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Towards precision medicine in Lynch Syndrome

Mev Dominguez-Valentin, PhD, on behalf of the Prospective Lynch Syndrome Database (PLSD)

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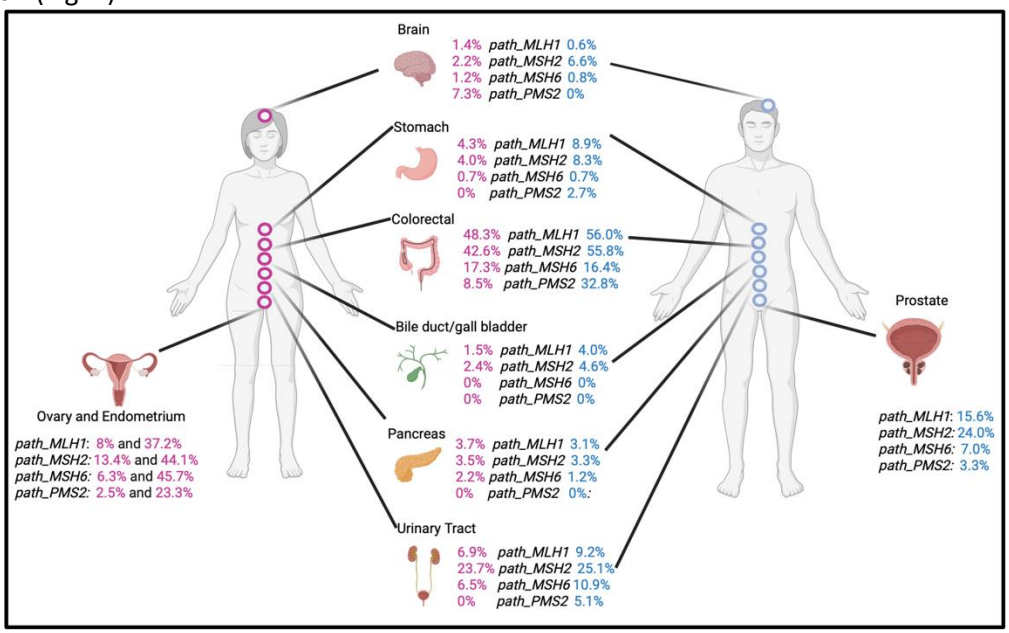
Lynch syndrome (LS) is an autosomal dominant cancer syndrome caused by pathogenic germline variants in one of the DNA mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6* or *PMS2,* or epigenetic silencing of *MSH2* caused by a deletion in the *EPCAM* gene (1). It is the most common hereditary cancer worldwide. LS has a prevalence of ~1 in 440 in European ancestry populations (2). However, most people do not know they have it. In **Europe around 2 million carriers of pathogenic MMR variants are estimated,** only 5% of which are under clinical surveillance. Carriers of *path_MMR* variants have a high lifetime risk of developing **colorectal (CRC), gynaecological, urinary trac**t and other cancers (3).

The **Prospective Lynch Syndrome Database** is the **largest worldwide prospective observational database in LS** and has provided more precise cancer risk according to gene, gender and organ (Fig. 1).



PLSD has assimilated an unprecedented resource of prospective data that informed the development of the updated European Guidelines for surveillance and management of LS. PLSD has contributed to provide a personalised precision medicine to LS by providing knowledge about:

1) Cancer risk within the LS-causing gene cohorts and even within a single pathogenic variant (across families) is substantially heterogeneous with very high risks and close-to-none (4), indicating that there are risk modifiers that are not computationally attributed directly to polygenic risk scores (5),

2) Colonoscopy prevention of CRC is suboptimal (6,7),

3) Prognosis of gynaecological cancers is better than for their sporadic counterparts (3),

4) Current diagnosis and management practices do not follow the guidance or observational evidence of the risks (8),

5) Cancer spectrum of LS is very wide and varies by gene (3), and that there are numerous clinically observed nuances in the cancer development of LS that are summoned by molecular phenotypes of the cancers (9).

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High-throughput Combination Drug Screening to Overcome Resistance in Breast Cancer

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Abstract: Despite the various breast cancer treatments currently available, a sub-population of cancer patients does not respond to treatment. More importantly, the resistance gets increasingly significant during the course of treatment, thus further reducing the chances of a progression free survival. This is mainly caused by the genetic instability of cancer cells coupled with the pressure exerted by both the immune system and the drugs which select for resistant variants that are harder to treat. These variants use multiple evasion mechanisms to resist killing by either drugs or the immune system. Hence the importance of combination therapies that allow targeting several of these resistance mechanisms concurrently therefore limiting tumour escape.

The aim of our work is to explore new treatment options for the various breast cancer subtypes through the conduction of high-throughput screenings and the identification of synergizing drugs. The large-scale drug sensitivity screening allows the simultaneous testing of a multitude of drug combinations. This is especially important in a highly active research field where the number of new candidate therapeutic compounds is constantly on the rise. The screening maximizes our chances to pinpoint interesting combination candidates, that might have otherwise gone overlooked, and then gives us the opportunity to advance them a step closer to the clinic. For this purpose, we designed a library of 64 drugs which we first tested as single therapies on 12 cell lines representing the different breast cancer sub-types. Accordingly, 53 drugs were selected for the combination drug screen which are being performed on at least 20 breast cancer cell lines. In addition, we explored the effect of some triple drug combinations in a smaller screen.

After the identification of selected promising combination therapies, a screening will be designed to validate the results on patient derived xenografts using the same experimental protocol. This will guide further in vivo experiments and contribute to the development of the in-silico tumour cell simulation models. In addition, the generated data will be used to identify possible drug response biomarkers.

This project is part of RESCUER, a consortium of 15 organizations from 10 different countries, funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 847912. It aims to identify novel characterization methods for breast cancer drug resistance and new knowledge on effective combinatorial treatments. To this end, RESCUER brings together a multidisciplinary group of partners (clinical, scientific, technical, industrial) who express diverse exploitation interests, aimed at bringing results to actual use in several different areas and generating a wider impact within and beyond the core project objectives.

Patient-derived organoids from metastatic colorectal cancer for functional precision oncology

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Most colorectal cancer (CRC) patients lack actionable targets identified through cancer genome sequencing. To address this, *ex vivo* drug sensitivity testing using tumor organoids has emerged as a promising approach in precision oncology.
We have developed a living biobank consisting of 370 organoids derived from liver metastases of 190 patients who underwent hepatic resection. Additionally, multiple lesions from 107 patients (56%) and longitudinal lesions from 13 patients (7%) were modeled to study disease heterogeneity.
Ongoing molecular profiling of these tumor organoids and corresponding tumor tissues involves RNA sequencing, targeted DNA sequencing of 20 CRC-critical genes, and fluorescence-based multiplex immunohistochemistry targeting 14 proteins. Our data confirm that the organoids accurately replicate their original tumors.
Organoids were categorized by morphological phenotypes, reflecting their differentiation status as determined by hematoxylin and eosin staining. Well-differentiated organoids exhibited high CDX2 expression, while poorly differentiated ones showed CK20 expression and were enriched with *KRAS/NRAS* and $BRAF^{V600E}$ mutations. Drug sensitivity testing using a customized panel of 40-47 drugs over a 10,000-fold concentration range indicated that well-differentiated organoids had the most robust treatment responses, particularly to standard chemotherapies like 5-fluorouracil. Notably, high expression of ABC transporter proteins, drug-metabolizing enzymes, and HSF1 was associated with resistance to multiple drugs.
The living biobank serves as a reference for drug nominations in an ongoing phase II umbrella trial for pharmacogenomics-guided treatment of metastatic CRC in the third line (EVIDENT; ClinicalTrials.gov Identifier: NCT05725200). As of July 2024, 85 patients have undergone pharmacogenomics pre-screening, with treatments nominated for 50 patients, including 30 recommended for experimental agents such as PARP, MEK1/2, and BCL-2 inhibitors. Preliminary co-clinical cases from the trial indicate potential clinical benefits from this functional oncology approach.

Biomarker guided ADC therapy

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Antibody Drug Conjugates (ADCs) are therapeutic compounds designed to exert targeted toxicity as compared to conventional chemotherapy. These drugs are highly effective; however, variable therapeutic outcome has been experienced, and the response rate is often as low as 20%. Thus, there is a desperate need for improved predictive biomarkers in the ADC field.

The tumor-targeted activity of ADCs depend on the presence of target molecules on the tumor cell surfaces, and the overall response of ADCs are in general dependent on the expression level of these surface receptors. However, in order to exert therapeutic effect, ADCs are also dependent on cellular uptake and intracellular trafficking for the cytotoxic payload to reach its target within the cell. ADCs are taken up into the cell by endocytosis, regulated by small molecular switches called Rab GTPases, and subjected to endocytic trafficking. Today predictive biomarkers for ADC therapy are limited to expression of the target antigen on the cell surface. We have hypothesized that proteins regulating endocytic processing can serve as predictive biomarkers for ADC response.

We have documented preclinical and in clinical studies of HER2-positive breast cancer that the efficacy of the HER2 targeted ADC trastuzumab emtansine (T-DM1) correlates with the expression of the early endocytic marker RAB5A, suggesting RAB5A as a predictive biomarker for T-DM1 response. Furthermore, we observed that cellular response to trastuzumab deruxtecan (T-DXd), another HER2 targeted ADC, is correlated to the expression of RAB5A, but not to HER2 expression. By comparing the two HER2-based ADCs, we investigated endocytic markers as predictive biomarkers for ADC response and show that T-DXd and T-DM1 are trafficked differently within the cell. Our data indicate that the uptake and intracellular transport of the ADC depends on the exact design, thereby dictating which intracellular markers are suited for predicting treatment response.

Dissecting the mutational drivers behind interclonal interactions in Cancer

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Abstract:

During cancer evolution, accumulation of random mutations can create highly diverse tumor subpopulations over time. Besides having differential intrinsic fitness properties, these heterogeneous tumor subclones are believed to engage in complex interclonal communications that influence their collective fate. Mutations in the oncogene RAS are prevalent across various cancers. Cornerstone studies have established that a single oncogenic insult to this gene is insufficient for sustained tumour growth and malignancy, typically requiring a secondary insult such as a tumor suppressor mutation or tissue inflammation. Notably, previous studies have shown that Ras tumour growth can be boosted by neighbouring Src oncogene mutant cells. However, the interclonal interaction outcomes of Ras mutants and other prevalent gene mutations in Cancer are not characterized. In this work, using a novel *Drosophila* genetic tool, EyaHOST, which allows for precise manipulation of two neighboring epithelial populations *in vivo,* we screened for oncogenes and tumour suppressors which can interclonally affect the growth of a Ras^{V12} tumor. The genes identified in our screen revealed two distinct patterns of interclonal interactions: 1) Interclonal cooperativity, where neighboring tumor subclones promote the overgrowth of Ras^{V12} mutant tumors, and 2) Interclonal competition, where Ras^{V12} tumors decrease in size in the presence of other mutated subclones, which surprisingly leads to increased animal survival.
This study elucidates the subclonal interactions that drive or inhibit tumor growth, offering new insights into the cooperative and competitive dynamics within tumors.

