated with p53 reactivation.

54124 - Multi-Magnification Analysis of Hematoxylin and Eosin Slides Improves Deep Learning Model for Breast Cancer Grading

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Abstract text

Introduction: Digital analysis of histopathology slides through Artificial Intelligence methods has emerged as a very exciting research field. Due to the gigapixel-nature of whole-slide images (WSI), it is not feasible to train a model end-to-end. Instead, WSIs must be cropped into smaller tiles at a specific magnification level before preprocessing and model training. However, at least for some tasks, it's unintuitive to analyze tissue slides at a single scaling. One example is breast cancer grading, for which pathologists take into consideration both architectural features of the tissue (degree of tubular formation) and cytological features (nuclear pleomorphism and number of mitoses). In this study, we hypothesize that a deep learning model can capture and synthesize information at multiple magnifications in a superior manner than a uni-magnification alternative and we use breast cancer grading as a use case to show this.

Material & Methods: We used data from 202 biopsies taken before treatment start in the neoadjuvant multicenter phase-two clinical trial, PREDIX-HER2. The patients were diagnosed with invasive ERBB2-positive breast cancer as primary diagnosis. The tissue slides were scanned with a Hamamatsu Nanozoomer scanner at a maximum of 40x magnification, with a spatial resolution of 0.2277 µm/pixel. After performing quality control, data from 62 patients were excluded due to missing grading score, unavailability of invasive cancer or suboptimal image quality. Each slide was patched using a window of 512x512 pixels at a spatial resolution of 0.4555 µm/pixel for the 20x magnification and at 1.8219 µm/pixel for the 5x magnification. A Vision Transformer was utilized to extract features from these tiles, which were fed to an attention-based multiple-instance-learning classifier for training. A held-out test set corresponding to 20% of the cohort's dataset was utilized to evaluate the model's performance which was trained using a 5-fold cross validation schema on the rest 80% of the dataset.

Results: The multi-magnification model integrating deep features from two magnifications (i.e. one corresponding to a 5x lens and one to a 20x lens) has achieved an AUC value of 0.864 for predicting breast cancer grading. In contrast, the uni-magnification models have achieved an AUC value of 0.669 and 0.713 at a respective magnification of 5x and 20x. Multi-magnification AI analysis of digital slides shows a great improvement in terms of AUC for predicting breast cancer grading when compared to two single magnification models.

Discussion: In this study we focused on breast cancer grading, for which multi-magnification analysis feels an intuitive course of action. However, other tasks for which one would intuitively focus only on cellular characteristics, such as mutation prediction, may not benefit from multi-magnification analysis. It is thus important to investigate whether our proposed model can benefit performance on a variety of tasks and datasets, which would indicate that we should assess not only the cellular features that can be observed at high magnifications but also the architectural features of the tissue compartments that can be observed at lower magnifications.

54128 - VEGF-C expressing Tumor-Associated Macrophages redirect cancer cells to preferentially disseminate to lymph nodes by normalizing tumor blood vessels.

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Abstract text

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 LYVE-1 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 VEGF-C expressing TAMs in redirecting cancer cells to preferentially disseminate to the lymph nodes, at least in part, by normalizing tumor blood vessels.

54155 - Intestinal estrogen receptor beta modulates the inflammatory immune microenvironment during colitis and early carcinogenesis

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Abstract text

Background: Chronic inflammation promotes the development of colorectal cancer (CRC). Studies show a protective effect of estrogen, mediated by estrogen receptor beta (ERβ), against the development of CRC. We have shown that intestinal ERβ protects against colitis and CRC by modulating inflammatory signaling. Our preliminary data further show that deletion of iERβ impaired tumor infiltration of natural killer cells, decreased T cell infiltration, and increased macrophage infiltration. The aim of this study was to investigate how iERβ affects the colonic immune microenvironment during colitis.

Methods: Wild-type (WT) mice and mice that lack iERβ (ERβKO^{Vil}) were treated with vehicle or AOM/DSS to induce colonic inflammation and early carcinogenesis and analyzed at 9 weeks after the start of the treatment. Using the novel COMET platform (Lunaphore Technologies) for multiplex immunofluorescence (available at the Spatial Proteomics Facility at SciLifeLab), we stained formalin-fixed, paraffin-embedded Swiss-rolled colons from 32 mice for a panel of 10 markers. QuPath was used to analyze the images and quantify the results. We profiled the numbers of infiltrating natural killer cells (Klrb1c+), M1 (F4/80+CD86+) and M2 (F4/80+CD206+) macrophages, regulatory (FOXP3+), helper (CD3+CD4+), and cytotoxic (CD3+CD8+) T cells, neutrophils (Ly-6G+), and dendritic cells (CD11c+).

Results: Immune cell infiltration into the colon increased overall during colitis. Dendritic cell infiltration into the mucosa significantly increased in WT mice, whereas ERβKO^{Vil} mice displayed a significant increase in pro-inflammatory M1 macrophage infiltration. ERβKO^{Vil} mice had significantly increased infiltration of all immune cell populations, among them M1 and M2 macrophages, into the muscular layer during colitis, which was not seen in WT mice. Further, sex differences in immune cell infiltration were seen. Infiltration of M2 macrophages and NK cells, which ameliorate colitis, increased more in WT females than males, while infiltration of helper and cytotoxic T cells increased more in ERβKO^{Vil}males than females. During colitis, ERβKO^{Vil} males also had significantly higher numbers of NK cells and cytotoxic and regulatory T cells infiltrating the muscular layer compared to WT males. We also observed correlations between infiltrating immune cell populations during colitis that were genotype specific.

Conclusions: We conclude that AOM/DSS-induced colitis increases infiltration of all immune cell populations studied in WT mice. ERβKO^{Vil} mice displayed a stronger response, and we show that iERβ modulates macrophage infiltration in vivo during colitis. Our findings indicate more severe colitis and early carcinogenesis in male mice that is augmented upon iERβ deletion. Activation of iERβ could be of therapeutic value for preventing and treating colitis and CRC.

54158 - Multiplexed proximity ligation-based method uncovers immune checkpoint activation in the context of the tumor microenvironment

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Abstract text
The interaction of immune checkpoint (IC) proteins PD1 and PD-L1 plays a pivotal role in initiating T cell inhibition and preventing autoimmune responses in healthy cells. In cancer, it facilitates tumor evasion from the immune system, which makes ICs promising immunotherapy targets. However, the success of IC inhibition has been limited to a poorly defined subset of patients. Identifying responders may better correlate with high levels of PD1/PD-L1 interaction than with the overexpression of either protein alone, which makes *in situ* proximity ligation technology a valuable tool in patient stratification. Here, we combine the power of PD1/PD-L1 interaction detection with the concomitant visualization of relevant biomarkers (for mature/cytotoxic/helper T cells, or tumor markers e.g., Ki67, cytokeratin) to create an immune profile. The detection of the IC interaction has the potential to make a difference in immunotherapy as its presence indicates activation of the IC axis in the tumor, not merely IC protein expression. Since the communication between PD1 and PD-L1 is not strictly limited to immune cells but can also be used by cancer cells, the addition of relevant biomarkers is useful in demonstrating whether T cell inhibition has occurred as part of the normal immune process (when interaction-positive cells are two immune cells) or is a sign of tumor evasion (when communicating cells are positive for tumor markers too).

54159 - Extracellular vesicles - non-invasive protein biomarker analysis in EGFR-TKI treated non-small cell lung cancer patients

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Abstract text

Background: Non-small cell lung cancer (NSCLC) tumors are highly heterogenous and biopsies are difficult to obtain. The use of small extracellular vesicles (sEVs) as liquid biopsies for non-invasive analysis of biomarkers (BMs) is of great interest as they contain material from their cell of origin. We collected sEVs from serum of *EGFR*-mutant NSCLC patients with the aim to reveal BMs associated to response.

Methods: sEVs were isolated from serum of NSCLC patients (n=21) included in the investigator-initiated clinical phase II trial TREM-study [1, 2]. The sEVs were isolated using size exclusion chromatography and examined using Nanoparticle Tracking Analysis (NTA). sEVs were fractionized into EpCam positive vs total sEVs using magnetic beads. Their protein content was profiled using proximity extension assay (PEA). Qlucore® Omics Explorer was applied for data analysis and visualization. Western blotting and ELISA were used to characterize sEV markers and for validation of putative BMs.

Results: Serum contained 10^9 to 10^{11} sEVs/ml that were 100-300 nm. The sEVs expressed CD9 and TSG101. PEA protein profiling revealed that 140 out of totally 185 proteins were expressed over limit of detection (LOD) with heterogeneity seen among individual samples. Protein signatures that correlated to osimertinib treatment response were identified and validated. Among them were PD-L1, EphA2, CD73 and SYND-1.

Conclusions: We show that sEVs isolated from serum can be used as non-invasive liquid biopsies for NSCLC patients to monitor alterations in oncogenic signaling and/or tumor microenvironment as a way towards non-invasive BMs.

References:

(1). Eide, I.J.Z., et al., *Intracranial effect of osimertinib in relapsed EGFR-mutated T790M-positive and -negative non-small cell lung cancer patients: results from a phase II study.* Acta Oncol, 2021. **60**(12): p. 1565-1571.(2).
(2). Eide, I.J.Z., et al., *Osimertinib in T790M-positive and -negative patients with EGFR-mutated advanced non-small cell lung cancer (the TREM-study).* Lung Cancer, 2020. **143**: p. 27-35.

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54163 - The expanding universe of CuET anti-cancer properties

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Abstract text

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

54168 - Hypoxia-induced epigenetic changes coordinate transcription start site selection and translational control through remodeling of 5’UTRs

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Abstract text

Emerging data suggest that adaptation of cancer cells to hypoxia enhances malignancy and negatively impacts patient outcomes. Reprogramming of mRNA translation represents an essential component of the hypoxia-adaptive response. During hypoxia, due to alterations in translation machinery in part driven by repression of mTORC1 signaling and induction of eIF2alpha phosphorylation, cells undergo a global decrease in protein synthesis. However, translation of subsets of transcripts encoding stress-response, survival and stemness factors is increased. This selective regulation is thought to be governed largely via the interactions between specific mRNA features and remodeling of the translational apparatus. However, our understanding of the regulatory mechanisms that govern translational perturbations under hypoxia remains incomplete. To address this, we interrogated the effects of hypoxia on global changes in histone methylation and transcription site selection, coupled with monitoring corresponding alterations in total mRNA levels and translation efficiencies on a genome-wide scale in T47D breast cancer cells and H9 human embryonic stem cells. This allowed us to map the impact of specific 5’UTR features on translation efficiency under hypoxia, and revealed widespread hypoxia-induced epigenetic alterations leading to TSS switching and extensive remodeling of 5’UTRs. As a consequence of TSS switching, a number of transcripts gain or lose specific mRNA features, allowing preferential translation under hypoxia. Among the genes that underwent hypoxia-induced 5’UTR remodeling was Pyruvate Dehydrogenase Kinase 1 (PDK1), an enzyme that is key in orchestrating metabolic adaption to hypoxia wherein cells shift from oxidative phosphorylation towards glycolysis. PDK1 manifests hypoxia-inducible 5’UTR isoforms that are preferentially translated under hypoxia, and we show that this effect is due to epigenetically-driven changes in transcription start site usage. Therefore, our findings provide a previously unappreciated mechanism driving translational reprogramming under hypoxia, whereby alterations in translational apparatus are orchestrated with epigenetic perturbations that result in 5’UTR remodeling of the transcriptome.

54169 - Microglia secrete extracellular matrix modulators that are essential for the invasive nature of diffuse midline gliomas (DMG)

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Abstract text
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.

54177 - Hypoxia-induced Complement Component 3 Promotes Aggressive Tumor Growth in the Glioblastoma Microenvironment

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Abstract text

Glioblastoma is the most aggressive primary adult brain tumor. Treatment includes surgery, irradiation and chemotherapy. Still, virtually all patients suffer a treatment resistant recurrent tumor. In previous studies from our lab, we have investigated the tumor microenvironment contribution to tumor progression, showing that stromal astrocytes become reactive upon irradiation and hypoxia and generate tumor-supportive conditions for glioblastoma cells. Common features of astrocyte reactivity in several neurological diseases, include upregulation of complement proteins including complement component 3 (C3). However, it remains unexplored if these complement proteins are expressed in stromal astrocytes in glioblastoma.

In a genetically engineered glioma mouse model, C3 was expressed in the invasive front, hypoxic, and perivascular tumor areas. TCGA data analysis revealed an overall strong correlation between hypoxia and complement gene expression signatures in glioblastoma patients. Astrocytes and glioma cells cultured in hypoxic conditions upregulated C3, as well as other genes associated with an infiltrative phenotype of GBM. In hypoxia, glioma cell lines, but not astrocytes, upregulated the C3a Receptor. GBM tumor single-cell sequencing data confirmed a subpopulation of highly C3-expressing astrocytes, enriched for cellular pathways comprising epithelial mesenchymal transition (EMT), TNF-alpha, hypoxia, and IFN-signaling. *In vitro*, C3 enhanced proliferation of glioma cells specifically under hypoxic conditions. Blocking C3a-C3aR signaling *in vivo* prolonged survival of glioma-bearing mice both alone and in combination with radiotherapy. Overall, our data indicate a strong link between hypoxia and complement expression in the brain tumor microenvironment of glioblastoma, where local expression of complement proteins lead to tumor promoting signaling. Inhibition of C3a-C3aR enhanced survival of glioma-bearing mice and could represent a promising avenue for further investigations.

54188 - Fine-needle-aspiration based immune profiling of tumor microenvironments

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Abstract text

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.

References:

1. Schoenberg SO, Attenberger UI, Solomon SB, Weissleder R. Developing a roadmap for

1. Schoenberg SO, Attenberger UI, Solomon SB, Weissleder R. Developing a roadmap for interventional oncology. Oncologist. 2018 Oct;23(10):1162-1170.
2. Franzén B, Kamali-Moghaddam M, Alexeyenko A, et al. A fine-needle aspiration-based protein signature discriminates benign from malignant breast lesions. Mol Oncol. 2018 Sep;12(9):1415-1428.
3. Franzén B, Alexeyenko A, Kamali-Moghaddam M, et al. Protein profiling of fine-needle aspirates reveals subtype-associated immune signatures and involvement of chemokines in breast cancer. Mol Oncol. 2019 Feb;13(2):376-391.
4. Franzén B, Viktorsson K, Kamali C, et al. Multiplex immune protein profiling of fine-needle aspirates from patients with non-small-cell lung cancer reveals signatures associated with PD-L1 expression and tumor stage. Mol Oncol. 2021 Nov;15(11):2941-2957.
5. Röbeck P, Franzén B, Cantera-Ahlman R, et al. Multiplex protein analysis and ensemble machine learning methods of fine needle-aspirates from prostate cancer patients reveal potential diagnostic signatures associated with tumor grade. Cytopathology. 2023 Feb 24.

54189 - Copy number alterations in sequential biopsies from a patient with recurrent oral leukoplakia that progressed to cancer

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Abstract text

Oral squamous cell carcinoma (OSCC) severely impacts patient morbidity and mortality. Early detection and treatment of OSCC is of outmost importance for patient survival and quality of life. Since a significant fraction of OSCCs is preceded by oral leukoplakias (OL), it might potentially be possible for early detection of patients with an increased risk of developing OSCC. However, currently there is a lack of knowledge about driving genomic alterations and biomarkers that can predict the risk of malignant transformation of OLs. We have recently identified copy number alterations (CNAs) involving the tumor suppressor gene *CDKN2A* and the oncogenes *EGFR* and *CCND1* in OLs, and particularly in OLs progressing to OSCC. Here, we have studied CNAs from 5 sequential biopsies taken from recurrent OLs over a period of 22 years from a patient that subsequently developed OSCC. Formalin-fixed paraffin-embedded and fresh frozen tissues, were obtained from a female patient with multiple recurrent OLs at the right lateral border of the tongue that subsequently developed OSCC. The patient initially presented with a homogenous OL that progressed to non-homogenous OLs with mild to severe epithelial dysplasia. CNAs involving the known OSCC driver genes *CDKN2A*, *CCND1*, *EGFR*, and *MYC*were detected by FISH. ArrayCGH was done on genomic DNA isolated from frozen intralesional tissue and clinically healthy mucosa from one of the OL recurrences. Cytogenetic and spectral karyotype analyses were also done on one of the OL biopsies. Loss of *CDKN2A* was the most prominent CNA detected in all OL biopsies as well as in the OSCC. Amplification/gain of *CCND1* and *EGFR* was detected in 4 and 3 OLs, repectively. Gain of *MYC* was seen in 3 OLs. In contrast, the OSCC specimen showed no evidence of amplification or gain of any of these three genes. Cytogenetic and spectral karyotype analyses of one OL biopsy revealed a hypodiploid karyotype with t(3;8) and t(X;9) translocations, loss of chromosomes 8, 13, and 21, and several double minute (dmin) chromosomes. Collectively, our findings indicate that loss of *CDKN2A* is an early event in the pathogenesis of the OLs and OSCC in this case, which is in line with our previous observations. Amplification/gain of *CCND1* and/or *EGFR* do also occur in OLs but did not contribute to the development of OSCC in the present case. Our findings also demonstrate for the first time that chromosome translocations may contribute to the development of OL. Further molecular analyses are needed to unveil the landscape of genomic alterations in OLs and their role in malignant transformation of these lesions.

54190 - Combined functional drug response and single-cell transcriptomic read-out in pediatric acute lymphocytic leukemia cells

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Abstract text

Functional precision medicine involves ex vivo measurement of drug response in order to tailor treatments for individual patients. Here, we functionally annotated drug resistance using the glucocorticoid-resistant E/R+ REH acute lymphoblastic leukemia (ALL) cell line as a cellular model in response to 25 drugs. We evaluated threedifferent technologies for barcoding selected drug conditions with a single-cell gene expression (scRNA-seq)readout. We sequenced >30,000 single cells with three current scRNA-seq methods and benchmarked the technologies against each other. Each of the scRNA-seq approaches accurately reflected gene expression changes in the system, with high cell recovery and reproducible tagging of the different drug conditions. However, differences in sensitivity and implementation feasibility were discerned. Furthermore, we identified a substantial and reproducible transcriptional response to one of the drugs, clinically of particular interest for treatment of high-risk ALL.

54191 - The anticancer compounds auranofin, TRi-1 and TRi-2 have distinct cytotoxicity profiles with regards to thioredoxin reductase inhibition

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Abstract text

Cancer cells' reliance on the thioredoxin (Trx) and thioredoxin reductase (TrxR) system for sustained proliferation has led to the development of TrxR inhibitors, such as auranofin, TRi-1 and TRi-2, with pronounced anticancer effects and varying target specificity (1). TRi-1 and auranofin additionally create prooxidant TrxR forms known as SecTRAPs, which potentiates their effects. The cellular signaling programs triggered by these inhibitors, however, are still poorly understood. We report three observations regarding the kinetics of intracellular TrxR inhibition, the compounds' cytotoxicity relative to cellular uptake and their broad effects on proteostasis.

Whereas cell death following auranofin treatment is swift, TRi-1 and TRi-2 treatment produces distinctly delayed cytotoxicity (2). Nevertheless, TRi-1 produces the fastest and most pronounced inhibition of TrxR activity which returns to pretreatment levels well before the onset of cell death, likely through Nrf2-mediated compensatory mechanisms. All three compounds deplete TrxR activity but not protein abundance, creating a window for additional cell damage via potential SecTRAPs.

The molecular mechanisms governing the inhibitors' cellular uptake are not known but we have found dose-dependent delayed cytotoxicity seen even with 5-10 min treatment and subsequent compound removal. This suggests rapid uptake before cytotoxicity can be detected. These effects are more prominent for TRi-1, suggesting the importance of distinct uptake and signaling pathways for these inhibitors' activity.

Outlining the differences in the specificity, speed, and uptake of TrxR inhibitors is crucial towards understanding the effects of antioxidant system inhibition and furthering its therapeutic potential.

1. Gencheva R, Arner ESJ. Annu Rev Pharmacol Toxicol. 2021. doi.org/ gm3q2z.
2. Sabatier P, Beusch CM, Gencheva R, Cheng Q, Zubarev R, Arnér ESJ. Redox Biology. 2021;48:102184. doi.org/gnprb5.

54193 - Targeting of astrocyte-driven enhancement of glioblastoma growth

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Abstract text

Glioblastoma (GBM) is a deadly disease with an urgent need for deeper understanding and new therapeutic approaches. The microenvironment of glioblastoma has previously been shown to guide glioblastoma progression. Previous efforts in our group have highlighted the astrocytes as enhancers of glioblastoma proliferation through correlative analyses of clinical samples and experimental *in vitro* and *in vivo* studies.

A first effort to establish a high-throughput screening pipeline on our astrocyte/glioblastoma co-culture system, used 1200 compounds belonging to the “Prestwick library”. This led to the identification of two compounds that specifically blocked the astrocyte-dependent proliferation of U343MG GBM cells. These findings confirmed the possibility of affecting GBM growth targeting their crosstalk with astrocytes. Based on these findings an additional drug screen was implemented taking advantage of a well-annotated compound library from AstraZeneca.

The drug screen, using luciferase assay as endpoint, was performed on co-cultured GBM cells and astrocytes, with the aim of identifying compounds that specifically interfere with the astrocyte-driven enhancement of GBM growth. Briefly, 14000 compounds from the AstraZeneca phenotypic collection were screened on GBM cells and astrocytes in co-culture. Following multiple steps of the screening cascade, from primary hits identification to near neighbor testing, interesting compounds were identified targeting relevant pathways in GBM biology. Around 130 compounds were ultimately identified as specific hits, 60 of these showing more than 10-fold higher activity in co-culture than in either of the mono-cultures. Several putative targets for our candidate compounds were identified through bioinformatics approaches, including casein kinase 2 (CK2) and fatty acid synthase (FASN).

Preliminarily analyses of the TCGA GBM data set indicated that upregulation of candidate targets is linked to a trend for shorter survival. Notably, FASN knock-down in astrocytes reduced the ability of astrocytes to support glioblastoma proliferation. Furthermore, FASN expression was detected in astrocyte-like cells of glioblastoma specimens by antibody staining.

Hit compounds not strongly associated with targets are being explored by ongoing proteomics analyses for target identification. In addition, a CRISPR/Cas9 genome-wide screen is being performed with the aim of identifying pathways responsible for the GBM/astrocyte crosstalk.

In summary, an assay system has been developed and used for identification of candidate drugs targeting astrocyte-mediated support of glioblastoma. Screening results, tissue profiling and knock-down experiments suggest FASN in astrocytes as a critical component of astrocyte-driven glioblastoma growth. These findings are developed in ongoing mechanistic studies and will be validated in orthotopic mouse GBM models.

54200 - Plasma and tumor levels of leucine-rich repeats and immunoglobulin-like domains protein 1 (LRIG1) are associated with survival in ovarian carcinoma

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Abstract text

BACKGROUND: Ovarian carcinoma is the eighth most common cause of cancer death in women worldwide. Current biomarkers for early detection, prognosis, and treatment prediction are suboptimal. Hence, there is a need for new and better biomarkers in ovarian carcinoma. Leucine-rich repeats and immunoglobulin-like domains protein 1 (LRIG1) is a transmembrane protein that functions as a tumor suppressor and regulator of growth factor signaling. Possible associations between LRIG1 protein levels and clinical parameters have not been investigated in ovarian carcinoma, previously.

OBJECTIVE: To investigate the possible diagnostic, prognostic, or predictive biomarker role of LRIG1 in ovarian carcinoma by evaluating the protein levels both in plasma and tumor tissue from the same cohort.

MATERIALS AND METHODS: A series of monoclonal LRIG1 antibodies was developed and used to establish both an LRIG1-specific single molecule array assay for the quantification of LRIG1 in biological fluids and an immunohistochemical protocol for the analysis of LRIG1 in tissue sections. LRIG1 levels were quantified in plasma and tumor tissue from 486 patients with suspicious ovarian masses. Statistical analyses were performed to assess the possible associations between LRIG1 levels and clinical parameters.

RESULTS: LRIG1 plasma levels were significantly elevated among women with ovarian carcinoma compared to women with benign or borderline type tumors. High LRIG1 plasma levels were associated with worse overall survival and shorter disease-free survival both in the group of all malignant cases and among the stage 3 cases only. In stage 3 ovarian carcinoma, high LRIG1 was an independent prognostic factor associated with worse survival. In fact, in type 1 stage 3 ovarian carcinoma, plasma LRIG1 outperformed all the other tested prognostic factors. LRIG1 immunohistochemistry showed partly opposite clinical associations compared to LRIG1in plasma. Intriguingly, in type 1, stage 3- 4, ovarian carcinoma, a high LRIG1 immunohistochemistry score was associated with superior survival.

CONCLUSION: LRIG1 plasma levels were elevated in patients with ovarian carcinoma, and high LRIG1 plasma levels were associated with poor prognosis, whereas a high LRIG1 immunohistochemistry score was associated with a good prognosis, especially in type 1 ovarian carcinoma. Taken together, our results suggest that LRIG1 might be an etiologic factor as well as a potentially useful biomarker in ovarian carcinoma.

54204 - scCircle-seq unveils the diversity and complexity of circular DNAs in single cells

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Abstract text

Extrachromosomal circular DNAs (circDNAs) have emerged as important intra-cellular mobile genetic elements that affect gene copy number and exert in trans regulatory roles within the cell nucleus. Here, we describe scCircle-seq, a method for genomically profiling circDNAs and unraveling their diversity and complexity in single cells. We implemented and validated scCircle-seq in normal and cancer cell lines, demonstrating that most circDNA species vary between cells and are stochastically inherited during cell division, although their genomic landscape is cell type-specific and can be used to accurately cluster cells of the same origin. circDNAs are preferentially produced from chromatin regions enriched in H3K9me3 histone mark and are induced during replication stress conditions. Concomitant sequencing of circDNA and RNA from the same cell uncovered the absence of correlation between circDNA copy number and gene expression levels, except for few oncogenes contained within large circDNAs in colorectal cancer cells, including MYC. scCircle-seq can be used to dissect the complexity of circDNAs across different cell types and further expands the potential of circDNAs for cancer diagnostics.

54210 - Drugging the undruggable target: The Ewing sarcoma fusion protein

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Abstract text

Ewing sarcoma (ES) is the second most-frequent bone tumour in childhood and adolescence with a peak incidence rate in the second decade of life. Localised ES has a five-year relapse-free survival rate of only around 55% which drops to 21% for metastatic ES. Survival has largely stagnated for the last decades despite several efforts of treatment intensification.

Resulting from a chromosomal translocation, ES is driven by a fusion protein (most frequently EWS-Fli1 or EWS-ERG) with aberrant transcription factor activity that reprogrammes the cellular transcriptome. Cytarabine, widely used for treatment of haematological malignancies, was identified as a drug that can induce degradation of the fusion protein and thereby reverse its transcriptomic effects, and its anti-tumour efficacy was confirmed in animal experiments. However, a phase-II trial with relapsed and refractory ES patients failed to demonstrate meaningful clinical efficacy of cytarabine. We hypothesize that the cytarabine resistance factor SAMHD1 may explain this discrepancy.

Here, we demonstrated differential SAMHD1 expression in a panel of ES cell lines ranging from absent to high, and SAMHD1 levels correlated with sensitivity to cytarabine. In addition, we used immunoblotting to demonstrate the dose-dependent ability of cytarabine to deplete the ES fusion protein. More important, gemcitabine, a drug used for treatment of relapsed bone sarcomas and a substance that we have previously described as a SAMHD1 inhibitor sensitized ES cells to cytarabine in a SAMHD1-dependent manner and dramatically increased cytarabine-induced ES fusion protein depletion. In a next step, we seek to validate these findings in mouse models of ES. Giving the longstanding use of cytarabine and gemcitabine in paediatric oncology, this research promises to lay the ground for a novel treatment strategy of ES that targets its hitherto undruggable cancer-driving fusion protein which could quickly be translated into a clinical trial.

54212 - Understand and target astrocyte reactivity in brain tumors to prevent radioresistance

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Abstract text

Glioblastoma multiforme (GBM) is the most aggressive type of glioma and deadliest brain tumor. Despite aggressive treatments including surgery, chemo-, and radiotherapy, tumors invariably recur as incurable lesions, and the median survival is approximately 15 months after diagnosis. Recurrence is tightly linked to tumor cell resistance to ionizing radiation (IR), a feature that in turn is linked to stem cell characteristics of tumor cells. Previous results from our lab indicate that stromal astrocytes respond to IR with a reactive phenotype that in turn promotes therapeutic resistance of neighboring tumor cells. Our objectives were to i) identify pathways involved in IR-induced astrocyte reactivity; ii) identify compounds able to inhibit IR-induced astrocyte reactivity.

We used two complementary approaches to meet our objectives. Using an antibody array we found that JNK and ABL pathways were activated following IR in primary human astrocytes. To validate their involvement in the induction of astrocyte reactive phenotype we used an image-based readout combining the changes in the expression of reactivity markers and in the morphology of astrocytes.

For the second approach, we used a similar readout to perform an image-based drug screen which aimed to identify compounds able to inhibit IR-induced astrocyte reactivity. The drug libraries used consisted of approved drugs to get candidates with potential for drug repurposing. We identified 119 compounds inhibiting IR-induced astrocyte reactivity. Most of the compounds belong to 6 different drug classes. Interestingly, amongst them 4 classes are composed of brain-penetrant compounds that could make them good candidates for repurposing in GBM. A confirmation screen is now ongoing for further validation of the compounds and the most efficient at inhibiting IR-induced astrocyte reactivity will be tested *in vivo* in a PDGFA/PDGFB-driven mouse model of GBM.

54214 - Contribution of extracellular vesicles in the communication of microglia to pediatric versus adult brain tumors

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Abstract text
High-grade gliomas are highly aggressive primary brain tumors that originate from glial cells. They can occur at any age, but there are differences in incidence when studied at a population basis. Even if they seem identical, the clinical research data strongly suggests that tumors are different in adults and children. The molecular composition of childhood high-grade glioma does not fit neatly into the subgroupings used for adults and they are likely biologically different tumors. In a tumor environment, **microglia**, the resident immune cells of the brain, are recruited by glioma cells which in turn change their original functions, toward tumor-supportive functions to promote tumor growth, invasiveness, and chemotherapy resistance. Therefore, the glioma-microglia communication leads to the formation and preservation of an immunosuppressive tumor environment. Elucidating the mechanisms by which brain tumors interact with microglia can uncover potential targets for clinical applications.
Extracellular vesicles (EVs) have been recognized as critical intercellular communicators that can contribute to cancer progression and have shown to be promising cancer biomarkers and therapeutics. The different subtypes of EVs vary in size and origin, but are similar in terms of function. They can be taken up by other cells, transfer their protein, RNA and DNA cargo, and change the behavior of the recipient cells. This work aims to determine the impact of glioma EVs on microglia. Since adult and pediatric glioma are related, but not as closely as was believed, we are interested to analyses the content of EVs released by both adult and pediatric high-grade gliomas and to observe how these EVs influence microglial cells.

The main objectives are:

- To study EVs derived from pediatric and adult brain tumor cells and to determine the tumor specific signature of released EVs
- To find how cancer cells modulate microglial phenotype subsequent after exposure to pediatric and adult derived EVs

These results will lead to find if tumor EVs play a role in tumor-associated microglia formation and if yes, they will open future direction to manipulate this process.

Current therapies are ineffective for glioma, with high levels of mortality. Pediatric tumors are more receptive to different treatment strategies, but with negative consequences regarding the brain development of affected children. Distinct molecular heterogeneity exists between different ages and brain location, even within a tumor. Our work will fill in the gap in understanding the effects of tumor EVs on microglia cells, with an extended comparison of pediatric versus adult tumor EVs. We work to elucidate the mechanisms of tumor-microglia communication that mediate the cancer growth and invasion. We believe that this study will lead to the identification of potential new targets and the development of new EV-based diagnostic/prognostic tools for patients affected by high grade glioma.

54215 - The impact of mesenchymal niche in human skin on drug response of acute myeloid leukemia

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Abstract text

Leukemia cutis (LC) or leukemic cell infiltration in skin is one of the common extramedullary manifestations in acute myeloid leukemia (AML) and signifies a poorer prognosis. However, the pathogenesis and maintenance of LC have been understudied, which has limited our understanding of its impact on AML progression and relapse. Mesenchymal stem cells (MSCs) in bone marrow (BM) have been shown to be critical for normal hematopoiesis and leukemia. Recently, in a transplantation-induced AML mouse model, we have shown that the AML cells infiltrated in the mouse skin during steady state and after chemotherapy were capable to regenerate AML after serial transplantation. Furthermore, similar to BM MSCs, skin MSCs could support the growth of AML-initiating stem cells (LSCs) and protect them from chemotherapy, even to a better degree than BM MSCs. These findings provide evidence for the important role of skin mesenchymal niche in AML cell survival and chemotherapy response, and may serve as a reservoir for AML cells, leading to potential AML relapse. However, it remains unexplored whether human skin MSCs exert the same functional impact in AML patients.

We here have successfully isolated and characterized human skin MSCs (CD45-CD235a-CD31-CD44+CD146-) from healthy donors. Q-PCR results indicated that human skin MSCs also express key hematopoiesis-regulatory genes, similar to human BM MSCs. Functionally, they displayed a superior protective effect on human AML cell line THP-1 than their BM cell counterparts, reflected in the increased residual chemo-resistant AML cells expressing CD36+ marker after chemotherapy treatment using cytarabine (Ara-C) in the co-cultures of human skin compared to that with BM MSCs. Further cobblestone-area forming colony (CAFC) assay showed increased residual CAFCs from THP-1 cocultured with human skin MSCs after chemotherapy treatment, indicating a stronger support of human skin MSCs for AML LSCs compared to BM MSCs. This notion was further supported by serial colony-forming unit in culture (CFU-C) assay of the CAFCs. RNA-sequencing of the skin MSCs before after co- culture with AML cells revealed that human skin MSCs are more resistant to AML-remodeling, which has been considered as one of the mechanisms underlying AML cell proliferation, providing a potential mechanism by which human skin MSCs maintain and protect AML cells.

Taken together, our preliminary data indicate a potentially important role of skin MSCs in supporting and protecting AML cells. Further investigations will be to unravel the molecular and function features of skin mesenchymal niche for AML cell chemoresistance and relapse in patients with AML.

54216 - in situ proximity ligation assays applied in fine needle aspirates provide a valuable complement to immunohistochemistry

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Abstract text
Histopathology and immunohistochemistry (IHC) are the gold standards to diagnose different cancer types, both solid tumors and hematologic malignancies. Routinely, the process takes a few days to prepare before the pathologist can evaluate the slides in the microscope.

Fine needle aspiration (FNA) is a method where a few hundred to thousands of cells are aspirated from the tumor with a needle and distributed on a glass slide. The cells are stained and evaluated by bright field microscopy. The molecular pattern by IHC makes it possible to phenotype the tumor.

The in situ proximity ligation assay (isPLA) is an advanced method to investigate proteins in tissues and cells. isPLA uses pairs of antibodies coupled to oligonucleotides directed against the protein targets of interest (Söderberg et al., 2008). Upon binding by the antibody pair to their targets in close proximity, a localized DNA amplification is initiated via rolling-circle amplification (RCA). The amplification products are detected using fluorophore-labeled oligonucleotides creating a strong, localized signals. isPLA is a highly specific and sensitive method due to its requirement for dual recognition and the localized amplification. The assay also allows detection of protein interactions and modifications, serving to identify protein activity states.

isPLA is performed on hematologic cells or tissues, however, to our knowledge, isPLA has not previously been applied to fine needle aspirates. The aim of our study was to apply different antibodies on lymphocytes and evaluate and validate their specificity. FNA samples from lymph nodes were first simulated by using cell cultures where antibodies against markers on B and T cells were validated by isPLA, and the assays were then applied to FNA samples, demonstrating excellent target detection.

isPLA for FNA makes it possible to access tumor tissue by a minimally invasive means, and to investigate states of the cells that may reflect responses to therapy or clonal evolution. isPLA applied on FNA may also decrease the turnaround time for cancer diagnostics compared to other types of biopsy material, and isPLA can be used as a complement to IHC. Further, unlike other tissue biopsies, FNA can be used to obtain samples repeatedly, in order to follow the progress of disease and introduction on new therapies.

Ref
Söderberg O, Gullberg M, Jarvius M, Ridderstråle K, Leuchowius KJ, Jarvius J, Wester K, Hydbring P, Bahram F, Larsson LG, Landegren U. Direct observation of individual endogenous protein complexes *in situ* by proximity ligation. *Nature Methods* **3**: 995-1000 (2006) (doi.org/10.1038/nmeth947)

54218 - Ex vivo expanded mucosa-associated invariant T cells respond robustly against pancreatic tumor-associated microbiota and bypass antibiotic resistance

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Abstract text

Pancreatic cancer (PC) is one of the most lethal malignancies, with little improvement in outcomes in recent decades. Emerging studies have revealed the association between PC progression and gut- and tumor microbiomes. We have recently identified metabolically active microbiota in cystic pancreas tumors (Gaiser et al. Gut 2019, Morgell et al. J Proteom Res. 2021) and isolated live bacteria from tumor-cyst fluid (Halimi et al. Gut Microbes 2021). We hypothesize that mucosa-associated invariant T (MAIT) cells, a class of innate-like T lymphocytes with a broad antimicrobial reactivity, can be employed to combat tumor microbes. The anti-tumor bacteria feature of MAIT cells is investigated in 2D and 3D pancreatic spheroids infected with various tumor bacteria. Our result indicates that blood MAIT cells from healthy donors were effectively activated by the bacterial metabolites, by showing a clear CD69 activation and TCR downregulation dependent on microbial riboflavin metabolism competence. Further, healthy donor MAIT cells prepared using an *ex vivo* expanded MAIT cell protocol developed for immunotherapeutic applications (Healy et al. JHepRep 2021), were able to respond to and clear the bacteria infection from pancreatic cells challenged with the tumor bacteria. Importantly, this was found even in the context of intracellular infection resistant to broad-spectrum antibiotic treatment. Moreover, MAIT cells were effective against bacteria lacking riboflavin competence, suggesting an MR1-independent activation mechanism may exist. Our results establish the potential of MAIT cells to target tumor-associated microbes and further benefit cancer immunotherapy by eliminating cancer-associated microbes.