Keywords:
#Tumour #Heterogeneity #Subclones #Cooperativity #Competition #KRAS

Immunological Changes during Tumor Growth in Triple-Negative Breast Cancer

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Introduction:

Breast cancer is the most common cancer among women, with 4200 new cases diagnosed in 2022. Approximately 10% is diagnosed with triple-negative breast cancer (TNBC), characterized by the absence ER, PgR and HER2. TNBC typically affects younger women, with a high proliferative index and an increased risk of recurrence and metastasis. Among breast cancer subtypes, TNBC has the lowest survival rate and limited targeted therapies. While tumor size is crucial in assessing severity and treatment, the immunological changes during TNBC remain unclear. In this study we investigated the immunological changes that occur during tumor growth in TNBC.

Material and Methods

This study analyzed 40 TNBC from the OSLO2 cohort, consisting of, 22 small tumors (T1) and 18 large (T2) tumors without distant metastasis, treated with chemotherapy post surgery. RNA was isolated from fresh frozen samples and sequenced on Illumina NovaSeq6000 sequencer. CibersortX was used to deconvolute bulk RNA into immune cell types, and differential gene expression analysis (DEGA) identified differences between tumor sizes. Immunofluorescence staining CD20, PanKER, CD163 and DAPI validated and visualized findings.

Results

CibersortX analysis revealed that Macrophages and CD8+ T-cells as the most abundant cell type across all TNBC samples. No significant difference in immune composition was observed between small and large TNBC tumors. Tumors with high immune infiltrations showed immune profile dominated by naïve B-cells, activated CD8+ and CD4 memory T-cells, follicular helper T-cells, and M1 macrophages. In contrast, tumors with low immune infiltration were dominated by M0 and M2 macrophages. DEG analysis between small and large tumors revealed down regulation of pathways involved in immune system, cell chemotaxis and signaling genes in larger tumors.

Conclusion

The study found no significant difference in immune cell infiltration or population dynamics during TNBC growth. However, the degree of immune infiltration shapes the tumor immune microenvironment with a down regulated immune response in larger tumors indicating reduced effectiveness of the immune response as tumor grows.

Pan-cancer Cartography of Human Tumors with CytoSPACE

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The tumor microenvironment (TME) plays critical roles in cancer development, tumor progression, and therapy response. However, the phenotypic states and spatial organization of TME cell types remain poorly understood. To address this, we recently developed CytoSPACE (Vahid/Brown/Steen et al., ***Nature Biotechnology* 2023**), a method for optimally aligning single-cell RNA-sequencing (scRNA-seq) data with spatial transcriptomics (ST) data, allowing for rapid and robust whole transcriptome analysis of the TME at both single-cell and spatial resolution.

To better understand the phenotypic and regional cell states within the TME at a pan-cancer level, we applied CytoSPACE to ST and scRNA-seq data from 10 distinct human neoplasms. We compiled ST data from 113 primary tumors, including both carcinomas and melanomas, and multiple spatial profiling platforms. Using CytoSPACE, we aligned scRNA-seq data from 135 tumor samples, covering the same 10 cancer types, creating a detailed pan-cancer map of over 5 million spatially resolved transcriptomes at cell type resolution, thereby surpassing previous studies limited to pre-defined gene panels or low-resolution ST data.

While our survey confirmed previously known spatial markers, it also uncovered new genes and transcriptional programs with regional variation across nine major TME cell types (CD4 and CD8 T cells, NK cells, B cells, plasma cells, macrophages, dendritic cells, fibroblasts, and endothelial cells). For example, we discovered gene expression differences between tumor and adjacent stroma that were strikingly conserved across neoplasms for each cell type and that were well-validated by single-cell ST (MERSCOPE). Surprisingly, we also discovered spatially distinct transcriptional programs conserved across cell types, revealing that many genes exhibit geographic heterogeneity independent of TME cell type or malignancy.

Our results provide an unprecedented atlas-scale overview of the spatial organization of the TME, revealing substantial regional plasticity. These data represent a new resource for defining novel heterotypic signaling axes, diagnostics, and therapeutic targets.

TIGIT modulates the mitochondrial-metabolism axis and preserves fitness in circulating effector memory T cells

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Bispecific T cell engager (BiTE) therapy is emerging as a promising therapeutic approach. However, TCR-redirected stimulation is prone to drive T cell differentiation and exhaustion by activating the P13K/AKT/FOXO1/mTOR pathway. Inhibition of this pathway counter metabolic rewiring and improves mitochondrial function of T cells. Thus, inhibitory signaling through checkpoint receptors could have crucial role in preserving effector potential during BiTE therapy. One promising immune check point is T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (TIGIT). Inhibition of TIGIT along with checkpoint protein PD-1, led to reverse T cell exhaustion. TIGIT signaling modulates the AKT/mTOR pathway, but the effects on metabolism and mitochondrial fitness remains poorly defined. Using mitochondrial probes combined with multi-color flow cytometry, we investigated how TIGIT expression on memory effector T cells can contribute towards mitochondrial fitness, retention of effector molecules and, consequently, T cell degranulation and target cell killing. TIGIT is a member of the immunoglobulin superfamily that binds to CD155/PVR. BiTE stimulation was used in combination with PVR+/- target cell lines or inhibitors of TIGIT-PVR interactions to explore therapeutic potential. We show that TIGIT expression on effector memory T cells is associated with enhanced mitochondrial fitness and retention of mitochondrial mass. Furthermore, transcription factors, Foxo1, involved in the regulation of mitochondrial biogenesis, and Tcf1, master regulator of T cell responses, increased, while mTOR activation is reduced in $TIGIT^+$ CD8 T cells during CD3-CD19 BiTE stimulation. $TIGIT^+$ T cells have increased granzyme B content and display enhanced degranulation. $TIGIT^+$ cells show comparable killing potential against PVR+Nalm6 cell line compared to $TIGIT^-$, while interference of TIGIT-PVR interaction unleashes greater killing capacity by $TIGIT^+$ cells than $TIGIT^-$, which is supported by their preserved mitochondrial fitness. Our results shed light on the importance of TIGIT in preserving effector potential through superior metabolic fitness that could unlock innovative therapies for cancer.

Impact of RAB4 and RAB5 on ADC efficacy in lung cancer

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Antibody drug conjugates (ADCs) are targeted chemotherapy consisting of an antibody conjugated to a cytotoxic drug. The antibody backbone provides selectivity towards the cancer cells, allowing the cytotoxic drug to exert its intracellular action in a more targeted manner. Thus, the mechanism of action of ADCs are reliant on not only the extracellular target, but uptake and processing inside the cell. Little is known on how the mechanisms of endocytosis and intracellular trafficking impact ADC efficacy. We have previously shown both in vitro and in clinical cohorts, that the efficacy of HER2 targeted ADCs in breast cancer is dependent on RAB5, a protein involved in early endocytosis. In the present project we are evaluating the impact of RAB5 and RAB4 on ADC efficacy in lung cancer cell lines.

Three different ADCs are included in this study (i) trastuzumab emtansine (T-DM1), (ii) trastuzumab deruxtecan (T-DXd), both targeting HER2, and sacituzumab govitecan (IMMU-132), targeting TROP-2. To generate a model system, the lung cancer cell line H1975 was genetically modified to stably express excess RAB5. siRNA knock down was also used to evaluated ADC dependency on RAB5. We show here the first results on this master project.

Spatial transcriptomics reveals cancer cell and stromal heterogeneity between center and periphery of pancreatic cancer

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Introduction:

We have performed comparative analyses of the RNA expression profiles of pancreatic cancer cells and surrounding cancer-associated fibroblasts (CAFs) specifically examining the tumor in the center vs periphery using spatial transcriptomic profiling. It is assumed that cancer cells at the invasive front have enhanced migratory and invasive properties, however, evidence is scanty, and the CAFs in this location have not been characterized.

Patients/Methods:

Four well-differentiated pancreatic ductal adenocarcinomas (PDAC) with conventional morphology were investigated with the GeoMx digital spatial profiling system. Five regions of interest were analyzed in the tumor center and periphery using panCK and αSMA as morphology markers. Three cases had confirmed *KRAS* mutation, the fourth was *KRAS* wild type.

Results:

Substantial spatial heterogeneity was identified, characterized by heightened activity in pathways associated with cellular stress, including TNFα-signaling via NFkB, hypoxia, and the P53 pathway, as well as proliferation markers such as MYC targets and mitotic spindle, and epithelial-mesenchymal transition (EMT) markers within both cancer cells and CAFs at the invasive front compared to the central region. Notably, this heterogeneity was less pronounced and frequently exhibited opposite trend in the PDAC case with wild-type *KRAS* compared to those harboring *KRAS* mutation.

Conclusion/Discussion:

Our results show that transcriptional spatial heterogeneity is not random but relates to specific regions, i.e., the center and invasive tumor front. Furthermore, this spatial heterogeneity is manifested in both the cancer cell population and CAFs and seems to be linked to the presence of mutated *KRAS*.

Abstract Title

Identification of clinically relevant drug combinations for melanoma treatment using *ex vivo* drug sensitivity screening

AUTHORS

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Abstract body

Melanoma, an aggressive form of skin cancer, is a **malignant disease** that starts in melanocytes and involves mutations in the Ras-Raf-MEK-ERK, cell cycle regulation, and DNA damage repair pathways.

Despite advances in melanoma treatment, therapy resistance development is common and the survival rates for late-diagnosed melanomas are low. There is **a need for new treatments**, and a promising strategy is to use **drug combinations, especially if they show synergistic interactions**.

Identifying drug combinations, together with **biomarkers** to stratify patients, is a difficult task. The design of combination courses is an empirical exercise based on molecular and clinical knowledge, but the occurrence of **synergy is rare**, and the effects observed in clinic, even for well-established combination courses, are mainly due to additive effect. In this scenario, we aim to establish a **standardized design and analysis method for finding synergistic drug combinations**.

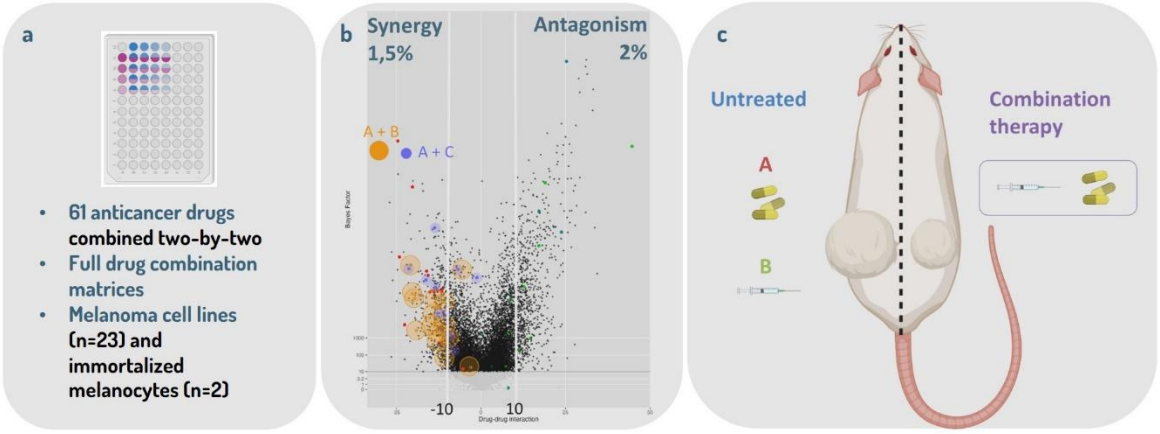
We have developed **a high-throughput *ex-vivo* drug sensitivity screen** which **combined 61 anticancer drugs in full drug combination matrices** and tested them against **melanoma cell lines (n=23)** (Fig. a). The drug effect was calculated as **relative viability** using a metabolic readout.