54224 - HER2-specific positron emission tomography for quantification of HER2 status in breast cancer with low HER2 expression - preliminary results

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Abstract text

Purpose: Tumors with low expression of Human Epidermal growth factor Receptor 2 (HER2) account for about 50% of all breast cancers and benefit from treatment with HER2-drug antibody conjugates (ADC). It is unknown to what extent HER2-low lesions can be imaged with HER2-specific positron emission tomography (PET) tracers. The purpose of this pilot study is to investigate the feasibility of HER2-specific PET imaging with the affibody molecule [^{68}Ga]Ga-ABY-025 (hereafter HER2 PET) for visualization of lesions with HER2-low status.

Methods and Materials: In this pilot, 10 participants with metastatic HER2-low breast cancer undergo a HER2 PET followed by a tumor biopsy guided by imaging results. The pilot is deemed successful when HER2 PET identifies all lesions with HER2 expression and no false-negative [^{68}Ga]Ga-ABY-025 uptake is detected in biopsy-verified HER2-expressing lesions according to immunohistochemistry or in situ hybridization.

Results: As of March 17th, six patients had undergone HER2 PET. Five of these underwent biopsies of either hepatic metastatic lesions (n=4) or lymph nodes (n=1). Maximum standardized uptake values in lesions biopsied varied between 6.0 and 28.7 and correlated to immunohistochemistry results of either 1+ or 2+, i. e HER2-low. Central pathology review is ongoing.

Conclusion: Preliminary results suggest HER2 PET with [^{68}Ga]Ga-ABY-025 is feasible for the detection of HER2-low tumors. Patient accrual will continue, and the study will be expanded to investigate the role of HER2 PET as a selection tool of patients who would benefit from HER2-specific ADC’s.

Clinical Relevance/Application: Given the confirmation of preliminary results the use of HER2 PET may be of significant value as a non-invasive tool to identify patients with HER2-low breast cancer that would benefit from treatment with HER2-specific ADC’s.

54226 - Patient-derived scaffolds; an in vivo-like model to study the impact of the tumor microenvironment in colorectal cancer

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Abstract text

Colorectal cancer is one of the most common and deadly cancer forms worldwide. The clinical outcome is profoundly influenced by the tumor microenvironment of the individual patient. The tumor microenvironment is a complex system consisting of many different components, including blood and lymph vessels, fibroblast, endothelial cells, immune cells but also cytokines, extra cellular vesicles and an extracellular matrix. A large body of evidence now exists that the individual patient specific microenvironment influences the chemotherapy response in many forms of cancer. Three-dimensional cell culture models are increasing in popularity as drug screening platforms due to their ability to more accurately mimic physiological conditions compared with traditional two-dimensional cultures; however, few or any can capture critical elements of the tumor microenvironment. To distinguish malign properties in the tumor microenvironment that can influence treatment response we used the Patient-derived scaffolds model that mimic in vivo-like growth conditions with an active tumor microenvironment. Patient-derived scaffolds was obtained via decellularization of surgically resected colorectal tumors from colorectal cancer patients with known clinical data. The patient-derived scaffolds were then used as growth substrate for the colon HT-29 cell line and cultured together for 3 weeks. Gene expression changes of HT-29 cells were analyzed by qPCR and compared to clinical information as response or progressive disease. HT-29 cells grown with patient derived scaffolds induced significant differences in gene expression that correlated to the clinical response from which the patient -derived scaffolds originate and may be used to predict treatment response. To investigate the protein composition of the cell-free patient derived scaffolds we performed mass spectrometry on tumor material from colorectal cancer patients with known clinical data. Proteomic analysis on decellularized tumors generated large-scale proteome data and quantified 5400 proteins in 56 primary colorectal tumors. The data support that Patient derived scaffolds model can reveal unique information how individual patient specific microenvironments influence the chemotherapy response in colorectal cancer and, in the future, as aid in personalized medicine. Mass spectrometry on patient derived scaffolds has revealed proteomic data of the composition of the tumor microenvironment and future analysis might reveal potential novel drug targets.

54227 - In situ identification of human multi-marker-defined colon CAFs with distinct and differential associations to T-cell and cancer cell properties

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Abstract text

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.

To identify characteristics indicative of differantial functions, explorative multiplex staing was performed on 6 stage II/III colon cancer tumors where 5-10 high-power-fields was 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 $PDGFRA^{low}$). 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. These fibroblast subset/cancer cell interactions were in general stronger for A3 than for A1 cells. 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 potential survival associations of the A-cell subsets. For this purpose FAP/PDGFRA ratio was used as a proxy of relative abundance of A2 cells. Analyses of the TCGA colon cancer dataset indicated a significant poor prognosis associations of this metric (p=0.015; optimed cut-off).

Quantitative spatial analyses of the fibroblast subsets, and T-cells, are ongoing in the well-annotated U-CAN cohort of approximately 400 cases. For mechanistic unserstanding secretomes of the four subsets are expolerd with particular focus on ligands relevant for the cell type-specific interactions.

Conclusion: Four novel multi-marker-defined, and spatially distinct, human colon cancer fibroblasts subsets were identified. Tumor-restraining functions were associated with to A1 and A3 subsets, with A1 predominatly linked to T-cell features and A3 to cancer cell features. In contrast, PDGFRA^{high}A2 was associated high cancer cell proliferation and PDGFRA^{low}A2 with inhibitory effects on T-cells. Findings suggest a series of novel paracrine interactions with biomarker and drug target potential that should be further explored.

54228 - Patient-derived scaffolds as a model for studying tumor microenvironment interactions and immune response modulation in breast cancer.

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Abstract text

Introduction: Tumor microenvironment (TME) and the interaction with the tumour cells play a crucial role in the progression of the disease and drug response. For better understanding of these interactions, our group have developed patient-derived scaffolds (PDSs), consisting of decellularized breast cancer samples that are used as 3D growth platforms for cancer cells studies. We have shown that decellularized PDS keeps relevant and unique information from the tumor microenvironment of each patient, including the preservation of molecules involved in the immune response of the tumor, and they can be repopulated with standardized breast cancer cell lines promoting epithelial-mesenchymal transition (EMT)-like and cancer stem cell (CSC) properties and decreasing proliferation, similarly to in vivo conditions (Landberg et al., 2020). Several of the expression changes in EMT markers and CSC related genes were also correlated to relevant clinical parameters such as grade, lymph node metastasis and patient disease-free survival (Garre et al., 2022). Moreover, cancer cells cultured in PDSs increased their resistance and modified their response to chemotherapy agents and endocrine therapies (Leiva et al., 2021; Gustafsson et al., 2021). Thus, we hypothesize that the characterization of the cellular and molecular interactions within the tumor microenvironment may be able to identify specific immune responses or modulations that are associated with better outcomes or resistance to treatment, which could lead to the development of more effective and targeted therapies. **Material and methods:** Here, we evaluated the immune tumor environment in different patient-derived scaffolds and the suitability of our model for testing immunotherapies. Breast cancer samples were decellularized by sequential detergent washes and the cell-free scaffolds were repopulated with standardized breast cancer cell lines for 21 days and the effect of the provided tumour microenvironment in the expression of relevant immune markers was monitored by qPCR. Additionally, we performed co-cultures in PDSs of cancer cells and T cells, and we analysed T lymphocytes infiltration by immunohistochemistry, and T lymphocytes killing capacity by viability assays. **Results:** We observed that the microenvironment provided by the PDSs clearly induced expression changes of immune markers, and they were associated to clinical parameters from the original tumour. Also, they had an impact on the ability of T lymphocytes to kill the cancer cells. **Conclusion**: Our data support that PDSs can reveal distinctive insights about immune response modulating properties of specific tumor microenvironments, making them a promising platform for immunotherapies testing.

References:

Landberg G, Fitzpatrick P, Isakson P, et al. Patient-derived scaffolds uncover breast cancer promoting properties of the microenvironment. *Biomaterials.* 2020. doi: 10.1016/j.biomaterials.2019.119705.

Garre E, Gustafsson A, Leiva MC, et al. Breast Cancer Patient-Derived Scaffolds Can Expose Unique Individual Cancer Progressing Properties of the Cancer Microenvironment Associated with Clinical Characteristics. *Cancers*. 2022. doi: 10.3390/cancers14092172.

Leiva MC, Garre E, Gustafsson A, et al. Breast cancer patient-derived scaffolds as a tool to monitor chemotherapy responses in human tumor microenvironments. *Journal of cellular Physiology*. 2021. doi:10.1002/jcp.30191

Gustafsson A, Garre E, Leiva MC, et al. Patient-derived scaffolds as a drug-testing platform for endocrine therapies in breast cancer. *Scientific Reports*. 2021. doi:10.1038/s41598-021-92724-9

54232 - Disinfection by-products in Swedish drinking water and risk of colorectal cancer – a population-based cohort study

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Abstract text

Background: Colorectal cancer is the third most common malignancy worldwide and is strongly linked to lifestyle and environmental risk factors. Trihalomethanes (THMs) are reactive chemical by-products that are formed during drinking water disinfection. Several disinfection by-products are rodent carcinogens, and exposure to these compounds has been associated with increased risk of colorectal cancer in case-control studies. However, confirmation from prospective studies is lacking.

Methods: We assessed the association of THMs, a proxy for disinfection by-product exposure, with incidence of colorectal cancer among 58,672 men and women enrolled in two Swedish population-based prospective cohorts. Exposure was assessed by combining information of residential history with municipal tap drinking water monitoring data, and participants were separated into categories of no exposure (no chlorination), low exposure (<15µg/L) and high exposure (≥15µg/L). During 22 years of follow-up (1998-2020), 1,928 incident cases of colorectal cancer (International classification of disease 10th revision, ICD-10, codes: C18-20) were ascertained from the Swedish cancer register. Cox proportional hazards regression models were used to assess hazard ratio (HR) and 95% confidence interval (CI) while adjusting for relevant confounders.

Results: Drinking water THM concentrations ≥15 µg/L was associated with an increased risk of colorectal cancer in men (HR: 1.25, 95% CI: 1.04 – 1.50) compared to no exposure. The association was observed for cancer of the proximal colon (HR: 1.52, 95% CI: 1.08 – 2.15) but not distal colon and rectum (HR: 1.09, 95% CI: 0.76 – 1.55, and HR: 1.15, 95% CI: 0.86 – 1.54, respectively). We observed overall no association of drinking water THM with colorectal cancer or subtypes in women.

Conclusion: Our results confirmed previous indications of a link between disinfection by-products in drinking water and colorectal cancer and suggest differences in risk between sexes and sites.

54233 - MYC inhibition induces lipid droplet accumulation in Clear Cell Renal Cell Carcinoma

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Abstract text
Clear Cell Renal Cell Carcinoma (ccRCC) is the most common type of kidney cancer, representing up to 75% of all cases. It is characterized by the loss of the *VHL (Von Hippel Lindau)* gene resulting in a pseudo-hypoxic state where hypoxia inducible factors (HIFs) are active even in the presence of oxygen. It is well known that these cells accumulate lipid droplets (LDs) and glycogen, which are usually dissolved during histopathological preparations, giving them the clear aspect. Lipid droplets are organelles formed with a central core of neutral lipids (cholesterol and triglycerides) surrounded by a monolayer of phospholipids with embedded proteins.

We previously demonstrated that inhibition of MYCN in neuroblastoma cell lines results in LD accumulation. Studying the effect of MYC inhibition in other cancer cell lines, we observed clear differences in RCC4 ccRCC cells depending on *VHL* status. While RCC4 *VHL-* cells were accumulating LDs, RCC4 *VHL+* cells did not show any signs of lipid deposits upon MYC inhibition. To further investigate this phenotype, MYC levels were downregulated by transduction with lentivirus carrying Omomyc, a dominant negative peptide. Our results showed that LDs were accumulated in RCC4 *VHL-* but not in RCC4 *VHL+* cells after MYC suppression both by genetic as well as pharmacological inhibition of MYC.

We next studied this phenomenon in hypoxia (1% O_2) employing two different strategies, treatment with cobalt chloride ($CoCl_2$), which produces pseudohypoxia, as well as incubating cells in a hypoxia chamber. In contrast to in normoxia, RCC4 *VHL+* cells accumulated LDs under hypoxic conditions. Considering that RCC4 cells express both HIF-1 α and HIF-2 α, we asked whether this effect was observed in ccRCC cells expressing HIF-2 α. Thus, we expanded our studies to the 786-and A-498 ccRCC cells. Notably, we found that in normoxia only *VHL-* cells accumulated LDs while both *VHL-* and *VHL+* cells showed LD formation under conditions of hypoxia.

In conclusion, our data revealed that simultaneous activation of HIF together with MYC inhibition is necessary for LD accumulation in ccRCC during hypoxia. Lipid droplets have been described to support cancer cell survival, proliferation, as well as resistance to chemotherapy. Identification of the mechanisms of how MYC and HIF regulate this process could potentially be exploited to develop novel therapeutic strategies for ccRCC patients.

54235 - Targeting autophagy as a therapeutic strategy in pediatric acute lymphoblastic leukemia

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Abstract text

Background: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children, accounting for approximately 25% of cancer cases in children below 15 years of age. The disease is heterogeneous with more than ten different genetic subgroups characterized by recurrent genetic abnormalities, such as chromosomal translocations, gene amplifications, and mutations. Glucocorticoids (GCs), such as dexamethasone (Dex) have long been used as the gold standard in clinical practice for treating ALL; however, the high doses are often associated with long-term toxicities. Also, resistance represents a major hurdle. The purpose of this study was to develop novel therapeutic approaches to selectively target cellular pathways that are activated in leukemic cells of different genetic subgroup of ALL, and to create treatment combinations to overcome resistance to GC therapies.

Methods: To identify the key pathways involved in the antitumor response of the ALL subtypes, we have analyzed our proteomics data, also publicly available at the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD023662. RNA-seq data can be also retrieved from NCBI’s Gene Expression Omnibus accessible through GEO Series accession number GSE168386 for a total of 41 pre-B ALL cell lines. We identified autophagy and lysosomal pathways as highly enriched in a genetic subgroup of ALL bearing a recurrent t(12;21), resulting in the ETV6-RUNX1 fusion protein. Using a smaller panel of pre-B ALL cell lines, we performed drug sensitivity screens to examine the impact of autophagy/lysosomal inhibition on ETV6-RUNX1 bearing cell lines in comparison with other pre-B ALL cells. To determine whether inhibition of autophagy/lysosomal pathways can potentiate the cytotoxic effects of GCs on ALL cells, we have performed synergy drug screens on a panel of pre-B ALL and T-ALL cell lines using combination treatment with Dexa and autophagy/lysosomal inhibitors and assessed using Synergy finder software.

Results: ETV6-RUNX1 bearing cell lines were significantly more sensitive to lysosomal and to one of the more selective autophagy inhibitors as compared to cell lines with other genetic rearrangements. Based on proteomics and drug-screening data, we have chosen 5 pre-B and 5 T-ALL cell lines that expressed GCR but had intermediate sensitivity to GC dexamethasone. The combination of dexamethasone and lysosomal/autophagy inhibitors was synergistic/additive, respectively, in killing both the B-ALL and T-ALL cells.

Conclusions: Activity of autophagy and lysosomal pathways may represent a valid target for treatment in the t(12;21) ALL subgroup. These pathways may be cytoprotective to ALL cells and even involved in developing resistance against treatment. Inhibition of these pathways using either existing drugs (drug repurposing) or commercially available inhibitors may increase susceptibility and synergize with convectional therapies such as GCs in ALL.

54236 - FET fusion oncoprotein binding sites, chromatin landscape and gene expression patterns in sarcoma

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Abstract text

Sarcomas constitute a heterogeneous group of tumors, where a subset of tumor types are characterized by FET (FUS, EWSR1 and TAF15) fusion oncogenes. FET fusion oncoproteins are considered to be causative and most tumors have few additional mutations. FET sarcomas affect mostly children and teenagers and no targeted treatments exist. FET fusion oncoproteins have been shown to influence global gene expression, in part by disrupting vital epigenetic regulators. However, the exact oncogenic mechanism remains unknown. Here, we applied a multi-omics approach to investigate the effect of FET oncoproteins on an epigenetic and transcriptional level. Utilizing ChIP-Seq, we compared the DNA binding profile of FUS-DDIT3, specific for myxoid liposarcoma, with the stress-induced transcription factor partner DDIT3. We identified unique properties of FUS-DDIT3 that were related to both FUS and DDIT3. Interestingly, we found a repetitive sequence that was highly enriched in FUS-DDIT3 but not in DDIT3. This could constitute a generic oncogenic function of FET oncoproteins since EWR1-FLI1 in Ewing sarcoma is known to bind repetitive microsatellites. Furthermore, by applying ATAC-Seq and RNA-Seq we found that FUS-DDIT3 expression substantially affected chromatin accessibility and gene expression and identified a core set of target genes. Our data contribute to our knowledge of FET fusion oncogenes, especially FUS-DDIT3 in tumor development. Detailed understanding of FET fusion oncogenes opens up new means for targeted therapies that ultimately result in improved treatment protocols.

54237 - Rewiring of promoter-enhancer interactome and regulatory landscape in glioblastoma underlying neurogliomal synaptic communication

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Abstract text

Chromatin organization controls transcription by modulating 3D-interactions between enhancers and promoters in the nucleus. Alterations in epigenetic states and 3D-chromatin organization result in gene expression changes contributing to cancer pathogenesis. Here, we mapped the promoter-enhancer interactome and regulatory landscape of glioblastoma, the most aggressive primary brain tumour. Our data reveals profound rewiring of promoter-enhancer interactions, chromatin accessibility and redistribution of histone marks across the four glioblastoma subtypes. This leads to loss of long-range regulatory interactions and overall activation of promoters, which orchestrate changes in the expression of genes associated to glutamatergic synapses, axon guidance, axonogenesis and chromatin remodelling. SMAD3 and PITX1 emerge as the major transcription factors controlling genes related to synapse organization and axon guidance. Inhibition of SMAD3 and neuronal activity stimulation cooperate to promote cell proliferation of glioblastoma cells in co-culture with glutamatergic neurons. Our findings provide mechanistic insight into the regulatory networks that mediate neurogliomal synaptic communication.

54240 - Development of affibody-based drug conjugates for targeted cytotoxic therapy of HER2-overexpressing cancers

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Abstract text

Background

Targeted delivery of drugs selectively to cancer cells might improve the efficacy and safety of cancer treatment. Human epidermal growth factor receptor 2 (HER2) is overexpressed in breast, gastric and ovarian cancers with low expression in normal tissues. Two antibody-drug conjugates are approved by FDA for the treatment of HER2-positive breast cancer. However, antibodies have several drawbacks: limited penetration into tissues; challenging construction of therapeutic agents; methods of drug conjugation provide heterogeneous mixtures with variable drug number and toxicity. Novel targeting agents, affibody molecules, are advantageous for the construction of drug conjugates. Controlled attachment of drugs provides uniform constructs with a well-defined structure, pharmacokinetics, and toxicity profile. High robustness makes them more tolerant to drug conjugation and radiolabeling conditions. Facile engineering enables the production of multifunctional constructs with a tunable half-life in the blood. We performed a series of studies to identify the optimal molecular design of affibody-based drug conjugates providing the highest uptake in tumors and the lowest uptake in normal tissues for the purpose of targeted cytotoxic therapy (1-5).

Methods

Affibody molecule targeting HER2 was fused to an albumin-binding domain for extension of half-life and site-specifically conjugated to a cytotoxic drug DM1. Several aspects of the molecular design were investigated: the number and position of targeting domains, linkers between domains and between protein and a cytotoxic payload, and various cytotoxic payloads. The conjugates were radiolabeled with $^{99m}Tc(CO)_3$ for quantitative characterization in vitro (binding affinity, specificity, internalization, cytotoxicity), and their biodistribution and tumor-targeting properties were evaluated in vivo. The most promising candidates were evaluated in experimental therapy studies in mice bearing HER2-expressing SKOV-3 xenografts. Tumor targeting specificity was evaluated using HER2-negative Ramos xenografts.

Results

We found that the introduction of a monomeric affibody domain instead of a dimeric and the use of a glutamate-containing linker minimized liver uptake and improved tumor accumulation. Increasing the drug-to-affibody ratio from one to three increased undesirable liver uptake. The therapy study using an optimized construct carrying DM1 at a dose of 10.3 mg/kg resulted in complete regression of SKOV-3 tumors in mice. When the DM1 drug was replaced by a more potent MMAF drug, further improvement in tumor-specific cytotoxicity was observed. In the following therapy study, the MMAF-conjugate provided complete tumor regression in 50% of mice at a reduced dose of 2.9 mg/kg, while the same dose of the DM1-conjugate provided a moderate anti-tumor effect.

Conclusion

Optimization of molecular design enabled the selection of several conjugates, which

demonstrated potent anti-tumor efficacy in mice without toxicities to normal organs. Valuable information about the structure-property relationship and pharmacokinetic profile of a novel class of targeting agents was obtained. This could aid the development of more effective and safe therapies for cancer.

References

1) Altai et al. J. Control. Release. 2018; 288:84-95;
2) Xu et al. Cancers. 2021; 13(1):85;
3) Ding et al. Pharmaceutics. 2021; 13(3):430;
4) Xu et al. Pharmaceutics. 2022; 14(3):522;
5) Yin et al. J. Control. Release. 2023; 355:515-527.

54243 - An FBXL12-FANCD2 regulatory axis links replication fork progression to immune evasion in neuroblastoma

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Abstract text

Replication stress (RS), caused by the activation of oncogenes such as cyclin E or MYC, constitutes an early obstacle for pre-cancerous cells in progressing towards malignancy. Genomic maintenance pathways including the Fanconi anaemia (FA) pathway are activated to alleviate the deleterious effects of excessive RS and promote cell survival. The high reliance of cancer cells on such pathways provides a rationale for development of new therapies.

Regulation of the FANCD2-FANCI (ID2) complex which functions as a central relay within the FA pathway has garnered much attention due to its pivotal role in coordinating DNA replication and repair, in addition to the links between FA and cancer predisposition. However, while ID2 activation and DNA binding is well studied, how the fully engaged ID2 complex is removed again remains obscure.

We report the SCF ubiquitin ligase receptor FBXL12 as a novel regulator of the RS response and reveal an unanticipated mechanism for the spatiotemporal clearance of FANCD2 from chromatin through its (poly-)ubiquitin-mediated proteasomal degradation by SCF-FBXL12, crucial for survival of cancer cells exhibiting high levels of replication stress. This dependency is reflected in different malignancies, including high-risk MYCN-amplified neuroblastoma (NB) where upregulation of FBXL12-FANCD2 is associated with poor patient outcome.

Despite the potential of MYCN to induce severe replication stress and thereby fuel genomic instability and the generation of neoantigens, MYCN-amplified tumours are immunologically ¨cold¨, largely devoid of infiltrating inflammatory cells. Strikingly, depleting FBXL12 reveals significant upregulation of multiple immune response pathways and an FBXL12-associated gene expression signature links inactivation of FBXL12 to a more favourable immune microenvironment. Collectively, our results indicate that FBXL12 constitutes a novel cancer vulnerability and hypothesise that targeting this pathway may unleash antitumour immunity in high-risk MYCN-amplified NB.

54244 - Tumor growth rate can serve as a measure of immune evasion.

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Abstract text
The development of theoretical models of tumor growth is an important step in advancing our conceptualization and mechanistic understanding of cancer development. Recent developments in imaging, sequencing and gene editing have shed a light on a large variety of previously unknown mechanisms. The impact of the tumor microenvironment (TME), especially the immune system has been found to play a pivotal role in tumor emergence and progression. Those findings have led to the development of treatment options, including new immunotherapies that show a large potential in cancer treatment. However, the highly adaptive and dynamic interplay of cancer and immune cells remains only partly understood. Current immunotherapies only show a response in a fraction of patients. Therefore, there is a need to develop theoretical and computational models that allow the quantitative description of the interaction of the tumor and the immune system. To develop and verify such models, detailed time-course data on tumor initiation and maturation of the TME would be ideal ground truth. Unfortunately, such data are impossible to obtain for clinical data and very elaborate in animal models. Clinical data is a precious resource of information and is obtained for all human tumor types across a wide variety of progression states.
Single-cell RNA sequencing (scRNAseq) is a popular emerging method and an ever-growing dataset of clinical and in-vivo tumor samples is freely available. Extracting the momentary dynamics of those samples can bring us one step closer to understanding tumor growth dynamics and the interactions with immune cells since it quantifies the tumor progression. Here, we developed a technique to extract the fraction of dying and dividing cells from scRNAseq data. Those values enable us to estimate the growth dynamics of the tumor, beyond the classical clinical measures (%KI67+ and immune hot/cold). We propose our newly introduced classifiers as an ordering parameter to find tumor-immune co-strategies. Slow tumor growth can be explained by slowly dividing cancer cells, but also by very quickly dividing cancer cells with equally elevated cell death. Discerning the different scenarios gives insights into the interplay of immune-driven cytotoxicity and immune evasion of tumor cells. Large-scale scRNAseq datasets of clinical tumor samples across a wide variety of tumors are available and we perform a metaanalysis across cancer types to determine cancer-immune strategies. We are able to correlate gene expression programs 136 with cell death and division rates across cancer types. Together with high-resolution spatial transcriptomics techniques, this allows the spatiotemporal ordering of tumor-immune interactions.

54248 - In Vivo Cell Fate Reprogramming Elicits Anti-tumor Immunity

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Abstract text

In recent years, immune checkpoint inhibitors (ICI) have revolutionized cancer treatment. However, only a small subset of patients responds to immunotherapy. Responsiveness to ICI has been associated with the presence of cross-presenting conventional dendritic cells type 1 (cDC1), but tumor-derived factors often limit cDC1 accumulation, survival, and functions in the tumor microenvironment. We previously demonstrated that overexpression of transcription factors PU.1, IRF8 and BATF3 (PIB) in fibroblasts and cancer cells imposes a cDC1 fate *in vitro*. Reprogrammed cells acquire high expression of antigen presentation complexes (MHC-I and -II) as well as co-stimulatory molecules at cell surface and cDC1 functions such as cross-presentation and cytokine/chemokine secretion. As such, we hypothesise that replenishing cDC1 in tumors by *in vivo* reprogramming of cancer cells into tumor-antigen presenting dendritic cells (tumor-APCs) will drive antigen-specific immunity.

In this study, we evaluated anti-tumor responses elicited firstly by *in vitro* generated tumor-APC through intratumoral injection and secondly by reprogramming cells inside tumors implanted in mice. Injection of TLR3-stimulated tumor-APCs in low immunogenic B16-OVA melanoma tumors extended animal survival, reduced tumor growth, and promoted lymphoid infiltration. To assess efficacy elicited by *in vivo* reprogramming in the tumor, we implanted B16 cells 16h after PIB-transduction mixed with non-transduced cancer cells. All animals were tumor-free for 40 days while controls displayed median survival of 27 days demonstrating the capacity of reprogrammed tumor cells to elicit anti-tumor immunity *in vivo* within the suppressive microenvironment and in absence of exogenous TLR triggering. Tumor-free mice showed increased antigen-specific T-cells in peripheral blood and median survival increase of 5 days post B16 re-challenge. Reduced tumor growth after *in vivo* reprogramming of $BRAF^{V600E}$ melanoma in cDC1-deficient $BATF3^{KO}$animals further confirmed the direct effect of replenishing cDC1 in tumors. Indeed, by collecting tumors after 5 days of reprogramming, we confirmed the acquisition of a cDC1 phenotype (CD45+MHC-II+XCR1+) in mouse tumor cells. Importantly, human glioblastoma (T98G) and lung (A549) cancer cells also underwent phenotypic reprogramming *in vivo* in immunodeficient animals. Finally, we combined *in vivo* reprogramming with either aPD-1 or aCTLA-4 treatment in ICI-resistant or sensitive melanoma (B16, YUMM1.7, B2905, $BRAF^{V600E}$). Remarkably, in all four models blocking PD-1 or CTLA-4 signaling was translated into further reduction of tumor growth or complete regression and high levels of systemic tumor-antigen specific CD8+ and CD4+ T-cells in the blood.

Collectively, we demonstrated that cDC1-reprogramming *in vivo* elicits immunogenic reprogramming of tumor cells that is translated into durable anti-tumor immunity as monotherapy or in combination with ICI. This study pioneers *in vivo* cell fate reprogramming for immunotherapy and paves the way for induction of a cDC1-fate *in situ* by delivering reprogramming factors directly to the tumor as a novel off-the-shelf gene therapy for cancer.

54249 - Unraveling cellular crosstalk in liver metastases of gastrointestinal cancer: Multimodal analysis of metastatic invasion

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Abstract text

Background

Pancreatic ductal adenocarcinoma (PDAC) has a dismal prognosis with a 5-year-overall survival around 10%. In most patients, liver metastases are found at initial diagnosis. Once pancreatic cancer liver metastases (PCLM) are diagnosed, therapeutic options are limited to palliative chemotherapy.

In primary pancreatic tumors, cellular interactions between tumor cells and their microenvironment shape tumor aggressiveness. However, little is known about the role of tumor-microenvironment interactions in PCLM.

Results

We have used single-cell RNA sequencing (scRNAseq) and cellular interaction profiling to globally analyze the tumor microenvironment of PCLM in a mouse model of aggressive metastatic growth. Single-cell interaction profiling identified novel tumor-liver interactions, which we spatially mapped in situ on RNA and protein level in a unique cohort of PCLM patients and in patients with colorectal cancer liver metastases. Our data suggest that metastases-liver crosstalk in both tumor types is driven by perimetastatic liver injury, induced by interleukin 6 (IL-6) signaling.To study the functional importance of the perimetastatic injury for tumor invasion, we treated PCLM-bearing mice with (i) a monoclonal IL-6 antibody (MP5-20F3) and (ii) a decoy receptor blocking IL-6-trans signaling (sgp130Fc, olamkicept). While we did not observe an effect on tumor size, we observed perimetastatic sinusoidal dilatation and haemorrhage accompanied by an increased immune cell infiltration. The impact of these observations on e.g. tumor cell fitness and the hepatic injury reaction are currently investigated.

Discoveries & Future directions

Using scRNAseq and cellular interaction profiling, we have identified a novel subset of tumor-adjacent liver cells in liver metastases and charted the interactome between these injury-induced liver cells and the invading metastases. Based on our data, we propose that the invasion of tumor cells into the liver parenchyma is associated with activation of acute-phase proteins in adjacent hepatocytes, regulated by IL-6 signaling, offering a potential lever to counteract metastasis invasion.

In the future, we will combine IL-6 inhibition with chemotherapy to target the hepatic injury reaction. In addition, we will combine CRISPR/Cas9 gene editing with our in vivo PCLM model. In a tumor cell-based genetic screen we will identify targets regulating perimetastatic injury and tumor cell aggressiveness.

54273 - Longitudinal proteogenomic profiling elucidates immunometabolism dynamics in breast cancer

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Abstract text

Background: Metabolic reprogramming exists within tumor cells and tumor microenvironment (TME) in breast cancer (BC), but little is known about how the immunometabolic interplay of BC evolves during treatment. Using temporal proteogenomic profiling, we studied in-depth BC immunometabolism and its potential therapeutic vulnerabilities.

Methods: BC tissue (pre/on/post neoadjuvant chemotherapy; NAC) was longitudinally collected from the PROMIX trial (NCT00957125) of NAC (n=150 patients) in HER2-negative BC and analyzed by: bulk RNA microarray (n=122), single nucleus RNA-seq (snRNA-seq) (n=8), whole-exome sequencing (WES) (n=20), and mass spectrometry-based proteomics (n=29), including bulk/single-cell immunometabolic phenotype/cluster deconvolution, protein correlation network and clonal evolution analyses.

Results: Baseline and dynamic change of immunometabolic phenotype based on bulk gene expression profiling suggested tumors with hot TME or downregulation of tricarboxylic acid (TCA) cycle, amino acid or nucleotide metabolism were associated with higher pathologic complete response in multivariable analysis. BC proteomes showed TCA cycle-related protein module was starkly elevated within cold tumors, and vice versa. Potential drug targets (FASN, LDHB, LDHA, IDH2, MDH2) in metabolic pathways regulating the TME were revealed through unbiased proteogenomic differential abundance analyses (cold vs hot). Fewer subclones were identified in hot (28.3%) tumors than cold (43.1%) tumors (P<.001), which were more likely to have accelerated growth relative to their parent if included known metabolic drivers (SDHA, CACNA1D, ACSL3, ATIC, MED12). Metabolic flexibility of breast epithelial cells was dissected by five snRNA-based metabolic clusters (C): C0 (normal and tumor cells, lowest global metabolic activity); C1 (OXPHOS and glycolysis, cold tumor exclusively); C2 (OXPHOS but mutually with glycolysis); C3 (glutathione); C4 (Notch signaling). Clonal analyses depicted the intra-tumoral heterogeneity in cancer evolution under therapy, and more subclones were identified in cold (43.1%) tumors than warm (37.8%) or hot (28.3) tumors (P<.001). Moreover, patients with positive immune phenotype change tended to reach clonal extinction (7/8), whereas those with negative phenotype change is associated with clonal persistence (9/11). Interestingly, subclones with known metabolic drivers (SDHA, CACNA1D, ACSL3, ATIC, MED12, SDHC, CCNC) were more likely to have accelerated growth relative to their parent, and those variants were pretreatment existed and persistent during treatment, or acquired after neo-adjuvant therapy, which mainly occurred to patients with negative TME switch.

Conclusion: This longitudinal proteogenomic study shows that interaction of tumor intrinsic metabolic states and TME is associated with treatment outcome, shedding light on the importance to target tumor metabolism for immunoregulation.

54280 - The role of an atypical chain-specific deubiquitinase in colorectal cancer

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Abstract text
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 play a key role in regulating stemness signaling pathways in intestinal and colorectal cancer stem cells, which offer potential cancer therapy targets. Yet, the molecular mechanisms remain poorly understood. Trabid is a K29 and K33-ubiquitin linkage-specific deubiquitinase. The function of these two ubiquitin linkages has been seldom studied and the role of Trabid also warrants further investigation.

Aim: To study the role of Trabid deubiquitinase in colorectal cancer.

Methods: Utilizing online sequencing and CRISPR/Cas9 screens data, 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 patient survival. To evaluate the growth capability of CRC cell lines following Trabid loss *in vivo*, after Trabid knockdown using shRNA, we inoculated CRC cell line into C57BL/6 mice and monitored tumor size. Additionally, we used APC, Kras, and TP53 gene-edited mice in conjunction with intestine-specific 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 crypt 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 lineage populations using qPCR.

Results: We found that most tumor cell lines, particularly CRC cell lines, are Trabid-dependent. Trabid knockout has negative survival effects on tumor cell lines. Trabid expression was elevated in the intestinal mucosa of early-stage CRC patients compared to normal tissues. High Trabid expression correlated with poorer overall and relapse-free survival in CRC patients. In xenograft tumor models, MC38 cells with Trabid knockdown exhibited smaller tumor sizes than those expressing the control shRNA. In the Apc^{min} mice model, intestinal Trabid knockout mice exhibited significantly longer survival times. In $Kras^{LSL\text{-}G12D}p53^{f/f}$, APC^{Min} $p53^{f/f}$, and APC^{Min} $Kras^{LSL\text{-}G12D}$ $p53^{f/f}$ mice models, intestinal-specific Trabid knockout mice tended to have smaller tumors and fewer distant metastasis. In organoids from systemic, intestinal-specific and intestinal stem cell-specific Trabid knockout mice, we observed that 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 functions as an oncogene, promoting tumorigenesis and metastasis. In the absence of Trabid, the intestinal stem cell population decreases, while the enterocyte and Paneth cell populations increase.

Key words: Colorectal cancer; deubiquitinase; Trabid; metastasis

54284 - Custom-designed liquid biopsy analyses - a potential biomarker in upper tract urothelial cancer

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Abstract text

Purpose: The prognosis of upper urinary tract urothelial carcinoma (UTUC) is associated with tumour grade (G) and stage. Despite preoperative risk-stratification and radical treatment, recurrence and progression are common. Thus, additional prognostic and monitoring biomarkers are needed. The study aimed to investigate if targeted analyses on circulating tumour DNA (ctDNA) in plasma could identify tumour-specific gene mutations and if the specific variants may contribute to predict disease progression.

Methods: Nine UTUC patients were included in this prospective pilot study. All tumours were previously genetically characterised. Two known tumour-specific variants were chosen for targeted analyses with multiplex droplet digital PCR on cell-free DNA (cfDNA) from plasma.

Results: Of six patients with diagnostic plasma samples, ctDNA was detected in four patients with G2 or G3 tumours. Three of these patients later progressed in their disease. The remaining patient had a large G3 tumour at sampling, which could explain the presence of detectable ctDNA. ctDNA in plasma was also associated with tumour size at sampling (P=0.045). In contrast, two patients with undetectable ctDNA in plasma at diagnosis had G1 tumour and G3 carcinoma in situ (CIS) respectively. The patient with G3 CIS had detectable ctDNA later during follow-up and progressed thereafter with aggressive intravesical recurrence and CT-scan verified CIS progression in the upper urinary tract. In four additional patients with sampling during follow-up, none had detectable ctDNA and none progressed.

Conclusion: Results indicate that ctDNA may be a prognostic and monitoring biomarker of aggressive UTUC. Further studies are needed to validate these results.

54287 - Ensembles for improved detection of invasive breast cancer in histological images

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Abstract text
Introduction: 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.

Materials and methods: Three datasets are used in this work. The first two (Clinseq (n=232);SöS (n=355)) were used to train and calibrate, and a third external dataset to validate (TCGA-BC (n=157)). Both training datasets include WSI level annotations of routine clinicopathological factors, including the associated Nottingham Histological Grade (NHG) and routine IHC biomarker status for ER, HER2 and Ki67 proteins. The Clinseq dataset contains exhaustive spatial annotations of IC specifically, and the SöS dataset contains exhaustive annotations for IC and Ductal Carcinoma In-Situ (DCIS) and partial annotations for other types of BC and artifacts. Such annotations are overlapped and a single tile can contain several labels which have to be mapped to IC vs the non-IC. TCGA IC annotations were available from a previously published study. All annotations were converted to binary tile level labels (IC vs non-IC) and an ensemble of inception V3 CNN networks were trained with cross validation. Composed of ten networks, the ensemble can provide a measurement of agreement with the ground truth that when visualized can point quickly to areas that could benefit from human intervention as well as providing insight into the confusion with other of the labels present in the SöS dataset, particularly DCIS. Additionally we explore important characteristics of ensembles such as aggregation methods, data sampling and the choice of architecture for each member of the ensemble.

Conclusion: The ensemble gives in fact better accuracy than the single model. There is a general increase in accuracy and dice score for IC in both training datasets and the external dataset. As an additional benefit, a quick qualitative observation of confusion areas is possible when the result of each member of the ensemble is visualized directly on top of the tissue.

54289 - Myeloid and T cell subsets defined in bladder cancer using single-cell RNA sequencing and their evaluation in a novel ex vivo model

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Abstract text

Background Immuno-oncological (I-O) treatment methods have revolutionized cancer therapy. Despite that bladder cancer has characteristics generally associated with response to I-O treatment, i.e. high immune infiltration and tumor mutational burden, only around 15-30% of the patients benefit from current immune checkpoint blockade. The absence of response in the majority of patients indicate that there are additional immunosuppressive hurdles to be overcome. We need to further identify and exploit novel drug targets that enable the immune system to efficiently eradicate cancer cells and improve survival for more patients.

Methods We performed single-cell RNA sequencing (scRNAseq) of tumor-infiltrating immune cells from non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) biopsies as well as full-length scRNAseq with parallel protein profiling (index sorting) of T cells in MIBC. $CD45^+$ or $CD3^+$ cells were sorted from tumor biopsies obtained from untreated patients with MIBC (N= 14) and NMIBC (N=4). In total, 30 000 $CD45^+$ and 4061 $CD3^+$ cells were processed according to the 10x Genomics and Smart-seq3 protocols, respectively. The publicly available sequencing data of the IMvigor210 cohort was used to investigate predictive effects of specific immune subsets. To validate findings from the scRNAseq, including evaluation of candidate targets expressed by specific immune cell populations, an *ex vivo* bladder tumor model is established by using precision cut bladder slices (PCBS). Fresh tumor tissue was cut into multiple slices with the same thickness using a vibratome, covering 20-40 cell layers and maintaining the original tissue composition. Cell viability and composition were assessed using flow cytometry and hematoxylin and eosin (H&E) staining of formalin-fixed paraffin-embedded (FFPE) tissue slices.

Results The transcriptomic immune cell landscape in NMIBC and MIBC could be successfully analyzed. In the myeloid compartment, $SPP1^+$ tumor associated macrophages (TAMs) were identified which correlated with response to checkpoint blockade in the IMvigor210 cohort. Within the T-cell compartment, populations including cytotoxic $CD4^+$ and $CD8^+$ T cells and exhausted $CD8^+$ T cells were detected. Two populations of cytotoxic $CD4^+$ T cells showed high similarity to their $CD8^+$ counterparts, underlining their potential role in anti-tumor activity. Furthermore, in Treg and cytotoxic T cell clusters, $CD56^+$ natural killer-like T cells were found. The gene expression profile was defined for each individual immune cell population and is used to further explore cell functionality and to identify novel surface markers potentially useful as drug targets for immune cell manipulation.

In the PCBS, we could maintain a cell viability of up to 80 % and identify cancer cells as well as several immune cell subsets even after seven days of cultivation. These included specific markers for T cells, B cells, and myeloid cells which are crucial in regulating a possible I-O response.

Conclusions Our results provide new and detailed insights into the myeloid and lymphocyte compartment in the microenvironment of bladder cancer. Understanding the functionality of specific immune subsets can give us important indications for designing novel treatment strategies. The implementation of PCBS conserves the tumor and its

surrounding microenvironment and enables a fast and personalized investigation of novel I-O treatments.

54292 - DNA-PK inhibition augment the cancer specific cytotoxicity of mitotic MTH1 inhibitor OXC-101

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Abstract text

DNA-dependent protein kinase (DNA-PK) plays an important role in the repair of DNA double-strand breaks via the non-homologous end joining (NHEJ) pathway. In addition to its role in the NHEJ pathway, DNA-PK is also known to have an emerging role in cell cycle progression and mitosis. Some studies have shown that depletion or inhibition of DNA-PK inhibitors leads to a delay in the transition from metaphase to anaphase. The mitotic MTH1 inhibitor OXC-101 (TH1579, karonudib) is a dual inhibitor that both targets MTH1 and disrupts tubulin polymerization in cancer cells, causing DNA damage and mitotic catastrophe in cancer cells. Based on the involvement of DNA-PK in mitosis, we hypothesized that depletion/inhibition of DNA-PK might enhance the DNA damage and mitotic catastrophic effect of OXC-101.

To test our hypothesis, we performed an in vitro drug combination experiment using the DNA-PK inhibitor nedisertib with OXC-101 in non-small cell lung carcinoma NSCLC (H460, A549), uveal melanoma (MP-38, MP-41 and MP-46), osteosarcoma (U2OS) and) and neuroblastoma (IMR32, Kelly, SKNFI) cancer cell lines and non-cancerous foreskin fibroblasts (VH-10, BjhTERT). We found cancer-specific synergistic drug interactions between nedisertib and OXC-101. Combined treatment of nedisertib and OXC-101 to H460 and MP46 increased expression of s10H3 (mitotic marker) and the apoptosis marker (cPARP) compared with single treatment. However, there was no additional increase in expression of the DNA damage marker γH2AX with combined treatment. We also performed annexin V staining by FACS on MP-38,MP-41 and MP-46 cells and observed a significant increase in the apoptotic population with combination treatment compared with single treatment. Imaging live cells, we observed a significant delay or arrest in the mitotic phase followed by death of cancer cells in mitosis in response to combination treatment, whereas cells treated individually with nedisertib or OXC-101 continued to proliferate and progress to the next phase of the cell cycle.

These data suggest that the DNA-PK inhibitor may enhance the cytotoxic effect of OXC-101 specifically in cancer cells. In addition, we will conduct an in-depth mechanistic study of how depletion/inhibition of DNA-PK enhances the cytotoxicity of OXC-101 and validate the drug combination in an appropriate preclinical mouse model.

54293 - Cancer-Associated Fibroblasts Demonstrate Significant Impact in Ovarian Cancer Cell Proliferation and Drug Response

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Abstract text

Ovarian cancer (OC) is a leading cause of gynecological cancers in women. Despite treatment advances, most patients succumb to disease. Little is known of how different microenvironmental features contribute to OC progression and response to treatment. Cancer-associated fibroblasts (CAFs) play a crucial role in tumor microenvironment, where they establish a complex network of cellular and molecular interactions. Studies suggest that CAFs can both suppress and support disease progression. Hence, here we sought to systematically investigate the impact of CAFs on cell proliferation and drug response in high-grade OC.

To capture the impact of CAFs and CAFs conditioned media on OC cell proliferation we developed co-culture system where pre-stained cells were continuously imaged for 72 h. Additionally, cytokine profiling was conducted using Olink proteomics to evaluate changes in signaling molecule secretion. To characterize alterations in drug response induced by co-culture, we employed a library of 528 different drugs and imaged after 72 h of treatment.

Our data shows significant impact of CAFs on OC cells proliferation, both in a contact-dependent and independent manner. Of the five cancer cell lines tested, one (TykNu) demonstrated unaltered proliferation rate regardless of environmental changes. Cytokine profiling analysis indicated altered secretion of molecules between cancer and fibroblast, with distinct profiles depending on each pair. HGF, IL-6, VEGFA, TFPI-2 were among the most altered cytokines under different experimental conditions. Interestingly, patient-derived fibroblasts had higher cytokine secretion compared to normal fibroblasts. In our drug screen, ~70% of the drugs had a similar effect on cancer cells regardless of culturing conditions. However, ~25% of the drugs were more effective in cells grown in co-culture, with only few showing higher effect in monoculture (~1%). Interestingly, drugs commonly used in ovarian cancer treatment (Carboplatin, Paclitaxel, Gemcitabine) exhibited stronger effect on co-culture. Hence, these results highlight the crucial need for further research to uncover the mechanisms causing the impact of stroma on OC response to the drugs and disease development.

These findings demonstrate impact of fibroblasts in cancer cell proliferation, cytokine production and drug response. This study highlights the importance of taking the microenvironment into account in drug discovery and functional precision medicine applications.

54294 - Cell-lineage controlled epigenetic regulation in glioblastoma stem cells determines functionally distinct subgroups and predicts patient survival

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Abstract text

There is ample support for developmental regulation of glioblastoma stem cells. To examine how cell lineage controls glioblastoma stem cell function, we present a cross-species epigenome analysis of mouse and human glioblastoma stem cells. We analyze and compare the chromatin-accessibility landscape of nine mouse glioblastoma stem cell cultures of three defined origins and 60 patient-derived glioblastoma stem cell cultures by assay for transposase-accessible chromatin using sequencing. This separates the mouse cultures according to cell of origin and identifies three human glioblastoma stem cell clusters that show overlapping characteristics with each of the mouse groups, and a distribution along an axis of proneural to mesenchymal phenotypes. The epigenetic-based human glioblastoma stem cell clusters display distinct functional properties and can separate patient survival. Cross-species analyses reveals conserved epigenetic regulation of mouse and human glioblastoma stem cells. We conclude that epigenetic control of glioblastoma stem cells primarily is dictated by developmental origin which impacts clinically relevant glioblastoma stem cell properties and patient survival.

54295 - Evolutionary constraint identifies candidate non-coding drivers in glioblastoma

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Abstract text

Glioblastoma (GBM) is the most common malignant brain tumor in adults. The disease is characterized by poor prognosis and a lack of effective treatment options. Non-coding mutations drive cancer by targeting regulatory elements and can be valuable biomarkers and therapeutic targets. However, their role in GBM remains poorly understood. We apply evolutionary constraint to whole-genome sequencing (WGS) data of 136 GBM patients to predict the functional impact of non-coding mutations. Non-coding mutations in evolutionary constraint positions are termed non-coding constraint mutations (NCCMs) and cluster as hotspots with regulatory potential in *PTEN, LRFN5* and *MIR99AHG.* We observe an enrichment of NCCMs in the regulatory regions of both novel and cancer genes, including *FOXA1, HOXA9* and *EBF1*. The genes with the strongest enrichment are *DLX5* and *DLX6* and NCCMs around them as well as *SHFM1* and *SSTR1* are proposed as novel candidate non-coding drivers. Patients with NCCMs in *SHFM1* have significantly poorer prognosis, suggesting that NCCMs could be useful biomarkers. Using WGS, we also describe the mutational landscape of 63 GBM cell lines that were established from tumors in our cohort. Most tumor mutations are replicated in the matching cell lines and mutational concordance between tumors and cell lines is high in coding and non-coding regions. The cell lines are confirmed as suitable models to study the non-coding space in GBM and used to validate candidate genes and mutations. We conclude that evolutionary constraint is a powerful tool to identify non-coding mutations that have the potential to drive GBM. Their discovery could lead to more robust patient stratification and better treatment in the future.

54296 - Tumor-associated microbiome composition and response to neoadjuvant chemotherapy (NACT) in early triple negative breast cancer (TNBC)

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Abstract text

Background

Breast cancer-associated microbiome and its role in treatment efficacy are poorly understood. We aimed to study tumor-associated microbiome composition in TNBC, its dynamics upon NACT, and its correlation with TNBC outcomes.

Methods

Diagnostic biopsies and surgery samples from patients with TNBC treated with NACT were selected. A 16S rRNA subunit gene analysis was performed to describe α-diversity with Shannon Index and study taxonomic profiles at genus levels. To control for differences related to the type of sample we also analyzed diagnostic biopsies and surgical samples from patients that had surgery without NACT.

Results

We analyzed 115 TNBC samples from: i) Diagnostic biopsies (n=87) from patients with either subsequent NACT (n=48, mean age 57) or upfront surgery (n=39, mean age 78); ii) Surgery samples (n=28) from patients with prior NACT (n=14, all unpaired; mean age 47) or upfront surgery (n=14, all paired with diagnostic biopsies; mean age 85). α-diversity was significantly lower in post-treatment surgery samples (mean 1.5) compared to pretreatment biopsy samples (mean 1.19, p=0.027), with a significant reduction of Prevotella abudance (p<0.001). Abundance of Blautia and Kocuria were significantly higher among patients that did not achieve pCR ($_{padj}$ <0.001). In patients with NACT, increased microbiome α-diversity at baseline correlated to worse event-free survival (HR 6.45, 95% CI 1.20 – 34.56) and overall survival. Abundance of Prevotella and Eubacterium at baseline, significantly correlated negative to overall survival and event-free survival and abundance of Lactobacillus was significantly higher among patients with relapse (p=0.004). Among the paired biopsy-surgery samples, biopsies correlated to an increased number of genera (p=0.026), but Shannon Index analysis did not yield significant differences.

Conclusions

Our results suggest that in TNBC, NACT has a significant impact in microbiome composition and some genera are correlated to outcomes. The type of sample (biopsy/surgical) must be taken into account in breast cancer-associated microbiome studies. This work provides the rationale for expanding microbiome analysis in order to find novel putative biomarkers of response to neoadjuvant therapy in early TNBC.

54298 - Improving leukemia clearance - Redirecting NK cells to the leukemic niche by genetic engineering of the cells

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Abstract text

Background

Infusion of Natural killer (NK) cells can induce anti-tumor responses in patients. Currently, most efforts to further improve the efficacy focuses on augmenting the NK cell cytotoxicity, while few addresses redirection of the NK cells to the tumor, which is critical for proper tumor targeting *in vivo*. Previous studies have shown that tumor development in the bone marrow (BM) can impair the BM homing capacity of cytotoxic lymphocytes. However, this has not been explored in acute myeloid leukemia (AML). Here, we aim to decipher how AML alter the BM niche to impair NK cell infiltration and how these insights can be utilized to solve this issue.

Methods

Using NSG-SGM3 mice inoculated with AML cells, we assessed the microenvironmental changes in the BM during AML development using flow cytometry, immunohistochemistry and ELISA. We genetically engineered NK cells using a clinically-compliant method to equip them with selected homing molecules. Finally, we evaluated NK cell function *in vitro* and assessed their ability to home *in vivo* to different tissues in AML-bearing mice.

Results

We demonstrate that NK cell BM infiltration is impaired in AML-bearing mice, which was closely linked to a decrease in the CXCR4 ligand SDF-1α. Additionally, E-selectin^{+} endothelial cells were found increased within the BM during AML progression. NK cells engineered to express fucosyltransferase-7 (FUT7), thereby expressing functional E-selectin ligands, show stronger adhesion to endothelial cells and a moderate increased BM homing in healthy mice. Co-expression of gain-of-function CXCR4 (CXCR4^{R334X}) with FUT7 triggered robust NK cell homing to the AML BM niche of mice.

Conclusions

AML development alters the BM microenvironment, impairing NK cell infiltration. Genetic engineering of NK cells to express key homing molecules can help overcoming this and enhance NK cell infiltration of the AML BM niche. We speculate that this could enhance *in vivo* leukemia clearance.

54299 - Reprograming of glutamine metabolism drives MYC-mediated lipid droplet accumulation during hypoxia in clear cell renal cell carcinoma

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Abstract text

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. But, in recent years, they have gained recognition as emerging regulators of tumorigenesis. Our group previously demonstrated that MYCN inhibition induces LD accumulation in neuroblastoma cells. However, the detailed mechanism of MYC in this process is still unknown.

Here, we studied the molecular mechanism underlying lipid droplet accumulation in ccRCC after MYC inhibition, as well as its correlation to hypoxia signaling. Using a combination of lipidomics and metabolic tracing, we found that constitutive HIF expression combined with MYC inhibition induces reprogramming of glutamine metabolism, which was directed towards accumulation of triglycerides, the main component of LDs. However, VHL expression and MYC inhibition resulted in an increase in inositol-related lipid species, and thus, LD formation was not observed. Importantly, concomitant inhibition of both MYC and glutamine metabolism using BPTES reduced tumor burden and impaired LD accumulation *in vivo*. Moreover, using RNAseq analysis, we identified that MYC inhibition induces the expression of specific genes participating in lipid droplet accumulation during hypoxia.

Taken together, our study characterizes the molecular interplay between hypoxia and MYC signaling resulting in LD accumulation. This data provides new insights on the regulation of metabolism in ccRCC, which could be exploited for therapeutic applications.

54301 - PERSONALISED TUMOUR-TRAINED LYMPHOCYTES DERIVED FROM REGIONAL LYMPH NODES FOR TREATMENT OF COLORECTAL CANCER

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Abstract text

Adoptive T cell therapies targeting cancer neoantigens is gaining increasing interest as a treatment for solid tumours. Personalised tumour trained lymphocytes (pTTL) is a novel autologous T cell therapy targeting patient-specific neoantigens. A phase I/II First in Human (FIH) clinical trial of pTTL in Stage IV colorectal cancer (CRC) patients is opening presently.

pTTL is produced through in vitro expansion of T cells derived from tumour-adjacent lymph nodes. The T cells are stimulated with an array of personalized neoantigen epitopes coupled to paramagnetic beads, produced using PIOR®, a software developed in-house, and with EpiTCer® technology. The beads are readily phagocytosed by antigen-presenting cells, resulting in presentation of the neoantigen epitopes in an HLA type-independent process.

pTTL can be manufactured with a high rate of success despite the product's personalized nature. The majority of pTTL cells are T cells, with small proportions of NK and B cells. The CD4 and CD8 T cell ratio is variable. pTTL includes a significant proportion of memory T cells. Phenotypic markers indicate maintained T cell functionality, with limited levels of Temra (late-stage memory cells) and maintained expression of CD28. TCR sequencing have demonstrated increased clonality in pTTL compared to RLNs, indicating antigen-specific expansion.

The planned FIH trial will include patients with Stage IV CRC and limited remaining standard care therapies. pTTL is administered as a single dose monotherapy after chemotherapy-based preconditioning with cyclophosphamide and fludarabine. The primary endpoint is safety. Response, clinical outcome and biomarker analysis for pTTL persistence and characteristics will also be evaluated.

Trial Registration EUDRA CT #2022-000394-96.

54303 - S100A9 positive immune cells during prostate tumor progression

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Abstract text

Increasing evidence indicates calcium-binding S100 protein involvement in inflammation and tumor progression. We have recently shown that in prostate cancer patients with metastases at diagnosis, high levels of *S100A9* and *S100A12* and high monocyte count were significantly associated with short progression free survival (PFS) after androgen deprivation therapy (ADT). High *S100A9* levels and monocyte count were also associated with short cancer-specific survival. To investigate the functional role of S100A9 positive immune cells in tumor progression we use the Dunning R-3327 prostate tumor model. Low-metastatic AT1 or high-metastatic MatLyLu (MLL) rat prostate cancer cells were subcutaneously injected in immunocompetent rats. Results show that peripheral blood mononuclear cells (PBMCs) from MLL tumor-bearing rats had higher mRNA levels of *S100a9* compared to PBMCs from AT1 tumor-bearing rats and tumor-free rats. Immunohistochemistry results also show that S100A9 positive immune cells are significantly increased in the tumor, liver and lung in MLL tumor-bearing rats compared to AT1 tumor-bearing rats. Furthermore, monocytes stimulated *in vitro* with serum from MLL tumor-bearing rats show significantly higher levels of *S100a9* compared to monocytes stimulated with serum from AT1 tumor-bearing rats with the same tumor size. The expression of *S100a9* also increased with tumor size. These results show that the Dunning R-3327 prostate tumor model is a good model for further functional studies of S100A9 and inflammation during tumor progression.

54304 - Whole-genome informed circulating tumor DNA analysis by multiplex digital PCR for disease monitoring in B-cell lymphomas: a proof-of-concept study

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Abstract text

Analyzing liquid biopsies for tumor-specific aberrations can facilitate detection of measurable residual disease (MRD) during treatment and at follow-up. In this study, we assessed the clinical potential of using whole-genome sequencing (WGS) of lymphomas at diagnosis to identify patient-specific structural (SVs) and single nucleotide variants (SNVs) to enable longitudinal, multi-targeted droplet digital PCR analysis (ddPCR) of cell-free DNA (cfDNA). In 9 patients with B-cell lymphomas (diffuse large B-cell lymphomas and follicular lymphomas), comprehensive genomic profiling at diagnosis was performed by 30X WGS of paired tumor and normal specimens. A total of 164 SNVs/indels were identified including 30 variants known to be functionally relevant in lymphoma pathogenesis. The frequently mutated genes included *KMT2D, PIM1, SOCS1* and *BCL2*. WGS analysis further identified recurrent SVs including t(14;18)(q32;q21) (*IGH::BCL2)*, and t(6;14)(p25;q32) (*IGH::IRF4*) as well as copy-number gains of chromosome arms 1q, 2p (*REL*) and 18q (*BCL2*). Patient-specific multiplex ddPCR (m-ddPCR) assays were designed, allowing simultaneous detection of multiple SNVs, indels and/or SVs including *IGH::BCL2*. With a detection sensitivity of 0.0025% for SV assays and 0.02% for SNVs/indel assays, m-ddPCR was applied to analyze cfDNA isolated from serially collected plasma at clinically critical timepoints during primary and/or relapse treatment and at follow-up. Plasma analysis at diagnosis showed positive circulating tumor DNA (ctDNA) levels in 88% of patients and the ctDNA burden correlated with baseline clinical parameters (LDH and sedimentation rate, p-value <0.01). While clearance of ctDNA levels after primary treatment cycle 1 was observed in 3/6 patients, all patients analyzed at final evaluation of primary treatment showed negative ctDNA, hence correlating with PET-CT imaging. One patient with positive ctDNA at interim also displayed detectable ctDNA (average variant allele frequency (VAF) 6.9%) in the follow-up plasma sample collected 2 years after final evaluation of primary treatment and 25 weeks before clinical manifestation of relapse. Plasma analysis at interim evaluation during relapse treatment showed MRD (VAF 0.58%) whereas PET-CT reported complete remission. In summary, we demonstrate that multi-targeted cfDNA analysis, using a combination of SNVs/indels and SVs candidates identified by WGS analysis, provides a sensitive tool for MRD monitoring and can detect relapse earlier than clinical manifestation.

54305 - Potential of liquid biopsy for detection of the KIAA1549-BRAF fusion in circulating tumor DNA from children with pilocytic astrocytoma

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Abstract text

Central neural system tumors are the most common solid tumors in children and adults, with considerable heterogeneity in localization, pathological characteristics, and clinical outcomes. Low-grade glial tumors (LGGs) prevail in the paediatric population, and despite excellent overall survival and prognosis, the treatment has many side effects that cause a significant decrease in life quality. The widespread introduction of sequencing methods promoted the identification of primary molecular alterations in pLGGs. The tandem duplication in chromosome 7q34 locus is a frequent aberration in pilocytic astrocytoma (PA), but also can be recognized in diffuse leptomeningeal glioneuronal tumors and rarely in ganglioglioma. This rearrangement results in the formation of a KIAA1549-BRAF fusion protein and leads to constitutive activation of the RAS/MAPK signaling route due to a lack of a regulatory domain in the BRAF protein. Patients with this rearrangement have the lowest risk of subsequent malignant transformation, so reliable detection of KIAA1549-BRAF fusion is beneficial for better prognosis and treatment.

Over the past years, liquid biopsy has shown its potential as a convenient source of circulating tumor DNA (ctDNA). Molecular characterization of ctDNA is slowly entering into clinical practice and can be applied for diagnostic and longitudinal MRD monitoring for different types of cancers. Cerebrospinal fluid (CSF) ctDNA has become the center of attention for brain tumors. Recently, the utility of sequencing and ddPCR methods to detect ctDNA in CSF samples has been demonstrated for high-grade gliomas and medulloblastoma. In addition, ctDNA in CSF could provide prognostic information and a possibility for adaptive treatment. For LGGs, ctDNA analysis can be challenging and requires highly sensitive techniques since the likelihood of detection of ctDNA correlates with active disease and high tumor burden.

For our research, we aim to analyze CSF cfDNA from patients with an established PA diagnosis to test the possibility of detecting ctDNA derived from the KIAA1549-BRAF fusion. Assessment of copy number variation by ddPCR for different BRAF exons can be successfully performed on low tumor DNA concentrations, as shown previously. With a tumor informed approach, we also plan to complement ctDNA analysis with patient-specific assays for detecting fusion protein junction sequences. WGS was used for molecular profiling of a small cohort of PA patients. Most patients (12/14) harbor the same variant of KIAA1549-BRAF fusion with a 1-16/9-18 exon combination, but all had unique breakpoints on the DNA level. The others had either a NTRK1 fusion or FGFR1/PTPN11 mutations that have also been described in PA patients. We design custom multiplexed ddPCR assays for simultaneous quantification of BRAF exon copy number variation and patient-specific sequences in ctDNA. This approach can be a starting point or relevant addition to the current protocol of molecular characterization of tumor samples. It also can have a significant value in cases where tissue biopsy cannot be performed.

54306 - The IL-33/ST2 signaling pathway in glioma microenvironment.

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Abstract text

The presence of non-cancerous cells in the GBM microenvironment, including astrocytes, microglia, and macrophages, help to accelerate tumor progression. The presence of activated inflammatory cells can be a major contributing factor to the growth of GBM. These cells produce a range of pro-inflammatory molecules, such as cytokines, chemokines, and growth factors, which can stimulate the proliferation of tumor cells and are an area of intense study. IL-33, an IL-1 family cytokine, has previously been shown by several laboratories, including our own, to be tightly regulated during neurodevelopment and neuroinflammation. The main sources of IL-33 in the mouse brain are astrocytes and oligodendrocytes, and in mice with a brain injury that are deficient for the IL-33 receptor, *St2*, infiltration of microglia/macrophage is reduced. We here address the roles of IL-33, and its receptor ST2, in the inflammatory tumor environment in GBM. As a syngenic glioma model, we use the stereotactic injection of mouse glioma cells into C57Bl/6 striatum, either wt or $St2^{-/-}$ knockout mice. We find that the IL-33/St2 pathway regulates the recruitment and infiltration of bone marrow-derived macrophages, brain-resident microglia, and other immune cells, which may promote tumor growth by influencing the tumor microenvironment. Furthermore, using neural cell cultures, we investigate the induction of IL-33 by simulating stress factors associated with the tumor microenvironment. In summary, these experiments will show how IL-33/ST2 signaling is involved in the host brain response to malignant brain tumors, and if this axis could be exploited in drug target identification.

54307 - Mechanism of histamine-mediated inhibition of the NADPH oxidase 2 enzyme

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Abstract text
The NADPH oxidase 2 (NOX2) enzyme is mainly expressed by myeloid cells and its only known role is to catalyze the reduction of molecular oxygen to generate superoxide, which is further converted to hydrogen peroxide and additional reactive oxygen species (ROS). While NOX2-derived ROS contributes to the host's defense against invading microbes, it has also been implicated in cancerogenesis, as ROS may act as a mutagen and may suppress effector functions of T cells and NK cells.

Histamine is an endogenous hormone and a neurotransmitter, that additionally has been implicated in immune regulation. Hence, histamine has been found to inhibit NOX2-derived ROS formation via binding to H2R on myeloid cells. Thereby, adjacent NK cells and T cells are preserved from inactivation by NOX2-derived ROS and thus remain functional to eradicate malignant cells. However, the precise mechanism by which histamine prevents NOX2-derived ROS production is not understood, the goal of this study is to define the intracellular pathways involved in this action.

NOX2-derived ROS formation in peripheral blood mononuclear cells (PBMC) and human monocytes was determined using a continuous chemiluminescence assay. The cells were stimulated with plate-bound rituximab (RTX), N-formyl-methionyl-leucyl-phenylalanine (fMLF), or Phorbol myristate acetate (PMA) in the presence or absence of pathway inhibitors, including histamine, GSK2795039 (direct NOX2 inhibitor), N-Methyl Histamine (NMH, H2-receptor agonist), Ibrutinib (BTK inhibitor), Idelalisib (PI3K inhibitor) and Bisindolmaleimide I (PKC inhibitor).

All three stimuli triggered robust ROS formation in PBMC and monocytes. The direct NOX2 inhibitor GSK2795039 as well as the PKC inhibitor efficiently reduces ROS formation induced by all three stimuli, while histamine, similarly to the H2-receptor agonist and the BTK- and PI3K- inhibitors only significantly reduced ROS-formation induced by RTX and fMLF. This implies that histamine may target similar intracellular pathways as BTK- or PI3K-inhibitors.

To further define the intracellular pathway utilized by histamine to inhibit NOX2, myeloid cells that are treated with RTX and the presence or absence of histamine and/or the other inhibitors are assessed by Western blot for phosphorylation of various intracellular signaling proteins, including AKT.

Furthermore, a gene enrichment analysis will be conducted on microarray data from myeloid PLB-985 cells that have been cultured in the presence and absence of histamine, with the aim of further understanding which pathways are impacted by histamine in myeloid cells.

54310 - The ACROBAT 2022 Challenge: Automatic Registration Of Breast Cancer Tissue

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Abstract text

Introduction

Whole-slide-image (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. 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. The ACROBAT data set is currently the largest publicly available WSI data set of corresponding H&E and IHC WSIs that originate from sections from the same tumors. 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 90th 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 and potentially clinical applications.

54311 - Characterization of exome variants in ovarian endometriosis and paired endometriosis-associated carcinomas

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Abstract text

Background

Endometriosis is a molecularly complex disease that affects roughly 10% of females in reproductive age. Although being pathologically benign, endometriosis shares several traits with cancer, including the ability to metastasize, resist apoptosis, and induce angiogenesis. Several studies have shown that ovarian endometriosis (OE) is associated with an increased risk of developing endometriosis-associated ovarian carcinoma (EAOC), predominantly of clear cell or endometrioid histotype.

Aim

The aim of the present study was to assess genomic alterations in OE and subsequent EAOC in an attempted to identify key events linking the two diseases.

Material method

Eleven patients with OE and subsequent EAOC (clear cell carcinoma n=4, and Endometrioid n=7) was enrolled for paired sample whole-exome sequencing. Data was evaluated for somatic mutations and copy number alterations (CNAs). Findings were validated with MSI-analysis and immunohistochemistry.

Results

The OE group displayed an overall high mutational burden with a median of 2.8 non-silent mutations per Mb (range 0.2–73.5). In line, mutational signatures suggested faulty DNA repair mechanisms, such as homologous recombination and mismatch repair. There was a significant correlation between high median variant allele frequency (VAF) in OE, and prolonged time between OE and EAOC diagnosis. All cases showed one or more conserved mutations between paired samples. Genes associated with an immune response were the most frequently affected by conserved non-silent mutations and CNAs. Eight OEs, and nine EAOCs harboured at least one non-silent mutation in a known cancer-associated gene. However, no conserved cancer-driver mutations were observed. CNA analysis showed large chromosomal aberrations affecting numerous cancer-associated genes, occasional overlap was observed in paired samples. KRAS was found mutated in two OEs and was the only recurrently mutated cancer-associated gene. In EAOC, the most frequently mutated genes were TP53 (n=5), KRAS, PIK3CA and AFF3 (n=3).

Conclusion

Paired endometriosis and carcinoma showed shared genetic variations suggesting a common origin. Conserved aberrations proposed that alterations of the immune response were early and significant occurrences. Absence of shared mutations in cancer-associated genes suggested that the incident leading to the malignant transformation was a later event. Furthermore, the data suggested that a dominant endometriosis may delay malignant transformation; that is, prevent tumour development.

54312 - Discovery of druggable cancer-specific pathways with application in acute myeloid leukemia

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Abstract text

An individualized cancer therapy is ideally chosen to target the cancer's driving biological pathways, but identifying such pathways is challenging because of their underlying heterogeneity and there is no guarantee that they are druggable. We hypothesize that a cancer with an activated druggable cancer-specific pathway (DCSP) is more likely to respond to the relevant drug. Here we develop and validate a systematic method to search for such DCSPs, by (i) introducing a pathway activation score (PAS) that integrates cancer-specific driver mutations and gene expression profile and drug-specific gene targets, (ii) applying the method to identify DCSPs from pan-cancer datasets, and (iii) analyzing the correlation between PAS and the response to relevant drugs. In total, 4,794 DCSPs from 23 different cancers have been discovered in the Genomics of Drug Sensitivity in Cancer database and validated in The Cancer Genome Atlas database. Supporting the hypothesis, for the DCSPs in acute myeloid leukemia, cancers with higher PASs are shown to have stronger drug response, and this is validated in the BeatAML cohort. All DCSPs are publicly available at https://www.meb.ki.se/shiny/truvu/DCSP/.

54313 - Defective ribosome assembly impairs leukemia stem cell function in a murine model of acute myeloid leukemia

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Abstract text

Acute myeloid leukemia (AML) is a heterogenous hematologic malignancy characterized by clonal expansion of aberrant myeloid precursor cells and bone marrow failure. The vast majority of patients with AML harbor mutations that lead to constitutive activation of oncogenic signal transduction pathways. These mutations typically arise late during disease progression and promote the conversion of pre-leukemic myeloid progenitor cells into AML leukemia stem cells (LSCs). Most oncogenic signal transduction pathways converge on protein synthesis machinery to regulate its function. However, the biological and therapeutic role of deregulated protein synthesis in LSC function and leukemia propagation remains incompletely understood.

In the present study, we address the role of protein synthesis control in AML. We apply a murine model of mixed-lineage leukemia-rearranged AML to demonstrate that LSCs are characterized by high global protein synthesis rate. Using a genetic model that permits inducible and graded regulation of ribosomal subunit joining (Jaako et al, Nat Commun, 2022), we show that defective ribosome assembly promotes survival in leukemia-engrafted mice by selectively eradicating LSCs but not normal hematopoietic stem and progenitor cells. Finally, transcriptomic and proteomic analyses identify a rare subset of LSCs with immature stem cell signature and high ribosome content that promotes the resistance to defective ribosome assembly. Collectively, our study unveils a critical requirement of high protein synthesis rate for LSC function and highlights ribosome assembly as a therapeutic target in AML.

54314 - Ultra-sensitive detection of structural and single nucleotide variants using droplet digital PCR in liquid biopsies from children with medulloblastoma

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Abstract text

Medulloblastoma (MB) is a malignant embryonal tumour of the central nervous system (CNS) that mainly affects infants and children. Treatment includes surgery, adjuvant chemotherapy and in most cases cranial or craniospinal radiotherapy (RT), depending on the age of the child, the histopathological and molecular features of the tumour, the radicality of surgery and the metastatic status of the disease. Although a majority of children with MB survive their disease many survivors have severe long-term toxicity effects of treatment impacting their quality of life.

Molecular biomarkers for measurable residual disease (MRD) detection are lacking in MB. Patients are thus monitored by clinical assessment, imaging (magnetic resonance imaging, MRI) and cerebrospinal fluid (CSF) cytology. In short, residual disease is assessed by visualization of malignant cells. Disadvantages of MRI include the frequent need for general anaesthesia and the occasional inability to discriminate between residual tumour tissue and post-therapeutic changes (fluids, blood or contrast-enhanced reactive tissue in the surgical cavity or irradiation artefacts). CSF sampling requires less anaesthesia, but cytology has low sensitivity. These methodological shortcomings sometimes leave clinicians in the dark and may lead to the under or over treatment of disease, both scenarios associated with their own detrimental consequences. Molecular biomarkers could help resolve some of these issues.

Analysis of cell-free DNA (cfDNA) in cerebrospinal fluid (CSF) using broad genomic approaches, such as low-coverage whole-genome sequencing, has shown promising prognostic value as an MRD marker. However, more sensitive methods are needed for accurate MRD analysis. Here, we show the technical feasibility of capturing medulloblastoma associated structural variants and point mutations simultaneously in cfDNA using a tumour informed approach and multiplexed droplet digital PCR (ddPCR). Assay sensitivity was assessed with a dilution series of tumour in normal genomic DNA and the limit of detection was below 100 pg. of input DNA for all assays. False positive rates were zero for structural variant assays. Liquid biopsies (CSF and plasma, n=47) were analysed from 12 children with medulloblastoma, all with negative CSF cytology. MRD was detected in 75% (9/12) of patients overall. In CSF samples taken before or within 21 days of surgery MRD was detected in 88% (7/8) of patients with localized disease and in the one patient with metastasized disease. Detection was possible in low volume samples (<1 ml) as well as in samples with elevated cell counts.

Our results suggest that this approach could expand the utility of ddPCR and complement broader analyses of cfDNA for MRD detection in MB. Larger studies of the approach at standardized timepoints are warranted.

54315 - A methodology for direct selection of image areas important for classification in weakly supervised CNN-based modelling of cancer pathology images

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Abstract text
In computational pathology, there is a rise of weakly supervised learning models where slide-level labels are used for training, since pixel or tile level annotations may not be available, or in cases where the label is on patient or tumour level, for example, histological grade classification or patient outcome. However, spatial interpretability is often desirable to understand which tissue areas are important in the classification but is not intrinsically available in weakly supervised tile-based models. In this study, we have proposed a direct methodology based on backward selection to determine WSI regions that are necessary for the classification of a whole slide image (WSI) in the context of a weakly supervised CNN model.

We optimised and validated ResNet-18 CNN models for the breast cancer histological grade 1 vs 3 classifications on the training set of the SöS-BC-4 cohort (n = 1695 WSIs) using a 5-fold CV. Two different tile-to-slide level aggregator functions were evaluated: the 75th percentile of the tile-level predictions probabilities as the slide-level score, and an attention layer trained on tile-level feature vectors derived from the CNN model to provide the slide-level score. In both modelling strategies, for each slide, we iteratively remove the highest-ranking tile and recalculate the slide-level score until the slide-level score reaches the classification threshold, which we define as the set of tiles, and corresponding tissue area, that drives the classification by the CNN model.