We also developed new bioinformatics tools to design and analyze drug combination screenings (**screenwerk**) and synergy assessments (**bayesynergy**), getting a classification of **synergistic, antagonistic, or non-interactive**.

Our results confirm that **synergy and antagonism are rare** (Fig. b). Although rare, we have identified some interesting drug combinations and their link to biomarkers. One example is the high synergy observed in the **combination of DNA-damaging agents and Chk1 inhibitors on cells that have a**

compromised DNA damage response. Experiments in xenografted mice confirm that this combination is efficient also *in vivo*, showing no toxicity for the mice (Fig. c).

Altogether, our results have the capacity to improve treatment development and patient stratification for melanoma and other cancer treatments.



Title: **DEVELOPEMENT OF A TGFβ-IL2/15 SWITCH RECEPTOR FOR THE TREATMENT OF SOLID TUMORS.**

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CAR-T cell therapy has demonstrated to be a successful approach in hematological malignancies, but not yet in solid cancers. CAR-T cell activity in solid tumors is limited by the immunosuppressive tumor microenvironment, characterized by the presence of multiple inhibitory factors, including transforming growth factor β (TGFβ). TGFβ has been found to be secreted at high levels in many cancer types, including prostate cancer, which is the most common cancer among males and a leading cause of cancer-related death. In order to enable CAR-T cells more effective against solid tumors, we developed a TGFβ switch receptor (SwR), in which the extracellular domains of the TGFβ receptor are fused to the intracellular domains from the IL-2/15 receptors. We evaluated the SwR in tandem with a CAR that we have previously developed and characterized targeting STEAP1, a cell surface protein highly expressed in prostate cancer.

The SwR-CAR-T cell activity was assessed *in vitro* against STEAP1 positive and negative prostate cancer cell lines, with or without added rhTGFβ, by flow cytometry cytokine and killing assays, real-time cytotoxic assay, multiplex cytokines profiling, proliferation and flow cytometry phenotyping. We showed that the SwR-CAR construct improved the functionality of CAR-T cells in TGFβ-rich environment, as shown by increased T cell proliferation and survival, high cytokine response and enhanced cytotoxicity. In repeated antigen-stimulation *in vitro* assays, the SwR-CAR-T cells showed a higher anti-tumor efficacy characterized by an increased proliferation, killing of tumors cells, cytokines secretion and polyfunctionality in TGFβ-rich environments. We are currently optimizing the *in vivo* SwR CAR-T activity in subcutaneous xenograft mouse model of prostate cancer. In conclusion, the SwR-STEAP1 CAR can be envisaged as a new therapeutic strategy for prostate cancer. The SwR may also be used as an add-on construct for other CAR-T cells or other forms of adoptive cell therapy.

Single cell spatial proteomics of HER2-positive ductal carcinoma in situ

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The molecular phenotype of tumor cells in ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) is surprisingly similar. However, more than half of DCIS cases never become invasive. This points to the microenvironment as a potential regulator of invasion, and immune cells may play important roles. The overrepresentation of HER2-positive lesions in DCIS compared to IDC indicates that the microenvironment may be especially important in this subtype.

In this study, we performed single cell spatial proteomics of two HER2-positive breast tumors; one DCIS and one mixed DCIS/IDC using Nanostring's CosMx platform and the Immuno-oncology protein panel. Twenty fields of view (FOVs) were analyzed for each sample obtaining protein expression for up to 64 protein markers, including tumor and immune cell markers. Cell typing was performed using the CELESTA algorithm which takes into consideration both the protein expression and spatial neighborhood information.

We found heterogeneity in tumor growth pattern, immune cell infiltration and protein expression within tumors. For instance, in the mixed tumor, there was considerable variation of HER2 expression in tumor cells, with lower HER2 intensity in invasive tumor cells compared to DCIS. The immune cell profile was distinctly different between the two samples, but also varied between FOVs within the same sample. There was strikingly higher abundance of B cells in the pure DCIS tumor compared to the mixed tumor, while CD8+ T cells were more abundant in the mixed tumor. Centrally located FOVs in the invasive areas of the mixed tumor showed different immune cell profiles compared to peripheral FOVs. Furthermore, DCIS lesions in the mixed tumor were commonly surrounded by many immune cells. In the pure DCIS, B cells and CD4+ T cells were the most abundant immune cells, and these were frequently located in distinct clusters in the interductal space, possibly representing tertiary lymphoid structures.

This study implies a central role of immune cells and in particular B cells in breast tumor progression. Although it includes only two samples, it highlights B cells as important players in the immune landscape of DCIS and provides a unique possibility to explore more in-depth the heterogeneity of the immune infiltration on a single-cell level.

MATRIX – Norwegian Centre for Clinical Cancer Research

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The Norwegian Centre for Clinical Cancer Research, MATRIX, is a national research centre with an overall ambition to extend the lives and improve the quality of life of patients with hard-to-treat cancers. The centre opened in 2022 and has a broad range of activities. MATRIX develops next-generation precision diagnostics and treatment as well as new, digital cancer care tools that secure treatment and follow-up tailored to individual patients. Moreover, the centre contributes to training of study personnel.

The centre has partners and study sites across Norway. Altogether, fifteen hospitals with cancer departments as well as the University of Oslo and Oslo Metropolitan University are partners in MATRIX. MATRIX is hosted by the Division of Cancer Medicine and the Institute for Cancer Research (ICR) at Oslo University Hospital and is closely linked to activities at ICR as well as national initiatives such as InPreD (national network for precision diagnostics) and IMPRESS-Norway (national clinical trial evaluating efficacy of anti-cancer drugs on new indications). Furthermore, the centre has a broad network of international collaborators and is strongly involved in several ongoing EU-funded projects within the areas of precision cancer medicine (PCM4EU and PRIME-ROSE) and patient-centred and palliative care (MyPath, EUonQOL, JANE and JANE2).

MATRIX is one of four Centres for Clinical Treatment Research (FKB) in Norway. This funding scheme aims to establish and strengthen clinical research environments, and through outstanding research, the aim is to contribute to improved outcomes for Norwegian patients. MATRIX receives funding from the Norwegian Cancer Society and the Research Council of Norway.

Cell migration drug screening in rhabdomyosarcoma cells using CellTraxx, a novel automatic cell tracking tool

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Rhabdomyosarcoma (RMS) is a malignant soft tissue tumour usually occurring in young children. Alterations in the receptor tyrosine kinase, FGFR4 (fibroblast growth factor receptor), resulting in increased signalling have been associated with metastasis and poor prognosis. One important trait of metastatic disease is the ability of the cancer cells to migrate and invade nearby and distant tissue. In this project we aim to investigate which signalling pathways downstream of FGFR4 are important for RMS cell migration and proliferation. To study cell migration, we have developed a computational tool called CellTraxx that automatically tracks and measures the velocity of migrating cells. RMS-derived cells overexpressing a constitutively active FGFR4 (RMS559 cells) were subjected to a highly selective inhibitor screen consisting of 462 compounds targeting a wide variety of signalling pathways including MAPK, PLCγ, and PI3K. The cell velocity was measured using CellTraxx 8-10 hours after treatment. Each inhibitor was tested at four different concentrations, resulting in the analysis of nearly 2000 films across approximately 500 000 cells. Validation of the hits from the screen, revealed that inhibition of proteins in several common signalling pathways such as the MAPK and the PI3K pathways reduced cell migration and often also cell viability. Surprisingly, inhibition of FAK and ROCK, two proteins involved in assembly/disassembly of focal adhesions, led to increased migration. We are now investigating if there is a link between FGFR4 signalling and FAK and ROCK.

The role of simaphagy and the ESCRT-I subunit VPS37 in cellular signaling and carcinogenesis

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External signal cues arriving at the plasma membrane are transduced into the cell by a variety of membrane-residual receptors. Ligand-bound receptors get endocytosed and segregated into endosomal sub compartments in an ESCRT machinery dependent manner. This sorting eventually leads to signaling termination. Defects in the ESCRT machinery result in prolonged receptor activation on endosomes which is associated with various types of cancer.

Recently, we identified simaphagy as an autophagic failsafe mechanism to remove these hyper-signaling endosomes. Upon simaphagy induction, the autophagy machinery gets recruited, followed by engulfment of endosomes by autophagic structures and lysosomal degradation. In the absence of the ESCRT-I components VPS37A/B this process is perturbed, resulting in sustained cellular signaling from endosomes and increased cell migration. However, molecular details of factors involved in simaphagy regulation and the consequences of simaphagy stalling on signaling pathways and carcinogenesis have not been investigated so far.

This project aims to provide details on these processes. Initially, a mass spectrometry-based secretome analysis is performed to identify changes in secretion upon simaphagy stalling. Candidate factors and the associated pathways are currently dissected in detail for ESCRT-I dependent effects on cell signaling and migratory behavior. To address the relevance of simaphagy for carcinogenesis, cancer cells such as prostate cancer cells, expressing low levels of VPS37, are used as model system.

This project will help to identify regulatory elements for simaphagy initiation and to reveal pathways affected by simaphagy stalling upon ESCRT-I subunit VPS37A/B depletion. On the long term, it aims to identify new therapeutic targets to manage the effects of prolonged receptor signaling and to provide treatment options of cancers with reduced VPS37 expression.

Core facility for Advanced Electron Microscopy

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The core facility for Advanced Electron Microscopy (EM) provides users with a large variety of methods within conventional EM, such as epon-embeding for ultrastructural studies as well as immunocytochemical localization of antigens on ultrathin cryosections. More advanced techniques such as high-pressure freezing followed by freeze substitution, correlative light electron microscopy (CLEM) and electron tomography are also available. We are continuously expanding into new methods which our users can benefit from. Our latest technological addition is the use of STEM-tomography, that allows for the acquisition of tomograms from very thick slices of biological samples. We help our users study a vast variety of questions and samples; all the way from small samples such as proteins and extracellular vesicles, through different cell culture samples and organoids, via model organisms such as D. Melanogaster. We also have experience from work in mice and rat tissues, such as tumors or heart muscle. We offer full- service packages and/or training for users in both specimen preparation and microscopy. Software for analysis, tomogram reconstruction and segmentation of 3D structures, such as iTEM, 3D mod and Amira is available for user.

PoDCall – Positive Droplet Caller – a tool for automated calling of droplets in droplet digital PCR experiments

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Oslo University Hospital, The Norwegian Radium Hospital, Institute for Cancer Research, Department of Molecular Oncology, Epigenetics Group (Lind lab)

Abstract

Droplet digital PCR (ddPCR) holds great promise as a highly sensitive method for absolute quantification of nucleic acids, and can detect rare targets in a complex background of other sequences. The method has been most frequently used for detection of rare sequences, copy number variation and gene expression, but has great potential also for DNA methylation analysis. Yet, there has been a lack of standardized methods for analyzing data from methylation-specific ddPCR. Thus, we developed PoDCall, Positive Droplet Caller, which is an R package that provides automated and robust calling of positive droplets in ddPCR experiments (Jeanmougin et al. 2023) performed on the Bio-Rad platform.