The proposed methodology provides means for spatial visualisation of the regions of interest. We observed an average of 32.34% (95% CI: 29.69% - 34.99%) and 44.97% (95% CI: 41.69% - 48.26%) of the WSI regions that contributed to the classification of Grade 3 in the CNN with a 75th percentile and attention layer based tile-to-slide level aggregator respectively. The two different aggregation functions had a similar 49% of areas driving the classification.

There is a need for interpretability and understanding of the decision-making of weakly supervised deep CNN models for cancer histopathology images in both research and clinical applications. Here we proposed and evaluated a methodology for interpretability that is directly linked to predictions provided by weakly supervised tile-based deep learning models, which can improve understanding of the classification decisions.

54316 - Identifying novel non-coding driver mutations in canine and human diffuse large B-cell lymphoma using evolutionary constraint

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Abstract text

Diffuse large B-cell lymphoma (DLBCL) is a highly aggressive form of lymphoma that results from the neoplastic transformation of B lymphocytes. While numerous genomic studies have identified mutations, genes, and pathways associated with DLBCL, the majority have focused on the protein-coding regions of the genome, leaving the non-coding portion relatively unexplored. However, non-coding elements are vital for gene regulation, and thus alterations in these sites can have a significant impact on gene expression, cellular processes, and may contribute to cancer development. Moreover, most somatic mutations occur in these regions. This being the case, further study of such non-coding regions may lead to the identification of novel etiological factors and molecular mechanisms involved in tumorigenesis. In light of this, the aim of this study is to investigate the non-coding genome to identify novel driver mutations in DLBCL. To achieve this, we employed evolutionary constraint as a tool to identify genomic positions that have been conserved throughout evolution and are likely to have functional significance. This approach was motivated by the hypothesis that disruptions in said regions may have a negative impact in the individual and possibly contribute to cancer.

Lymphoma is not a disease restricted to humans, as it can also manifest in other species, including the dog. In fact, DLBCL, which is the most frequent and aggressive subtype of lymphoma in humans, is also one of the most common malignancies in dogs. Additionally, dogs develop similar symptoms as humans and have been successfully used in previous studies to pinpoint shared driver mutations for this disease. As a result, we chose the dog as animal model for this study.

We used whole-genome sequencing data of paired tumor/normal samples from 73 canine and 41 human DLBCL patients. To validate our data, we conducted a “significantly mutated genes” (SMGs) analysis using coding mutations. This revealed that the top SMGs in both canine (*TRAF3, POT1, SETD2, FBXW7,* and *TP53*) and human (*B2M, TMSB4X, MYD88, TMEM30A,* and *TP53*) cohorts were consistent with previously established genes implicated in cancer, raising confidence in our subsequent analysis. Next, we investigated the non-coding constraint mutations (NCCMs) landscape and identified a total of 108 and 82 NCCM-enriched top genes (≥2NCCMs/100 Kbp) for each cohort. The *BCL6-RTP2-SST* locus (*BCL6*-locus) exhibited the highest degree of NCCMs in both species. By cross-referencing these outcomes, we discovered that 10 of the NCCM top genes were common to both cohorts. In addition to the *BCL6*-locus, shared genes included *BCL7A, BCL11A,* and *PAX5* which have previously been linked to lymphoma. Finally, a pathway analysis on the top NCCM genes revealed that cytokine signaling pathways were among the most significant. These preliminary findings suggest that leveraging evolutionary constraint could serve as a valuable approach for detecting regulatory mutations within non-coding regions. As a next step, further analysis will be done in the canine and human *BCL6*-locus and other NCCM-enriched regions. Once the *in-silico* analysis has been completed, the most promising variants will be further investigated and validated by wet-lab techniques.

54317 - Analysis of extracellular vesicle cargo in the context of treatment of mutated epidermal growth factor receptor-driven Non-small cell lung cancer

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Abstract text

Background: Extracellular vesicles (EVs) are released by all cell types, have been associated with several cancer hallmarks and may provide non-invasive information about tumor progression or response to therapy. In this work, we investigated EVs and their protein cargo in mutated Epidermal growth factor receptor (EGFR)-driven Non-small cell lung cancer (NSCLC) cells prior and post treatment with the clinically used EGFR-tyrosine kinase inhibitor (TKI) osimertinib. Our group and others have shown that Ephrin-B3/EphA2 signaling cascade promotes invasion[1] and is a bypass resistance driver[2] to erlotinib trearment, in this context, it is important to evaluate the contribution of Ephrin-B3/EphA2 pathway in response to osimertinib in a resistant manner.

Methods: EVs were isolated from the *EGFR* mutant cell line H1975 and from the osimertinib resistant subline H1975/OR[3] in response to osimertinib using size exclusion chromatography. EVs characterization was performed by Nano Tracking Analysis and western blot. Proteomic profiling was carried out using Proximity Extension Assays. Ephrin-B3 silencing, was made to assess osimertinib sensitivity and effect on EV cargo.

Results: We show sensitization of H1975 cells to EGFR-TKIs when Ephrin-B3 was silenced. Both Ephrin-B3 and EphA2 were confirmed in EVs prior and post treatment. A comparison PEA protein profiles shows differentially expressed proteins in the H1975 cells and EVs prior and post osimertinib treatment including CXCL10 and FGF-BP1. These are currently validated and explored in context of Ephrin-B3 and EphA2 signaling.

Conclusion: Ephrin-B3/EphA2 are emerging as potential targets to sensitize for osimertinib. EV-containing proteins (e.g., CXCL10 and FGF-BP1) could be a source biomarker for monitoring osimertinib response.

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References:

1 Efazat G et al., Ephrin B3 interacts with multiple EphA receptors and drives migration and invasion in non-small cell lung cancer. Oncotarget. 2016 Sep 13;7(37):60332-60347. doi: 10.18632/oncotarget.11219. PMID: 27533087; PMCID: PMC531238

2 Amato KR et al., EPHA2 Blockade Overcomes Acquired Resistance to EGFR Kinase Inhibitors in Lung Cancer. Cancer Res. 2016 Jan 15;76(2):305-18. doi: 10.1158/0008-5472.CAN-15-0717. Epub 2016 Jan 7. PMID: 26744526; PMCID: PMC4715957.

3 McGowan M, Kleinberg L, Halvorsen AR, Helland Å, Brustugun OT. NSCLC depend upon YAP expression and nuclear localization after acquiring resistance to EGFR inhibitors. Genes Cancer. 2017 Mar;8(3-4):497-504. doi: 10.18632/genesandcancer.136. PMID: 28680534; PMCID: PMC5489647.

54319 - Pan-cancer analyses of whole genomes using evolutionary constraint to identify non-coding regulatory drivers

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Abstract text

Whole-genome sequencing (WGS) of thousands of cancer genomes across multiple cancer types has made it possible to identify genetic factors that contribute to cancer development and progression at the pan-cancer level. An average tumor develops as a result of at least 4-5 driver mutations, but the majority of these alterations are found in the coding part of the genome. The role of non-coding regulatory mutations has only begun to emerge. However, while the consequence of a mutation in the coding can be evaluated by its direct effect on the coding sequence, this approach is not translatable for the noncoding genome. Here, we propose that the identification of evolutionary constraint positions in the genome can be leveraged to identify somatic mutations in cancer genomes. We analyzed WGS data from 2,539 cancer genomes from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium of the International Cancer Genome Consortium (ICGC), and intersected the non-coding regions of the genome with phyloP scores, a constraint metric from sequence alignment of 240 mammalian species, to identify non-coding constraint mutations (NCCMs) with regulatory potential. The genes with the highest number of NCCMs were transcription factors *ZEB2*, *ZIC4*, *ZFHX4* and *BCL6* all showing a rate of over 0.2 NCCMs/100kbp/patient. Overall, the majority, 65%, of the top genes with highest number of NCCMs (n = 103) were transcription factors, and 30% of these top genes belonged to the *HOX*-gene clusters. We also identified 459 genes which had a significantly higher NCCM load compared to the overall background mutation rate of the locus. Of these genes, *NOG*, *EBF3*, *LRTM1* and the *BLC6*-locus showed the highest normalized patient count with NCCMs in on average 334 patients/gene. In addition, we found 457 hotspots of recurrent NCCMs with eight or more patients sharing per hotspot either in one or multiple cancers. These results show that evolutionary constraint can be used as a powerful indicator of critical molecular function that may, when mutated, lead to cancer.

54320 - Chromaffin-to-neuroblast cell state transitions drive tumor plasticity in NF1 and KIF1Bb deficient neuroblastoma,pheochromocytoma and composite tumors

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Abstract text

Introduction:
Intratumor heterogeneity and high-plasticity account for therapy resistance and poor clinical outcome in neuroblastoma (NB) and paraganglioma (PPGL). Causes of plasticity during tumor progression remain poorly understood.

Aims:
Here, we profile tumor cell state transitions of genetically engineered mouse sympatho-adrenal tumors at several stages, from embryonic, pre-neoplastic hyperplasia to pheochromocytoma (PCC), neuroblastoma and composite tumors.

Methods:
We have generated conditional gene target of *Kif1bb* (tumor suppressor gene) in the mouse sympatho-adrenal system, and crossed this mouse line with mice expressing Cre-recombinase under the control of the gene for dopamine beta hydroxylase (DBH-Cre). The offspring we crossed with $Nf1^{fl/fl}$ mice to generate double knockout (referred as DKO) mice. H&E staining, immunofluorescence, *in situ* hybridization (RNAscope) and single-cell transcriptome analysis have been combined with RNA velocity to explore chromaffin-to-neuroblast tumor state transitions.

Results:
Loss of *Kif1bb* potentiated the oncogenic activity of *Nf1* loss causing the development of large, bulky, and locally invasive masses that arose in the adrenals. Histopathology revealed pheochromocytoma, neuroblastoma and composite tumours in aged mice. We observed abundant embryonic neuroblast hyperplasia in mutant embryonic medulla that transit to a neuroplastic chromaffin state postnatally. We found chromaffin tumor cells obtained a neuroblastic feature postnatally (3 month or older) and continued to form neuroblastoma, pheochromocytoma and composite tumors of both types. Meanwhile, these tumors have a remarkable heterogeneity. In early tumor development at 3 month old, a distinctive three segment structure has been found breaking through the cortex from medulla, which shows the transitional state of chromaffin cells to neuroblasts. We further validate these transitions in human PPGL and NB. The transitions are consistent with the single-cell RNA velocity prediction.

Conclusions:
Deep single cell RNA sequencing combined with immunohistochemistry and RNAscope revealed chromaffin-neuroblast cell state transitions at embryonic and postnatal stages driving tumor plasticity. Cancer cells progressively adopt neuroblast lineage identity, computationally predicted to be mediated through a common chromaffin-neuroblast transitional, high-plasticity cell state. Such newly discovered lineage transitions suggest important implications for understanding neuroblastoma and pheochromocytoma heterogeneity.

54321 - Aging and the immune landscape of lung cancer

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Abstract text

The general population is growing older. As an age-related pathology, cancer incidence increases with age. Lung cancer, for which the median age at diagnosis is 75, is the deadliest cancer worldwide. Although it is established that physiological aging alters the immune landscape of the body, the connection between the aging immune system and cancer progression has not yet been studied in the context of lung cancer. Distinct characteristics of the aging immune system include decreased competence of the adaptive immune system, clonal skewing of hematopoietic stem cells (HSCs) towards the myeloid lineage, and chronic inflammation (inflammaging). To test whether age-related changes in the lung tumor microenvironment (TME) contribute to cancer progression and immune evasion, we utilized single cell transcriptomics and investigated changes in the lung TME in our genetically engineered mouse model (GEMM). Moreover, we performed high parameter immunophenotyping on blood, spleen, and bone marrow of the same mice to identify potential differences in systemic immune response to cancer using spectral flow cytometry (SFC). Similarly, we profiled the immune landscape of the tumors formed by subcutaneous injections of aged and young lung cancer cells into young recipient mice to investigate how young and aged cancer cells influence the immune microenvironment. We observed alterations in lymphocyte infiltration and shifted proportions of immunosuppressive and pro-inflammatory immune populations with age. Our study underlines the importance of the age-related alterations in the lung TME and lays the groundwork for new ways of targeting immune system in the aged patients.

54323 - Understanding neural-cancer interactions and invasiveness in glioblastoma

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Abstract text

Glioblastoma (GB) is the most frequently occurring malignant type of primary brain tumor, comprising 12%–15% of all intracranial tumors. GB is known for its capacity to spread locally in the brain and thrives in the microenvironment of which neurons are the biggest constituent, while extracranial metastasis is rare. Several studies showed that denervation can lead to suppressed tumor growth and metastasis of certain tumour types, indicating nerve dependence mechanisms in cancer. Furthermore, the recent discovery of neurogliomal synapses has shown that these synaptic contacts can relay neuronal activity to glioma cells to drive tumor progression. GB is also characterized by networks of interconnected brain tumour cells that communicate via Ca^{2+} transient and have a positive effect on tumour growth. In order to describe the neuron-to-glioma communication in GB mouse models, we determined the presence of neurons in GB tumors induced either genetically or by orthotopic transplantation. Furthermore, we are studying how the proliferation of GB cells and other cancer cells is affected in the presence or absence neurons, for which we are using mouse primary cortical neurons and plan to use iPSCs-derived neurons in the near future. Our study aims to help understand the communication between neurons and migrating glioma cells and its contribution to invasiveness.

54324 - Gamma Delta T cell recognition and activation potential in Medulloblastoma

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Abstract text

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 plan to explore 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.

54326 - CD8+ T cells co-expressing CD39+ and CD103+ in pancreatic cancer tissues present an exhausted phenotype and a unique chemokine receptor profile

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Abstract text

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 identified a subset of $CD8^+$ 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. 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.

54330 - Mechanistic basis of atypical TERT promoter mutations

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Abstract text
Non-coding mutations in the *TERT* promoter are among the most prevalent driver mutations in cancer and are found in more than 50 cancer types. These mutations normally occur at one of two bases -124 and -146 bp upstream of the start codon, in both cases leading to the creation of a de novo ETS transcription factor binding site that proposedly activates transcription through binding of GABPA and interaction with a preexisting native ETS site. However, specifically in melanoma and other UV exposed cancers, several additional recurrent *TERT* promoter mutations have been reported, although their functions and origins remain unexplained. These include a CC>TT mutation at -138/139 that also introduces an ETS motif, and several recurrent mutations in close proximity to the canonical driver mutations. Here we show, through analysis of targeted sequencing of the *TERT* promoter from 22,000 cancer patients in GENIE v11, that, in addition to the known driver mutations, up to 11% of UV-exposed cancers with canonical *TERT* driver mutations possess additional co-occuring mutations at cytosines upstream of the introduced ETS sites. It has recently been shown that ETS binding sites are hotspots for UV induced mutations, as the binding of ETS transcription factors to DNA generates favorable conditions for UV damage and blocks efficient repair. Indeed, recurrent mutations are also detected at the cytosines upstream of the native ETS sites only in tumors with *TERT* driver mutations, confirming the highly mutagenic nature of ETS factor binding, and supporting that GABPA binds directly to the introduced ETS site in combination with the upstream native ETS binding site. The -138/139 CC>TT mutation, as well as the known driver mutations -124 and -146 appear even in tumors with low UV burden, indicating positive selection. The additional recurrent ETS-induced mutations however, correlate with UV burden and are absent in non-UV-exposed cancers, indicating they result from increased UV mutagenesis rather than positive selection. Additionally, we show that these mutations arise sub-clonally specifically in the mutant DNA strand of *TERT*-mutant A375 cells treated with low dose daily UV, and not in the wild type strand. This study represents the most extensive analysis of *TERT* promoter mutations to date, and provides a mechanistic explanation for the presence of additional *TERT* promoter mutations in UV exposed cancers.

54331 - Evaluation of TP53 mutations in paired liquid and solid clinical specimens from High-Grade Serous Ovarian Carcinoma

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Abstract text

Background: Ovarian carcinoma (OC) has the highest mortality rate among gynaecological malignancies. The underlying cause is associated with late-stage diagnosis due to absent or unspecific symptoms. The high-grade serous ovarian carcinoma (HGSC) accounts for over 60% of the OC associated deaths. The most frequent genetic aberrations in HGSC are mutations in the *TP53* gene, detected in approximately 96% of the tumours. Previous studies have proposed the potential diagnostic utility of detecting mutations in circulating tumour DNA (ctDNA) from OC patients. However, the need for diagnostic methods capable of detecting asymptomatic HGSC is unmet.

Objectives: The primary aims of this study were to design a screening tool for early identification of HGSC and to evaluate the suitability of various clinical specimen types for diagnostic applications.

Method: The current study involved the analysis of various clinical specimens, including primary tumours, plasma, ascites fluid, and both liquid and solid samples from cystic-, cervical-, and uterine- origin. Additionally, self-collected cervical specimens (FTA elute micro cards™) were examined. These analyses focused on detecting *TP53* mutations in DNA from patients with HGSC (n=11).

The *TP53* panel was constructed by applying the simple, multiplexed, PCR-based barcoding of DNA for sensitive mutation detection using sequencing (SiMSen-seq) technique, which utilizes straightforward, multiplexed PCR-based DNA barcoding for the sensitive identification of mutations by next-generation sequencing (NGS). The panel amplified 17 regions, encompassing 619 nucleotide positions within the *TP53* gene.

Results: The panel identified variants in 10/11 (91%) of the primary tumours, with a VAF ≥ 9% (range: 9-91). Preliminary data indicated that all (100%) paired samples obtained corresponding mutations between one or more of the different samples from the same patient**.**
In total 8/8 (100%) plasma-, 10/10 (100%) ascites-, 16/16 (100%) cystic-, 18/21 (86%) cervical-, and 16/19 (84%) uterine samples showed mutations at a consensus read depth of 3 and a minimum VAF ≥ 0.1%.

Conclusion: The present study suggests that the SiMSen-seq technology can identify *TP53* mutations in non-invasive liquid biopsies. Additionally, the assessment of the HGSC diagnostic panel exhibits potential for forthcoming clinical utilization as a diagnostic tool.

54338 - Introducing Proteomics Biomedicum Core Facility

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Abstract text
Proteomics Biomedicum is a proteomics core facility at the Department of Medical Biochemistry and Biophysics located in the Biomedicum building. We provide a broad range of services using best available proteomics techniques and methods with focus on quantitative proteomics, both label-free and multiplexed TMT-labeling. Based on our experiences accumulated in many years, we are always glad to discuss projects related to identification and quantification of proteins and assist with experimental design as well as to deliver high quality results.

As a latest addition to our services, we have recently established an experimental workflow that allows us to analyze extremely low amounts of proteins extracted from single cells and generate quantitative results with reasonable depth of the proteome. Similarly, we are able now to work with low number of cells that has been a challenge earlier when samples were available in limited amounts.

Services of Proteomics Biomedicum:

- Single cell proteomics by mass spectrometry
- Large scale quantitative proteomics experiments using label-free or isobaric labeling (TMT and TMTpro) quantification strategies
- Targeted proteome analysis, including SureQuant analysis of plasma/serum samples
- Accurate molecular mass determination of peptides and small proteins
- Detection of post-translational modification and their localization on the amino acid sequence
- General protein identification in gel and solution

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54339 - Predicting tumor growth-driving interactions from transcriptomics data using machine learning

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Abstract text
The project is about finding immune-cancer interactions that drive tumor growth in breast cancer by using a machine learning model. This project is important since we want to know how cancer cells escape the immune system. One famous example of immune suppression is the PD1 PDL1 immune checkpoint. By blocking this interaction, patients can be treated - but not all of them. That is why we want to find other similar interactions which drive tumor growth in order to find new treatments of cancer in the near future.

Single-cell RNA sequencing data and spatial transcriptomics data is utilized in this project and the machine learning model is created in order to learn a low-dimensional feature space which is predictive of tumor growth and captures the genetic variability of both cancer and immune cells.

54342 - SAMHD1 is a major resistance factor for nelarabine treatment in T-ALL that can be alleviated by hydroxyurea

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Abstract text

Although the 5-year overall survival rate for T-cell Acute Lymphoblastic Leukemia (T-ALL) patients has improved to over 80%, prognosis for relapsed and refractory T-ALL remains very poor. Nelarabine can be used in those settings, but complete response rates are below 40%. Nelarabine is a pro-drug of ara-G, a guanosine analogue, that has been shown to be selective for T-lymphoblasts, and its active metabolite is the triphosphate ara-GTP.

We have previously shown that the triphosphohydrolase SAMHD1 inactivates ara-CTP, the active metabolite of cytarabine (ara-C) which confers ara-C resistance in acute myeloid leukaemia. Furthermore, we have shown in the clinical HEAT-AML trial that SAMHD1 can be inhibited by hydroxyurea (HU), thereby effectively sensitising leukemic cells to ara-C.

Here, we investigated SAMHD1 protein expression in a panel of T-ALL cell lines, and generated SAMHD1 knockout clones for the paediatric MOLT16 and the adult SUP-T11 cell lines using CRISPR/Cas9. Knockout of SAMHD1 dramatically sensitised T-ALL cells to nelarabine by up to 100-fold. Those results were confirmed by transient SAMHD1 depletion through Viral protein X-mediated proteasomal degradation. Importantly, addition of HU could reverse SAMHD1-mediated nelarabine resistance. We are currently validating the effect of SAMHD1 protein expression in a set of (n=10) relapsed adult and paediatric T-ALL patients treated with nelarabine at Karolinska University Hospital. Furthermore, we are examining the effect *SAMHD1* mRNA expression on outcomes of T-ALL patients treated upfront with nelarabine in the COG AALL0434 trial (n=300).

Collectively, we provide clinical and pre-clinical evidence that leukemic SAMHD1 expression could be a predictive biomarker for nelarabine treatment in T-ALL and that addition of HU might clinically overcome SAMHD1-mediated nelarabine resistance.

54344 - Using evolutionary constraint to define novel candidate driver genes in medulloblastoma

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Abstract text

Current knowledge of cancer genomics is biased against non-coding mutations. Here, we use whole genome sequencing data from pediatric brain tumors, combined with evolutionary constraint inferred from 240 mammals to identify genes enriched in non-coding constraint mutations (NCCMs). We compare medulloblastoma (MB, malignant) to pilocytic astrocytoma (PA, benign) and find drastically different NCCM frequencies between the two. In PA, a high NCCM frequency only affects the *BRAF* locus, while in MB, >500 genes have high levels of NCCMs. Intriguingly, many genes are associated with different age of onset, such as *HOXB1* in young patients and *NUAK1* in adult patients. Our analysis points to different molecular pathways in different patient groups. These novel candidate driver genes may assist patient stratification in MB and may be useful for treatment options.

54346 - Personalized analysis of circulating tumor DNA: A novel biomarker in childhood malignancies

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Abstract text

Despite improved management of pediatric malignancies during the last decades, 15% of children diagnosed with cancer in Sweden today die from their disease. The treatment is associated with severe acute and chronic complications, and accurate evaluation of treatment response is crucial in order to balance the treatment intensity for each child. However, clinical biomarkers are lacking for most childhood tumors and those that exist are hampered by limited sensitivity and specificity.

In this study, we develop personalized analysis of circulating tumor DNA (ctDNA) as a marker of treatment response and disease relapse in children with different types of cancer. We generate targeted sequencing panels for ultra-sensitive detection of 10 tumor-specific single nucleotide variants for each patient. We have so far collected more than 2,100 blood samples at different timepoints before, during and after the treatment in 285 children; 118 with leukemia, 54 with lymphoma, and 126 with non-hematological tumors.

We detected ctDNA in all of 12 children with neuroblastoma, and the levels at diagnosis correlated with clinical risk group stratification. CtDNA showed a stepwise reduction during treatment and was detected after neoadjuvant chemotherapy at levels down to 1:100,000 of the pre-treatment sample. Patient-specific ctDNA analysis showed a wider range of positivity and appeared to be more sensitive than five urine and blood biomarkers currently used in the clinic. Preliminary data from children with other tumor types showed lower ctDNA levels at diagnosis than in neuroblastoma. Still, longitudinal ctDNA analysis confirmed association with treatment response and detection of disease relapse.

Our findings raise the possibility of using personalized ctDNA analysis as a novel tool for disease monitoring in childhood malignancies. This could reduce the number of invasive and potentially harmful procedures, such as repeated radiology examinations in children with solid tumors and bone marrow aspirations in those with leukemia. However, the results need to be reproduced in larger cohorts before the method can be implemented in the clinical management of pediatric cancers.

54347 - Multimodal classification resolves the molecular subtype of pediatric undefined/B-other acute lymphoblastic leukemia

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Abstract text

Genomic analyses have redefined the molecular subgrouping of pediatric acute lymphoblastic leukemia (ALL). Molecular subgroups guide risk-stratification and targeted therapies, but outcomes of recently identified subtypes are often unclear, owing to limited cases with comprehensive profiling and cross-protocol studies. We developed a machine learning tool (ALLIUM) for the molecular subclassification of ALL in retrospective cohorts as well as for up-front diagnostics. ALLIUM uses DNA methylation and gene expression data from 1131 Nordic ALL patients to predict 17 ALL subtypes with high accuracy. ALLIUM was used to revise and verify the molecular subtype of 280 cases with undefined/B-other molecular phenotype, resulting in a single revised subtype for 85.4% of these cases. Our study shows the power of combining DNA methylation and gene expression data for resolving ALL subtypes and provides the first comprehensive population-based retrospective cohort study of molecular subtype frequencies in the Nordic countries, identifying subgroups with differential survival outcomes.

54349 - Cannabidiol inhibits cancer cell proliferation and motility induced by extracellular High Mobility Group Box 1 protein

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Abstract text
Cannabidiol (CBD) is a phytocannabinoid discovered in 1940. Clinical research on CBD includes studies on anxiety, cognition, movement disorders, chronic pain and cancer, but there is insufficient knowledge on the underlying mechanisms that make cannabidiol a potential effective treatment for these disorders Nevertheless, CBD is an herbal dietary supplement advertised with unproven claims of specific therapeutic effects. In recent years the main focus on the studies with cannabidiol is its profound anti-tumor activity.

That is why we decided to test how cannabidiol would affect cancer cells *in vitro* by using model lung cancer cells. We evaluated its effect on cell proliferation and on the epithelial to mesenchymal transition process (EMT), leading to the activation of cell migration. One of the main processes for the occurrence of metastases is the transformation of cancer cells phenotype from epithelial to mesenchymal ones. EMT has been proposed to be a crucial early event in cancer metastasis.

Our results showed that cannabidiol inhibits cell proliferation in a dose-depending manner. Using wound healing assay, we also demonstrated that it effectively blocks cancer cell migration. Western blot analysis of key epithelial and mesenchymal markers showed altered protein levels upon CBD treatment.

High mobility group box 1 (HMGB1) protein is highly expressed in different cancer cells and its levels are positively associated with to tumor cell proliferation and metastasis. HMGB1 can be dislocated into the cytosol under stress conditions or even secreted into the extracellular space. Its extracellular role has been subject of interest because the interaction with several key receptors triggers a cascade of biochemical changes in the cells, including EMT. We observed that a recombinant HMGB1 (rHMGB1) enhanced the proliferation of lung cancer cells. After treatment with high concentrations of rHMGB1, molecular phenotype alterations of epithelial-to-mesenchymal transition and elevated expression levels of vimentin and N-catherin were observed.

Encouraged by the results above we tested if CBD is also able to inhibit cell proliferation and migration when cancer cells are stimulated by extracellular HMGB1. We observed a reduction in cell proliferation as well as inhibition of cell movement. The expression levels and subcellular distribution of the EMT markers were also altered.

Taken together, these results suggest that treatment with CBD effectively blocks epithelial-to-mesenchymal transition and cell migration, including those induced by HMGB1. These findings suggest that CBD might have a beneficial effect in advanced cancers with high HMGB1 expression levels.

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54350 - Dissecting the role of AKT signaling in oncogenic MYB activation in adenoid cystic carcinoma

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Abstract text

Adenoid cystic carcinoma (ACC) is one of the most common malignant tumors of the salivary glands but may also arise in other exocrine glands such as for example in the breast, skin, and lung. It is a slow growing yet aggressive tumor with a poor long-term prognosis due to frequent late recurrences and/or metastasis. There are no useful systemic treatments available for patients with advanced disease. The genomic hallmark of ACC is a t(6;9)(q22-23;p23-24) chromosomal translocation leading to gene fusion events with activation of the *MYB* oncogene [1]. In a subset of cases, *MYB* is alternatively activated by enhancer hijacking or gene amplification. MYB overexpression leads to transformation of glandular cells and increased cell proliferation and is seen in more than 90% of ACCs. This makes MYB the main oncogenic driver in ACC. We have previously shown that MYB is regulated by IGF1R in an AKT-dependent manner [2]. AKT is part of the PI3K signaling pathway and regulates various cellular processes such as proliferation, survival, and metabolism. To further investigate the importance of AKT signaling in ACC, we have now treated primary t(6;9)-positive ACC cells with the AKT inhibitor MK-2206 and assayed the effects on global gene expression. MYB downstream targets were significantly affected by the treatment, indicating that AKT signaling is a major regulator of MYB function in ACC. To further dissect the roles of the individual AKT isoforms in human cells (AKT1-3), we performed knockdown experiments with isoform-specific siRNAs. Preliminary results indicate that AKT3 regulates *MYB* expression in ACC cells and that knockdown of this isoform results in decreased ACC cell proliferation. Importantly, the *AKT3* gene was significantly overexpressed in a subset of ACCs with poor prognosis. Taken together, our study identifies key players in the PI3K-AKT pathway that are responsible for *MYB* regulation and are novel therapeutic targets in ACC.

References

1. Persson M, Andrén Y, Mark J, Horlings HM, Persson F, Stenman G. Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. *Proc Natl Acad Sci U S A*. 2009. 106:18740-4.
2. Andersson MK, Afshari MK, Andrén Y, Wick MJ, Stenman G. Targeting the Oncogenic Transcriptional Regulator MYB in Adenoid Cystic Carcinoma by Inhibition of IGF1R/AKT Signaling. *J Natl Cancer Inst*. 2017. 109.

54351 - Evaluation of treatment response in paediatric leukaemia using patient specific circulating tumour DNA analysis

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Abstract text

Leukaemia is the most common paediatric cancer, accounting for almost 1 out of 3 childhood malignancies. As per gold standard, to diagnose and evaluate treatment response a bone marrow aspirate is required. Bone marrow aspiration is an invasive procedure that when done in children requires general anaesthesia, which is associated with a significant risk of complications. In contrast, blood samples are less invasive and entail less risks than bone marrow aspirates.

In this study, circulating tumour DNA (ctDNA) analysis using SimSenSeq is presented as a candidate for minimal residual disease (MRD) evaluations during treatment. Personalised multiplex assays are used for tracking multiple patient specific single nucleotide variants (SNVs) over the course of treatment in children with acute lymphoblastic leukaemia. The analysed SNVs are selected from those present in the diagnostic bone marrow aspirate and followed up in longitudinal paired bone marrow aspirates, plasma samples and peripheral blood cells samples.

Preliminary results for 9 patients show that the targeted SNVs levels in ctDNA are comparable to those in bone marrow. In addition, the total amount of ctDNA follows a similar trend as the percentage of cells with leukaemia-associated immunophenotype in the bone marrow measured by flow cytometry as part of the clinical MRD evaluation. Similar results are observed when qPCR is used to evaluate the treatment response in T-ALL patients.

Further analyses are needed to determine a ctDNA cut-off value for MRD classification to be able to influence treatment strategy. However, these results show the potential use of patient-specific ctDNA analysis in peripheral blood as a marker for treatment response in childhood ALL.

54354 - High-content screening of drug combinations of an mPGES-1 Inhibitor in Multicellular Tumor Spheroids

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Abstract text

High-throughput drug screening is undoubtedly a valuable resource for the discovery of new anticancer drugs. To date, monolayer cell cultures have been the most widely used models for drug screening. However, these models are unable to reconstruct the complex microenvironment of solid tumors and have low bench-to-bedside translational efficiency. In contrast, three-dimensional (3D) cell cultures, such as multicellular tumor spheroids (MCTS), are emerging as an important tool in cancer research because they mimic the architecture of in vivo tumors. These models offer great potential for studying the biological properties of cancers and provide a promising platform for drug discovery. In this work, we aimed to develop a neuroblastoma (NB) MCTS model compatible with high-content drug screening. We also aimed to elucidate the potential mechanisms of action of different drug combinations that are highly synergistic in this disease model. Several conventional agents from different therapeutic categories and with different mechanisms of action were tested alone or in combination with selective inhibition of PGE_2, by pharmacologic inhibition of mPGES-1. After a systematic investigation of the sensitivity of individual agents and the effects of pairwise combinations, GFP-transfected MCTS were used in a confirmatory screen to validate hits. Finally, caspase 3/7-mediated apoptosis and the inhibitory effects of efflux pumps were kinetically examined in real-time. In summary, this work demonstrates how NB MCTS-based high-throughput drug screening has the potential to uncover effective drug combinations and provide insights into the mechanism of synergy between an mPGES-1 inhibitor and chemotherapeutic agents.

54355 - Amino acid deprivation renders lung adenocarcinoma cells dependent on the antioxidant glutathione to prevent lipid peroxidation and ferroptosis

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Abstract text

Progression and metastasis of lung adenocarcinomas are restricted by oxidative stress, indicating that lung tumor patients may benefit from pro-oxidant therapies. Glutathione is the most abundant intracellular antioxidant and a major determinant of the redox environment in cells, and thus an attractive target for pro-oxidant therapies. Depletion of glutathione by buthionine sulfoximine (BSO), a glutamyl-cysteine ligase inhibitor that blocks *de novo* synthesis of glutathione, sensitizes cancer cells to ferroptosis, a regulated form of cell death mediated by iron-dependent lipid peroxidation. Ferroptosis is triggered by reductions in cysteine, which is the rate-limiting substrate for glutathione synthesis, or glutamine that regulates cystine uptake. The impact of other amino acids on ferroptosis has largely been overlooked. Herein we show that a mild reduction of amino acids other than cysteine or glutamine, have a major sensitizing effect to BSO in multiple lung adenocarcinoma cell lines, from human and mice. BSO-treated cells showed increased lipid peroxidation and were rescued by lipid peroxide scavengers or iron chelators. Thus, reduction of amino acids sensitizes lung adenocarcinoma cells to ferroptosis. Of note, reduction of amino acids did not lower the concentration of glutathione in BSO-treated cells, but inflicted a strict dependency on the antioxidant that was not present in control cells. The amino acid sensor p-GCN2, and the downstream integrated stress response proteins p-eIF2α/ATF4/CHOP, were upregulated in amino acid deprived cells, and knockdown of ATF4 efficiently rescued BSO-treated cells from ferroptosis. We conclude that a mild reduction of amino acids dramatically increases the cells' need for glutathione to prevent iron-dependent lipid peroxidation in response to the integrated stress response pathway. The result warrants further investigation of ferroptosis under conditions of mild amino acid deprivation, a state that is not uncommon in solid tumors that are characterized by poor vascularization and rapid growth. The finding raises new opportunities for BSO-based lung cancer therapies, possibly in combination with protein-restricted diets.

54356 - The microRNA landscape of prostate cancer bone metastasis and the role of microRNA-375 in modulating the metastasis subtype MetA, B and C

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Abstract text

Introduction
Bone metastatic prostate cancer (PC) display a substantial heterogeneity, and stratification into molecular subtypes is important to predict treatment response and prognosis. Recently, we identified and validated the transcriptomic-based subtypes, termed MetA, B and C (MetA-C) in PC bone metastases (Thysell et. al., Mol. Oncol., 2019; 2022). Features of the MetA subtype include a high androgen receptor (AR) activity and a low proliferation rate. MetB displays contrasting characteristics and associates with a poor survival after androgen deprivation therapy. Preliminary results indicate some plasticity in-between subtypes, and we hypothesize that it is possible to modulate MetB to a less aggressive phenotype with a better response to AR targeting therapies and, thus, a more favorable prognosis.

MicroRNAs (miRNAs) are short non-coding RNAs regulating gene expression by guiding Argonaute proteins to complementary messenger RNA (mRNA) transcripts. This leads to translational inhibition and mRNA degradation. The miRNA expression profile is broadly altered in PC and specific miRNAs may have oncogenic or suppressive functions. Restoring levels, or inhibiting such miRNAs, constitutes a possible therapeutic strategy. miRNA-375 has previously been reported as upregulated in PC, and is suggested to regulate epithelial plasticity.

Aims
To examine the miRNA signatures of the transcriptomic subtypes MetA-C of bone metastatic PC. Furthermore, the study aimed to investigate if specific miRNAs could affect phenotypic subtype characteristics of metastatic PC cell lines *in vitro*, such as proliferation and AR activity.

Methods
An integrated mRNA and miRNA microarray analysis of bone metastatic biopsies (n=103) and metastatic PC cell lines (n=4) was performed. Based on mRNA expression (Clariom™ D array), the material was classified according to a panel of predefined transcripts (n=157), differentiating MetA-C. Expression levels of miRNAs (GeneChip™ miRNA 4.0 arrays) were correlated to the MetA-C content in the biopsies, to identify subtype-associated miRNAs. PC cell lines overexpressing specific miRNAs were constructed by lentiviral transduction. Effects of overexpression on proliferation and AR activity were evaluated using a luminescent cell viability assay and by quantifying expression of prostate specific antigen (PSA) by quantitative polymerase chain reactions.

Results and conclusions
The study analyzed the expression of 2560 miRNAs in PC bone metastases, of which 483 showed levels that correlated with the MetA, B or C subtypes (p<0.05). This finding suggests that the MetA-C subtypes might in part be epigenetically regulated by miRNAs. miRNA-375 correlated positively with MetA (r=0.45). In the cell line profiling, the phenotype of LNCaP-C4-2B cells shifted from being predominantly MetB to obtain more MetA features following dihydrotestosterone stimulation. The results indicated a possibility to modify the MetA-B subtype of LNCaP-C4-2B cells, and this cell line was selected as a suitable model system to study subtype plasticity. Forced expression of miRNA-375 reduced proliferation of the LNCaP-C4-2B cells, suggesting a tumor suppressive role. Furthermore, the PSA transcript levels were decreased following miRNA-375 overexpression, indicating a functional link to AR activity. Such a relationship needs to be further explored.