PoDCall reads raw two channel fluorescence amplitude data exported from either QuantaSoft or QX Manager, and based on a Gaussian mixture model approach, sets well-specific thresholds to call positive droplets. Based on the number of positive droplets, PoDCall returns quantification and, if relevant, normalization of the target of interest. All of PoDCall's functionality is accessible in the accompanying Shiny application, which provides an interactive and user-friendly interface for data analysis, including visual inspection of droplet plots and manual adjustment of thresholds. PoDCall is freely available as an R package in the Bioconductor repository at https://bioconductor.org/packages/PoDCall/.

Although PoDCall was originally developed for ddPCR DNA methylation analyses, it has been shown to perform well also on other types of ddPCR data. In a recent publication, PoDCall was presented as one of the better overall performing tools for calling positive droplets in ddPCR experiments, using mutation data (Vynck et al. 2023).

In conclusion, we have developed the PoDCall algorithm, which is a user-friendly tool for automated calling of positive droplets in ddPCR analyses. Importantly, PoDCall contributes

to increased robustness and standardization of the ddPCR data, and thus ensures consistency across experiments.

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Enhancing BCG immunotherapy with photochemical treatment

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Intravesical immunotherapy with Bacille Calmette-Guérin (BCG) is the standard of care for high-risk non-muscle-invasive bladder cancer. Although its mechanism of action is not fully understood, BCG immunotherapy significantly delays tumour recurrence in most patients. However, 25-45% of them do not respond to the therapy and an additional 40% eventually relapse.

In this project, we hypothesize that delivering BCG immunotherapy by photochemical internalization (PCI) enhances the efficacy and durability of the antitumour response.

PCI is a drug delivery technique that involves bursting the cell's endosomes upon light-activation of the photosensitizer TPCS2a, thereby releasing entrapped therapeutic agents into the cytosol. PCI can improve immunotherapies by forcing cross-presentation of tumour-specific epitopes as they get released in the cytosol of antigen-presenting cells (APCs). This in turn enhances recruitment and activation of cytotoxic T cells. Recent evidence suggests that PCI delivery of BCG enhances its immunogenicity in tuberculosis vaccination by inducing local inflammation and stronger CD4 and CD8 T-cell response. In the context of bladder cancer, this could lead to a more robust antitumour response.

Here, BCG-TPCS2a complexes were prepared for *in vitro* and *in vivo* experiments. In vitro, the complexes were localized in APCs, but not in cancer cells, with no observed direct cytotoxicity upon illumination. Potential immunological marker changes caused by the complexes are under investigation in bone marrow-derived models. *In vivo*, the complex was injected intratumorally in a subcutaneous bladder cancer model in immunocompetent mice, followed by tumour illumination and growth monitoring.

Preliminary results show that PCI-delivered BCG immunotherapy delays tumour growth and induces tumour remission in some tested regimes. The optimal regime will be further investigated by analysing tumour and lymphoid tissues for immunophenotyping and a selection of T-cell- and APC-specific immunological markers using a large full spectrum flow cytometry panel.

Unraveling the role of transcriptional networks in pancreatic cancer cell states using machine learning prediction algorithms

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancer types often diagnosed late with little information about the early stages of cancer initiation. Cancer heterogeneity and chemotherapeutic resistance further compound the problem, causing a major obstacle in improving the survival of PDAC patients. The mechanisms of drug resistance in PDAC are not clear and are partially attributed to epithelial-mesenchymal transitions (EMT) and cancer cell plasticity in a complex tumor micro-environment (TME). The state and identity of a cell is controlled by lineage-specific transcription factors (TF). Hence, it is important to study these fundamental mechanisms in PDAC development.

In this project, we utilized state-of-the-art machine learning methods to model robust relations between TFs and gene regulatory networks (GRNs) from treatment-naïve and neoadjuvant-treated PDAC patients. In our analysis, we can recover well-known and new TFs related to pancreatic cell identity and PDAC progression. These TFs were further perturbed in-silico and the cells were examined for their expression profile and role in controlling cell state. The predictions reported a strong activity from both well-known and newly predicted TFs regarding the cell state transition and their regulatory role. This is critical for further validation and in establishing their role in delineating PDAC disease progression and therapy resistance. The findings from our analysis will be presented and discussed in this meeting.

KA103, a novel targeted toxin for Head and Neck Cancer

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world, with over 878,000 new cases diagnosed and over 440,000 deaths in 2020. Despite the heterogeneity of HNSCCs, it has been found that the transmembrane protein epidermal growth factor receptor (EGFR) is overexpressed in nearly 90% of cases, making it a potential therapeutic target. The EGFR monoclonal antibody cetuximab is the only targeting drug approved for this indication. However, intrinsic and acquired resistances provide considerable limitations for treatment efficacy.

The anticancer properties of targeting molecules can be enhanced by linking them to a toxin, generating a targeting toxin (TT). Moreover, we have designed and recombinantly produced KA103, a novel EGFR-targeted fusion protein based on EGF and gelonin, a ribosome inactivation protein toxin (RIP).

Here, we show that KA103 has similar efficacy as gelonin in a cell-free reticulocyte system but exerts targeted cytotoxicity, as shown in EGFR-positive and negative cell lines. Moreover, KA103 is highly effective against EGFR-expressing HSCC *in vitro*, especially against cetuximab-resistant SCC040 cells, indicating KA103 to overcome cetuximab resistance. In addition, *in vivo* data depicts that KA103 induces SCC040 tumor growth delay without detecting severe toxicity. Furthermore, we have established that fluorescently labeled KA103 can be used to evaluate *in vivo* biodistribution, showing the highest labeled KA103 uptake retention at the tumor.

In conclusion, our findings show that KA103 production is highly reproducible and induces EGFR-targeted toxicity in HNSCC cell lines. In addition, KA103 promotes tumor growth delay in HNSCC models without general toxicity and with high KA103 uptake at the tumor. The presented results warrant further preclinical evaluation of KA103.

Peripheral blood Immune cell phenotypes in metastatic breast cancer patients

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Immune checkpoint inhibition has shown some efficacy in metastatic breast cancer (mBC), but often hindered by immunosuppressive mechanisms. Understanding these mechanisms is crucial for developing personalized treatments, and monitoring peripheral blood could offer a less invasive alternative to repeated biopsies. In this study, we conducted a detailed mass cytometry analysis of peripheral blood immune cells from 104 patients with HER2-negative mBC and 20 healthy donors (HD). Results showed higher monocyte levels and lower CD4+ T cells and plasmacytoid dendritic cells in mBC patients compared to HD. mBC patients also had more effector and regulatory T cells, with increased expression of immune checkpoint markers, and a shift towards a Th2/Th17 phenotype. T cell phenotypes correlated with T-cell functionality, as measured by IFN-γ production. Additional analysis showed that previous chemotherapy and CDK4/6 inhibition influenced immune cell counts and phenotype. Paired PBMCs and tumor analysis from 63 mBC patients revealed moderate correlations between peripheral CD4+T and NK cells with their counterparts in tumors. A specific cluster of CD4+ T cells in peripheral blood, characterized by co-expression of multiple immune checkpoint receptors, was inversely related to CD4+ T cell infiltration in tumors. The identified immune signatures highlight an immunosuppressed environment in mBC patients progressed or relapsed on standard treatments, and were consistent with ongoing chronic inflammation. These immuno-suppressive mechanisms may be investigated as therapeutic targets, and for use as biomarkers of therapeutic response.

Characterizing the localization and function of the BEACH domain containing protein NBEAL2.

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The Beige and Chediak-Higashi (BEACH) domain is a highly conserved protein domain with unknown function, found in nine human proteins called BEACH domain containing proteins (BDCPs). Function of several members of this family, exemplified by autophagy-linked FYVE protein, neutral sphingomyelinase activation associated factor and lipopolysaccharide-responsive beige-like anchor protein have been associated with autophagy. Loss of function mutations in seven BDCPs are linked to different genetic disorders. Gray Platelet Syndrome (GPS) is a rare congenital recessive autosomal disorder caused by mutation in BDCP neurobeachin like 2 (NBEAL2). It is characterized by mild to moderate bleeding due to decrease or absence of alpha-granules in platelets. Although NBEAL2 has been linked to GSP, the structure and function of this protein as well as other BDCPs is still unknown. In this project NBEAL2, as well as closely related Neurobeachin like 1 (NBEAL1), have been cloned and stably transfected into HeLa TRex Flp-in cells. Surprisingly, despite high sequence homology, both proteins localizes to different subcellular compartments that have been mapped by co-localization studies. Using deletion mutants, we identified protein domains essential for distinct subcellular localizations of NBEAL1 and NBEAL2. Utilizing stable HeLa cell line we purified the full-length recombinant NBEAL2 with intention to resolve its 3D structure with Cryo-EM approach.

Biobanking and Sample Processing in the IMPRESS-Norway Study

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Abstract

IMPRESS Norway is a national, clinical cancer research study. The study offers all patients with advanced, incurable cancer a targeted treatment based on the patient's molecular gene changes in the tumor. This is precision diagnostics; focusing on the molecular profile instead of the location of the cancer. The IMPRESS study has close collaboration with the diagnostics, InPreD. Patients are offered an extended gene panel analysis which maps 523 genes in the cancer tumour. This panel is called TSO500 (TrueSight Oncology 500) and covers the majority of the most common gene changes in cancer tumors for which there is treatment.

Our role in this study is to receive, process, analyze and biobank study material. In addition, we coordinate shipment of blood test kits to the study centers. The sample materials we analyze are blood samples and fresh-frozen tumor biopsies. At screening level, plasma from Cell-Free DNA BCT STRECK tubes is used for ctDNA analysis. This is compared with the DNA profile analyzed by the TSO500 gene panel. Whole-genome sequencing is analyzed on the biopsies taken at various times in the study. Our task will be to slice, isolate DNA and RNA before it is prepared for sequencing. Quality assurance of the pre-analytical value chain is important to achieve reliable results and is therefore a large part of our everyday work.

Autophagic clearance of protein aggregates – an imaged-based approach –

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Neurodegenerative diseases (NDs) are devastating diseases of the central nervous system which are caused, or accompanied, by the formation of protein aggregates. While these aggregates are also substrates of selective autophagy, the mechanistic role of autophagy in NDs is far from being understood. We have established a workflow that aims at identifying novel regulators of autophagic clearance of protein aggregates (aggrephagy) and elucidating their role in development of NDs.