54357 - Plasma protein profiles predictive of metastatic prostate cancer

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Abstract text
Introduction: Prostate cancer (PC) is a common male cancer and a dominant cause of cancer-related deaths. In Sweden, approximately 2500 men per year develop metastatic PC that ultimately leads to death, while others live for a long time with a cancer that does not cause any main problem. Diagnosis of PC is based on measuring the serum level of prostate-specific antigen (PSA) followed by magnetic resonance imaging of the prostate and tissue biopsy examination under light microscopy. Unfortunately, these methods cannot predict with sufficient certainty neither the disease course, nor the treatment needed. This leads to overtreatment of many patients, while others receive inadequate treatment too late. Patients diagnosed with localized PC can be cured by surgery or radiation therapy. For patients where the cancer has already metastasized, no curative treatment exists. Standard treatment for metastatic disease is castration therapy followed by complementary treatments of various kinds. It is of outmost importance to detect potentially fatal disease as early as possible, at a stage where optimal treatment can make a big difference for survival.

Aim: To find plasma biomarkers predictable of metastatic PC, which could be used to identify patients in need of intensive treatment at an early stage.

Methods: Blood samples were obtained from men examined due to elevated serum levels of PSA and suspicion of PC (n=350, Urology clinic, Umeå University Hospital, 2003-2011). At the time for blood drawn, 314 patients were diagnosed with PC, 19 were diagnosed with metastatic cancer (M1) and 46 developed metastases over time. Blood samples from 36 age-matched men who did not develop PC during follow up (11 years) were included as benign controls. Protein levels were assessed using the Olink Explore 1536 platform (Olink Proteomics, Uppsala). Logistic regression analysis was performed to identify proteins showing different plasma levels between M1 patients and patients with benign disease as well as between M1 and M0 patients at diagnosis ($p<0.05$, adjusted for serum PSA levels and age). Furthermore, proteins being associated with time to metastasis were identified (Cox regression analysis). Proteins being statistically associated with metastases in all three comparisons were evaluated by internal cross-validation (1000 repetitions for prediction of 10% randomly selected cases). Validated proteins were included together with age and serum PSA levels in models for predicting metastasis development within 5 and 10 years from diagnosis.

Results and conclusions: The study identified plasma proteins (n=211) differing between M1 patients and patients without cancer as well as proteins (n=63) differing between M1 and M0 patients at diagnosis. Furthermore, plasma proteins (n=40) were identified being associated with time to metastasis development. Nineteen proteins were identified through all three comparisons, and 12 could be verified by internal cross-validation. The 12 verified proteins together with serum PSA level and age provided more reliable models for prediction of metastasis development within 5 and 10 years from diagnosis (AUC 0.84 and 0.81) compared to models based solely on PSA and age (AUC 0.72 and 0.74). Further validation of the 12 metastasis associated proteins is ongoing in separate patient cohorts.

54358 - Polo-like kinase 1-targeting RNA interference prodrugs against childhood cancer

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Abstract text

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.

54359 - Sequential drug treatment targeting cell cycle and cell fate regulatory programs blocks non-genetic cancer evolution in acute lymphoblastic leukemia

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Abstract text

Targeted therapies exploiting vulnerabilities of cancer cells hold promise for improving patient outcome and reducing side effects of chemotherapy. Despite a high response rate to induction chemotherapy in acute lymphoblastic leukemia (ALL) there is an urgent need to identify effective combination therapy to overcome treatment resistance that associates with disease relapse.

ALL cells are acutely highly sensitive to the WEE1 inhibitor AZD1775 that compromises the G2/M-phase cell cycle checkpoint. Here, we show that AZD1775 selectively inhibits recovery of proliferation in KMT2A/MLL-rearranged (MLLr-) ALL, a group of leukemias that respond poorly to current chemotherapy and have dismal prognosis compared to other leukemia subgroups. Extensive genomics profiling of the drug response by joint single-cell transcriptome and chromatin accessibility profiling revealed that AZD1775 led to a strong p53-driven gene regulatory response resulting in induction of apoptosis and senescence and disrupts a crucial hematopoietic TF-network involving RUNX1, MYC and GATA2.

Leukemia relapse may arise from rare cells that escape treatment through genetic mutations, transcriptional reprogramming or signaling rewiring. We here demonstrate that upon the pre-mature mitosis entry resulting from AZD1775 treatment, a rapid transcriptional reprogramming is triggered involving activation of NfKB and transcription factors regulating lipid metabolism, BCL6 and pre-BCR signaling that resulted in a small surviving cell population. Single-cell characterization of AZD1775 response in a MLL-r patient-derived xenograft (PDX) model supported the *in vivo* relevance of this drug resistance mechanism. By sequential treatment targeting the drug tolerant cell state phenotype with BCR-signaling inhibitors dasatinib, ibrutinib, or perturbing metabolism by fatostatin or AZD2014 after AZD1775 administration, this cell state evolution underlying recovery of leukemic cells could be blocked. Collectively, our findings provide new insights into the tight connectivity of gene regulatory programs associated with cell cycle and cell fate regulation, and a rationale for sequential administration of WEE1 inhibitors with low toxicity inhibitors of pre-BCR signaling or metabolism.

54360 - Inhibiting SAMHD1 enhances the efficacy of fludarabine/cytarabine-based salvage treatments against acute myeloid leukemia

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Abstract text

Acute myeloid leukemia (AML) has an overall survival rate of approximately 30%, depending on age and risk group at diagnosis, with younger patients generally faring better. About half of patients that reach complete remission will experience relapse, and only one-third of relapsed AML patients will become long-term survivors, indicating a need for improved relapse therapies. One hurdle for these patients is resistance to the nucleoside analog cytarabine (ara-C), a backbone drug for primary and relapse therapy. Based on preclinical data indicating an increase in ara-CTP, the active metabolite of ara-C, in leukemic cells when pre-treated with the nucleoside analog fludarabine (F-ara-A), combinations of ara-C and F-ara-A (FLA), with or without idarubicin, have become standard relapse regimens. In adults, addition of the Bcl-2-inhibitor venetoclax has recently been demonstrated to further increase efficacy of FLA-based regimens. We have previously reported a lack of synergy between ara-C and F-ara-A, questioning the clinical rationale of FLA combinations in relapsed AML. We have also demonstrated that addition of the ribonuclease reductase inhibitor hydroxyurea (HU) can overcome ara-C resistance in primary AML by inhibiting SAMHD1. As SAMHD1 mediates resistance both to F-ara-A and ara-C, we investigated whether addition of HU could enhance the effect of FLA.

First, we explored the ability of HU to enhance F-ara-A cytotoxicity using cell viability assays. We found that HU sensitized an AML cell line to F-ara-A, reducing its half-maximal effective concentration by a factor of 20. More importantly, AML cells were more susceptible to FLA combinations in the presence of HU, as indicated by increased levels of cytotoxicity and apoptosis. These findings were validated in adult and pediatric primary AML samples. Second, to understand the molecular basis of this HU-FLA synergy, we measured the active metabolites of F-ara-A and ara-C under different conditions. In line with our previous findings, the FLA combinations increased neither ara-C nor F-ara-A active metabolites in AML cells. Addition of HU, however, increased both ara-C and F-ara-A active metabolites when combined with the single drugs and with FLA. Third, we performed four-drug experiments adding either venetoclax or idarubicin to HU-FLA combinations and successfully excluded a negative interference with HU-mediated FLA synergy.

Together, we demonstrated that HU potentiates the efficacy of FLA against AML through SAMHD1 inhibition that increases leukemic exposure to active F-ara-A and ara-C metabolites. Based on these findings, we are currently preparing a randomized clinical trial protocol for salvage therapy in pediatric and adult AML.

54361 - Investigating the haematopoietic landscape of Systemic Mastocytosis through single-cell multi-omics

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Abstract text

Mast cells are critical components of the human immune system. They arise in adults as a product of proliferation and differentiation of haematopoietic stem cells. Functional abnormalities in haematopoiesis and mast cell development processes can lead to diseases such as systemic mastocytosis (SM), marked by high numbers of mast cell infiltrates in tissues such as the bone marrow. Clinical phenotypes of SM include the genetic mutation *KIT* D816V, as well as abnormal expression of CD2 and CD25 surface markers on aberrant mast cells. Advances in single-cell technologies allow us to simultaneously measure individual cell transcriptomes with corresponding surface expression through oligo-tagged surface antibodies. Harnessing this method, a sorted spectrum of haematopoietic progenitor cells including oligo-tagged mast cells can be sequenced, generating multi-dimensional datasets which facilitate a variety of exploratory analysis. Integration of gene and surface expression phenotypes allowed us to accurately chart haematopoiesis in SM, delineating aberrant and phenotypically normal populations of mast cells in patient bone marrow. This revealed a novel transcriptomic signature in aberrant mast cells across several SM bone marrow datasets. Additionally, long-read targeted sequencing of *KIT* transcripts of the same single-cell dataset produced high-fidelity reads from which individual cells could be genotyped for the D816V mutation – demonstrating that both mutant and wild-type cells can be called throughout an individual's haematopoietic landscape. Comparative analysis of mast cells using both conventionally defined surface phenotypes and mutation genotypes uncovered several known and novel characteristics of SM. Downstream analysis of these novel clues could provide new mechanistic insight into disease development of SM, and the haematopoietic origin of aberrant mast cells.

54362 - Identification of novel factors controlling non genetic cell plasticity in Chronic Myeloid Leukemia

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Abstract text

In cancer cell communities the dynamic fluctuation of gene expression can lead some cells to acquire rare drug resistant phenotypes. These cells can survive even targeted therapies impairing our ability to eradicate the disease. This phenotypic plasticity, a hallmark of cancer, has been observed in several tumors as chronic myeloid leukaemia (CML). Indeed, a previous study identified a CD24+ subpopulation in K562 cells displaying drug resistance and increased tumorigenicity. Importantly, K562 cells were shown to switch between the CD24+ and CD24- state as both cell types were able to originate a heterogeneous culture over time. In this study we leveraged the CD24 as a marker to track these phenotypic switches in K562 CML cells and identify genes regulating state transition by CRISPR screening. We firstly characterized the CD24+ phenotype reanalysing publicly available K562 scRNA-Seq. In these cells, the CD24 mRNA levels were inversely correlated with those of several genes involved in the oxidative metabolism. To further investigate this association, we performed real time extracellular flux analysis by Seahorse cell energy phenotype assay confirming the reduced metabolic activity of CD24+ cells. To identify genes regulating the phenotypic switch, we developed a genome-wide CRISPR-KO screening line in K562 cells and studied the distribution of KO events in the cells changing their phenotype. We leveraged two different approaches to isolate the switching cells. Firstly, we isolated CD24+ and - cells and cultured them for 24H. Afterwards, we sorted again the two lines based on the CD24 expression levels isolating those cells changing their phenotype over the time in culture. The second approach was instead based on the temporal delay between mRNA and protein levels variations upon both gene overexpression and repression. Briefly, we measured CD24 mRNA and protein levels in the same cells by combining mRNA fluorescence *in situ* hybridization (FISH) and immune staining. Those cells showing an inconsistent level of mRNA and protein were identified as transitioning cells. In the two screenings, we identified a signature of 49 genes associated with state transition, many of those were involved in the manteinance of cell energetic homeostasis and cell cycle progression. Given the association between cell plasticity and drug resistance, we then investigated whether the expression levels of these genes were associated with drug sensitivity in human CML. To this extent we leveraged publicly available data from 96 CML patients profiled by microarray in PBMC at the diagnosis and then starting a therapy with Imatinib. Early molecular response (EMR) failure at month 3 of therapy was used as a proxy for Imatinib resistance. In these patients, we confirmed the association of 7 genes with drug sensitivity. Interestingly, most of them were involved in the regulation of cell energetic metabolism including a key enzyme of glycolysis and three genes involved in oxidative metabolism and mitochondrial homeostasis. Overall, we identified a gene signature associated with the regulation of cell plasticity in CML models and drug resistance in patients. Our preliminary results suggest a link between cell plasticity and the regulation of cell energetic metabolism in CML.

54363 - Primary tumor PSA and Ki-67 predict the effectiveness of addition of docetaxel in patients with metastatic hormone naïve prostate cancer.

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Abstract text

Purpose

For metastatic hormone naïve prostate cancer patients, androgen deprivation therapy (ADT) is the standard therapy, together with adjuvant addition of docetaxel or androgen targeting drugs, but markers for patient selection are lacking. The purpose of the present study was to investigate if PSA and Ki67 immunoreactive scores can identify patients who benefit from ADT alone and those who need additional therapy.

Experimental design

Prostate biopsies from 92 patients with metastatic hormone naïve PC were immunohistochemically evaluated for PSA and Ki67. Gene expression analysis was performed with Clariom D microarrays to identify the phenotypic profile associated with the immunohistochemistry scores.

Results

Cox regression analysis after adjustment for age, ISUP and serum PSA value demonstrated that Ki67 labeling index was positively, and PSA immunoreactive score negatively associated with progression-free and overall survival. Patients with a high Ki67/PSA ratio significantly benefitted from docetaxel treatment added to ADT, while no benefit was seen for those with a low Ki67/PSA ratio. Accordingly, mRNA expression data analysis showed an association between high Ki67/PSA ratio, cell-cycle regulation and DNA damage repair.

Conclusion

PSA and Ki67 immunoreactive scores are prognostic in the metastatic hormone sensitive setting. Their combined evaluation can distinguish patients with metastatic prostate cancer who benefit from early addition of docetaxel as adjuvant therapy to ADT. Since the number of patients treated with docetaxel was small, further studies are warranted to establish these results.

54364 - Immuno-oncological effects of conventional anticancer agents and concomitant drugs: an in vitro assessment.

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Abstract text
In the field of oncology, it has become evident that treatment outcome is dictated by the combined effect exerted on both cancer- and immune cells. Some of the chemotherapeutics that are used in the clinic have severe immunosuppressive adverse effects while others have the ability to enhance anti-tumor immunity. Furthermore, as oncology patients often suffer from cancer symptoms, treatment complications, and/or additional diseases, concomitant drugs such as pain killers, corticosteroids, statins, and antibiotics are commonly administered during the course of cancer treatment. We evaluated potential immunological effects of 46 conventional anticancer drugs and 22 commonly administered concomitants drugs, selected to cover a broad range of mechanisms of actions, using an in vitro tumor-immune model.

We utilized a miniaturized model system comprised of fluorescently labeled colon and lung cancer cell lines grown as monocultures or co-cultured with activated peripheral blood mononuclear cells (PBMCs). The drug panel was screened in mono- and co-cultures and the Bliss Independence Model was applied to detect synergy and antagonism between drugs and activated PBMCs. As expected, the immunosuppressive tyrosine kinase inhibitors (TKIs) ruxolitinib and dasatinib generated the lowest Bliss Scores. On the contrary to the other TKIs, the multi-kinase inhibitor sorafenib was shown to synergize with activated PBMCs. In the HCT116-GFP model, the only anticancer agent that generated a higher Bliss Score than sorafenib was thioguanine. As for the concomitant drugs, the immunosuppressive corticosteroids betamethasone and prednisolone had the greatest antagonistic effect. Finally, in HCT116-GFP, the statins mevastatin and simvastatin were uniquely shown to synergize with activated PBMC at all tested drug concentrations. This observation is in line with previous findings in our group; aiming to identify immunomodulatory small molecule drugs with a potential application in cancer treatment, we recently performed a drug repurposing screen. Among the 1,280 FDA-approved drugs that were screened, mevastatin stood out as one of few drugs with a potent and reproducible ability to enhance immune cell-induced cancer cell death. Upon further investigation it was demonstrated that this feature was shared by other lipophilic statins such as simvastatin and pitavastatin.

In conclusion, we utilized a miniaturized tumor-immune model to enable time and cost-effective evaluation of a broad panel of drugs in an immuno-oncology setting in vitro. The model was used to identify drugs that stimulate or inhibit the ability of immune cells to attack cancer cells. Among the conventional anticancer agents, sorafenib and thioguanine stood out as the two drugs with the highest Bliss scores and as for the concomitant drugs, statins were uniquely shown to synergize with activated PBMC at all tested drug concentrations.

54365 - Small molecules targeting the intrinsically disordered MYC oncoprotein

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Abstract text
Deregulated expression of *MYC* family oncogenes occurs frequently in human cancer and is often associated with aggressive disease and poor prognosis. While MYC is a highly desired target, it has been considered "undruggable" as it does not have any druggable pockets or enzymatic activity, and no specific anti-MYC drugs are available in the clinic.

In a screening campaign to find potential molecule probes for targeting the interaction between MYC and its essential partner MAX, we identified small molecules named MYCMIs that directly target MYC. Two molecules, MYCMI-6 and MYCMI-7, efficiently inhibit MYC:MAX and MYCN:MAX interactions in cells, bind directly to recombinant MYC, and reduce MYC-driven transcription at single-digit micromolar concentrations, as validated by split *Gaussia* luciferase-, *in situ* proximity ligation-, microscale thermophoresis- and surface plasmon resonance-assays.

The MYCMI molecules inhibit tumor cell growth in a MYC-dependent manner with IC_{50} concentrations as low as 0.5 µM, while sparing normal cells. The response to the MYCMIs correlate with MYC expression based on data from 60 human tumor cell lines and is abrogated by MYC depletion. Further, they inhibit MYC:MAX interaction, reduce tumor growth and induce massive apoptosis in tumor tissue in MYC-driven xenograft mouse tumor models without causing severe side effects.

The MYCMIs are unique molecular tools to specifically target MYC:MAX *in vitro* and *in vivo*, and they have good potential for clinical drug development.

54367 - Determinants of Translation – a literature review on the determining factors for the implementation of Personalised Cancer Medicine

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Abstract text
Background: The introduction of Personalised medicine entails a paradigmatic shift in the medical profession and organisations providing healthcare. Although more research is needed to fully employ the different aspects of personalised medicine, several advancements have been made during the past decades. At the forefront are research groups and organisations that have succeeded in creating the necessary conditions. Throughout the published scientific literature on Personalised medicine and other associated concepts, few articles disclose the determining factors to accomplish a project with a personalised medicine profile.

Methods: This review article examines recent articles that provide insights into some of the determining factors for the success of personalised cancer medicine programs.

Results: The emergence of personalised medicine entails rapidly increasing access to complex biological information of the individual patient and the cause of disease. Healthcare providers and organisations aiming to be at the forefront of personalised medicine have met three fundamental challenges: coordinating multiple sources for funding, overcoming organizational differences and leadership with the capacity to facilitate translational processes and support driven investigators. Research funding is often oriented towards specific investigators or research groups. Investment in common infrastructures often needs complex governmental and multi-stakeholder collaborations that take time to organise. Fragmented and poorly informed leadership is a significant obstacle to implementing a multidisciplinary approach to personalised treatment.

Conclusions: Personalised medicine requires a well-coordinated collaborative environment across several disciplines. With personalised medicine, new professions and expertise have emerged, which require dedicated training programs and organisations that foster team science. The organisational and cultural transformation necessary for the efficient introduction of personalised medicine within institutions built on older medical paradigms requires strong leadership with the ability to bring together a multitude of actors towards a shared vision.

There is a need for a more comprehensive understanding of possible generalisable determining factors for translation of projects with a personalised medicine profile.

54368 - Socioeconomic Consequences in Adult Life after Childhood Cancer in Scandinavia (SALiCCS)- a Nordic register-based cohort and research programme

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Abstract text

Background: Survival rates in childhood cancer have increased substantially over the past 60 years due to better diagnostics and treatments. As a result, there is a growing population of childhood cancer survivors with a long life ahead of them. So far, survivorship research has mainly been focusing on somatic late effects, but survivors may also face socioeconomic consequences due to their disease or treatment. These outcomes are difficult to study because of the rarity of childhood cancers and the methodological challenges in studies involving social factors; for example, participation rates in studies are likely to be affected by both health and socioeconomic factors, distorting the study validity. The Nordic population- and health registers, where the whole population is included, provide a unique opportunity to study socioeconomic outcomes longitudinally, also taking somatic late effects into account. To enrich understanding of the mental, social and socioeconomic difficulties that childhood cancer survivors may face during their life course, the research programme Socioeconomic Consequences in Adult Life after Childhood Cancer in Scandinavia (SALiCCS) was initiated.

Methods: The SALiCCS research programme is based on a register-based matched cohort study comprising childhood cancer survivors from Sweden, Denmark, and Finland, and two comparison groups; matched individuals from the general population (ratio 1:5), and siblings as a second comparison group controlling for confounding by familial and genetic factors.

All survivors diagnosed at ages 0-19 years, since 1971 and onwards, have been included and followed. Information from national health- and population registers in the three countries have been gathered. For specific studies, information from the Nordic Society of Pediatric Hematology and Oncology (NOPHO) register for acute lymphoblastic leukaemia could be included for children diagnosed in Denmark and Sweden. The pseudonymized data have been harmonized and pooled across the three countries, and are analysed at a secure server at Statistics Denmark, reached by remote access for involved researchers.

Results: The SALiCCS core population includes 21,292 five-year survivors, 103,303 population comparisons and 29,644 siblings. Key findings include that, despite a small absolute difference, survivors are at increased risk of psychiatric disorders, especially survivors treated in young ages. Survivors are also at increased risk of delays in attainment of upper secondary education, although many had caught up with regard to this education level at age 25. Health-related unemployment is more common among survivors than in the comparison group, although we observed no large difference in unemployment for reasons unrelated to health. Ongoing projects are investigating income patterns, educational achievements at tertiary level, and other social outcomes among survivors such as moving from parental home. Furthermore, analyses of somatic late effects using more recently diagnosed patients and updated follow-up data are ongoing.

Discussion/Conclusion: The process of collecting and pooling Nordic micro data on both health and socioeconomic factors has been demanding but has resulted in a unique three-country wide collaboration with a large population-based cohort of childhood cancer survivors where important late effects and socioeconomic outcomes can be assessed with high validity—findings that are of upmost relevance for evidence-based survivorship care.

54369 - Role of polymorphisms in CYBA for NOX2-derived reactive oxygen species production by myeloid cells

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Abstract text
Myeloid cells express high levels of the multi-subunit enzyme complex NADPH oxidase 2 (NOX2), which when activated generates reactive oxygen species (ROS). Upon activation, the membrane bound subunits p22phox (CYBA) and gp91phox (CYBB) associate with the cytosolic subunits to form a superoxide forming enzyme. Within the CYBA gene there are several single nucleotide polymorphisms (SNP), including rs4673 and rs1049254, that have been suggested to alter ROS production. Our group has demonstrated that multiple sclerosis patients carrying the rs1049254/G and the rs4673/A alleles show reduced disease severity along with a delayed development to secondary progressive disease (Törnell et al Eur J Neurol, 2022). Furthermore, our results imply that the rs1049254/G and the rs4673/A alleles are associated with reduced ROS formation in human monocytes. However, other studies show partly divergent results regarding the relation between these SNP and ROS production. To clearly define if variation at rs1049254 and the rs4673 alters NOX2-derived ROS formation in monocytes, we utilize a CRISPR/Cas9 approach to insert allelic variations at rs4673 and rs1049254 in the CYBA gene of the myeloid cell line PLB-985. Thus far, the region around SNP rs4673 has been sequenced and CRISPR RNA (crRNA) and single-stranded DNA (ssDNA) designed and transfected into PLB-985 cells. Transfected cells have been single cell sorted and four clones with altered rs4673 genotype were obtained. These clones will be differentiated using dimethyl sulfoxide (DMSO) and their ROS production following stimulation with N-formyl-methionyl-leucyl-phenylalanine (fMLF) or Phorbol 12-Myristate 13-Acetate (PMA) will be assessed using a highly sensitive chemiluminescence-based assay. A similar approach will be applied to determine the role of rs1049254 variations for NOX2-derived ROS production. All in all, this project may yield a set of new biological tools to study NOX2-generated ROS formation. Furthermore, it may clarify if alterations at rs4673 and rs1049254 do affect NOX2-derived ROS formation.

54370 - High yield isolation of circulating tumour cells to identify adaptive treatment responses in prostate cancer

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Abstract text

Background

Liquid biopsies utilizing circulating tumour cells (CTCs) - which reflect the metastases - show great potential as a tool for precision medicine. Current limitations include the low concentration of CTCs in blood and the marker-biased capture of antibody-based isolation methods. The overall goal is to overcome current limitations and implement CTC-based precision medicine in prostate cancer. The specific aims are to isolate and molecularly subtype CTCs in patients at first relapse after curative treatment and to identify early resistance mechanisms for first line systemic therapy for metastatic prostate cancer.

Method

Patients at the Urology clinic at University Hospital of Umeå with either metastasized disease at diagnosis or PSA relapse following surgery are included in this prospective clinical study.

By using the clinically available apheresis methodology, nucleated cells including CTCs are “harvested” from the whole blood volume. Aliquots of the fresh apheresis product is processed for CTC isolation by a label free acoustophoresis method (AcouWash) for separating cells. As read-outs we use 1) CTC count by CellSearch, 2) immunomagnetic isolation by AdnaTest and 3) whole transcriptomic profiling from apheresis product, acoustophoresis product and AdnaTest isolation. This will be evaluated to single cell RNA and DNA sequencing performed from CellSearch isolated cells and acoustophoresis product.Results

The optimization AcouWash protocol was performed by using spiked-in GFP transfected PC3-cells at different concentration. The recovery rate is between 60-80% and purity in the center outlet is increased at least 35x compared to the inlet sample.

The apheresis product (50-100 ml) from the whole volume contains 1000-10000 times more CTCs compared to the CellSearch protocol using only 5 ml blood sample. We identified CTCs by apheresis and acoustophoresis also in the PSA relapse situation where imaging by PSMA-PET and CTC detection with CellSearch were negative.

We are by the protocol able to freeze and thaw apheresis product for acoustophoresis and/or FACS with possibility to achieve transcriptomic profiling. CTCs isolated by acoustophoresis and AdnaSelect show similar profiles by whole transcriptomics. Single cell

RNA sequencing needs further optimization but single cell DNA sequencing using DEP array system has shown good profiling capacity.

Ongoing analysis of samples taken before and 4 weeks after initiation of first line systemic treatment will indicate if we are able to identify early resistance mechanisms to be further explored as possible treatment targets.

Conclusion

We have, in this study, combined apheresis with a subsequent acoustic separation and thereby increased both the yield of collected CTCs from blood, and the CTC purity by using a label free method, reducing the background of immune cells. We show the first results of a method using clinically available apheresis combined with a scalable label-free method, indicating the possibility to achieve molecular subtyping of CTCs from a patient before metastases are detectable, enabling precision medicine at an early state of metastatic disease.

54371 - mPGES-1 inhibitor enhances the cytotoxic effect of paclitaxel and vincristine in multicellular tumor spheroids

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Abstract text

The stroma surrounding the tumor is usually characterized by the presence of fibroblasts. Cancer cells can activate fibroblasts to perform various functions, ultimately resulting in a tumor microenvironment that actively promotes tumor growth. Most nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase enzymes (COX), resulting in decreased synthesis of prostaglandins (PGs). This causes not only an anti-inflammatory effect, but also a reduction in tumor growth by inhibiting the synthesis of the tumor-promoting factor prostaglandin E2 (PGE2). To avoid various adverse effects of COX inhibitors associated with nonspecific PG reduction, inhibition of microsomal PGE synthase-1 (mPGES-1) may be an interesting target, specifically to block PGE2 formation. In this study, we investigated the effect of inhibiting mPGES-1 in combination with cytotoxic drugs on multicellular tumor spheroids (MCTS). Specifically, we tested the effect of combined treatment of pancreatic cancer cells (PaTu-8988T) and cervical cancer cells (HeLa) cocultured with human dermal fibroblasts (HDF) in MCTS on ATP-based cell viability and PGE2 production. Immunohistochemical analysis revealed an activated COX /mPGES-1 pathway, particularly in the cocultured HeLa/HDF spheroids. Combined treatment of HeLa/HDF spheroids with paclitaxel and the mPGES-1 inhibitor 934 resulted in increased cell death compared with mono-treatment with paclitaxel. Similarly, combined treatment of PaTu-8988T/HDF spheroids with vincristine and the mPGES-1 inhibitor resulted in increased cell death compared with vincristine as a single agent. In HeLa/HDF spheroids, PGE2 levels were increased after paclitaxel treatment. The results suggest that inhibition of mPGES-1 increases the efficacy of cytotoxic drugs, so the use of mPGES-1 inhibitors may be a novel therapeutic approach for various cancers.

54372 - Delineating genome instability dynamics in breast cancer by time-course single-cell copy number profiling

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Abstract text

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

54373 - Identifying cell type-specific proliferation signatures in spatial transcriptomics data and inferring interactions driving tumor growth

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Abstract text
In this project I will use scRNAseq and ST data to find cancer-immune interactions that correlate to tumor growth. The project aims at finding single-cell and multicellular gene programs that drive tumor proliferation from spatial transcriptomics data. The approach uses bias-free experimental methods and does not depend on pre-defined pathways and ligand-receptor interactions. Therefore, the approach can determine unknown interactions between cell types. Furthermore, as it is designed in a quantitative manner, the impact that different interactions have can be quantified and validated experimentally.

Two methods are used for finding gene programs, principal component analysis (PCA) and non-negative matrix factorization (NNMF). The components produced by the two methods is what defines a particular gene program. By selecting the sample cells which have the highest contribution to each program found we are able to construct a library of annotated cells. This annotated cell library is then used to construct artificial spots, mimicking the ST we have, with the exception that we know the gene program proportions in our artificial spots. Since we know the proportions this allows us to optimize our gene program finding methods for different deconvolution methods. These combined methods can subsequently be used on real ST data to correlate tumor growth to interactions between different gene programs in a spot. Hopefully this will provide us with a tool to deconvolute ST data with a higher resolution than contemporary methods and give us insight to tumor microenvironment interactions.

54374 - Investigation of the DNA profile and suitable preanalytical handling of cervical samples for use in liquid biopsy-based diagnostics

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Abstract text

Background: Ovarian carcinoma (OC) is the leading cause of death from gynecologic malignancies and strategies for earlier detection are urgently needed. Liquid biopsies are an emerging research field and previous studies have shown that OC-derived mutations can be detected in transvaginally obtained samples. Cervical samples are routinely collected as part of the nation-wide screening program for cervical dysplasia and cervical cancer and provide a suitable specimen for cell-free tumor DNA analysis using ultrasensitive methods. However, little is known about the DNA profile of cervical samples.

Aim: Herein, we aimed to evaluate the DNA profile of cervical samples. Furthermore, we wanted to evaluate if storage condition affected the DNA integrity.

Method: Cervical samples were simulated by addition of preservative solution to an ovarian cancer cell line, followed by 48h of storage at room temperature (RT) leaving cells to sediment, mimicking standard sample handling at the clinic prior to analysis and biobanking. DNA was extracted directly or after storage at 4°C, -20°C or -80°C. DNA integrity was evaluated using automated electrophoresis and qPCR. A series of simulated cervical samples were set up and DNA was extracted at fixed timepoints (0h-96h). Cervical samples were collected from patients enrolled at Sahlgrenska University Hospital in Gothenburg. A duplicate of cervical samples was collected from patients undergoing surgery due to endometriosis (n=10), according to routine sampling procedures. One sample was immediately placed at 4°C and the other was kept at RT. For all samples, DNA was extracted within 4h from sample collection and after 48h.

Results: Simulated cervical samples showed an accumulation of DNA fragments with a mean fragment size of 269bp. The DNA fragmentation was significantly increased within the regions of 100-2500bp and 100-350bp after storage at 4°C for a month. A decline in DNA integrity number (DIN) was observed in these samples, although this was not statistically significant. Fragment analysis showed that the relative quantity of small-size fragments (<2500bp) gradually increased with time during the initial 48h. Gating of the regions 100-2500bp and 100-350bp showed a mean percentage of total of 6.0 and 0.5 at t=0h. After 48h, the percentage of total had increased to 23.0 and 4.2. DNA degradation could be reduced by decreasing the temperature at which samples were stored immediately after addition of cells to preservative solution. Up to 84h, there was no significant increase in fragments of 100-350bp in samples stored at 4°C. Preliminary data from cervical samples collected from patients indicates similar trends. More importantly, these clinical samples provide an insight into the DNA profile of cervical samples in close proximity to collection (<4h), and how the DNA profile potentially changes during the first 48h.

Conclusion: Temperature-dependent DNA fragmentation occurs in cells within the first 48h of methanol-based cell preservation. To some extent, DNA seems to be degraded in cells in preservative solution stored at 4°C for a longer period (1 month). However, short term storage (≤48h) comparing RT and 4°C showed that DNA fragmentation significantly decelerated at a lower temperature.

54375 - From Inflammation to Invasion: Unraveling Myeloid-Derived-Reactive-Oxygen-Species-SNAI2 Signaling in Metastasis

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Abstract text
Background:

Metastasis is a leading cause of incurable disease and poor prognosis (1, 2). One of the processes driving the metastatic cascade is epithelial to mesenchymal transition (EMT), which involves activation of epigenetically regulated transcription programs within cancer cells (3). Recent research has highlighted a potential link between hypoxia-induced intracellular reactive oxygen species (ROS) and EMT, but a direct link between extracellular ROS released from inflammatory cells and EMT has not previously been demonstrated (4) (5). In this study, we utilize *in vitro*, *in vivo*, and *in silico* analyses to show that myeloid-cell derived ROS has the potential to induce EMT in cancer cells, with ensuing metastasis formation.

Methods:

Extracellular ROS in the form of hydrogen peroxide (H_2O_2) was added to MCF-7 and 4T1 cells, followed by analysis of expression of EMT markers by RT-PCR and wound healing in the scratch assay. In addition, RNA-seq data of H_2O_2-treated MCF-7 cells from Gene Expression Omnibus (GEO) were analyzed for markers of EMT. Alternatively, monocytes were added in cell culture inserts to wells containing scratched cancer cells in the presence or absence of ROS-inducers or inhibitors. The healing of the scratched area in the presence or absence of monocytes, as well as expression of EMT-related genes was then determined.

To evaluate the effect of extracellular ROS on cancer metastasis *in vivo*, luciferase-tagged 4T1-breast cancer cells were orthotopically implanted in mouse breast pads followed by intratumoral injections of H_2O_2 or the myeloid cell ROS-inducer D-peptide. Formation of lymph node metastasis was then followed using bioluminescence imaging. Furthermore, data from The Cancer Genome Atlas (TCGA) was analyzed via gene set enrichment and Xcell to correlate myeloid cell portions in tumors and EMT scores in real patient data.

Results:

Our *in vitro* and *in vivo* experiments consistently showed that myeloid-cell derived ROS and H_2O_2 induced EMT in cancer cells via enhanced expression of the SNAI transcription factor family. Interestingly, the SNAI family has previously been shown to induce a quasi-mesenchymal phenotype of cancer cells, which is more metastatic compared with the complete mesenchymal phenotype (6). In accordance, intratumoral injections of H_2O_2 or the ROS-inducer D-peptide into orthotopically implanted 4T1 tumors increased primary tumor growth, augmented expression of EMT markers and aggravated metastasis formation to draining lymph nodes. Furthermore, bioinformatic analysis of TCGA data showed a strong correlation between Xcell macrophage scores in the tumor microenvironment and EMT-related gene expression.

Conclusion:

This study provides evidence that myeloid cell derived ROS stimulates the SNAI transcription family in tumor cells, which may induce a quasi-mesenchymal tumor phenotype leading to more aggressive and metastatic tumors. Our findings suggests that therapies targeting myeloid cell derived ROS may limit EMT and metastasis formation in cancer patients.

54376 - Serum thymidine kinase 1 and its kinetics in HER2-positive breast cancer: Results from the Swedish phase II PREDIX HER2 trial

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Abstract text

Background: Thymidine kinase 1 (TK1) plays a pivotal role in DNA synthesis and cellular proliferation. TK1 has been studied as a prognostic marker and as an early indicator of treatment response in human epithelial growth factor 2(HER2)-negative early and metastatic breast cancer (BC). However, the prognostic and predictive value of serial TK1 activity in HER2-positive BC remains unknown.

Methods: In the PREDIX HER2 trial, 202 patients with HER2-positive BC were randomized to 6 cycles of neoadjuvant trastuzumab, pertuzumab and docetaxel or trastuzumab emtansine every three weeks followed by surgery and adjuvant epirubicin and cyclophosphamide. Serum was prospectively collected from all participants at multiple timepoints: At baseline, after cycles 2, 4 and 6, at end of adjuvant therapy and then annually for 5 years and in case of recurrence. TK1 activity was measured by DiviTum assay (Biovica, Sweden), blinded to treatment allocation, patient characteristics and outcome. sTK1 level as categorical variable are explored by dividing patients into three groups based on median value at baseline: undetectable (<100 Du/L), low(below median), high(above median). At subsequent timepoints, patients were categorized into three groups based on the median value of sTK1 at each timepoint: low (below median), high(above median), and out of range(>2000 Du/L). TK1 activity was correlated with baseline characteristics, pathologic complete response (pCR), event-free survival (EFS) and disease-free survival (DFS).

Results: Baseline sTK1 was significantly associated with a higher histological grade(p=0.03), but not correlated with other clinicopathologic characteristics. An increase of TK1 activity from baseline to visit cycle 2 was seen in all cases with available data. sTK1 level at baseline), visit 2) and visit 4 ()did not significantly correlate with pCR status in and adjusted logistic regression models((baseline: estimates β[95% CI]= 0.202[1.122; 1.531], p=0.765; visit 2: estimates β[95% CI]= 0.508[-0.806; 1.841],p=0.449; visit 4: estimates β[95% CI]= 0.419[-0.942; 1.796] p=0.546). After a median follow-up of 58 months, 21 patients had EFS events. There was no significant correlation between baseline or visit 2 sTK1 activity and EFS in multivariable Cox regression analysis (high vs low sTK1 at basline: HR[95% CI]=0.921[0.275-3.085], p=0.894; high vs undetectable sTK1 at baseline: HR[95% CI]=1.587 [0.49-5.132], p=0.442; high vs low sTK1 at visit 2: HR[95% CI]= 0.782[0.233-2.623], p=0.69; high vs undetectable sTK1 at visit 2: HR[95% CI]=2.199[0.691-7], p=0.182). When using the median value as a cut-off, high versus low sTK1 activity at the

visit end of treatment did not predict DFS in a multivariable Cox regression analysis (HR [95% CI] = 1.464[0.392-5.471], p=0.571).

Conclusions:

Serum TK1 activity in HER2-positiv e BC increased following treatment with neoadjuvant therapy but was not correlated to pCR rates, EFS or DFS.

54377 - An in vivo model to mimic radiation-induced skeletal late-complications reveals the mechanism of growth plate regeneration.

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Abstract text

Background: Radiotherapy is used to treat approximately a third of childhood cancer patients in Sweden. Children´s growing bones are particularly sensitive to radiation-induced damage, which can impair bone growth over time and lead to skeletal late-complications. One reason radiotherapy impairs bone growth is that ionizing radiation directly damages cells that are responsible for bone elongation: growth plate chondrocytes. Growth plate damage can result in skeletal late complications including leg length differences (typically for children treated for soft tissue sarcomas such as synovial sarcoma, rhabdomyosarcoma, and Ewing's sarcoma), irregular body proportions, short stature or spinal curvature (typically following spinal irradiation to treat Wilms´ tumor, or CSI tumors such as medulloblastoma, neuroblastoma and pineoblastoma). Depending on their severity, skeletal late complications can impair the routine activities of childhood cancer survivors and persist throughout their adult lives.

Purpose: After irradiation, bone growth can continue to a limited extent, but the underlying mechanisms of this recovery process are poorly understood. We aimed to compare radiation applied in single fractions to multiple fractions, within the same biological effective dose (BED) range and reveal the cellular recovery processes in the growth plate.

Methods: The left proximal tibia of one-month old mice were (x-ray) irradiated dorso-ventrally and the right leg used as a contra-lateral control. The effects of irradiation were characterized by measuring bone lengths and rates of new bone formation at tissue collection. Legs were collected 28 days after irradiation and recovery was analyzed using clonal genetic tracing (using the Confetti mouse model: Col_2Cre^{ERT}:R26R-Confetti); cell growth and differentiation (using immunofluorescence and RNAscope) imaging was performed by confocal microscopy.

Results: We first conducted a radiation-dose-response study from 5Gy (BED=12.1, using an α/β ratio of 3.5) to 15Gy (BED=79.3). We then fractioned radiation in a clinically relevant way (twice per day with a 6-hour interval) and determined that fractionation (8 doses of 2Gy, BED=25.14) caused less damage than the highest single dose (15Gy). We used clonal genetic tracing with mice to visualize the clonal recovery 28 days after irradiation and made three inter-connected observations: (i) radiation dose-dependently prevented chondrocytes from further dividing, thus reducing the number of clonal-columns; (ii) some chondrocytes dose-dependently produced an increased number of columns leading to regeneration of the growth plate, and (iii) the same patterns were evident when radiation was fractionated.

Re-populating chondrocytes underwent normal differentiation and bone growth rates were lower after irradiation compared to contra-lateral control and sham by a significant reduction after 15Gy and fractioned 16Gy, following regeneration (5Gy: 96.9% ± 9.7% n=6; 10Gy: 91.3% ± 5.1% n=6; 15Gy: 80.4% ± 9.4% n=4; 16Gy (8*2Gy fraction): 71.8% ± 1.7% n=5), suggesting that the observed re-population was capable of regenerating growth plate function to some extent in order to induce new bone formation.

Conclusion: After radiation-induced damage, some growth plate chondrocytes can functionally compensate for the damaged cells and produce more than twice the expected number of columns, which contribute to growth plate regeneration after irradiation. This mechanism of regeneration occurs even when radiation doses are broken into clinically relevant fractions.

54379 - Deletion of the TMEM30A gene allows leukemic cell evasion of natural killer cell cytotoxicity

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Abstract text

Natural killer cells are key innate effector cells in cancer surveillance and in the defence against virus infections. However, much is still unknown about how natural killer cells distinguish between malignant and healthy cells. To learn more about what structures that determine the outcome of interactions between natural killer cells and target cells, we performed a genome-wide CRISPR screen in K562 cells. The screen identified genes that provide protection or susceptibility to natural killer cell cytotoxicity. We could confirm the major role of the NKp30 ligand B7-H6 for natural killer cell recognition, along with CD58 that ligates the co-stimulatory receptor CD2. Knock-out cell lines with depleted B7-H6 and/or CD58 expression displayed altered interactions with natural killer cells with regards to degranulation, cytotoxicity and cytokine production. Deletion of the gene *TMEM30A* provided protection against natural killer cell cytotoxicity. The encoded protein is a subunit of P4 ATPase flippase, which is involved in transfer of phosphatidylserine to the inner leaflet of the cell membrane. Accordingly, *TMEM30A* knock-out cells displayed increased levels of phosphatidylserine on the surface as well as lower susceptibility to natural killer cell cytotoxicity and induced lower natural killer cell degranulation and cytokine production. Blockade of phosphatidylserine by Annexin-V or blocking the inhibitory receptors IRp60/CD300a or TIM-3 on natural killer cells restored natural killer cell killing of *TMEM30A*-deficient cells. Notably, *TMEM30A* is commonly mutated in diffuse large B cell lymphoma and this may serve as an escape mechanism for natural killer cell immunosurveillance. Our study highlights the potential role for agents targeting the interaction between phosphatidylserine and IRp60/TIM-3 in cancer immunotherapy.

54380 - Preclinical Imaging Facility (PIF)

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Abstract text
Preclinical Imaging Facility (PIF) is an imaging hub for small animals located at Karolinska Institutet Flemingsberg campus. PIF is a joint core facility between the Department of Laboratory Medicine (LABMED, KI) and the Preclinical Laboratory (PKL, FoUUI, Karolinska University Hospital, Huddinge; K). PIF is supported by CIMED Infrastructure Grant, closely collaborating with Jonasson Center for Medical Imaging at KTH. PIF is one of the few imaging facilities that cover a wide range of preclinical imaging modalities within the same barrier.

PIF is equipped with advanced noninvasive preclinical imaging instruments as follows:

- IVIS Spectrum (PerkinElmer)
- Quantum FX micro-CT (PerkinElmer)
- Vevo® LAZR-X (Visualsonics)
- Naoscan PET/MRI (Mediso)
- MOMENTUM Magnetic Particle Imaging (Magnetic Insight)

PIF aims to:

1. ***Provide one-stop complete multimodal-imaging services.***
2. ***Develop new methods and techniques that can be applied in research areas specific for K-Huddinge / KI-Syd incl. regenerative medicine, transplantation, molecular therapy and cell therapy***.

PIF together with PKL provides a full range of preclinical study services for both academia and industry sectors to reach and provide high-quality preclinical data. **At PIF we provide the following services**:

- Assist researchers in the design, performance, and analysis of their experiments and collected.
- Produce high-quality preclinical data at PIF to promote translational research at Flemingsberg campus.
- Provide researchers with “the state–of–the–art” imaging techniques to facilitate the translation of the scientific findings at the laboratory bench to be-side.
- Provide a combination of multiple imaging modalities in order to understand the molecular, cellular, and physiological changes in living animals and to predict the outcome following new treatments in patients.
- PIF also helps researchers to fulfill the 3R (Refine, Reduce, and Replace) requirement in their animal studies, via repeated measurements in the same living animals.
- PIF actively organizes doctoral courses, preclinical imaging workshops, and training in image acquisition and analysis to increase knowledge about the use of imaging in research.

The existing five instruments provide eight different imaging modalities that facilitate the application of multimodality imaging. Furthermore, the data from the different modalities can be combined by co-registration:

- Bioluminescence Imaging (BLI)
- Fluorescence Imaging (FI)
- Micro-Computer Tomography (µ-CT)

- High-Frequency Micro-Ultrasound (μ-US)
- Photoacoustic Imaging (PAI)
- Positron Emission Tomography (PET)
- Magnetic Resonance Imaging (MRI)
- Magnetic Particle Imaging (MPI)
- Multimodality Co-Registration

The following methods/techniques are established at PIF and can be applied easily as a service to different researchers according to the purpose and goal of their projects:

- Ultrasound-guided injection
- Tumor progression and evaluation of treatment efficacy: BLI; FI; μ-US; MRI
- Tumor perfusion/vascularity: μ-US with contrast; CT with contrast; MRI with contrast
- Tumor hypoxia: Oxy-Hemo PAI
- Lung cancer/fibrosis progression and metastasis analysis: μ-CT
- Bone / adipose tissue analysis: μ-CT
- Biodistribution of nanoparticles: BLI, FI, and MPI
- Heart and artery function analysis: Echocardiography and strain analysis
- Cell (CAR-T, NK cell, stromal cell, stem cell) tracking: BLI, FI, and MPI
- Liver function evaluation: CT with contrast; MRI with contrast

You are always welcome to us for a discussion about your project.

54381 - Cancer-associated fibroblast heterogeneity in pancreatic ductal adenocarcinoma

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Abstract text

Pancreatic cancer is characterized by an abundant stroma that comprises a variety of cancer associated fibroblasts (CAF). CAF subtypes may differentially mediate disease progression and therefore represent novel and specific therapeutic targets. Despite recent advances the regulatory mechanisms, tumorigenic functions and diversity of CAF subtypes in PDAC remain incompletely defined.

We performed single cell RNA sequencing analysis of a large number of CAF purified from PDAC samples. This confirmed the presence of two previously described CAF subtypes, myCAFs and iCAFs (myofibroblasts and inflammatory cancer-associated fibroblasts). In concordance with recent findings, myCAF and iCAF subtypes can be further delineated into two lineage dependent subpopulations. We characterized the biological processes specific to each of these populations. In addition, we also identify a novel subpopulation of CAFs in PDAC defined by an interferon-responsive gene signature (ifCAFs), which we validate in human PDAC using *in situ* hybridization.

To better understand the process by which functionally distinct CAF subtypes arise from common progenitors, we characterised the heterogeneity of fibroblast-like cells in an in vitro 3-dimensional organoid-based model of CAF differentiation. Cluster and trajectory analysis revealed the sequential induction of iCAFs and myCAFs. Interestingly, tumour cell-derived signals are sufficient in vitro to induce ifCAF differentiation. We explored possible endogenous triggers of the interferon pathway such as splicing overload and activation of endogenous retroviruses. A distinct CAF subpopulation defined by antigen presentation (apCAFs) could be formed when fibroblast-like cells were exposed to immune cell-derived signals. Using pharmacological agonists and transcriptomic analysis, we show that antigen presentation and ifCAF characteristics are induced by distinct branches of the interferon signaling pathway.

Finally, comparison with publicly available single cell data confirms the relevance of ifCAF in a pan-cancer context.

Taken together, through interrogating CAF heterogeneity in PDAC we identify a novel interferon-responsive CAF subtype (ifCAFs) and demonstrate the capacity of an organoid co-culture model system to recapitulate differentiation of defined CAF subtypes detected *in vivo*.

54383 - Ex vivo evaluation of mRNA lipid nanoparticle cancer vaccines

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Abstract text

Lipid nanoparticle vaccines have recently been developed broadly against pathogens and recently, in precision medicine targeting mutations in different tumors. Evaluating vaccine candidates typically require animal models using orthotopic or allogenic transplantations to evaluate efficiency. Evaluating vaccine efficacy using ex vivo systems is faster and less technically demanding.

Here we aim to develop individualized cancer vaccines based on neoantigen identified within established human organoids or directly from patient tissue. The selected neoantigens are enriched for epitopes identified on extrachromosomal DNA molecules. The neoantigen candidates are then evaluated using a personalized ex vivo testbed.

Within the ex-vivo testbed personal tumor neoantigens are tested on the patients own isolated CD8+ T cells and monocytes. The monocytes are *in vitro* differentiated into mature dendritic cells (DCs) using established cytokine cocktails and inoculated with LNPs carrying the mRNA vaccine or loaded with peptides. The neoantigen presenting mature DCs are then co-cultured with syngeneic T cells. Activity and cytotoxicity of co-cultured CD8+ T cells, and tumor organoids are evaluated to determine the effect of vaccine candidates.

54385 - Deciphering the microglia transcriptional regulation on an early response to glioblastoma.

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Abstract text
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 investigated the changes in the transcriptional profile of microglia in response to a glioblastoma cell line using a segregated coculture system. We exposed BV2 microglia to the C6 glioblastoma cell line and analyzed the transcriptional changes using RNA sequencing. We found that C6 cells induced significant changes in the expression of microglia transcription factors, including the upregulation of the inhibitors of DNA binding ID1 and ID2. Furthermore, we identified several gene sets and pathways that were enriched in the C6 glioblastoma-induced microglia transcriptome, like an early inflammatory or immune response. Our results support that C6 glioblastoma cells can reprogram microglia transcriptional profile to create a tumor-supportive environment. This study provides new insights into the molecular mechanisms of microglia transformation and highlights potential targets for therapeutic intervention.

54387 - IBRUTINIB RESCUES NATURAL KILLER CELLS FROM APOPTOSIS AND IMMUNOSUPPRESSION INDUCED BY MONOCYTE-DERIVED REACTIVE OXYGEN SPECIES

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Abstract text
Chronic lymphocytic leukemia (CLL) is a slowly progressing cancer characterized by uncontrolled expansion of mature malignant B cells in blood, lymph nodes and bone marrow. CLL is commonly treated with targeted therapies such as the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib that limits B cell proliferation, and the anti-CD20 antibody rituximab (RTX) that induces apoptosis and NK cell-mediated antibody-dependent cellular cytotoxicity of CD20-expressing B cells. When exposed to immobilized antibodies such as RTX, monocytes bind to the FC-portion of the antibody, which stimulates formation of reactive oxygen species (ROS) via the enzyme complex NADPH oxidase 2 (NOX2). NOX2-derived ROS are critical for microbial defense but may, when released extracellularly, exert cytotoxicity and immunosuppression to neighboring NK cells and T cells. BTK was first explored in B cells but is now considered a central activating kinase also in monocytes. We hypothesized that a complementary clinical effect of ibrutinib in CLL may be a reduction in monocytic immunosuppression leading to enhanced NK cell mediated killing of malignant cells. We thus determined the potential NOX2-inhibitory efficacy of ibrutinib *in vitro* and *in vivo*.

Monocytes and NK cells were isolated from healthy blood donors and purified for use in *in vitro* assays. First, ibrutinib was found to potently inhibit monocyte NOX2-derived spontaneous and RTX-induced ROS formation at 1mM and 0.1mM. Next, monocytes and NK cells were isolated from seven healthy donors and co-cultured at a 1:2 ratio overnight in the presence or absence of plate-bound RTX. NK cell apoptosis was then determined by flow cytometry. Monocytes induced apoptosis in NK cells in the presence ($P<0.0001$), but not absence of plate-bound RTX. Ibrutinib efficiently inhibited monocyte-induced NK apoptosis at concentrations ranging from 1mM ($P<0.0001$) to 10nM ($P<0.05$). The co-culture experiments were repeated using the lymphoblastoid cell line 221 coated with RTX and added as target cells during the last 4h of incubation at a ratio of 1:4 (221:NK) (N=5-6). NK cell degranulation (CD107a surface expression) and 221 cell apoptosis were determined using flow cytometry. NK cell degranulation and 221 cell apoptosis were significantly decreased in co-cultures with monocytes in the presence but not absence of plate-bound RTX, and increased significantly with the addition of ibrutinib at 0.1mM. A similar trend was observed using primary human CLL cells as target cells. All statistical comparisons were made by mixed-effects analysis with Bonferroni's multiple comparisons test.

To determine effects of ibrutinib on NOX2 *in vivo*, myeloid cells were first isolated from bone marrow of five B6 mice and purified, after which ROS formation was determined *ex vivo* in the presence or absence of ibrutinib. In these experiments, ibrutinib reduced spontaneous ROS formation at concentrations from 5mM to 10nM (N=5). An animal model using the lymphoma cell line YAC-1 is currently being optimized to determine the NOX2-reductive efficacy of ibrutinib *in vivo*.

In conclusion, ibrutinib limited NOX2-derived ROS formation in human and murine myeloid cells, and promoted NK cell viability and cytotoxic function in co-cultures with ROS-forming monocytes. These findings may point towards new uses for BTK inhibitors.

54388 - By increasing transferrin uptake in lung tumor cells, WNT-signaling is synthetic lethal with buthionine sulfoximine, a glutathione synthesis inhibitor

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Abstract text

Lung tumors must overcome barriers of oxidative stress to progress and metastasize, suggesting that pro-oxidant cancer therapies might be efficient against lung cancers. Glutathione is the most abundant intracellular antioxidant and an attractive target for pro-oxidant therapies. We performed a genome-wide CRISPR-CAS9 knockout screen in human lung cancer cells to identify knockouts that eliminate cancer cells in the presence of buthionine sulfoximine (BSO), a glutathione biosynthesis inhibitor that has been used in phase-I trials.

The screen showed that knockouts leading to WNT-pathway activation were depleted from BSO-treated samples, indicating that WNT-signaling causes a strong dependency on glutathione. The screen result was validated in a range of lung adenocarcinoma cell lines, from human and mice. By stimulating endocytosis of iron-bound transferrin, WNT-signaling causes lipid peroxidation and ferroptosis, in a reaction catalyzed by iron. Lipid peroxidation can be reversed by the GPX4 enzyme in a reaction that utilizes glutathione, thus explaining the need for glutathione.

Since activation of WNT-signaling is a common feature of human cancer cells, the result opens new opportunities for precision medicine therapies. To resolve which branch of the WNT-signaling pathways that causes transferrin uptake and lipid peroxidation will be important to identify the patients that has the greatest chance to benefit from BSO-therapy. Our preliminary results point towards a β-catenin independent branch of the canonical WNT signaling pathway involving the transcription factor ATF4. The pathway is potently activated by the GSK3-inhibitor CHIR99021, thus indicating a potential partner for combination therapies with BSO.

54389 - Histamine dihydrochloride induces myeloid cell production of the anti-metastatic protein thrombospondin-1

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Abstract text
Primary tumor cells may disseminate and colonize distant organs, a process known as metastasis. Metastatic disease accounts for the majority of deaths among cancer patients and new treatment options for patients with metastatic disease are highly warranted. Anti-angiogenic treatments may prevent the outgrowth of new blood vessels and thereby limit further tumor growth and establishment of new metastasis.

Thrombospondin-1 (TSP-1) a natural endogenous angiogenic inhibitor, has been shown to suppress metastasis in cancer patients. In addition, low circulating levels of TSP-1 correlate with adverse survival rates in some types of cancer patients. TSP-1 has been shown to modulate the tumor microenvironment via effects on macrophage polarization. TSP-1 is produced by platelets, but may also be produced by other cells, including myeloid cells. Preclinical studies suggest that tumor cells may secrete factors that stimulate or inhibit TSP-1 production by GR1+ myeloid cells in the pre-metastatic niche, with pronounced consequences for metastasis formation. Hence, non-metastatic tumors were shown to secrete factors that enhanced TSP-1 production, while highly metastatic tumors secreted TSP-1 inhibitory factors. In agreement, myeloid-cell-derived TSP-1 was shown to limit the metastatic burden of mice orthotopically injected with metastatic tumor cells.

We have found that the endogenous amine histamine dihydrochloride (HDC) stimulates the production of TSP-1 from myeloid cells. The TSP-1-inducing effect of HDC was mediated via histamine type 2 receptors (H2R), and the induction was observed in primary myeloid cells from human and mice and cancer cell lines from human.

To investigate the potential anti-metastatic mechanism of HDC-induced TSP-1 production, we utilized the B16F10 melanoma metastasis model. Hence, WT and TSP-1 knock-out (KO) mice were treated systemically with HDC before and after intravenous injection with B16F10 cells, and the number of lung metastasis formed was enumerated three weeks later. Systemic HDC treatment was found to suppress lung metastases in WT mice. In contrast, there was no effect of HDC on metastasis formation in TSP-1 KO mice. Western blot analysis of lung tissues of HDC-treated WT mice confirmed a higher level of TSP-1 production compared with the untreated counterpart. All in all, our data suggest that HDC can trigger the production of TSP-1 from myeloid cells which may contribute to metastasis prevention. Further studies to define the effects of HDC-induced TSP-1 production on neo-angiogenesis and macrophage polarization within the tumor microenvironment are warranted.

Keywords: Thrombospondin-1, angiogenesis, histamine dihydrochloride, myeloid-derived suppressor cells, immunotherapy

54390 - Extracellular matrix and tumor microenvironment dynamics in glioblastoma treatment

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Abstract text

Glioblastoma (GBM) is the most aggressive type of brain tumor and nevertheless the tremendous steps on GBM research the first standard-of-care treatment is, if applicable, the maximal surgical resection followed by irradiation and adjuvant chemotherapy with temozolomide, while advanced therapies such as immunotherapy exhibit limited efficacy. The median survival rate of patients with primary GBM is 15 months, while after the first-line treatment GBM can recur with poorer prognosis. The extracellular matrix (ECM) is a dynamic network of macromolecules with deformation and degradability properties. ECM displays an interconnectivity with resident cells in the tumor microenvironment (TME) oriented cellular phenotypes, states, activation, differentiation, stemness and immune system regulation. ECM landscape is distinct for each stage of the disease; GBM vs normal brain, primary GBM vs irradiated GBM, primary/irradiated GBM vs recurrent GBM, with the last category be the least studied. For all those reasons, the goal of our study is to decipher the interplay of the ECM with the TME upon treatment interventions in GBM, aiming for an opportunity window for combinational ECM-targeted therapies.

54391 - The role of CCN1 in the crosstalk between pancreatic stellate cells and Panc1 cancers cells in 3D heterospheroids

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Abstract text
Pancreatic ductal adenocarcinoma is a deadly disease that is almost completely resistant to conventional chemo- and radiation therapy. A major reason for this resistance seems to lie in the dense desmoplastic stroma, which includes abundant heterogeneous cancer-associated fibroblast (CAF) populations.

Previously, we used a 3D heterospecies heterospheroid co-culture model to examine the crosstalk between human pancreatic tumor Panc1 and mouse pancreatic stellate cells (mPSCs) by global expression profiling. Since we found CCN1 strongly upregulated in Panc1 cells by coculture, we decided to study the role of CCN1 by CRISPR-Cas9 knockout technology.

CCN1-KO cell lines were generated by CRISPRCas9 and verified with western blot. Viability of cells grown in 2D and 3D to gemcitabine, paclitaxel and SN38 was measured with CelltiterGlo3D and Apoptosense CK18. RT-PCR and Western blotting was performed on selected genes and proteins for phenotypical characterization of the cells.

Panc1 cells lacking CCN1 were more de-differentiated and less sensitive to gemcitabine, the latter due to the lower expression of gemcitabine transporting and metabolizing genes.

Based on the previous observation of increased mRNA expression of TGFB and the LPA generating enzyme (Enpp2) in heterospheroids, we treated cells with TGFB1 and lysophosphatidic acid. These stimuli not only upregulated the CCN1 expression in Panc1 cells but also shifted mPSCs to a more myCAF-like phenotype.

CCN1 renders cancer cells more sensitive to gemcitabine. The identification of pathways shifting CAFs from immunosuppressive iCAFs to more tumor suppressive myofibroblastic myCAFs may represent a new therapeutic opportunity for PDAC intervention.

54395 - Digital Assessment of Cancer Cellularity and Purity in HER2+ Breast Cancer: Correlating with Neoadjuvant Treatment Response and Survival Outcomes

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Abstract text

Introduction: The morphological assessment of tumor cellularity in hematoxylin and eosin (H&E) sections has been widely incorporated in evaluating neoadjuvant treatment responses in breast cancer (BC), such as residual cancer burden (RCB) and cellularity - tumor infiltrating lymphocytes (TILs) score (CelTIL). Although tumor cellularity is a simple metric to assess, its semi-quantitative nature and subjective assessment can pose significant reproducibility problems. The aim of this study is to digitally calculate cancer cell associated metrics in scanned H&E tissue sections and correlate with treatment response and survival outcomes after neoadjuvant therapy in HER2-positive BC.

Method: The PREDIX HER2 phase II randomized clinical trial (NCT02568839) included 202 patients with HER2-positive BC treated with neoadjuvant docetaxel, trastuzumab, pertuzumab or trastuzumab emtansine. A tissue biopsy was obtained at baseline, after second cycle of treatment (on-treatment) and at surgery. H&E-stained tissue sections from all timepoints were stained centrally and scanned using Hamamatsu Nanozoomer at 40x resolution. Slides with confirmed invasive cancer were included in this study and manually annotated (C.B., B.A.), according to established guidelines, while also manual cellularity (W.S.) was assessed for 49 patients. Subsequently, a machine learning model was used with QuPath to classify the cells in a biopsy into cancer cells, TILs, stromal and others. Based on these detections, digital cellularity and purity metrics were developed. Digital purity was defined as the sum of the tumor cells' area in the invasive cancer region divided by the sum of the area of all cells in the biopsy. Digital cellularity was defined as the sum of the tumor cells' area in the invasive cancer region divided by the area of that tumor region. All variables were univariately analyzed in both their continuous and dichotomized (upper quartile) forms.

Results: A total of 171 patients (171 baseline, 50 on-treatment, and 32 operational samples with residual disease) were evaluated. Both digital cellularity and purity metrics achieved high positive correlation with pathologist's reported cellularity (ρ_{cel}=0.77, p<0.001), (ρ_{pur}=0.63, p<0.001). On treatment digital purity was predictive of pCR (OR_{pur}=0.94, CI=[0.87-0.99], p=0.034), although digital cellularity was not (OR_{cel}=0.98, CI=[0.93-1.03], p=0.46). Regarding patients with residual disease, digital cellularity at surgery was prognostic for event-free survival (EFS) (HR=1.04, CI=[1.01,1.07], p=0.0194). The longitudinally assessed dichotomized difference of digital cellularity at the operation minus that at baseline in patients with matched samples indicated prognostic relevance regarding EFS (HR=8.34, CI=[1.02 – 68.05], p=0.0476).

Discussion: Using digital metrics to assess cancer cellularity and purity in scanned H&E tissue sections is feasible and can offer improved prognostication in BC, although further validation in larger and more diverse patient populations is necessary. The machine learning approach used to detect and classify cells provides an accurate, objective and reproducible calculation of these metrics, which can overcome the limitations of manual assessments.

54396 - High-throughput drug screening using a human neural stem cell model identifies therapeutic targets for SHH-driven medulloblastoma

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Abstract text

Background Medulloblastoma (MB) is one of most common malignant brain tumor in children, consisting of four molecular subgroups WNT, SHH, Group3 and Group4. Current treatment regimens for MB include surgical resection, multidrug chemotherapy and craniospinal irradiation. Though the combined treatment shows encouraging results of increasing overall survival, it can lead to devastating side effects in survivors such as cognitive deficit, neuroendocrine disorders and increasing risks of secondary cancer later in life. This shows the need for more effective and targeted therapy for patients with MB. **Methods** We have previously established a human neuroepithelial stem (NES) cell model by reprogramming of noncancerous somatic cells carrying a germline mutation in PTCH1 gene. Patient NES cells were undistinguishable from control NES unless stressed. However, upon orthotopic transplantation in mouse cerebellum patient NES formed tumors mimicking human SHH-MB. Re-injection of isolated tumor NES (tNES) cells showed accelerated tumor growth with increased malignancy. We have used our NES model (both naïve and after tumor formation) for an unbiased high-throughput screening of a library of compounds with known targets. Efficacy was assessed by comparing compound toxicity between normal human NES cells and tumor-derived NES cells. Selected compounds were further validated *in vitro* and *in vivo*. **Results** In total, we tested 172 compounds with known targets, 86 compounds showed efficacy towards tumor NES cell. However, majority of compounds also showed toxicity in normal neural stem cells. Importantly, Brivanib (VEGFR1/FGFR2 inhibitor) and PF-4708671 (p70S6k1 inhibitor) were shown to specifically target tumor NES cells compared to normal NES cells with the same genetic background. Brivanib treatment of tumor NES cells resulted in cell cycle arrest *in vitro* and delayed tumor growth *in vivo*. PF4708671 has interfered with tumor cell growth both in 2D and 3D. In addition, both Brivanib and PF4708671 showed synergistic effect with conventional chemotherapy. Importantly, inhibition of VEGFR1/FGFR2 and p70s6k1 had only mild effects on normal human neural stem cells. **Conclusions** Our data demonstrate that Brivanib and PF4708671 show potential selectivity toward SHH-MB compared to normal neural stem cells. The NES cell platform can be used to identify potentially effective new therapies for SHH-MB.

Keywords: Medulloblastoma, precision cancer medicine

54399 - Investigating BACH1 as a potential anti-metastatic therapeutic target in cancer

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Abstract text

The deadliest type of cancer is lung cancer, 85% of which is non-small cell lung cancer (NSCLC). Lung adenocarcinoma (LUAD), the most common subtype of NSCLC, is highly prone to metastatic growth, which is the cause of death in 90% of these patients. The KEAP1/NRF2 pathway is commonly mutated in LUAD, which leads to downstream stabilization of the transcription factor BACH1. BACH1 has been shown to promote the transcription of several pro-metastatic genes. Induction of the BACH1 has been shown to play an important role in lung cancer invasion by increasing the transcription of *GAPDH* and *HK2,* hexokinase2, resulting in increased glucose uptake and glycolysis rates, which then trigger cancer cell invasiveness.

There is an acute need for anti-metastatic treatments to control the invasiveness of cancer cells and potentially impede the spread of cancer cells to distant organs. Patients with metastasis can also utilize palliative treatment as a symptom-relieving and life-prolonging treatment. Thus, it's important to identify potent BACH1 inhibitors that have the potential to go from bench to bedside.

Very few compounds have shown to effectively inhibit BACH1 in cancer cells. Our studies have identified BACH1 inhibitors that can impede the invasiveness of human LUAD cell lines by inhibiting BACH1, the invasion capability of cancer cells is inhibited, which potentially will prevent metastatic growth *in vivo*. In the future, we hope to test the toxicity and effect of BACH1 inhibitors *in vivo*. Overall, BACH1 has potential as an anti-metastatic therapeutic target that we plan on investigating further.

54400 - Assessment of trophoblast cell surface antigen 2 (TROP2) mRNA and protein expression and prognostic significance in breast cancer

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Abstract text

Introduction: Trophoblast cell-surface antigen 2 (TROP2), also known as tumor-associated calcium transducer 2, is a cell surface glycoprotein receptor overexpressed in various carcinomas, including breast cancer. It is associated with cell proliferation and metastasis and is a target of novel cancer therapies, in particular antibody-drug conjugates. The aim of our study is to evaluate Trop2 gene (TACSTD2) and protein expression and associations with clinical-pathological characteristics and outcomes in an early breast cancer (BC) cohort.

Methods: The study cohort includes patients diagnosed with primary BC between 1997 and 2005 in healthcare region of Stockholm, Sweden that were retrospectively selected using the Stockholm-Gotland Breast Cancer Registry. Gene expression data were already available. Tissue microarrays from this cohort were immunohistochemically (IHC) stained for Trop2 using a mouse monoclonal anti-Trop2 antibody (clone: sc-376746) (Santa Cruz, Biotechnology, USA, dilution 1:50). Automated staining was performed by Intellipath stainer, and then histopathology slides were recorded from Hamamatsu Nanozoomer scanner. Evaluation was performed with QuPath software. Associations of Trop2 mRNA and protein with clinical-pathological characteristics (Kruskal-Wallis test), overall survival (OS, Cox proportional hazard model) and distant recurrence-free interval (DRFI, Cox proportional hazard model) across different BC subtypes were determined.

Results: In total, 564 patients were evaluated (median age 55 years, IQR 23-76). TACSTD2 expression was significantly higher in lower grade tumors (G1 vs G3 and G2 vs G3, $p < 0.001$). Median follow-up was 12.4 years for DRFI and 15 years for OS. Gene expression differed significantly between intrinsic subtypes but not in clinical subtypes. The upper quartile of Trop2 gene expression was significantly correlated with worse DRFI in HER2-enriched [Hazard Ratio (HR): 2.52; 95% Confidence Interval (CI), 1.25 to 5.07; p-value=0.010] and basal-like BC (HR: 2.27; 95% CI, 1.09 to 4.70; p= 0.028). TACSTD2 expression and Trop2 protein were weakly correlated (Correlation coefficient, R= -0.019; p=0.68). There was a trend for worse DRFI in cases where higher expression of Trop2 protein was assessed, compared to lower (p=0.065).

Discussion: Trop2 mRNA and protein were detectable in all breast cancer subtypes, with a wide range of expression. The results of this study support the potential independent prognostic role of Trop-2 in some BC subtypes. Overall, more studies are needed to provide a better understanding of Trop2 mRNA and protein expression and further inform on the use of Trop2-directed therapy.

54402 - Fat mass and obesity-associated (FTO) levels in relation to body composition, tumour characteristics and breast cancer prognosis

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Abstract text

Breast cancer (BC) patients with obesity are more likely to have a worse prognosis compared with healthy-weight patients. The fat mass and obesity-associated (FTO) protein is a potential mediator in the obesity-breast cancer link and an interesting new biomarker to predict disease outcome. The current study aims to evaluate FTO tumour levels in relation to body composition and its association with tumour characteristics and prognosis in BC patients of the Malmö Diet and Cancer Study. We hypothesise that a high abundance of FTO in the tumour tissue would indicate an unfavourable prognosis. Duplicate tissue samples obtained from patients with invasive BC (n=718) were collected in a tissue microarray (TMA), and immunohistochemistry (IHC) staining was performed targeting the FTO protein. All patients were divided into four categories based on the FTO staining intensity through nuclear positivity scoring. The final distribution indicated that 18% of the patients were represented in the negative group (score=0), 35% in the weak group (score=1), 25% in the moderate group (score=2), and 22% in the strong group (score= 3). In the next phase, descriptive analysis of the FTO levels according to patient and tumour clinical characteristics, including anthropometry (BMI, waist circumferences, body fat %), tumour size, lymph node positivity, histological grade, hormonal receptor status, and proliferation, will be performed. Finally, an analytical analysis linking the tumour-specific FTO abundance to disease outcome will be performed, discriminating three different endpoints: (I) BC recurrence, (II) BC-specific mortality, and (III) overall mortality. The data of this study will provide information on FTO as a possible biomarker for risk stratification in BC patients with obesity.

54403 - Genomics studies of small intestine neuroendocrine tumors

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Abstract text

Small intestine neuroendocrine tumor (SI-NET) is the most common cancer of the small bowel and has several unusual properties. SI-NETs are often multifocal (multiple clustered tumors), has low mutation burden, and lack known driver mutations that can explain tumor initiation and metastasis. Recently, we showed that multifocal SI-NETs arise from clonally independent precursor cells, and that multiple independent primary tumors can metastasize. While this gives a starting point for future research, the underlying tumorigenic mechanisms remain poorly understood and therapeutic options are limited. Here, we are using a “multi-omics” approach to help pinpoint driver mechanisms in SI-NET. Whole genome sequencing data from primary and metastatic lesions is coupled with matched transcriptome and methylome data, with the aim of uncovering non-mutational driver alterations. By generating life histories of genetic changes within individual patients, we are also gaining insights into the timing of molecular events during SI-NET progression, suggestive of earlier-than-expected time of tumor initiation in some cases.

54404 - Regulation of p53-dependent tumor suppression by the bacterial microbiota

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Abstract text
Increasing evidence highlights the role of bacteria in the physiopathology of cancer. Several cancer-associated bacteria have been shown to produce toxins which interfere with the host defense against tumorigenesis. However, the underlying molecular mechanisms linking bacterial systems and cancer remain poorly understood.

In this study, we investigated the impact of *Klebsiella pneumoniae*, an opportunistic pathogen of the digestive and upper respiratory tract which have been associated with colorectal cancer, on the host p53 pathway. We found that lipopolysaccharides from *Klebsiella pneumoniae* and other Enterobacteria strongly inhibit the host tumor suppressor p53 pathway through a novel mechanism of p53 regulation. We discovered that lipopolysaccharides destabilize TP53 mRNA through a TLR4-NF-κB-mediated inhibition of the RNA-binding factor Wig-1. Importantly, *K. pneumoniae* disables two major tumor barriers, DNA damage signaling and oncogene-induced senescence, by impairing p53 transcriptional activity. Furthermore, we found an inverse correlation between the levels of TLR4 and p53 mutation rate in colorectal tumors. Hence, our data suggest that the repression of p53 by Enterobacteria via TLR4 alleviates the selection pressure for p53 oncogenic mutations and shapes the genomic evolution of cancers.

On the bacterial side, we identified the *K. pneumoniae* type VI secretion system (T6SS) to be a major regulator of LPS secretion by Enterobacteria. We found that the biogenesis of the T6SS machinery increases the release of LPS and outer membrane vesicles by bacteria, leading to activation of the NF-κB pathway and repression of p53 in the host cells. Depletion of *K. pneumoniae* T6SS rescued p53 inhibition in the host cells, prevented NF-κB transcription program and alleviated inflammation *in vitro* and *in vivo* in mice. Altogether, our data suggest that targeting specific bacterial systems such as a T6SS could open novel opportunities for cancer therapies.