Radiosensitization of Prostate Cancer through Therapeutic Inhibition of BET Bromodomains

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The mainstay treatment for metastatic prostate cancer (PC) is radiation. The PARP inhibitors, including Olaparib, have shown promise in enhancing radiation therapy by disrupting DNA damage repair pathways. Epigenetic drugs such as BET inhibitors, including AZD5153 and JQ1, target transcriptional regulators and have potential as radiosensitizers. We find that combination treatment of Olaparib with BET inhibitors significantly enhances the radiosensitivity of PC cells. This combination leads to a substantial reduction in clonogenic survival and cell proliferation, primarily through inducing G1 cell cycle arrest. The presence of the androgen receptor (AR) and c-Myc expression levels further modulate the effectiveness of these treatments, highlighting the importance of personalized therapeutic approaches based on specific molecular profiles. In vivo studies corroborate these findings, demonstrating that these combinations lead to tumor growth arrest without significant toxicity. Collectively, we provide strong evidence for combining epigenetic drugs or Olaparib with radiation to induce irreversible tumor growth arrest, avoid toxicity, and increase radiation efficacy in PC treatment.

Unveiling the Role of ESCRT-III subunit in DNA Damage Response

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The endosomal sorting complex required for transport ESCRT-III is a machinery involved in critical functions, remodeling and repair of cytoplasmic and nuclear envelope membranes. Among these subunits, CHMP4B is the main component of the ESCRT-III machinery. Surprisingly, we found that CHMP4B exists also as puncta in the cell nucleus. These nuclear puncta increase significantly after the use of different DNA damaging agent treatments and they do not seem to be associated to nuclear envelope ruptures, but they rather correspond to areas of late DNA damage marked by γH2Ax. Our data show that CHMP4B co-localizes with the non-homologous end joining factor 53BP1, but it is excluded from DNA damage sites containing homologous recombination factors BRCA1 and Rad51. In addition, a knock-down of 53BP1 induces a total loss of CHMP4B nuclear puncta. Intriguingly, while we do not detect direct signs of nuclear envelope damage, we observe that the 53BP1-CHMP4B foci localize in close proximity to invaginations of the nuclear envelope. This is consistent with recent research showing that double strand breaks (DSB) localize to the periphery of nuclear envelope to trigger the end-resection step. However, the underlying mechanisms leading to the anchoring of DSBs to the nuclear envelope and the function of this remain unclear. The CHMP4B-53BP1 localization is exciting and raises the question of whether the nuclear envelope could be an anchor for specific DNA damage signaling and repair. DSB mobility of damaged chromatin is crucial for efficient DNA repair, and this mobility might require the CHMP4B protein. Finding evidence that CHMP proteins, and their interactions with the nuclear envelope, play a role in the spatial organization and dynamics of the DNA damage response will open new exciting approaches in cancer therapy.

FGFR1 in Hormone Receptor-Positive Breast Cancer Drives Endocrine Resistance and Suppresses Innate Immunity

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Fibroblast Growth Factor Receptor 1 (FGFR1) has emerged as a central player in breast cancer progression. Alterations in FGFR1, such as gene amplification and overexpression have been identified in breast cancers, driving aberrant signaling pathways that promote tumor growth, metastasis and drug resistance. Notably, in estrogen receptor-positive (ER+) breast cancer, FGFR1 amplification or overexpression has been linked to endocrine resistance and poor therapeutic outcomes, highlighting the potential of FGFR1-targeted therapies.

Our current research focuses on elucidating the dual role of FGFR1 in breast tumor progression, particularly its contribution to endocrine resistance and its regulatory influence on innate immune sensing and subsequent Type 1 interferon (IFN) responses.

We have previously demonstrated a direct association between FGFR1 and endocrine resistance in ER+ T47D breast cancer cells. Interestingly, we also identified an inverse correlation between FGFR1 expression and the Type 1 IFN response. Our studies revealed that stimulation of T47D cells with Type 1 IFNs induces FGFR1 and ESR1 expression, leading to suppression of interferon-stimulated genes (ISGs). Additionally, we observed that FGFR1-mediated suppression of the Type 1 IFN response is linked to intracellular nucleic acid sensors. Inhibition of FGFR1, either through the pharmacological agent erdafitinib or shRNA-mediated silencing, enhanced the expression of immune sensors and ISGs in T47D cells. Furthermore, conditioned media from FGFR1-expressing, tamoxifen-resistant (T47D-TAMR) cells suppressed Type 1 IFNs, immune sensors, and ISGs expression in monocyte-derived dendritic cells, suggesting that FGFR1 suppresses innate immunity. In vivo, FGFR1 inhibition in an FGFR1-amplified luminal PDX tumor model led to tumor regression by enhancing the tumor cell-intrinsic Type 1 IFN response.

These findings suggest that FGFR1 acts as an innate checkpoint, potentially exploited by tumor cells to dampen Type 1 IFN responses and evade antitumor immunity in hormone receptor-positive breast cancer.

“TCR-T cell therapy for the treatment of patients with multiple myeloma”

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Introduction

Multiple myeloma (MM) is one of the most common types of blood cancer, accounting for 10% of all hematologic malignancies in developed countries. It is characterized by the accumulation of malignant plasma cells in the bone marrow, which typically produce large quantities of abnormal, monoclonal immunoglobulins. Over the last decades, significant treatment improvements have been made, most notably with the addition of proteasome inhibitors, immunomodulatory drugs, monoclonal antibodies, and chimeric antigen receptor (CAR) T cells. The introduction of the B-cell maturation antigen (BCMA) (CAR-T) cells in clinical settings has shown great promise in the treatment of MM, however relapses, mainly driven by antigen loss, are common. This underlines the need for additional target discoveries for cell-based therapies and treatment modalities. T-cell receptor (TCR) therapies are a promising candidate for filling the gap in treatment as it allows us to expand the repertoire of targets; however, no TCR therapy is currently available for MM. Here, we describe the development of a novel TCR targeting immunoglobulin A (IgA), as a potential treatment for MM.

Methods: Peptides derived from IgA were identified using mass spectrometry. By using our established TCR identification platform, we identified several TCRs targeting our peptides of interest. These candidate TCRs were then evaluate for target specificity, sensitivity, safety, and efficacy through a series of in vitro assays.

Results: Our preliminary data demonstrate that the candidate TCRs are highly specific to their target, showing no signs of cross reactivity. In addition, CD8+ T-cells transduced with the relevant TCR exhibit robust killing of both MM cell lines and primary tumor samples.

Conclusion: We have identified IgA-derived peptides that are promising candidates for TCR-based therapy in MM. The TCRs targeting these peptides have shown a promising efficacy and safety profile *in vitro*.

Optimizing Incomplete Multi-view Clustering: From MATLAB to Python

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Abstract

Patient stratification is important because it enables tailored treatments and improves clinical outcomes by categorizing patients based on their similarities and specific characteristics. With the rise of **multi-omics** data, several sources (**views**) of molecular-based patient information can be used to augment the correct identification of such patient groups using **unsupervised Machine Learning** methods. However, the incompleteness of multi-omics data poses a significant challenge, as missing values can hinder the recognition of meaningful patterns.

Addressing this challenge, several incomplete multi-view clustering methods have been implemented. Originally implemented in MATLAB, the clustering methods from the survey [1] were inaccessible to many due to MATLAB's proprietary nature. To enhance accessibility and performance, we translated these algorithms into **Python**, leveraging its widespread use and powerful computational tools. This transition aims to improve execution times while maintaining or surpassing the original algorithms' accuracy.

We have successfully translated two of these clustering algorithms, namely the Doubly Aligned Incomplete Multi-view Clustering (DAIMC) and the Self-representation Subspace Clustering for Incomplete Multi-view Data (IMSR). We benchmarked our Python implementations against their MATLAB counterparts using the Adjusted Rand Index (ARI) and Adjusted Mutual Information (AMI) clustering metrics. Depending on the datasets and the percentage of missing values, our Python implementations provide a **2 - 100x speed-up** compared to the MATLAB versions, without compromising performance.

Our efforts to translate and optimize these clustering methods not only make them more accessible but also significantly enhance their performance, providing a powerful tool for precision medicine and the analysis of incomplete multi-omics data.

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TCR-engineered T cells recognizing a shared β-catenin mutation presented on two prevalent HLA-alleles eradicates solid tumors in vivo

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We aimed to identify T-cell receptors (TCRs) capable of recognizing recurrent mutations within β-catenin, a protein encoded by the CTNNB1 gene. β-catenin is mutated in 3.3% of cancers, with several recurrent gain-of-function mutations in the N-terminal domain. These mutations are known to contribute to cancer progression by driving cell cycle induction and proliferation, whereby offering attractive targets for TCR therapy. By performing immunopeptidomics on human leukocyte antigen (HLA)-monoallelic cell lines transduced to express CTNNB1 hotspot mutations, we found that a recurrent mutation in the CTNNB1 gene ($CTNNB1^{Mut}$) was presented in the context of the two frequent HLA-alleles, HLA-A*02:01 and HLA-A*24:02. Targeted immunopeptidomics was performed to confirm and quantify neopeptide processing and presentation on cell lines endogenously expressing the mutation. To investigate whether the identified peptides could induce neoantigen-reactive TCRs from healthy donor naïve T cell repertoires we utilized an *in vitro* screening pipeline to identify T cell clones capable of reacting to the peptide antigens. Here, we identified two T-cell receptors recognizing the recurrent CTNNB1 mutation, one restricted by HLA-A*02:01 and the other restricted by HLA-A*24:02. The neoantigen-reactive TCRs were sequenced to enable retroviral expression of TCRs in healthy donor T cells. Both the HLA-A*02:01 and the HLA-A*24:02 restricted $CTNNB1^{Mut}$ TCRs recognize their cognate peptides at high sensitivity while showing no reactivity to the corresponding $CTNNB1^{WT}$ peptides. T cells modified to express the $CTNNB1^{Mut}$ TCRs demonstrate promising efficacy, effectively eliminating endogenous $CTNNB1^{Mut}$ cell lines *in vitro* and inducing tumor eradication and prolonged survival in an *in vivo* melanoma mouse model. The promising results of the identified TCRs indicate their potential for targeting a recurrent mutation in CTNNB1 presented in the prevalent context of HLA-A*02:01 or HLA-A*24:02.

Identification of biomarkers for risk stratification and treatment response in colorectal cancer using a high-throughput digital pathology platform: The BIOMAN study

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Colorectal cancer (CRC) is a heterogeneous disease, both at the molecular level and with respect to disease outcome and treatment response. Development of robust biomarkers is needed to improve risk stratification and clinical decision-making. For this purpose, we have designed a nation-wide clinical resource for biomarker analyses in a population-representative cohort of 4,000 patients with stage I-III CRC. The Biomarker Norway (BIOMAN) series includes patients from all Health Regions in Norway diagnosed between 2008-2013. Detailed clinical information is gathered from hospital records by responsible clinicians at each participating hospital and implemented in a joint database. Representative tumor blocks from each patient have been evaluated by local pathologists and sent to the Department of Molecular Oncology at Oslo University Hospital. We have established a digital pathology platform for precise and high-throughput biomarker analyses, including tissue microarray (TMA) construction, multiplexed fluorescence-based immunohistochemistry and digital image analyses using the PhenoImager HT system from Akoya Biosciences (Lopes et al., Lab Invest 2020). In total, 28 TMAs have been completed consisting of 1 mm cores from 3,549 diagnostic tissue blocks from 3,384 patients. Several multiplexed biomarker cocktails with up to 7 protein markers have been optimized, including both cancer-intrinsic and tumor microenvironment-specific markers. In parallel, all corresponding diagnostic H&E-stained whole-tissue sections are imaged for machine learning of tumor morphology and integration with *in situ* fluorescence-based protein expression. So far, a major cohort of CRC patients from Oslo University Hospital has been used as test panel for selected epithelial markers and immune markers (Bergsland et al., ESMO Open 2020; Mod Pathol 2022; Majid et al., Cell Oncol 2024).

Using mtDNA mutations as biomarkers for lineage tracing in tumors and distant metastasis.

Senior Scientist Per Olaf Ekstrøm, in Eivind Hovig's research group, Department of Tumor Biology

The identification and monitoring of tumor cell lineages are crucial for understanding cancer progression and metastasis. Mitochondrial DNA (mtDNA) mutations are a well suited biomarker due to their high mutation rate and clonal inheritance. We have used somatic mtDNA mutations for lineage tracing in tissue from primary tumors like testis, prostate, colon, rectal, bladder and breast cancer. Additionally, some lymph nodes and metastases have also been analyzed. Mutations were detected by our own developed assay, cycling temperature capillary electrophoresis (CTCE), which exploit the physical properties of the DNA under temperature settings. The method has a limit of detection for a variant of about 1%. To enhance the precision of our mutation detection, laser capture micro dissection (LCM) was utilized to isolate smaller areas within the tumor and metastatic tissues. Areas selected were done in an objective grid like structure of 48 up to 384 samples from each section, with an average of 30 cell pr. LCM sample. This approach showed that low frequency mutation could be detected and quantified.
Our findings demonstrate that mtDNA mutations, detected using CTCE and LCM, can serve as reliable markers for tracking tumor cell lineage and offer a powerful tool for studying the dynamics of cancer metastasis. This approach could improve our understanding of metastatic patterns and potentially be used as biomarker for relapsing for example in urine.

The ER protein Protrudin protects against apoptosis

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Protrudin is an endoplasmic reticulum (ER) protein with multiple binding domains. This protein can promote neurite outgrowth, axonal regeneration in mature rodent central nervous system, and the formation of phagocytic cups in human retinal pigmented epithelial cells. In addition, Protrudin is associated with tumorigenic phenotypes such as invadopodium formation, angiogenesis, cell growth, extracellular matrix degradation, and transfer of invasiveness to surrounding cells. Most of these functions are facilitated by ER-endosome membrane contact sites formed by Protrudin and its various interaction partners.

By utilising an unbiased high-throughput drug screen with 528 small-molecule anticancer compounds, we identify a novel function for Protrudin, namely apoptosis protection. Here we show that cancer cells expressing Protrudin are less likely to undergo programmed cell death compared to cancer cells lacking Protrudin. Our data from advanced microscopy, suggests that in addition to forming contacts with endosomes, Protrudin in the ER localizes near mitochondria together with its binding partners PDZD8 and FKBP38. The latter protein is associated with apoptosis protection by stabilizing the anti-apoptotic protein BCL-2. Furthermore, we identify a new binding site for FKBP38 in Protrudin and we demonstrate that Protrudin knockout causes a relocation of FKBP38 from the ER to the mitochondria followed by destabilization. Taken together these data propose that Protrudin can protect cancer cells from apoptosis by forming ER-mitochondria contact sites and stabilise anti-apoptotic proteins. This discovery provides novel insight into future personalised medicine approaches for cancer patients with high Protrudin-expressing tumours.

PRIME-ROSE: Collaboration for implementation of precision cancer medicine in Europe

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PRIME-ROSE is a European Cancer Mission project focused on precision cancer medicine trials. Currently, it includes 11 ongoing or soon-to-start DRUP-like clinical trials across various countries (www.prime-rose.eu). These trials are investigating the efficacy of biomarker-driven treatments beyond their current labels across a wide range of tumor types. Such testing in new cancer subtypes requires the ability to find patients with rare biomarkers or tumors to offer treatment with matched drugs available in each trial's drug portfolio.

In PRIME-ROSE, the trials now share and aggregate data for each tumor type/biomarker/drug combination. This cooperation is expected to significantly enhance the recruitment rate for each cohort, thereby accelerating evidence-building and potentially impacting patient care. So far, 13 cohorts have been filled, enabling the analysis of clinical outcomes and the determination of whether these cohorts should progress to the next stage. The network currently serves a recruitment area encompassing 71 million inhabitants.

In addition to data merging, the PRIME-ROSE project is developing new methodologies for utilizing synthetic control arms by leveraging access to large datasets from national cancer registries across Europe. The use of synthetic control arms is key for enabling health economic evaluations necessary for reimbursement decisions. To support the implementation of new drugs, the project also

addresses critical issues related to on-label and off-label reimbursement and the pathways for approval of new precision cancer medicines in different European countries.

Photoactivable tetrachlorin-conjugated chitosan nanoparticles and the GPX4 inhibitor RSL3 affect the mitochondrial potential and metabolism in MDA-MB-231 breast cancer cells

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Ferroptosis has been described as an immunogenic form of cell death characterized by iron dependency, lipid peroxidation, and accumulation of reactive oxygen species (ROS) and recently gaining great attention for its role in diseases such as cancer. Tetrachlorin-conjugated chitosan nanoparticles (TPC-CS NPs) prepared in our laboratory have photosensitizer-inducing ROS production upon illumination and were already shown to induce ferroptosis in cancer cells [1]. Here, we aimed for combined treatment with TPC-CS NPs and the ferroptosis inducer RSL3 in breast cancer cell lines. We selected two phenotypically different breast cancer cell lines, MDA-MB-231 and MCF7. MDA-MB-231 is a triple-negative cell line with mesenchymal phenotype and high metastatic potential, whereas MCF7 is estrogen and progesterone receptor positive and considered as poorly aggressive with low metastatic potential. Using this model we have already shown that the treatment with TPC-CS NPs and RSL3 induce several changes in MDA-MB-231 cells but not in MCF7 cells, including changes in cell viability, GPX4 levels or lipid peroxidation (data not shown).

Since we observed such changes, we decided to investigate also the effect of TPC-CS NPs and RSL3 treatment on mitochondrial potential and cellular metabolism using Seahorse analyzer. We detected significantly lower mitochondrial potential in MDA-MB-231 cells upon illumination for TPC-CS NPs and RSL3 but no effect in MCF7 cells. Moreover, this effect was inhibited using a ferroptosis inhibitor. Metabolic experiments in the cell lines revealed significant differences in their response. While MCF7 cells showed no differences between the treatments, MDA-MB-231 displayed significantly decreased mitochondrial respiration upon illumination for TPC-CS NPs and RSL3 compared to treatments alone.

We show different responses in MDA-MB-231 and MCF7 to TPC-CS NPs and the ferroptosis inducer RSL3 in mitochondrial potential and cellular metabolism in connection with ferroptosis.

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Gene- and imaging-based biomarkers for hypoxia-targeted therapy in combination with radiotherapy in cervical cancer

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Tumor hypoxia is associated with radiation resistance and metastasis in many cancer types, including cervical cancer, and is both a predictive marker for risk of recurrence and a therapeutic drug target. Methods for classifying patients according to hypoxic status are important for reliable drug evaluation and to avoid added toxicity to patients with no expected benefit. Our aim is to develop robust hypoxia biomarkers that can be translated into clinical trials with radiotherapy and hypoxia-targeted therapy in cervical cancer.

Biopsy-based gene expression and medical imaging provide complementary information and could both be useful hypoxia biomarkers. We identified imaging features associated with outcome in 78 patients treated with conventional chemoradiotherapy. By combining these features with gene expression profiles of the same patients, imaging were shown to reflect a gene expression program activated under hypoxia. Based on these data, a 6-gene hypoxia classifier was developed. Classifier robustness was demonstrated by successful validation of prognostic value across two cohorts of 108 and 131 patients and across the Illumina beadarray and RT-qPCR platforms. The prognostic value was independent of existing clinical markers.

We further compared hypoxia classification by the two biomarkers in 118 patients with paired data. Totally 75% of the tumors were classified with the same hypoxia status by imaging and genes. Combining the biomarkers into a single hypoxia score, significantly improved the prognostic value compared to each biomarker alone.

Metformin is a low-cost, non-toxic, anti-diabetic drug that targets hypoxia pathways reflected by our gene classifier. We have run a randomized trial adding metformin prior to and during chemoradiotherapy in cervical cancer. Hypoxia surveillance by our imaging biomarker has ensured that no drug-induced increase in hypoxia occurred prior to radiotherapy. Based on serial biopsies and images collected in the trial, the hypoxia biomarkers are evaluated and the metformin effect is explored in ongoing work.

Abstract: Norwegian Cancer Symposium 2024

Title: Single cell analysis of paired lymph nodes and primary tumors in breast cancer patients

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A sentinel lymph node (SN) is the primary node draining the tumor and is assumed to be affected early in the metastatic process. The sentinel node holds a key position in the immune response against tumor in breast and represents a unique connection between the tumor and the host immune response. We hypothesize that the immune profile in the primary tumor and the paired lymph node (LN) is different during tumor progression.

3.6 million single cells from paired primary tumor and lymph nodes from 28 breast cancer patients (Oslo2 cohort) was analyzed by single cell mass cytometry (CyTOF) with a 47 antibody immune panel and characterized by a semi-automatic gating approach (FlowSOM).

We identified a skewing towards higher abundance of memory CD4 and CD8 T cells expressing an exhausted phenotype in LN with metastasis, and higher abundance of activated Tregs and significantly lower abundance of resting Tregs. The change in immune composition and exhaustion was correlated to the metastatic tumor burden. The skewing towards an exhausted immune profile was also found in larger primary tumors compared to smaller primary tumors. No link between the immune composition in primary tumors and corresponding lymph nodes were found.

Tumor cells from smaller metastases had an epithelial-mesenchymal transition phenotype compared to an epithelial phenotype in larger metastases, impacting the local immune response.

The immune profile of the primary tumor was not predictive of the lymph node immune profile or metastatic status. LNs with manifested metastasis had increased presence of CD4 and CD8 exhausted memory T cells and activated Tregs. The tumor size of primary tumors and metastatic lymph nodes are the main drivers of changes in respective immune cell compositions. Tumor cells in smaller metastatic tumors resembled a “mesenchymal like” phenotype compared to in the larger manifested tumors. These results suggest that the immune suppression is correlated with the tumor burden.

Causal Effect of Enhancer Methylation: Impact on Breast Cancer-Driving Pathways

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In breast cancer, lung cancer and pan-cancer, through integration of DNA methylation and gene expression data from primary tumors, we have shown that loss of enhancer methylation, facilitated by chromatin loops, is linked to the activity of important cancer-driving pathways, suggesting that that such functions are under epigenetic control (Fleischer, Tekpli et al. Nat Commun 2017; Ankill et al. NAR Cancer 2022; Brativnyk et al. IJC 2024; Ankill et al. bioRxiv 2023).