54405 - MYC-driven pineoblastoma, medulloblastoma and retinoblastoma share a common photoreceptor development program in the brain

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Abstract text

Medulloblastoma (MB), pineoblastoma (PB) and retinoblastoma (RB), three types of malignant neural pediatric brain tumors are anatomically situated in different parts of brain but share a common feature - elevated expression of a photoreceptor ‘program’. MYC-amplified MBs and PBs are often histologically indistinguishable. Both grow aggressively, often relapse after standard treatment and have a dismal prognosis. Trilateral RBs are rare but often present with aggressive eye tumors first and later on PBs. It is important to understand how these photoreceptor-positive tumors arise and to know if they could be similarly treated.CRX, a transcription factor critical for retinal and pineal cell development is also a master regulator for MB-Group3 tumor maintenance. To understand if MYC-driven MB, PB and RB can arise from specific photoreceptor-positive progenitors in the developing brain, we performed CRX-lineage tracing with tamoxifen injections. We could confirm that CRX-positive cells exist in retina and in pineal gland progenitors as expected. However, CRX-traced cells also marked granule neurons in the flocculonodular lobe of the cerebellum, which is a recently suggested site of MB-Group3 origin arising from the developing rhombic lip. To investigate if the MYC oncogene can generate different types of pediatric brain tumors in CRX-positive cells from retina, pineal gland and cerebellum, a XMYC(T58A)-Tomato mouse model was established by crossing CRX-CreERT2 strain with a LSL-MYC(T58A) strain and R26R-LSL-tdTomato stain. Here stabilized MYC (MYC(T58A)) oncogene was turned on with tamoxifen injection after birth and red tumor cell development could be followed. XMYC(T58A)-Tomato mice developed brain tumors from retina, pineal gland and cerebellum after 4-6 months. Histologically, tumors were non-glial (GFAP-, Olig2-), showed neuronal activity (Neurod1+) and stained positive for photoreceptor markers (CRX+, OTX2+) similar to both MB-Group3 and PB-MYC. Our data suggest that MYC-driven Group 3 MB, PB and RB all could originate photoreceptor positive cells, which has implications for future research and the development of novel treatments targeting these devastating childhood malignancies.

54406 - Proteome-wide profiling of subcellular localization: Clinical relevance of protein farnesylation in lung cancer

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Abstract text
Subcellular localization of proteins is a key determinant of their function in cells. Protein localization is not static, it is regulated and varies between cell types, cell states and pathophysiological processes. The dynamics of the localization is controlled, in part, by post-translational modifications (PTMs) of proteins. Farnesylation is a type of PTM where farnesyltransferase (FTase) enzyme catalyzes the addition of a lipid, farnesyl isoprenoid, to the target protein, thus preparing it for anchorage to intracellular membranes. The RAS family of oncogenic proteins is a notable example of proteins with functions regulated by farnesylation-dependent localization. This finding triggered the development of FTase inhibitors (FTIs) as potential cancer therapeutics and inhibitors of RAS signaling pathways. Despite so-far modest clinical trial results, FTIs continue to have potential in cancer therapy, as only a fraction of FTase targets have been functionally investigated. Among 600 theoretically predicted targets of farnesylation, only approximately 100 have been experimentally verified.

To expand the knowledge on protein farnesylation, we developed and performed a proteome-wide analysis of farnesylation-dependent protein localization in four human lung cancer cell lines. Specifically, we utilized a metabolic labeling approach for the pull-down of farnesylated proteins. Using our previously developed method for in-depth proteomics, HiRIEF-LC-MS, we identified 133 farnesylated proteins including 76 proteins previously not experimentally verified. Next, we performed a proteome-wide investigation of FTI-driven protein relocalization using our SubCellBarCode method and found significant relocalizations of 35–182 proteins per cell line.

To identify targets of clinical relevance, we cross-analyzed the candidate list with our previously generated in-depth proteome-wide analysis of non-small-cell lung cancer (NSCLC) tumors. Interestingly, one of the farnesylated proteins most affected by FTI treatment, the phosphatase PTP4A1 (Protein Tyrosine Phosphatase 4A1, also called PRL1/Phosphatase of Regenerating Liver 1), showed overexpression in one of the NSCLC subtypes characterized by liver-like signaling. We have previously shown a connection between inactivation of the tumor suppressor STK11 (LKB1) and high expression of liver-specific genes in this subtype. Also, PTP4A1 is specifically overexpressed in STK11-inactivated tumors in our clinical NSCLC analysis, and evaluation of PTP4A1 mRNA level in normal tissue based on public domain data indicate a liver-specific expression. Furthermore, we observed a poor correlation between PTP4A1 mRNA and protein levels, suggesting protein-level regulation and highlighting the importance of our protein-level analysis.

Investigating the functional relevance of PTP4A1 and its farnesylation-dependent activity in NSCLC may facilitate elucidating metabolic rewiring in NSCLC. Furthermore, the findings may identify a subtype of NSCLC responsive to FTI treatment. Thus, we are performing a protein-level mechanistic study of PTP4A1 signaling in lung cancer.

54407 - MNK1/2-eIF4E signaling in cancer-associated fibroblasts modulates the tumor microenvironment and promotes lung metastasis

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Abstract text

Background & Aims

The MNK1/2-eIF4E signaling axis is an important node of gene expression regulation in cancer cells, where eIF4E, when phosphorylated by MNK1/2 kinases, is known to promote selective translation of mRNAs with pro-metastatic functions. However, its involvement in supporting the pro-tumor function of tumor-associated cells has largely been unexplored. As the importance of the tumor microenvironment (TME) in regulating tumor progression is firmly documented, and as MNK1/2 inhibitors are entering clinical trials, we sought to characterize the effect of disrupted MNK1/2-eIF4E signaling specifically in cells of the TME on their ability to facilitate breast cancer metastasis.

Methods

We used an established mouse model of phospho-eIF4E-deficiency (eIF4E$^{S209A/S209A}$ mice) to study the consequences of host phospho-eIF4E-deficiency on the metastatic capacity of syngeneic wild-type 4T1 breast tumors and we performed single cell sequencing and 38-color multiplex spectral flow cytometry to study the composition of the TME with or without MNK1/2-eIF4E signaling. We further isolated phospho-eIF4E-deficiency to specific key TME subsets – immune cells and fibroblasts - through bone marrow transplants (BMT) and co-implantation of tumor cells with mammary gland-derived fibroblasts to delineate the consequences of disrupted MNK1/2-eIF4E signaling in major components of the TME.

Results

Our work shows that phospho-eIF4E deficiency in cells of the tumor microenvironment limits extravasation and metastasis of 4T1 tumors. Single cell sequencing of dissociated tumors identified cancer-associated fibroblasts (CAFs) as the most active component of the TME, as measured by receptor-ligand interaction analysis. Deep phenotypical profiling of the TME using spectral flow cytometry revealed alterations in the CAF compartment in the tumors grown in phospho-eIF4E-deficient mice. Limiting phospho-eIF4E deficiency specifically to infiltrating immune cells through BMT did not recapitulate the metastatic phenotype observed in eIF4E$^{S209A/S209A}$ animals; however, co-implantation of 4T1 cells with primary eIF4E$^{S209A/S209A}$ mammary gland fibroblasts showed a significant reduction in the metastatic burden and circulating tumor cells.

Conclusions

Our data support a model whereby MNK1/2-eIF4E signaling specifically in CAFs acts to create a microenvironment propitious for tumor cell dissemination, possibly through its regulation of fibroblast plasticity and CAF differentiation. The molecular mechanism for how this is achieved and whether other components of the TME, such as tumor vasculature, are involved as intermediaries remain to be determined.

54409 - Defining the role of physiological aging during lung cancer progression and metastasis

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Abstract text

Cancer is often defined as a disease of ageing. Of note, incidence of most cancers increases dramatically as we age. More than 50% of patients with lung cancer, deadliest cancer worldwide, are diagnosed after the age of 65, and 30% are older than age 70. As the population is growing older, the number of lung cancer patients will continue to increase, and its mortality toll with it.

Despite the clear link between ageing and cancer, lung cancer is rarely studied in a biological context where the age factor is considered. Aging is characterized by oxidative stress, mitochondrial dysfunction, immunologic deficiencies, and stem cell exhaustion among other.

The aim of the study is to investigate how ageing impacts lung cancer progression and response to anti-cancer therapy lung cancer. Using an *in vivo* aged mouse model of lung cancer, we observed that age decreased tumor burden and increased metastasis incidence. Primary cultures established from primary lung tumors from young and aged mice were used to further study the role of ageing in lung cancer progression. Transcriptomics, proteomics and metabolomics approaches identified strong enrichments of several metabolomic, cytokine and migration pathways in aged primary cultures compared to young primary cultures; led to the identification of several metabolic vulnerabilities observed in aged cells. We plan to explore further these findings *in vitro* and *in vivo.*

Our results highlight the importance of ageing on lung cancer progression. This is of importance as age is currently disregarded regarding treatment options.

54410 - Sex bias of targeted therapy in a BrafV600E-induced mouse model of papillary thyroid cancer

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Abstract text

Thyroid cancer is the most common endocrine malignancy, with papillary thyroid cancer (PTC) being the most prevalent subtype. The majority of patients harbor a BRAF-mutation, causing hyperactivity in the MAPK signaling pathway and deregulated cell growth. Treatment of advanced PTC battles against tumor growth and progression, and tumor cells' ability to dedifferentiate and resist mainstay treatment with surgery and radioactive iodine (RAI). Braf-specific kinase inhibitor vemurafenib is currently evaluated for treatment of radioactive iodine (RAI) refractory tumors. Human PTC has a 3:1 female/male incidence ratio, and is an example of sexual dimorphism in cancers of nonreproductive organs, poorly understood due to lack of reliable experimental models that recapitulate sporadic carcinogenesis.

We established a transgenic mouse model of sporadic PTC, based on stochastic activation of mutant *Braf*. The advantages are focal neoplasia in a natural microenvironment without elevated thyroid stimulating hormone (TSH).

Objectives: Evaluate the effect of Braf-kinase inhibitor PLX4720, prodrug of vemurafenib, on tumor development and progression in a PTC mouse model, and to elucidate the possible influence of sex.

Methods: Conditional $Braf^{V600E}$ induction in *TgCreBraf*^{CA} mice by spontaneous Cre activation. Mice administered with PLX4720 or control feed from 4 weeks or 6 months (mo) age. Tumor size longitudinally evaluated by Magnetic Resonance (MR) at 4-12 mo and thyroids excised. Tissue processed for HE staining and immunohistochemistry. Quantitative RT-PCR analysis of TSH-R, Tg, TPO and NIS at 6 mo. RNA sequencing of tumors and normal tissue.

Results: At 6 mo there were statistically significant sex differences (*p=0.0053*) in tumor volume; female/male ratio 2:1. Reduced expression of thyroid differentiation genes; by 86% in females and 70% in males. Recovery of gene expression by PLX4720; females 52% and males >90%.

Long-term kinase inhibition partially decreased (by 40-55%) tumor volume, female/male ratio 1.6:1, at 4 mo, but paradoxically accelerated tumor progression in 4-12 months old female mice. Histomorphologically 12 mo treated males exhibited a seminormalized follicular architecture staining positive for Tg. Corresponding female thyroids displayed papillary tumor phenotype of high heterogeneity and signs of dedifferentiation, with invasive cells overexpressing Estrogen Receptor (ER) α. Principal component analysis (PCA) after RNA sequencing reveals distinct transcriptomic profiles in treated and non-treated tumors, and between sexes.

Conclusions: Evident sex differences in tumor growth, loss of thyroid specific gene expression, and therapeutic response to PLX4720 in a murine model of sporadic PTC.

54411 - Galectin-3 regulates amino acid uptake and is essential for sonic hedgehog-driven medulloblastoma

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Abstract text

Background: 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.

Results: 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.

Conclusions: 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.

54413 - Single-cell profiling of leukemic stem and progenitor cells in patients receiving cytoreductive hydroxyurea in early-phase chronic myeloid leukemia

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Abstract text

Hydroxyurea is frequently used in the early phase of chronic myeloid leukemia (CML) to achieve cytoreduction prior to initiation of tyrosine kinase inhibitor (TKI) therapy. However, its potential impact on leukemic stem and progenitor cells (SPC) in CML remains largely unknown.

This study investigated the effects of short-term hydroxyurea treatment on SPC in CML peripheral blood. In addition, a comparison of SPC in peripheral blood and bone marrow samples obtained during hydroxyurea treatment was performed. Panels of 597 genes and 51 proteins were used for multiomic profiling of 26,000 single $CD14^{-}CD34^{+}$ cells from CML patient samples. The analysis revealed an enhanced frequency of relatively mature hemoglobin-expressing (*HBA1, HBA2, HBB*) erythroid progenitor cells in peripheral blood following hydroxyurea treatment. Surprisingly, the results additionally pointed to a reduction in a seemingly non-proliferating erythroid-biased cell subset, that displayed low expression of the G2M and S cell cycle phase-related genes *MKI67, NDC80, DTL* and *GINS2*. Parallel analysis of bone marrow samples obtained during hydroxyurea treatment showed similar proportional trends, with even higher levels of hemoglobin-expressing, and lower levels of non-cycling, erythroid-biased progenitors.

To achieve a higher resolution for analysis of the most immature stem cell compartment, $CD14^{-}CD34^{+}CD38^{-}$ cells were selected for further investigation. In line with the findings from the $CD14^{-}CD34^{+}$ compartment, we observed a shift towards a higher fraction of cells expressing markers of active proliferation within the relatively immature progenitor population. However, for the most immature clusters of leukemic stem cells (LSC), defined by high expression of CD90, CD26 and CD25 and low expression of CD38 and CD45RA, no consistent proportional differences or expressional changes were observed between cells obtained before and after hydroxyurea treatment or between peripheral blood and bone marrow.

In conclusion, the findings of this study suggest that hydroxyurea skews the CML SPC compartment from G0/G1 myeloid cells towards G2M/S phase and/or hemoglobin-expressing erythroid progenitors. However, no consistent effects were observed within the most immature LSC population, which may explain the lack of additional clinical benefit of combining hydroxyurea and TKI treatment in CML.

54414 - Expression profiling of circulating tumor cells in metastatic prostate cancer for identification of treatment predictive biomarkers

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Abstract text

Background

Several treatment options for metastatic prostate cancer have been introduced, and the need for predictive biomarkers is urgent. The molecular phenotypes of circulating tumor cells (CTCs) molecularly represent those of bone metastases (1), and therefore gives comprehensive information about the tumor volume to be targeted, compared to single biopsies of the primary tumor or metastases. We have previously shown that the androgen receptor (AR) splice variant AR-V7 can be detected in hormone naïve prostate cancer CTCs and that it has prognostic information (2). We have also identified MDK and CDH2 expression to be prognostic for response to androgen deprivation therapy (ADT) (3). The present study aims to validate these results in a larger patient cohort, and to perform a broader gene expression profiling in CTCs for evaluation of its prognostic and predictive potential for therapeutic options and evaluate the dynamic changes from hormone sensitive to castration-resistant prostate cancer (CRPC).

Methods

CTCs from prostate cancer patients were isolated with AdnaTest Prostate Cancer Select, and RNA was isolated with oligo(dT) coupled magnetic beads. RNA was preamplified with the target specific TATAA PreAmp®GrandMaster® Mix and qPCR for 158 assays were performed on a BioMarkTM (Fluidigm). Normalization was based on average signal strength of CTC- associated amplicons.

Results

Over 650 CTCs samples were collected from 89 hormone naïve and 146 CRPC patients, before and at regular intervals during different treatment modalities. At start of ADT for hormone naïve metastatic prostate cancer AR and AR-V7 were detected in 44.9 and 19.1 % of CTC samples, respectively, while MDK and CDH2 were detected in 68.5 and 15.7 % of samples. The fraction of AR-V7 positive samples increased to 25 at CRPC relapse and to 30.1 % at start of CRPC-therapy, while the detection rate of AR, MDK and CDH2 remained approximately the same. In a preliminary survival analysis, AR, AR-V7, and CDH2 were not statistically significantly associated to progression-free survival, while expression levels of MDK and two other splice forms of AR, AR-V1 and AR-V3, could prognosticate time to relapse into CRPC. A comprehensive analysis of the broader profiling data is under way as well as survival analysis in the CRPC treatment phase.

Conclusion

This preliminary analysis show that AR modifications increase during ADT and that specific constitutively active AR variants are associated to poor response to therapy. In this unique set of CTC samples from metastatic prostate cancer patients with long follow up we have the potential to both identify specific genes as biomarkers and reveal gene expression

patterns underlying resistance to certain therapeutic options.

References

1. Josefsson A, Larsson K, Mansson M, Bjorkman J, Rohlova E, Ahs D*, et al.* Circulating tumor cells mirror bone metastatic phenotype in prostate cancer. *Oncotarget* **2018**;9:29403-13
2. Josefsson A, Damber JE, Welen K. AR-V7 expression in circulating tumor cells as a potential prognostic marker in metastatic hormone-sensitive prostate cancer. *Acta Oncol* **2019**:1-5
3. Josefsson A, Larsson K, Freyhult E, Damber JE, Welen K. Gene Expression Alterations during Development of Castration-Resistant Prostate Cancer Are Detected in Circulating Tumor Cells. *Cancers (Basel)* **2019**;12

54415 - Drug-induced cancer cell secretome promotes resistance in colon cancer

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Abstract text
Colorectal cancer (CRC) is the third most common cancer in the world, with a projection of increased lethality in the upcoming years. Adjuvant chemotherapy with biological agents is the most practiced standard of care today. However, patients with metastatic CRC (mCRC) do not benefit from the wide range of therapies available for CRC, which either owe to unrespectability or co-morbidity or more importantly resistance to therapy. 5-fluorouracil (5-FU) has been routinely employed as the first-line therapy in colon cancer, but most patients develop resistance. Additionally, tumor cell dormancy plays a major role in tumor relapse which leads to poor prognosis in the patients. Despite many advances, the understanding of therapy-induced resistance is still in its infancy. Here, we show that 5-FU-induced tumor cell secretome helps in the outgrowth and metastasis of 5-FU-resistant clones in colon cancer.

In the preliminary observations, we found that 5-FU resistant (5-FU-R) colon cancer cells showed an increase in proliferation and survival when cultured with the secretome of 5-FU exposed colon cancer cells compared to the DMSO-induced secretome. Interestingly, we also observed elevated migration and invasion in the 5-FU-R colon cancer cells exposed to 5-FU-induced secretome compared to the DMSO counterpart. Additionally, in zebrafish xenograft, we found that 5-FU-R colon cancer cells cultured with 5-FU-induced secretome showed higher tail-vein metastatic burden compared to the 5-FU-R cells cultured with DMSO-induced secretome.

This provides a background to further study in detail the therapy-induced resistance as well as dormancy in colorectal cancer which will help to develop approaches to prevent or reverse chemoresistance in patients who receive systemic therapy for mCRC.

54416 - Deep histology: Artificial intelligence reveals what’s hidden in plain sight

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Abstract text

While single-cell and spatial omics analyses reveal detailed cellular phenotypes, they often fail to reveal the histopathological context of the tissue. This is of particular importance in investigations of tumours with great intratumoural heterogeneity, such as glioblastoma multiforme. In these tumours, the histopathological context of a given tumour niche as well as its cellular components determine the fate of cells.

Deep histology combines the use of histological stainings and deep learning to reveal histological tissue features and generate tissue-context maps. Said maps are aligned to and accompany spatially resolved single-cell transcriptomic data. The use of fluorescent dyes, click chemistry kits and immunohistochemistry allows for both a broad and specific insight into histological tissue features relevant for glioblastoma, while the use of convolutional neural networks, allows us to process a high volume of samples rapidly.

We have implemented deep histology using twelve distinct stains on six consecutive cryosections, adjacent to sections used for spatial transcriptomics. We have trained neural networks to identify features informative of the histopathological tissue context, such as microvasculature and its abnormalities, proliferating cells, apoptosis/necrosis and immune cells among others. Combining such features locally, we could identify distinct classes of tumour microenvironment. These classes mainly differed by the amount of apoptotic and proliferating cells, presence of dense microvascular proliferation or variably abnormal blood vessels, presence of pseudopalisading necrosis, cellular and nuclear size and shape, density of immune infiltration, as well as the composition of extracellular matrix.

Overall, deep histology is a cheap, fast, high-throughput method that helps us gain insight into the big picture of tumour tissues. Furthermore, it provides the ability to generate tissue context maps that can be used to visualise individual tissue features on top of spatially resolved single-cell transcriptomic data.

54418 - Comprehensive Analysis of Sexual Dimorphism in Pancreatic Cancer

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Abstract text
Sexual dimorphism in cancer has been reported to cause different rates of cancer incidences and mortalities in males and females across several types of cancer. Epidemiological research is well studied to determine relevant factors of sexual dimorphism influencing cancer development. Unfortunately, there is still a knowledge gap about differences in sex-specific immune responses and how these contribute to the response to immunotherapy. Our recent study has indicated that the accumulation of a subpopulation of myeloid cells in human pancreatic lesions is associated with an immune-exclusive tumor phenotype and effector T cell exhaustion by mechanisms involving the G-protein coupled receptor formyl peptide receptor 2 (FPR2). Furthermore, the accumulation of FPR2 had been observed in women with poor survival correlated with the genetic signature of M2 macrophages and T cell exhaustion. Sexual dimorphism also induces immune response discrepancy to conventional therapy. Sex-related hormone signaling makes gender differences in constituting the tumor microenvironments. In this present study, we investigate the phenotype-genotype correlation differences in gender across multiple cancer types to identify sex-specific therapeutic targets. We will use the publically available RNA-sequence datasets of cancer patients and normal tissues from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) Consortium, respectively. In the pancreatic ductal adenocarcinoma (PDAC) patient dataset, gene expression differences were observed in males and females in the preliminary analysis. In the male patient cohort, Macrophage migration inhibitory factor (MIF), DDT known as functional MIF homolog, and C19orf33 negatively regulate the myeloid cell population activities, theoretically, downregulation of those unleash suppressive myeloid pro-tumor function and lead to cancer progression. On the other hand, we rather observed genes involved in lymphocyte function regulation and metastasis such as ALDOA negatively correlates with lymphocyte infiltration, CD226 regulates NK cell activity, and MMP21 promotes the invasion of hepatocellular carcinoma in the female cohort. These differentially expressed gene profiles indicate immune regulation differences in males and females. We will further examine survival analysis for the effects of gender-specifically expressed genes, and prognostic ligand-receptor pair analysis to determine possible cancer-targeted genes in males and females. An overall expectation from this project is to identify gender-optimized therapeutic targets. Thus, the gender-specific approach is crucial to target tumor progression. Furthermore, a better comprehension of the gender-diverse molecular mechanisms in different types of cancer is essential to improve cancer prevention and treatment.

54419 - Cellular states are coupled to genomic and viral heterogeneity in human papillomavirus-related oropharyngeal carcinoma

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Abstract text

Introduction: Oropharyngeal squamous cell carcinoma (OPSCC) can be divided into two biologically distinct entities based on human papillomavirus (HPV) status, where most HPV-positive (HPV+) patients show dramatically better survival than patients with HPV-negative (HPV-) tumours. Neither the mechanisms behind why HPV+ patients have such a good prognosis, nor why a subset (~15%) of these tumours are still highly aggressive and respond poorly to treatment, are yet understood. Intra-tumour heterogeneity has been linked to treatment resistance and is a known feature of HPV+ tumours due to viral DNA integration. Since each tumour consists not only of multiple interacting cell types but also cancer cells with different roles, we hypothesized that studying intra-tumour heterogeneity could answer these questions.

Aim: Characterize the impact of HPV on tumour composition and cancer cell heterogeneity in order to explain differences in prognosis among HPV+ patients.

Methods: 16 OPSCC patients (11 HPV+, 5 HPV-) were included in the study. Samples were taken for dissociation and single-cell RNA sequencing (10x) at surgery. In total, over 70,000 cells were sequenced. Sequences were aligned to the human transcriptome as well as transcriptomes of the major high-risk HPV strains, quantifiying viral and human mRNA expression at the single-cell level.

Results: In malignant cells, we saw a high degree of both genetic and transcriptional heterogeneity. We identified signaling states such as cell cycle, senescence, interferon response and epithelial-mesenchymal transitions. These states were represented to different degrees between patients, e.g. epithelial-mesenchymal transitions were enriched in HPV- tumours, while cell cycle was enriched in HPV+.

In every single HPV+ tumour, we identified a previously unknown subset of cancer cells where HPV mRNA expression was completely lost (*HPVoff*). This novel population was highly enriched in a senescent state as opposed to HPV-expressing cells (*HPVon*) that were largely proliferating.

We validated these findings in HPV+ cell lines, also showing that *HPVoff* cells were less sensitive to chemotherapy and radiation due to not being actively proliferating, as well as more invasive. Through analyzing TCGA data from HPV+ OPSCC, we found that tumours with high levels of HPV expression had significantly improved survival compared to those with lower expression levels. We further showed that HPV DNA is present in *HPVoff* cells even when mRNA cannot be detected, and that treatment with methyltransferase inhibitors reverse the *HPVoff* state, suggesting epigenetic regulation.

Conclusion: These findings suggest a model where a subset of cells, originally becoming malignant through HPV infection, are able to epigenetically repress HPV gene expression and become persister cells that survive therapy through entering a senescent state. This shows that HPV expression diversity must be taken into account during diagnosis and treatment of HPV+ patients, with important ramifications for uncovering patients at risk for poor outcomes among an otherwise largely favourable prognosis.

54420 - Prognostic and predictive implications of sterile alpha motif and HD domain-containing protein 1 (SAMHD1) expression in breast cancer

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Abstract text

Introduction: The sterile alpha motif and HD domain-containing protein 1 (SAMHD1) is a deoxynucleoside triphosphate (dNTP) triphosphohydrolase that depletes the intracellular dNTP pool and has been described as a tumor suppressor in various cancer types and as a modulator of antimetabolite efficacy. However, the role of SAMHD1 expression, its biological correlates and prognostic and predictive implications in breast cancer (BC) remain largely unexplored.

Methods: Our study cohort consisted of 564 patients with early BC patients with available gene expression profiling (GEP) data. Whole-exome sequencing data were also available in a subset of patients (n=162). Immunohistochemical staining was performed using FFPE tissue microarrays while *in silico* analysis was carried out for deconvolution of immune cell subpopulations and other biology-related pathways. A large pooled transcriptomic early BC dataset (n=2402) was used for validation. GEP data from patients (n=120) enrolled the metastatic Swedish TEX randomized phase III clinical trial (NCT01433614) were used for the evaluation of SAMHD1 expression in response to capecitabine.

Results: SAMHD1 protein was expressed in 20% of patients in the study cohort and significantly correlated with mRNA levels ($p<0.01$). There was a low frequency of SAMHD1 mutations (1.2%) and gene amplifications/deletions (1.8%). SAMHD1 protein and gene levels were higher in HER2+/HER2-enriched and triple-negative BC/ basal-like (BL) subtypes. Both SAMHD1 gene and protein expression were independently associated with favorable distant recurrence-free interval (DRFI) in BL tumors in the early BC study cohort. SAMHD1 gene expression was independently associated with improved disease-free survival (DFS) in the entire population (HRadj =0.83, 95% CI 0.72 – 0.96, p=0.011) in the validation cohort and also within the HER2-enriched and BL molecular intrinsic subtypes. SAMHD1 was significantly correlated with proliferation and immune-related signatures. Furthermore, in metastatic BC SAMHD1 mRNA levels were enriched in responders receiving capecitabine (ORadj=1.78, 95% CI 95% 0.93-3.78, p=0.09).

Conclusions: SAMHD1 gene and protein expression represent promising prognostic biomarkers in basal-like early BC. Its potential predictive value to capecitabine treatment warrants further investigation in larger patient cohorts.

54421 - Dynamic tuning of the extracellular matrix stiffness promotes the induction of phenotypic changes in mammary epithelial cells

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Abstract text

To find better breast cancer treatments, it is important to understand the mechanisms that drive the tumorigenic transformation of normal mammary tissue. It has been well established that the microenvironment surrounding epithelial tissue is the key component that drives tumor growth and proliferation. Lately, mechanical changes to the microenvironment like increased ECM stiffness has been identified as a key driver of tumorigenesis and metastasis. Currently, no 3D physiological system of the epithelial microenvironment exists that allows dynamic tuning of its mechanical properties and permits long-term phenotyping and genotyping during tumorigenesis. To address this need, we have developed an in vitro model that mimics normal and cancerous mammary epithelial microenvironments (nMEME and cMEME) using an interpenetrating (IPN) hydrogel network system, composed of alginate and basement membrane extract (BME). Soft IPN gels were formed by combining alginate and BME followed by incubation at 37˚C. However stiff IPN gels were formed by mixing calcium, a divalent cation known to crosslink alginate, into the IPN gel mixture followed by incubation. Soft and stiff IPN gels matched the stiffnesses of native nMEME and cMEME and when MECs were encapsulated and cultured in them, native mammary acini and invasive clusters were formed respectively which illustrated the differential behavior of MECs to different stiffnesses. We then adopted the model to dynamically tune the stiffness by adding calcium to the soft IPN gel that had preformed mammary acini in them to mimic the ECM remodeling in nMEME during tumorigenesis. Most of the acini population in soft gel underwent phenotypical changes to result in invasive clusters once the gel was stiffened. In contrast, the MECs that formed invasive clusters in stiff IPN gels retracted their invasive processes and formed spherical clusters when the gel was softened using a calcium chelator. These results suggest that the sole effect of ECM stiffness modulation can drive phenotypical changes in previously established nMEME and cMEME. We also showed the basement membrane expressed in normal acini is disrupted and fibronectin is highly expressed when they turn into invasive clusters. However, the fibronectin is less expressed, and the basement membrane is restored in the spherical clusters that changed from being invasive clusters. We used flow cytometry and observed a shift in the luminal population of mammary acini towards basal type when the gel was stiffened and vice versa when the gel was softened. The use of small molecule inhibitors enabled us to explore the possible mechanistic pathways involved in ECM stiffness sensing. In the future, the use of bulk RNA and single-cell sequencing will help us in identifying key mechanosensitive genes involved during the phenotypical changes of MECs when the stiffness of IPN gel is dynamically modulated which could be potential cancer drug targets. The developed mechanically tunable model of nMEME is a new physiologically relevant epithelial system that could be extended to engineer any other cancer type and used as a platform for drug screening. The complexity of our model could be increased by adding other tumor microenvironment components to study their combinatorial effects.

54422 - The Application of Antioxidants to Reduce Stem Cell Transplantation Associated Cardiovascular Complications and Graft-Versus-Host Disease

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Abstract text

Background

Stem cell transplantation (SCT) is the only curative treatment for many patients suffering from hematologic malignancies including leukemia, lymphoma, and myeloma in addition to several solid tumors. Despite decades of successful SCT, graft-versus-host-disease (GvHD) remains one of the most significant causes of morbidity and mortality for transplanted patients. Several clinical studies showed that high-dose Cyclophosphamide (Cy) in a conditioning regimen can cause severe and life-threatening cardiovascular complications. Reactive oxygen species (ROS) are known to cause cellular excessive oxidative stress and are involved in the progression of GvHD as well as cardiovascular complications. In the current study, we aimed to apply a novel antioxidant N-acetylcysteine amide (NACA) as a prophylactic treatment to avoid GvHD and to prevent Cy-induced cardiovascular complications in preclinical models.

Methods

Using a well-established mouse GvHD model with complete MHC-mismatched allo-SCT (C57Bl/6 to Balb/c), the effect of NACA on the occurrence and severity of acute GvHD was evaluated by following clinical GvHD scores and survival after SCT. The mechanism of action was also investigated. in NACA-treated GvHD mice and compared with the same parameters observed in N-acetylcysteine (NAC)-treated GvHD mice.

The prophylactic efficacy of NACA was evaluated using human aortic endothelial cells (HAECs), human umbilical endothelial cells (HUVECs), and human cardiomyocyte cells. The efficacy of NACA was compared with the clinically approved derivative N-acetylcysteine (NAC). In addition, *in vivo* animal studies were performed to evaluate the protective effect of NACA /NAC on Cy-induced cardiovascular toxicity by left ventricle (LV) echocardiography followed by histological analysis.

Results

In allo-SCT and GvHD mice models, we found that NACA treatment significantly prolonged the survival and decreased GvHD severity score. Moreover, NACA downregulated the pro-inflammatory cytokines, such as IFNγ, TNFα, IL-1β, and IL-6, but upregulated the anti-inflammatory IL-4 in the mouse serum. Data also showed that NACA administration significantly reduced ROS accumulation in the spleen after transplantation. Importantly, NACA did not alter the engraftment. Opposite to NACA, the effect of NAC on GvHD was not significant.

In 4-OH-Cy treated HAECs, HUVECs, and human cardiomyocyte cells, pre-incubation with 5 mM NACA preserved the normal cell morphology and prevent cell death. Moreover, NACA was found to decrease 4-OH-Cy-induced DNA damage and cell death via caspase-dependent apoptosis. In addition, NACA significantly inhibited 4-OH-Cy-induced oxidative

stress by reducing the intracellular levels of reactive oxygen species (ROS) and by improving the cellular antioxidative capacity. Our results showed that in contrast to NAC, its amide derivative NACA showed remarkably superior ability in alleviating both endothelial damage and cardiac dysfunction.

Conclusions

In conclusion, the current study proved NACA, rather than NAC, has the potential to be applied as a prophylactic treatment to reduce two major complications during SCT: GvHD and Cy-induce cardiovascular toxicity. Further preclinical and clinical studies are warranted to systemically evaluate its efficacy and safety before clinical application.

54423 - MYC determines the outcome of pro-senescence cancer therapy by regulating immune surveillance

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Abstract text

Cellular senescence, defined as a state of permanent cell cycle arrest, is considered 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), which influence the microenvironment including immune cells, has been shown to possess both anti- and pro-tumorigenic properties in different tumor models. 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 induction in tumor cells in culture and in vivo. $BRAF^{V600E}$/MYC-ER/$CDK2^{FLOX/FLOX}$ mice were generated, and activation of $BRAF^{V600E}$ and MYC-ER, and simultaneous deletion of CDK2 in the lung epithelium of these mice were accomplished by inhalation of the Ad-CRE virus. In addition, the activity of MYC-ER can be regulated by administration of tamoxifen (TAM). While activation of MYC by TAM accelerated lung tumor development as previously reported, 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 several markers of senescence, with no apparent activation of apoptosis. 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 analysis of the CDK2-depleted lung tumor tissues from mice 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 production 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, decreased tumor-infiltrating macrophages and increased tumor-infiltrating T-cells populations. 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 by 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.

54424 - MYCN Amplification correlates with reduced expression of γ-secretase and NOTCH signaling activity in neuroblastoma

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Abstract text

Amplification of the MYCN oncogene is found in ~20% of neuroblastoma (NB) cases and correlates with high-risk disease and poor prognosis. Despite the plethora of studies describing the role of MYCN in NB, the exact molecular mechanisms underlying MYCN's contribution to high-risk disease are not completely understood. Herein, we implemented an integrative approach combining publicly available RNA-Seq and MYCN ChIP-Seq data derived from human NB cell lines to define biological processes directly regulated by MYCN in NB. Our approach revealed that MYCN-amplified NB cell lines are characterized by downregulation of genes involved in NOTCH signaling pathway. More specifically, we found genes encoding members of the γ-secretase complex that cleaves and activates NOTCH receptors to be underexpressed in MYCN-amplified NB cell lines. Analysis of MYCN ChIP-Seq revealed an enrichment of MYCN binding at the transcription start site of genes encoding γ-secretase complex subunits. Notably, using publicly available gene expression data from NB primary tumors, we revealed that the expression of γ-secretase subunits encoding genes and other components of the NOTCH pathway was also reduced in MYCN-amplified tumors and correlated with worse overall survival in NB patients. Genetic or pharmacological depletion of MYCN in NB cell lines induced the expression of γ-secretase genes and NOTCH-target genes, and chemical inhibition of the γ-secretase activity dampened the expression of NOTCH-target genes upon MYCN depletion in NB cells. In conclusion, this study defines a set of MYCN-regulated pathways that are specific to MYCN-amplified NB tumors and uncovers a novel role of MYCN as suppressor of γ-secretase expression with impact on NOTCH signaling activity in MYCN-amplified NB.

54433 - Identification of novel PDGFRalpha-dependent sarcoma-supportive functions of fibroblasts

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Abstract text
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 PDGFRa 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 was 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.

54469 - NEAR-INSTANT DETECTION OF GLIOMA STEM CELLS IN LIVE HUMAN GBM-TISSUE

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Abstract

Emerging research suggests that failure to target glioma stem cells (GSCs) rather than the inability to remove tumors through surgery, radiation, or chemotherapy, explains the poor survival of GBM patients. In this study, a luminescent conjugated oligothiophene (LCO), named GlioStem (p-HTMI), is used for non-invasive and non-amplified real-time detection of GSCs in live human GBM-tissue. More than 90 patient samples were stained, quantified, and analyzed by fluorescent microscopy for the presence of Gliostem-positive (GS+) cells. Approximately 30 of the samples were FACS-sorted for GS+ and GS- cells where quantification by FACS could verify microscopy results. In addition, GS+ cells were shown to express significantly higher levels of stem cell markers (CD271, CD133, PDGFRa, CD44) in FACS-experiments. Bulk RNA sequencing of 7 GBM patient samples with paired GS+ and GS- sorted cells revealed that GS+ patient samples clustered together, whereas the GS- populations did not cluster together neither with each other nor with the GS+ populations. These data suggest a distinct heterogeneity in the GS- samples and a certain level of homogeneity regarding the GS+ populations, independent of intra-patient or patient-to-patient heterogeneity. Moreover, the GS+ samples were found to express significantly higher levels of stem cell markers including SOX10, OLIG1/2, and ASCL1, compared to the GS- samples. More specifically, the GS+ samples exhibited significantly higher expression of 35 genes associated with stemness compared to the GS- samples, with most markers being associated with pre-oligodendrocyte precursor cells (pre-OPCs) and pro-neural subtypes. Our results suggest that GlioStem is a versatile tool for near-instant and selective detection of GSCs in live tumor tissue. Detecting and eliminating these cells during tumor resection may therefore be an important aim in efficiently preventing tumor regrowth and would mean a crucial step towards increased patient survival.