To assess the causal impact of these epigenetic alterations, we performed epigenetic editing using CRISPR. We designed a breast cancer cell line (MCF7) with stable and inducible expression of deactivated Cas9 fused with the catalytic domain of DNMT3 to induce methylation at specific enhancers in the breast cancer genome. To study estrogen signaling in breast cancer, we targeted enhancers of two estrogen receptor (ER) target genes (PGR and AGR2) using single guide RNAs. Following doxycycline-induced expression of dCas9-DNMT3 we observed increased methylation at the targeted enhancers, and this methylation was stable for at least 120 hours after removal of dCas9-DNMT3. Importantly, using ChIP-PCR, we observed that ER binding was almost completely inhibited by the increased DNA methylation. Further, using qPCR, we also observed a significant downregulation of PGR and AGR2 following the increase of DNA methylation.

Taken together, our results show that loss of enhancer methylation is linked to upregulation of crucial cancer-driving pathways, and functional experiments highlight examples where enhancer methylation is sufficient to regulate gene expression. In breast cancer, we show that estrogen signaling may be directly regulated by methylation at enhancers carrying ER binding sites.

Experimental hyperthermic intraperitoneal chemotherapy in mice - establishment of a novel ovarian cancer xenograft model

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Introduction: The peritoneum is a common metastatic site for several abdominal cancers, including ovarian and colorectal cancer. Peritoneal metastasis is associated with poor prognosis and poor response to therapy. A potentially curative treatment is cytoreductive surgery (CRS) to remove all visible tumor tissue, followed by perfusion with hyperthermic intraperitoneal chemotherapy (HIPEC) to eliminate small residual tumors and free-floating tumor cells. Hyperthermia can enhance the cytotoxic effect of the drug, inhibit DNA repair and activate the immune system. At the current time, there is no standardized protocol for performing HIPEC, and there is a large variation regarding key parameters, such as choice of drug, drug concentration, treatment duration, carrier solution, volume and temperature. The impact of these parameters is unknown, and in vivo models can be a helpful tool to better understand some of these parameters to improve this treatment strategy.

Methods: HIPEC was established in a murine xenograft model of ovarian cancer to evaluate the response to intraperitoneal perfusion of cisplatin and mitomycin C for 30 min at 41.5°C (HIPEC) and 37°C (NIPEC) compared to no treatment or intraperitoneal perfusion with saline at 41.5°C. The luciferase transfected B76 ovarian cancer cell line was injected intraperitoneally and treatment was performed on day 4 when the tumors were < 2 mm. Treatment efficacy was assessed by weekly luminescence measurement, tumor weight at an early time point (day 17), and overall survival.

Results: Intraperitoneal perfusion with cisplatin or mitomycin C significantly inhibited tumor growth as assessed by luminescence, tumor weight on day 17, as well as increased overall survival of the mice compared to control treatment from 25-27 days to 36 days for cisplatin and 37 days for mitomycin C. No significant differences in tumor growth or survival were observed by the addition of hyperthermia. However, a slight reduction of tumor weight at day 17 was observed in the HIPEC groups.

Conclusion: We have established a functional closed HIPEC model in mice bearing peritoneal metastases from the B76 ovarian cancer cell line which accurately mimics many features of the procedure in patients. Intraperitoneal perfusion of both cisplatin and mitomycin C

efficaciously inhibited tumor growth and improved overall survival of the mice. Addition of hyperthermia caused an additional modest, temporary growth inhibition which did not translate into improved long-term outcome.

Nuclear Membrane Dynamics in Cancer Progression

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The genome of an organism is contained within the cell nucleus that is compartmentalised by the nuclear membrane. The nuclear membrane in cancer cells is prone to rupture as cells migrate through small constrictions, however, these ruptures are rapidly repaired by the ESCRT-III membrane-remodelling machinery. In cancer cells, DNA damage and errors in mitosis can lead to the formation of aberrant chromosome containing structures, such as micronuclei, which can be found outside of the primary nucleus, enveloped by their own nuclear membrane. The nuclear membrane of micronuclei is defective, and this triggers uncontrolled ESCRT-III activity that leads to collapse of the micronucleus. Similarly, mitotic errors in cancer cells frequently lead to the formation of chromosome bridges which contain threads of an entire chromosome that connects two daughter nuclei. Chromosome bridges are also enveloped by a defective nuclear membrane. Ruptures in the defective nuclear membranes of both micronuclei and chromosome bridges are known to be upstream of cGas signalling and activation of innate immune response and of large-scale DNA damage, mutations and rearrangements by a process called chromothripsis. Characterisation of nuclear membrane dynamics at DNA containing structures such as chromosome bridges and dissection of the role of ESCRT-III at these nuclear membranes will provide crucial insight into a mechanism underlying chromothripsis, a key contributor to cancer progression.

High-throughput drug-repurposing screen identifies FAK as a potential modulator of the suppressive functions of regulatory T cells.

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Regulatory T cells (Tregs) are important controllers of the immune system homeostasis by preventing disproportionate effector T cells (Teffs) responses. Tregs contribute to tumor development by suppressing the immune cells in the tumor microenvironment (TME). In fact, infiltration of Tregs in the TME has been associated with poor prognosis in cancer patients. Thus, targeting Tregs could conceivably improve current cancer immunotherapies.

In this project, we stablished a high-throughput cell-based drug screening to identify compounds downregulating FoxP3 expression in human primary T cells. We conducted a first screening of a drug library composed of 1520 FDA/EMA-approved small molecules (Prestwick Chemical Library).

Among other hit compounds, we validated bosutinib, a multi-tyrosine kinase inhibitor, as a FoxP3 down-regulator. Then. we searched for chemical analogs *in silico*. However, bosutinib analogs failed at reducing FoxP3 expression more potently than the parental compound. Furthermore, bosutinib inhibited Treg-mediated suppression of Teffs proliferation and reduced the expression of surface markers associated with the suppressive function of Tregs.

To characterize the mechanisms whereby bosutinib inhibited the suppressive functions of Tregs, we analysed intracellular signalling pathways in T cells by phospho-flow cytometry. Interestingly, the bosutinib target focal adhesion kinase (FAK) was more potently activated and consequently more inhibited in Tregs compared to Teffs upon short T cell receptor (TCR) stimulation. By analysing different Treg subsets, FAK was more activated in resting Tregs (rTregs, low suppressive activity) compared to effector Tregs (eTregs, high suppressive activity) or non-Tregs (proinflammatory profile) upon short TCR stimulation. However, after 2 days of stimulation, eTregs were more bosutinib-sensitive, suggesting a potential role of FAK in the transition of rTregs to a more suppressive status (eTregs).

Although further studies are needed, our high-throughput screening for small molecule inhibitors of FoxP3 expression identified FAK as an important player in the modulation of the immunosuppressive activity of Tregs.

DETECT study: Detection of structural genomic variants causing hereditary cancer

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This study aims to plug an under-reported gap in contemporary genetic analyses: large structural genomic variants (insertions, deletions, translocations and inversions) are suspected to underlie a significant portion of heritable diseases, but are often missed with current diagnostic techniques. Despite their large size, these structural variants are paradoxically harder to detect using modern high-throughput whole genome sequencing than smaller genetic changes such as single nucleotide variants.

A large proportion of families with an apparently familial cancer predisposition remain without a genetic confirmation of their condition. Identifying the genetic cause of cancer enables predictive genetic testing of relatives, clarification of risk, and early detection and prevention of cancers. The result is improved survival and better use of healthcare resources.

Alternative sequencing (long-read) and optical genome-mapping techniques offer greatly improved opportunities for structural variant detection. To demonstrate the potential for these new technologies to increase the diagnostic yield of genetic testing, we will apply them to 30 families with a history of cancer strongly suggestive of hereditary cancer syndromes (breast & ovarian cancer / Lynch syndrome), but for whom a genetic diagnosis has remained elusive. It is hoped this will trigger larger studies and the subsequent introduction of these methods as a routine diagnostic tool.

GCN2 in and out of stress

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Cancer cells are often in an unfavourable microenvironment, experiencing several kinds of stress, which they have to adapt to in order to successfully proliferate and metastasize. These stresses include endogenous stresses due to oncogene activation and increased translation, as well as a exogenous stresses such as a shortage of amino acids and oxygen in a growing tumour. Therefore, interfering with stress-response pathways is thought to be a promising principle for an effective anti-cancer strategy and inhibitors are being developed for future clinical use.

Our research focuses on one of the stress-response kinases, GCN2. Inhibitors of GCN2 are being actively developed for therapy and some are in clinical trials. We have recently found important novel functions for this kinase, which are not dependent on its canonical role in stress responses. These functions are highly relevant for cancer development and need to be considered when designing strategies to interfere with GCN2 function. We are investigating the novel functions in detail, with the aim of understanding how the new knowledge can be exploited in therapeutic approaches.

Single-cell transcriptome analysis reveals the role of myofibroblastic cancer-associated fibroblasts in chemotherapy-resistance in pancreatic cancer

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Abstract

Pancreatic cancer remains one of the most lethal malignancies and understanding the tumor microenvironment (TME) is crucial for developing effective treatments. In this project, we performed in silico analysis of single-cell RNA sequencing data from more than 200,000 cells derived from pancreatic cancer patients with and without neoadjuvant treatment. We identified 12 distinct cell types and focused our analysis on fibroblast cell population due to their important role in TME. Fibroblasts were classified into three major subtypes: regular cancer-associated fibroblasts (CAF), inflammatory CAF (iCAF), and myofibroblastic CAF (myCAF). Our findings reveal that samples from patients with poor treatment response as well as from untreated patients exhibited a higher proportion of myCAFs compared to treatment-responsive patients. Using specific markers derived from each fibroblast subtype, we applied these signatures to the PAAD cohort of TCGA and found a significant association between the myCAF signature and worse prognosis as well as advanced TNM staging. Functional analyses further demonstrated elevated levels of Wnt/β-Catenin and Notch signaling pathways in the untreated and poor response groups. We are now further characterizing the cell states and associated transcriptional programs within the distinct TME subtypes. The findings and results from our analysis will be presented in the meeting.

The Core Facility for Preclinical Proton Therapy and Imaging

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Introduction

Small animal models are vital for studying cancer biology and testing out experimental treatments, like novel proton and immune therapies before progressing into clinical trials. A challenge in animal models is the inherent biological variability compared to the controlled in vitro systems. This necessitates vigilant monitoring throughout the experiment which can be achieved through non-invasive preclinical imaging. These images provide a window into the tumor biology prior to, during and following treatment, enabling better understanding of the treatment effect, thereby saving valuable time and reducing number of required animals. In 2025 we will have a dedicated proton treatment room for preclinical research (Varian Probeam^{360}).

Methods

The facility offers optical imaging through an IVIS system (IVIS Spectrum, Revvity), and a stereo microscope for higher resolution imaging. IVIS imaging is ideal for fast and high-volume animal experiments. Optical labelling makes it possible to track cells, proteins and drugs throughout the animal. Its largest limitation is the 2D technology which provides limited anatomical information. For full 3D characterization of the anatomy and tumor biology we offer a 7T & 3T Bruker MRI. Together with users we have developed tools to investigate cell density, treatment induced cell death and necrosis, detailed information on tumor vasculature and hypoxia. For bone imaging we offer a 2D X-ray imaging system (MultiRad 225) which can also be used for X-ray treatment. We have also developed novel tools for combining information from these complementary imaging modalities with co-registered histological sections for optimal biological insight. The upcoming proton research beam will utilize these imaging facilities and thereby provide unique opportunities for studying novel proton therapy treatments.