54470 - Spatial omics at KIGene core facility

Lars Selander[1] , *Ida Nilsson[1], Malin Alvehus[1], Katarina Gell[1], Annika Eriksson[1], Martin Schalling[1]*

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Abstract

KIGene is a well-established node at CMM with near to thirty years of experience of running a core facility. Traditionally we offer genetic analyses, covering DNA sequencing, gene expression, genotyping, miRNA profiling and copy number variation, as well as epigenetic analysis. When we a couple of years ago merged with the previous in situ core facility at CMM we acquired the GeoMX platform from Nanostring and created a node for Spatial Biology. Spring 2023 we will receive CosMx platform from the same company.

The GeoMx Digital Spatial Profiler (DSP) provides morphological context in spatial transcriptomics and spatial proteomics experiments from a regular tissue section, e.g., fresh, TMA or FFPE. By utilizing fluorescently labeled morphology markers researchers can precisely select which tissue compartments or cell types to profile based on the biology, and subsequently count expression levels using either the nCounter Analysis System (<96 plex) or an Illumina Sequencer (whole transcriptome, 18,000+ protein-coding genes). In addition to the morphology antibodies, the assay relies upon antibodies or probes coupled to photocleavable oligonucleotide tags. After hybridization of antibodies or probes to slide-mounted tissue sections, the oligonucleotide tags are released from the selected discrete regions of the tissue via UV exposure. Released tags are counted in a standard nCounter assay or sequenced using next-generation sequencing, and counts are mapped back to tissue location yielding a spatially resolved digital profile of protein or RNA abundance.

The CosMx Spatial Multiomics Single-Cell Imaging (SMI) s the first high-plex *in situ* analysis platform to provide spatial multiomics with FFPE and fresh frozen tissue samples at cellular and subcellular resolution. CosMx SMI enables rapid quantification and visualization of up to 1,000 RNA and 64 validated protein analytes. It is the flexible, spatial single-cell imaging platform that will drive deeper insights for cell atlasing, tissue phenotyping, cell-cell interactions, cellular processes, and biomarker discovery.

54473 - Effect on cell viability and metabolism upon treatment with BET bromodomain inhibitors in childhood medulloblastoma

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Abstract

Medulloblastoma (MB) is the most common malignant brain tumor in 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, the detailed mechanisms for their action have not been fully defined despite the number of investigations conducted.

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. Proliferation and viability assays revealed BETi treatment affects growth in all cell lines tested, independently of *MYC* status. Our results showed an increase in the expression of apoptotic markers and 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 that 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 Omomyc MYC inhibitor peptide.

Altogether, our results show that BETi treatment inhibits the glycolytic capacity of *MYC*-amplified MB cells and this effect could be attributed to increased TXNIP expression, leading to metabolic stress and decreased cell viability. The impact 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.

54496 - Side effects of low-dose tamoxifen

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Abstract

Background

Adherence to adjuvant tamoxifen therapy is suboptimal and acceptance of tamoxifen for primary prevention is poor. Published results indicate effect of low-dose tamoxifen therapy. Using questionnaire data from a randomized controlled trial, we describe side effects of standard and low-dose tamoxifen in healthy women.

Method

In the KARISMA trial, healthy women were randomized to six months of daily intake of 20, 10, 5, 2.5, 1 mg of tamoxifen or placebo. Participants completed a 48-item, five-graded Likert score symptom questionnaire at baseline and follow-up. Linear regression models were used to identify significant changes in severity levels across doses and by menopausal status.

Results

Out of 48 predefined symptoms, five were associated with tamoxifen exposure (hot flashes, night sweats, cold sweats, vaginal discharge and muscle cramps). When comparing these side effects in premenopausal women randomized to low-dose (2.5, 5 mg) versus high-dose (10, 20 mg), the mean change was 34% lower in the low-dose group. No dose dependent difference was seen in postmenopausal women.

Conclusions

Symptoms related to tamoxifen therapy is influenced by menopausal status. Low-dose tamoxifen, in contrast to high-dose was associated with less pronounced side effects, a finding restricted to premenopausal women.

55574 - Rewiring of the enhancer landscape and 3D chromatin organisation in glioblastoma underlie the neuron-to-glioma synaptic communication

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Abstract

Chromatin organization controls transcription by modulating 3D-interactions between enhancers and promoters in the nucleus. Alterations in epigenetic states and 3D- chromatin organization result in gene expression changes contributing to cancer pathogenesis. I will discuss our most recent findings: a study where we mapped the promoter-enhancer interactome and regulatory landscape of glioblastoma (GB), the most aggressive primary brain tumour. Our data reveals profound rewiring of promoter- enhancer interactions, chromatin accessibility and redistribution of histone marks across the four glioblastoma subtypes. This leads to loss of long-range regulatory interactions and overall activation of promoters, which orchestrate changes in the expression of genes associated to glutamatergic synapses, axon guidance, axonogenesis and chromatin remodeling. SMAD3 and PITX1 emerge as the major transcription factors controlling genes related to synapse organization and axon guidance. Inhibition of SMAD3 and neuronal activity stimulation cooperate to promote cell proliferation of glioblastoma cells in co-culture with glutamatergic neurons. Moreover, we demonstrated that inhibition of SMAD3 accelerates tumour growth in vivo in mouse models of glioblastoma. Our findings provide mechanistic insight into the gene regulatory networks that underlie neurogliomal synaptic communication.

55575 - Phenotypic characterization of spatial immune infiltration niches in non-small cell lung cancer

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Abstract

The immune microenvironment of non-small cell lung cancer (NSCLC) is heterogeneous, which impedes the prediction of response to immune checkpoint inhibitors. We have mapped the expression of 49 proteins to spatial immune niches in 33 NSCLC tumors and report key differences in phenotype and function associated to the spatial context of immune infiltration. Tumor-infiltrating leukocytes (TIL), identified in 42% of tumors, had a similar proportion of lymphocyte antigens compared to stromal leukocytes (SL) but displayed significantly higher levels of functional, mainly immune suppressive, markers including PD-L1, PD-L2, CTLA-4, B7-H3, OX40L, and IDO1. In contrast, SL expressed higher levels of the targetable T-cell activation marker CD27, which increased with a longer distance to the tumor. Correlation analysis confirmed that metabolic-driven immune regulatory mechanisms, including ARG1 and IDO1, are present in the TIL. Tertiary lymphoid structures (TLS) were identified in 30% of patients. They displayed less variation in the expression profile and with significantly higher levels of pan lymphocyte- and activation markers, dendritic cells, and antigen presentation compared to other immune niches. TLS also had higher CTLA-4 expression than non-structured SL, which may indicate immune dysfunction. Neither the presence of TIL nor TLS were associated with improved clinical outcomes. The apparent discrimination in functional profiles of distinct immune niches, independent of the overall level of leukocytes, illustrates the importance of spatial profiling to deconvolute how the immune microenvironment can dictate a therapeutic response and to identify biomarkers in the context of immunomodulatory treatment.

55576 - Radiotherapy- and hypoxia-induced remodeling of the brain tumor microenvironment

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Abstract

Inevitable therapeutic failure for patients with glioblastoma (GBM) is associated with tumor recurrence and intrinsic or acquired resistance to the standard of care, including radiotherapy. Recurrent tumors are generally incurable, and there is little evidence supporting that re-irradiation of recurrent GBM benefits patient survival. In contrast to many other tumor types, GBMs typically recur within the treatment field receiving high-dose radiotherapy during treatment of the primary tumor, i.e. in or overlapping with the original primary tumor volume. It is likely that acute and late, direct or indirect, effects of radiation affect the biology of the recurrent GBM microenvironment and recurrent GBM cells, but few studies have addressed the cellular and molecular composition of the recurrent brain tumor microenvironment in relation to the primary tumor. Using genetically engineered mouse models of GBM, we have combined highly multiplexed immunohistochemistry, single cell RNA sequencing, and experimental tumor biology to characterize GBM progression before, during, and after radiotherapy. We found that radiation generates a net tumor-supportive microenvironment in the mouse brain, and that the tumor-supportive effect can be linked in part to radiation-induced astrocyte reactivity. Reactive astrocytes remodeled the tumor microenvironment of recurrent GBM by altering the extracellular matrix as well as the immune landscape, and targeting these alterations prolonged survival in pre-clinical models of GBM. Our studies suggest that there is largely untapped therapeutic potential in targeting the irradiated brain tumor microenvironment.

55577 - Epigenetic mechanisms and vulnerabilities in acute myeloid leukemia

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Abstract

Acute myeloid leukemia (AML) is a clonal malignant disease characterized by accumulation of immature myeloid cells and an inability of the bone marrow to produce sufficient levels of mature blood cells. AML is characterized by early mutations in epigenetic regulators that are one of the most important contributions to the pathogenesis in AML. The epigenetic aberrations can either be involved in regulation of DNA methylation (such as mutations in *DNMT3A*, *TET2*, *IDH1* and *IDH2*) or in other types of chromatin regulatory mechanisms and histone modifications (*MLL* fusions, *MLL-PTD* and mutations in *ASXL1* and *EZH2*). It has been suggested that early aberrant epigenetic changes in stem or progenitor cells, caused by mutations in epigenetic regulators, create epigenetic plasticity and heterogeneity, resulting in a wide range of different phenotypes that in turn increase the probability for cancer development. We are investigating how the epigenetic dysregulation in AML cause a vulnerability that can be exploited therapeutically.

55578 - Decoding the cancer microenvironment in cancer drug development and optimized diagnostics

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Abstract

Patient-derived scaffolds and analyses of adapting cancer cell lines can monitor malignant properties of a cell-free cancer microenvironment highlighting distinct links between scaffold influences and clinical aggressiveness in cancer. The protein composition of the cell-free cancer microenvironments influencing adapting cancer cells have been defined by quantitative mass spectrometry and results indicate clear clustering of PDS differing in extracellular matrix related proteins as well as immunoregulatory and metabolic regulators. Interestingly both the defined clusters as well as individual imprinted proteins in the cell-free scaffolds are linked to clinical behaviours of the cancer and data from breast cancer and colorectal cancer will be presented. The in vivo identified proteins can be used for improved disease subtyping, cancer drug targeting and to construct optimal synthetic 3D-models that can be used for human-like drug discovery and validation of novel cancer treatments.

55579 - Uncovering the origin of chromosomal gains in childhood leukemia by single cell sequencing and in silico modeling

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Abstract

High hyperdiploid acute lymphoblastic leukemia (HeH ALL), one of the most common childhood malignancies, is driven by nonrandom aneuploidy (abnormal chromosome numbers) mainly comprising a specific pattern of chromosomal gains. In this study, we investigated how aneuploidy in HeH ALL arises. Single cell whole genome sequencing of 2,847 cells from nine primary cases and one normal bone marrow revealed that HeH ALL generally display low levels of chromosomal heterogeneity, indicating that they are not characterized by chromosomal instability and showing that aneuploidy-driven malignancies are not necessarily chromosomally heterogeneous. Furthermore, most chromosomal gains were present in all leukemic cells, suggesting that they arose early during leukemogenesis. Analysis of copy number data from 577 primary cases, investigations of paired diagnostic/relapse samples, and studies of the pattern of duplicated somatic mutations revealed selective pressures that were used for in silico modeling of aneuploidy development. This showed that the aneuploidy in HeH ALL likely arises by an initial tripolar mitosis in a diploid cell followed by clonal evolution, in line with a punctuated evolution model.

55580 - Childhood brain tumor development and mechanisms of therapy resistance

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Abstract
Pediatric brain tumors with elevated MYC levels are often coupled to aggressive growth and poor prognosis regardless of tumor diagnosis. We study how MYC genes generate childhood brain tumors like medulloblastoma, pineoblastoma and high-grade glioma in transgenic mice in order to understand timing of tumor onset and the distinct nature of the tumor cell of origin. It is evident that MYC-driven medulloblastomas are more biologically similar to pineoblastoma than to other medulloblastoma types or to high-grade gliomas. We show that this depends on if the tumor arises from a photoreceptor-positive progenitor and if an important suppressor gene like p53 or Arf is depleted during tumor initiation.

Tumor recurrence developing from therapy resistance, immune escape and metastasis is the leading cause of death of malignant pediatric brain tumors. We have established patient-derived xenografts from a number of matched primary-relapse samples, including pediatric high-grade glioma, medulloblastoma and ependymoma that we follow at the single cell level using scRNA- Seq to identify changes in distinct signaling pathways. By studying paired primary-recurrent MYC-driven medulloblastoma samples we identified accumulation of cells that express the transcription factor SOX9, that was similarly induced following long-term irradiation in our transgenic models. SOX9 was also accumulated in glioma cells that became resistant to temozolomide treatment. Unfortunately, specific SOX9 inhibitors do not exist. We therefore developed a cytotoxic gene therapy that specifically targets SOX9-positive cells that escape from standard treatment. This exemplifies how relapsing cells that express transcription factors that are often hard to treat, can in fact be efficiently targeted.

55583 - Molecular mechanisms underlying ATR/ALK inhibition in ALK- driven neuroblastoma models

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Abstract
The Anaplastic Lymphoma Kinase (ALK) receptor tyrosine kinase was originally identified as a transforming oncogene associated with lymphoma. To date, many chromosomal rearrangements leading to an activated ALK have been described and are implicated in a range of cancer types. ALK tyrosine kinase inhibitors (TKIs) are now employed clinically for treatment of a range of ALK driven tumors. In addition to a high-profile role in lung cancer, ALK is involved in numerous other tumor types, notably in both familial and sporadic neuroblastoma, a pediatric tumor arising from the neural crest. In the case of neuroblastoma, the full-length ALK RTK is often mutated in the kinase domain leading to activation of the receptor. Here, the ALKAL ligands, which were described in 2015, are potentially important and in mouse models have been shown to drive neuroblastoma together with MYCN. In recent years we have investigated ALK signaling in response to both ligand activation and mutation in a range of ALK driven models, mouse models and human cell lines. Our latest efforts in investigating ALK-driven signaling events through use of phosphor/proteomics and proximity labelling, have identified additional therapeutic opportunities for ALK-driven neuroblastoma. Data will be presented regarding misregulation of the ALKAL ligand in neuroblastoma as well for molecular targets, such as ATR, that together with ALK TKI treatment provide effective treatment of ALK-driven neuroblastoma in mouse models. Our recent data investigating the underlying mechanisms of ALKi/ATRi combination treatment in neuroblastoma will be presented.

55584 - Microbiome in Cancer

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Abstract
Our bodies are home to trillions of microbes, including those in the gastrointestinal, vaginal, and oral microbiomes. Recent research has highlighted the significant role that the microbiome plays in cancer development, progression, and response to treatment.

In our research, we studied the vaginal microbiota of 345 young Swedish women and found that non-*Lactobacillus*-dominant vaginal microbiota is strongly associated with HPV infection, particularly oncogenic types. We also conducted a meta-analysis study that found a significantly higher risk of cervical dysplasia with non-*Lactobacillus*-dominant vaginal microbiota than *Lactobacillus*-dominant vaginal microbiota, indicating the important role of vaginal microbiota in HPV infection and cervical cancer development.

Furthermore, we performed microRNA expression profiling on the same group of women and found that microRNAs were clustered into distinct groups according to vaginal microbiota composition. We also evaluated the salivary microbiota of participants with and without cervical dysplasia, finding that certain bacteria significantly increased in those with dysplasia, especially among smokers compared to never-smokers. Currently, we are exploring more immune response-related genes and investigating the mechanisms of vaginal microbiome-related inflammation using in vitro 2D and 3D models.

Additionally, we analyzed the whole-genome sequencing of gastric mucosa, which bolstered our knowledge on stomach physiology with respect to the gastric microbiome and microbial function. Moreover, we present a comprehensive immune cellular landscape of the human stomach, which will be a valuable resource to interrogate the pathology of gastric cancer.

Overall, our findings have significant implications for cancer development and surveillance with the microbiome in both research and clinical settings.

55585 - Immune microenvironment in cancer and microbes: a dangerous liaison?

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Abstract

Bacterial genotoxins are unusual effectors that cause DNA damage in eukaryotic cells. These toxins are produced by commensal and pathogenic bacteria that can be found with higher frequency in the microbiota of Inflammatory Bowel Disease (IBD) and colorectal cancer (CRC) patients. In vitro infection with genotoxigenic bacteria promotes genomic instability in pro- carcinogenic conditions. However, their role in the modulation of the host-microbial interaction in health and disease remains unclear.

Using isogenic strains of Salmonella enterica expressing or non-expressing a functional genotoxin, we have shown that infection with the genotoxigenic strain suppressed the intestinal inflammatory response. In the infected tissue, we observed predominantly alternatively activated macrophages, higher ration of anti-inflammatory cytokines, increased presence of Salmonella in proximity to cells with higher levels of DNA damage marker, higher number of cells with nuclear p53 and senescent cells. Some of the features observed in the infected intestine resembled the suppressive tumour microenvironments.

The suppressive effect was tissue specific and was not observed in the liver where infection promoted an inflammatory response, independently on the presence of the genotoxin.

We have further demonstrated that infection with the genotoxigenic Salmonella strain synergised with pro-inflammatory and pro-carcinogenic conditions to enhance intestinal colonization (as observed in CRC patients) and cancer development.

Conclusions: in healthy individuals this genotoxin promotes a stealth invasion and persistent infection in the host, while it exerts carcinogenic properties in already compromised individuals. Our work highlights the relevance of the tissue microenvironment in defining the host response to infection with genotoxigenic bacteria.

55588 - Modulation of cancer cell signalling by secreted bacterial factors

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Abstract
Recently, we demonstrated that a novel bacterial cytotoxin, the protein MakA released by Vibrio cholerae, is a virulence factor that kills Caenorhabditis elegans when the nematodes graze on the bacteria. Studies with mammalian cell cultures in vitro indicated that MakA could affect eukaryotic cell signalling pathways involved in apoptosis, autophagy, and lipid biosynthesis. Intriguingly, MakA displayed potent cytotoxic activity particularly against several tested cancer cell lines, whereas it appeared less toxic to non-transformed cells. MakA bound to the tumour cell surface was internalised into the endolysosomal compartment and caused endolysosomal membrane permeability, resulting in cytosolic release of cathepsins and in activation of pro-apoptotic proteins. Additionally, MakA altered the integrity of β-catenin in colon cancer cells via a caspase- and proteasome-dependent mechanism. The intratumour injection of MakA substantially diminished tumour development in a murine solid tumour model of colon cancer. Furthermore, it was discovered that MakA inhibits the PIP5K1 lipid-signalling pathway in cancer cells, resulting in a decrease in the expression of PIP5K1α and pAkt. MakA inhibited cyclin-dependent kinase 1 (CDK1) and induced p27 expression, which led to G2/M cell cycle arrest. These findings designate MakA as a novel candidate to be considered in developing new strategies for treating colon cancer.

55589 - Targeting tumor-stromal interactions in pancreatic cancer

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[2] Umeå University, Faculty of Medicine, Department of Radiation Sciences.

Abstract

Pancreatic cancer is a disease resistant to available therapies, hence novel therapeutics are urgently needed. The tumor is characterized by a dense tumor stroma consisting of a diverse extracellular matrix and different subtypes of cancer-associated fibroblasts (CAFs) that provide cancer cells with signals that both positively and negatively regulate cancer cell growth, and contribute to immune evasion.

To better understand the pathophysiological role of CAFs we have developed a co-culture system with tumor organoids and CAFs where the interactions between the celltypes can be studied in detail. In this model we have performed a high throughput compound screen, and can show that it is possible to pharmacologically shift the balance between different CAF subtypes and furthermore, that induction of certain CAF subtypes reduces cancer cell growth. We hypothesize that shifting the composition of CAFs in a tumor could turn tumors from being ”wounds that do not heal” to wounds that can heal.

Bio

I have a specific interest in pancreatic cancer and tumor microenvironment research. In my thesis I studied the role of the tumor stroma as a prognostic and diagnostic marker. During my postdoctoral in the Tuveson Laboratory at CSHL I continued to study the tumor microenvironment. In 2017 I became an assistant professor at Wallenberg Center for Molecular Medicine at Umeå University. Currently, I am focusing on deciphering the functional heterogeneity of stromal components within the tumor microenvironment aiming to identify drugable stromal interactions driving tumor initiation and progression of the disease, and to develop inhibitors for these interactions.

55591 - Targeting NOX2 and myeloid derived suppressor cells in cancer

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Abstract
The efficiency of anti-cancer immunotherapy depends on a balance between effector cells, in particular natural killer (NK) cells and T cells, and cancer-related immunosuppression. Inflammatory myeloid cells, including myeloid derived suppressor cells (MDSC), have been found to suppress immune responses via multiple mechanisms, including the formation of NOX2-derived reactive oxygen species (ROS).

My group studies NOX2-related immunosuppression and explores possibilities of targeting NOX2 therapeutically. We have found that genetic and pharmacological inhibition of NOX2 facilitates NK cell-mediated clearance of metastatic tumors and enhances the efficacy of checkpoint blockade (Aydin et al. Cancer Immunol Res 2017, Grauers Wiktorin et al. Cancer Immunol Immunother, 2019). Furthermore, we recently observed that the NOX2-related immunosuppression was aggravated in conditions of surgical stress. This inflammation-induced metastasis could be counteracted by NOX2-inhibition. In addition, our recent studies suggest that NOX2-derived ROS from myeloid cells exerts direct effects on tumor cells. Hence, myeloid cell-derived ROS stimulate epigenetic changes in tumor cells resulting in activation of epithelial-to-mesenchymal transition (EMT) programs with ensuing metastasis.

Bio
Anna Martner is group leader at the Sahlgrenska Center for Cancer Research, Gothenburg University, since 2012. She has a PhD in infectious immunology, and has studied tumor immunology with a focus on MDSC since her post doc studies in the laboratories of Prof. Kristoffer Hellstrand, University of Gothenburg, and Prof. Dmitry Gabrilovich, Moffitt Cancer Center, Tampa (2009-2012). In 2019 she conducted a sabbatical at MIT, Boston, hosted by Prof. Robert Weinberg with a focus on EMT.

55592 - CAR T-cell experiences in Sweden

Gunilla Enblad[1]
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Abstract
The talk will cover a short background on CAR T-cells and the experiences of our Uppsala academic CAR T-cells studies and the introduction of commercial CAR T-cells. Results, toxicity and resistance mechanisms will be covered.

Bio
Gunilla Enblad is professor of oncology at Uppsala university Hospital and Uppsala University. Her main research interests are lymphomas and CAR T-cells. She was PI for the first two academic CAR T-cell trials in Uppsala, started already 2014 which was first in Europe. She is the chairman of the Swedish CAR T-cell group and co-chairman of SWECARNET, a collaborative group on CAR T-cells. She is currently performing clinical and translational research on lymphomas and CAR T-cells.

55593 - Exploring the role of tertiary lymphoid structures in melanoma

Göran Jönsson[1]
[1] Lund University

Abstract
Recently, work by us and others have identified groundbreaking evidence that tertiary lymphoid structures (TLS) are crucial components of the anti-tumor immune response in melanoma and that such immune niches are critical for response to immunotherapy. Using spatial and single cell genomics we are now further analysing melanoma tumors and the tumor microenvironment from patients relapsing on PD1 or CTLA4 blockers. This and the role of TLSs in immunotherapy resistance will be discussed.

Bio
Göran Jönsson is a professor in molecular oncology at Lund University. His research has for many years been based on strong collaboration between basic- and clinician scientists in truly translational projects. Göran has also been the recipient of Young and Senior Investigator Awards from the Swedish Cancer Society and more recently was awarded with the Göran Gustafsson prize in medicine 2022 and Cancer Researcher of the year (2023) from the Swedish Cancer Society.

55595 - Harnessing the Power of Genomics for Improved Precision Medicine in AML

Thoas Fioretos[1]
[1] Lund University

Abstract
Acute myeloid leukemia (AML) is a genetically heterogenous disease, with multiple subtypes characterized by distinct disease-driving mutations. In recent years, several classification schemes have been developed to categorize AML based on genetic mutations. Genome-wide technologies are now entering the clinical diagnostic arena although several challenges remain. In a collaborative study, the SciLifeLab Clinical Genomics platform and Genomic Medicine Sweden (GMS) are investigating if whole-genome and whole transcriptome sequencing can replace current gold standard diagnostics.

Additional layers of genomic information, including multimodal single cell sequencing, allow an even higher resolution of the cellular and molecular basis of AML. Such studies may further improve current classification schemes and also reveal new therapeutic vulnerabilities. In this talk, I will present preliminary data on how we have used multimodal single cell sequencing to identify a new subtype of AML displaying reversible immune evasive properties.

The ability to specifically target the disease driving leukemia stem cells has remained the holy grail of AML research for decades. The final part of my talk will describe how we have used a combinatorial screening strategy to identify new targets on leukemia stem towards the development of antibody-based treatments.

Bio
Thoas Fioretos is a professor and senior consultant in Clinical Genetics at Lund university. His research is focused on improving diagnostics and treatment of acute leukemia. He is the platform Director of SciLifeLab Clinical Genomics, an investigator within GMS, and a Wallenberg Clinical Scholar. Fioretos is the co-founder of three spin-out companies active in visualization of genomics data (Qlucore AB) and in the development of antibody-based therapeutics of cancer (Cantargia AB and Lead Biologics Intl).

55596 - Advancing prostate cancer care

Johann De Bono[1]
[1] The Institute of Cancer Research, London

Abstract
Prostate cancer is the commonest cancer in men and one of the major causes of global cancer mortality. The complexity of prostate cancer's biology, and intra- and inter-patient heterogeneity, is being increasingly elucidated. This is leading to the development of novel therapeutic strategies that can improve prostate cancer care. This presentation will focus on recent advances in elucidating prostate cancer biology and the translation of this into therapies

55597 - Tumor intrinsic immunity: mismatch repair deficiency as a model system

Luis Diaz[1]
[1] Memorial Sloan-Kettering Cancer Center

Abstract
Dr. Diaz is a physician-scientist who leads the Division of Solid Tumor Oncology at Memorial Sloan Kettering Cancer Center. His teams have pioneered the use of circulating tumor DNA as a cancer biomarker for screening, monitoring, and detection of occult disease, and discovered the therapeutic link between cancer genetics and immunotherapy in patients with mismatch repair deficient tumors. This research led to the first tumor agnostic FDA approval for any solid tumor with this genetic lesion and the first cancer study that resulted in a 100% complete remission rate. He is the recipient of numerous awards and honors and in 2021, Dr. Diaz was appointed by President Biden to the National Cancer Advisory Board.

55882 - Introduction to Division of Pathology, Department of Laboratory Medicine, Karolinska Insitutet

Dhifaf Sarhan[1]
[1] Department of Laboratory Medicine, Karolinska Institutet

Abstract
A short introduction to Division of Pathology at the Department of Laboratory Medicine, Karolinska Institutet.

55883 - The future of cancer imaging

Regina Beets-Tan[1]
[1] Netherlands Cancer Institute

Abstract
Prof Regina Beets-Tan is professor of Radiology at the University of Maastricht, The Netherlands and Adjunct Professor of Abdominal & Oncological Radiology at the University of Southern Denmark. She is a passionate radiologist in oncologic and abdominal Imaging. She chairs the Department of Radiology at the Netherlands Cancer Institute, Amsterdam and leads the multidisciplinary research focusing on cancer imaging and AI. Her pioneering research work in colorectal cancer imaging has worldwide recognition.
Regina Beets Tan has demonstrated extraordinary leadership in Radiology throughout her entire career. This is evidenced in her positions as current chair of the Board of Directors of the European Society of Radiology (ESR), President of ESR 2021-2022 and President of the European Congress of Radiology 2022. She was the former President of the European Society of Gastrointestinal and Abdominal Radiology as well as the former President of the European Society of Oncologic Imaging. With her leadership in Radiology she is the perfect spar in strategic panels as demonstrated by her appointment as member of the 2019 EU Mission Board for Cancer and in 2018 as member of the Board of Directors of the European Cancer Organisation. Regina holds seats in research panels of Dutch Organization of Science and Dutch Cancer Society. She is part of strategic and scientific bodies of medical societies and acts as a liaison between radiology and other disciplines. With ECR 2022 ‘Building Bridges’ Regina aimed to give a multidisciplinary platform to professionals to connect in science and education. With her lecture “The future of cancer imaging” Regina will share her vision of how imaging will need to move forward to improve treatment decision making and patient outcome.
“Multidisciplinary coordination of research is essential for good patient care, and collaboration between the disciplines is more important than ever.”

55884 - The Evolution of the I-SPY 2 Platform Trial and the Transition to a More Patient Centered Design

Laura J. Esserman[1]
[1] University of California, San Francisco

Abstract
I-SPY 2, a neoadjuvant platform adaptive trial for molecularly-highrisk stage2/3 breast cancer, **shifts the paradigm** by: integrating early endpoints to shorten knowledge turns; changing the order of therapy so that efficacy is generated early in the course of care; moving the evaluation of novel therapeutic interventions to the early stage high-risk setting where good response translates into cures; improving the efficiency of the trial process and conduct; and promoting adaptation of treatment both over the course of the trial as well as within individual patients; and combining efficacy and toxicity endpoints so trials can be more patient centered**.** Complete pathological response (pCR) after neoadjuvant therapy (and the more granular residual cancer burden (RCB) at the time of surgery is the primary (early) endpoint. MRI functional tumor volume (FTV) serves as an early response assessment that informs “graduation” or designation of agents as likely to be successful. 24 agent combinations have completed testing over 12 years in I-SPY 2. Full biomarker profiling of every patient has allowed us to redefine cancer subtypes- Response Predictive Subtypes- changing our notion of who should get what therapy. By assigning patients to the right agents at the right time, we have increased the overall population pCR from 19% to 60%. pCR is a strong predictor of 3 and 5 year EFS and DRFS with a hazard rate of HR 0.18 regardless of subtype and treatment. The trial has evolved to I SPY 2.2, where we are introducing novel agents without standard chemotherapy as first line therapy, with subtype specific optimal therapy assigned as second line therapy. Patients get to go to the OR early and forego subsequent chemotherapy regimens if they are predicted to have a pCR. For those who have residual disease, they have multiple shots on goal to try to achieve a 90% overall pCR rate over the next five year, hopefully without standard chemotherapy. The I-SPY effort is a great example of team science. The model is exportable to other cancers and disease types. Together with the not-for-profit sponsor, Quantum Leap Healthcare Collaborative, there has been a huge advance in the efficiency of trial conduct. Multiple pharma and biotech companies have put agents and biomarkers into the trial. The database of closed arms is open to investigators who can submit concepts and the first 990 patient data is publicly available.

55886 - Data-driven cancer hallmarks for diagnostics and precision therapy

Olli Kallioniemi[1]
[1] SciLifeLab and Karolinska Institutet

Abstract
Translating multi-dimensional data from genomics, transcriptomics, proteomics, metabolomics, functional drug testing, or imaging into actionable precision cancer medicine insights poses significant challenges in terms of cost and complexity. We present a novel paradigm to facilitate cancer diagnostics and care using acute myeloid leukemia (AML) as a model.

Our approach introduces the concept of data-driven hallmarks (DDHMs) for cancer, inspired by the Weinberg-Hanahan cancer hallmark framework (Hanahan, 2022). The cancer hallmark concept, based on predefined biological properties, provides a useful theoretical framework but is not directly applicable for diagnostics and therapy. We distilled complex multi-omics properties of cancer into a limited number of unbiased, quantifiable properties, termed DDHMs. Applying this approach to AML samples profiled with four omics technologies and 500+ drugs in high-throughput ex-vivo testing (Erkers et al., submitted, 2023), we condensed nearly 100 million data points into 11 dimensions of variability, or DDHMs.

Individual cancer patients exhibit unique combinations of independent and potentially druggable DDHMs, contrasting with traditional precision cancer medicine stratifications based on genomic subgroups, each defined by specific driver signals. Most DDHMs are driven by data types beyond genomics, emphasizing the importance of multi-omics integration in cancer research and diagnostics. DDHMs can predict high-risk AML and specific drug response vulnerabilities, making the DDHM concept broadly applicable to cancer research and precision medicine interventions. Our proof of concept bridges the gap between data-intensive academic research studies and clinical precision medicine needs, and we hope that it will enable tailored drugs and drug combinations for individual cancer patients.

References:

1. Hanahan, D. (2022). Hallmarks of Cancer: New Dimensions. Cancer Discov 12, 31-46.
2. Erkers T, Struyf N, James T.… Orre L, Jafari R, Pawitan Y, Seashore-Ludlow B, Lehtiö J, Lehmann S, Östling P, Kallioniemi O. Data-driven hallmarks of acute myeloid leukemia, submitted, 2023.

56061 - Using in situ sequencing to spatially characterize the cellular, genetic and molecular heterogeneity of solid tumors

Mats Nilsson[1]
[1] SciLifeLab

Abstract
Abstract

56066 - Paediatric oncology in the omics era

Klas Blomgren[1]
[1] Karolinska Institutet

Abstract
Abstract

56067 - A Phase 1 First-in-human Study of the Cyclin-dependent Kinase-9 (CDK9) Inhibitor AZD4573 in Relapsed/Refractory Hematological Malignancies

Richard Olsson[1]
[1] Oncology R&D, AstraZeneca, Gothenburg, Sweden

Abstract
Tim H. Brümmendorf[1], Patrick Medd[2], Raphael Koch[3], Stephan Stilgenbauer[4], Shringi Sharma[5], Yun He[6], Stefanie Meyer[6], Margaret C. Wey[6], Jamal Saeh[6], Richard F. Olsson[7], Arnon P. Kater[8]

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[8]Cancer Center Amsterdam, Amsterdam, Netherlands

56069 - Quantify and characterize any protein interaction – even in complex backgrounds, even with challenging targets

Alex Spice[1]

[1] Fluidic Analytics, Unit A, The Paddocks Business Centre, Cherry Hinton Road, Cambridge CB1 8DH, United Kingdom

Abstract

Co-authors

Ian Watt, Roland Worth, Alison Ilsley, Monika Piziorska, Adam Halski, Alexey Morgunov, Sean Devenish, Sebastian Fiedler

Abstract

Microfluidic Diffusional Sizing (MDS) technology allows researchers to examine the most challenging protein interactions under native conditions and gain insights that were previously unattainable. MDS technology can be accessed on our new Fluidity One-M platform, providing accurate and reliable measurement of interactions based on changes in molecular size. This approach is purification-free, works directly in solution, comes with easy access and simple workflows, and has already advanced discoveries in the fields of infectious disease, oncology, and neurology.

56071 - BRINGING AUTOMATION TO LARGE SCALE MANUFACTURING OF TCR MODIFIED T CELLS FOR ADOPTIVE CELL THERAPY

Carola Barth[1]
[1] Miltenyi Biotec, Bergisch Gladbach, Germany

Abstract
Carola Barth, Daniela Gudert; Alexander Bohn, Sophia Lollies; Peter Maul, Marion Braun, Nadine Mockel-Tenbrinck; Andrew Kaiser

Adoptive immunotherapy using gene-modified T cells redirected against cancer has proven clinical efficacy and tremendous potential in several medical fields. However, such personalized medicine faces several challenges in the complexity associated with the current clinical manufacturing methods. Conventionally, the preparation of autologous gene-modified T cells comprises many open handling steps, is labor intensive and is not adapted to manufacture large numbers of gene-engineered T cells in a fully automated setting. Moreover, the cell-manufacturing process requires extensive training of personnel as well as a dedicated infrastructure, which restricts the wide spread use of these clinical procedures.

We have developed an automated process for lentiviral gene-modification and expansion of selected T cells enabling the manufacturing of large amounts of T cells. The CliniMACS Prodigy T Cell Engineering – Large Scale (TCT-LS) application allows purification and polyclonal T cell stimulation (using a newly developed TransAct format) followed by gene-modification and expansion of T cells in a single-use closed tubing set (TS 620). The large scale application is an advancement of the T Cell Transduction application (TCT) and allows the cultivation and expansion of higher cell numbers by using a larger cell preparation and cultivation unit.

Here, we demonstrate that the automated process enables the generation of larger numbers of gene-engineered T cells for cancer T cell therapy. In average $2x10^{10}$ T cells could be obtained, meaning a 4-fold higher number compared to the standard TCT process. Furthermore, the process yields a cell product comparable in respect to cellular composition, T cell subset ratio and differentiation. In addition, *in vitro* functionality of the gene-modified T cells is demonstrated.

Taken together, the automated TCT-LS application on the CliniMACS Prodigy is capable of yielding a cell dose compatible with the needs for higher infusion doses often applied clinically for TCR modified T cells.

56108 - From phenotype centered multi-omics analysis to precision medicine of childhood ALL.

Isabelle Rose Leo[1]

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Abstract

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Aim

Despite relatively high survival rates, childhood ALL patients continue to experience poor long-term health outcomes. The current standard treatment protocols are not appropriately calibrated to achieve specific disease management, and tolerability is often compromised.

Methods

The goal of our research is to improve treatment options for these patients by systematically identifying phenotypes that can be paired with sensitivity to drugs. We obtain detailed characterization of leukemia biology using mass-spectrometry based proteomics methods and pair these characteristics with drug sensitivity profiling to determine exploitable mechanisms for precision therapies.

Results

Our comprehensive methods using integrative multi-omics and drug response profiling of childhood ALL cell lines (https://proteomics.se/forall/) comprise a navigable public resource and have allowed us to obtain unprecedented molecular dissection of leukemia phenotypes and mechanisms of drug susceptibility {Leo, 2022 #3795}. Extending the proteomics profiling with thermal proteomics at baseline for 20 precursor B-ALL cell lines, we developed an approach to stratify the cellular proteome into functional proteoform groups. Using this new method, we identified differences in proteoform-proteoform interactions, and associations of proteoforms groups to specific drug responses {Kurzawa, 2022 #3793}. Together, these results have identified lineage-dependent drug sensitivity correlations and the specific indication of a TCF3 proteoform in a model of susceptibility to the diacylglycerol-analog bryostatin-1, which is proposed as a therapeutic candidate in MEF2D-rearranged leukemia {Leo, 2022 #3795}.

Conclusions

By connecting the phenotype level analysis using quantitative proteomics and thermal proteomics, we see great promise in augmenting the current precision medicine capabilities for childhood ALL patients.