Conclusion

Through expertise, novel in-house developed tools, and state of the art imaging equipment, the core facility aid users in getting the most out of every animal experiment, thereby speeding up translational cancer research and proton therapy.

MR hypoxia imaging of prostate cancer patients reveals a strong association between severe hypoxia, the metastatic cascade and poor outcome after prostatectomy

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Introduction
Radiation therapy (RT) is a major cancer treatment modality providing efficient therapy and cure for many patients. Some patients are not cured due to radioresistant or already metastatized tumors. Tumor hypoxia, areas of low oxygen concentration, is one of the major resistant mechanisms to RT and is associated with poor outcome after RT in prostate cancer (PC) patients. A lack of feasible methods for its assessment limits clinical investigations to study the biology of hypoxia driven RT resistance. We have previously biologically validated a MR imaging tool for visualizing hypoxia levels in PC patients. Here we aim to study the association between hypoxia, clinicopathological parameters of tumor aggressiveness and outcome after prostatectomy.

Methods
106 patients with intermediate/high risk PC underwent MR hypoxia imaging prior to prostatectomy. From the surgical specimens, pathological parameters like, Longest axis, Gleason score (GS), T stage (pT), lymph node metastasis (LN), lymphovascular invasion (LVI) and comedo necrosis was determined. Information that relates to the metastatic cascade was then extracted; pT, LVI, and LN represents local invasion, intravasation and extravasation respectively. Outcome to prostatectomy was determined as PSA relapse.

Results
MR hypoxia imaging revealed that patients with very low oxygen concentrations had poor outcome after prostatectomy. Areas of severe hypoxia was associated with PSA relapse ($p<0.0001$) and provided independent prognostic information in multivariate analysis including the current treatment decision variable D'Amico. Severe hypoxia was strongly related to presence of comedo necrosis, a well-known feature associated with aggressive disease and metastasis. Furthermore, severe hypoxia was associated with the parameters representing the metastatic cascade; pT, LVI and LN ($p<0.0001$).

Conclusions
Severe hypoxia is associated with the metastatic cascade in PC patients causing poor outcome after prostatectomy. MR hypoxia imaging may provide a biomarker for selecting patients in need of systemic treatment in addition to surgery or RT.

Differential Talin Cleavage in Transformed and Non-Transformed Cells and Its Consequences

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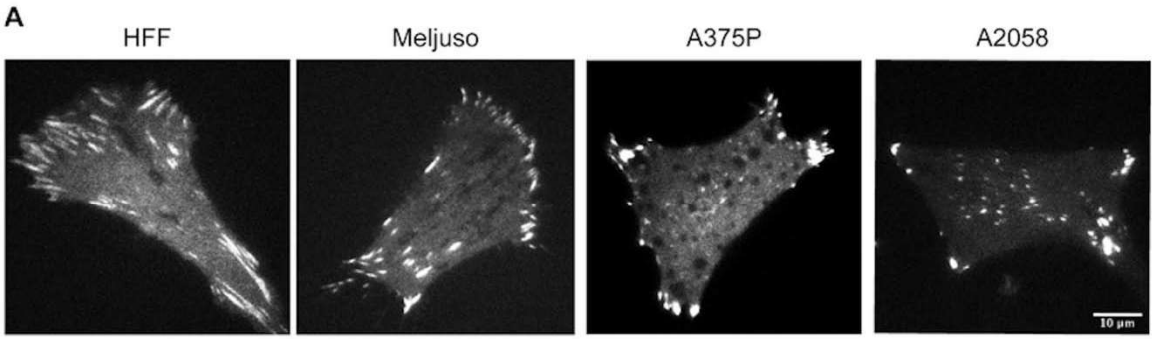
Abstract:

This study investigates differences in focal adhesion (FA) morphology and Talin cleavage levels between transformed and non-transformed cell lines. Utilizing fluorescently tagged wild-type Talin and Talin mutants with calpain cleavage site mutations, FA structures were visualized. Mutations in different Talin cleavage sites showed varying impacts on FA morphology and distribution across melanoma cell lines (Meljuso, A375P, A2058) and a non-transformed cell line (HFF). Western blot analysis, ratiometric fluorescence intensity-based measurements, and FRAP experiments revealed higher Talin cleavage levels within FAs of transformed cell lines compared to non-transformed cells. Additionally, growth assays indicated that reducing calpain cleavage levels attenuated transformed cell growth. These findings suggest that Talin cleavage level is crucial for FA morphology and assembly, with higher levels observed in transformed cells, influencing their growth dynamics.



Talin Expression in different cancer cell lines

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Reduced immune-cell infiltration in MHC class I negative DLBCL

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Diffuse large B-cell lymphoma (DLBCL) is an aggressive malignancy and the most common type of non-Hodgkin B-cell lymphoma. It is a genetically, biologically and clinically heterogeneous disease and current research focuses on identifying stratification biomarkers to identify high-risk patients and guide selection of therapy. The genetic landscape of DLBCL is well characterized, but how these mutations affect the lymphoma microenvironment is not fully understood. We aimed to categorize DLBCL cases based on tumor microenvironment (TME) features and to identify how loss of MHC expression shape the TME.

Diagnostic biopsies from 53 DLBCL patients were imaged by Hyperion imaging mass cytometry. Single-cell segmentation was followed by clustering of 14 lineage markers and manual annotation to identify eight cell types. Using CytoMAP (PMID: 32320656), the cells were divided into 20-µm-radius neighborhood and clustered to define four neighborhood classes. Unsupervised hierarchical clustering of the prevalence of neighborhood classes in each image revealed three types of TME; immune-cell depleted (43%), tumor/immune cell mixed (42%) and immune-cell rich (8%). The LymphGen algorithm (PMID: 32289277) was used to classify tumor genotypes, but the TME subclasses were not correlated with tumor genotypes. Loss of MHC class I was seen in 36% of the cases and was associated with the immune-cell depleted TME subclass. Furthermore, MHC class I loss correlated with lower infiltration of CD4 and CD8 T cells.

In conclusion, our study contributes to improved understanding of the DLBCL microenvironment through mapping of the diversity of immune-cell composition. The prominent loss of MHC has potential impact for the selection of patients for CAR T-cell therapy vs. bispecific antibodies/T-cell engagers. Together, refined classification tools of DLBCL based on genetic aberrations and microenvironment architecture may improve strategies of personalized medicine.

The Norwegian Cancer Symposium 2024

Title: Targeted alpha therapy with the bimodal ^{224}Ra and ^{212}Pb-TCMC-TP-3 technology in a multicellular tumor spheroid model of osteosarcoma

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Osteosarcoma (OS) is the most common primary bone malignancy in children and young adults, with a 60% survival rate for localized OS while <20% for metastatic OS. Our goal is to develop a bimodal targeted alpha radiotherapy for OS by combining osteoblastic stroma-seeking 224Ra and tumor cell-targeted 212Pb. A TCMC chelator conjugated to the TP-3 monoclonal antibody is added to a 224Ra solution in equilibrium with 212Pb. TP-3 targets an alkaline phosphatase (p80) isoform on the OS cell surface, potentially enhancing therapeutic efficacy and reducing radiotoxicity. This study investigates the efficacy of this technology using multicellular tumor spheroids.

Cellular binding and cytotoxicity were tested in the human OS cell line OHS. Therapeutic effects of 212Pb-TP-3 and 224Ra, alone or combined, were evaluated in OHS multicellular tumor spheroids at various activities and incubation times against a negative control (Rituximab (RTX)). Spheroid diameters were measured on days 0, 3, 7, 14, 21, and 28. Viability was assessed using the CellTiter-Glo 3D Cell Viability assay, while live and dead cells were visualized using fluorescein diacetate and propidium iodide staining.

212Pb-TP-3 specifically bound to the cell surface and inhibited OHS spheroid growth in an activity- and time-dependent manner. OHS spheroids treated with 14, 7, and 3.5 kBq/ml of 212Pb-TP-3 for 1, 4, and 24 hours, respectively, disintegrated by day 15. Spheroids treated with 212Pb-RTX disintegrated only at ≥110 kBq/ml. The cytotoxic effect of the 224Ra solution with 212Pb-TP-3 was significantly higher than 224Ra alone or combined with 212Pb-RTX. Spheroids treated with 10 and 5 kBq/ml of the 224Ra solution with 212Pb-TP-3 for 1 and 4 hours, respectively, disintegrated by day 15, with no viable cells observed.

These promising results warrant further exploration in preclinical animal models to evaluate the therapeutic efficacy and toxicity of the dual alpha 224Ra with 212Pb-TP-3 solution for treating OS.

Studies of cabazitaxel-loaded poly(2-alkyl cyanoacrylate) nanoparticles

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Biodegradable poly(alkyl cyanoacrylate) nanoparticles (NPs) are gaining increasing attention in the field of anti-cancer nanomedicine development. In a previous study (1), we reported promising outcomes using cabazitaxel (CBZ)-loaded poly(2-ethylbutyl cyanoacrylate) NPs (PEBCA-CBZ NPs) in a patient-derived xenograft (PDX) model of triple-negative breast cancer, which correlated with a reduction in M2 macrophages. This study delves into a comparative analysis of CBZ encapsulated in two PACA NP variants, namely poly(2-ethylbutyl cyanoacrylate) (PEBCA) and poly(2-ethylhexyl cyanoacrylate) (PEHCA) (2). These variants were explored both with and without surface functionalization using folate as a targeting ligand. In vitro assessments across different breast cancer cell lines revealed similar patterns of NP-induced cytotoxicity, despite variations in early NP internalization. Efficacy studies conducted in the HBCx39 PDX model of triple-negative breast cancer demonstrated an enhanced therapeutic effect of drug-loaded PEBCA variants when compared to free drug and PEHCA NP variants. Furthermore, analyses of immune cell population in tumors by multiparametric flow cytometry revealed that the treatment with drug-loaded PEBCA variants affected the myeloid cells, especially macrophages, which in turn contributed to an inflammatory and immune-activated tumor microenvironment. In summary, drug-loaded PEBCA NPs exhibit promising therapeutic potential in triple-negative breast cancer, showcasing a dual impact on both tumor cells and the tumor-immune microenvironment.

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Fusion transcripts in high-grade serous carcinoma

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Background

High-grade serous tubo-ovarian carcinoma (HGSC) is marked by extensive genomic instability. Chromosomal rearrangements result in structural variants, including gene fusions. Fusion transcripts have been identified as key oncogenic drivers in various cancers, offering potential as diagnostic, prognostic, and therapeutic targets. In HGSC the prevalence and potential role of fusion genes remain unknown. We have investigated fusion transcripts in HGSC and explored their potential as biomarkers in the context of tumor heterogeneity.

Methods

Twenty-three patients diagnosed with advanced HGSC between 2002 and 2012 provided 108 samples for this study. Tumor samples were collected from ovary and intraabdominal metastases (2-3 sites, 2-3 samples per site). Total RNA was sequenced using stranded libraries and 2x101 bp paired-end reads. STAR-Fusion was used for fusion predictions and FusionInspector for exclusion of artifacts and normal fusions. We prioritized fusion transcripts expressed in all samples per patient.

Results

A total of 3787 different fusion transcripts were predicted (18163 including recurrences and variants), of which 141 met the filtering criteria. To enrich for fusions with a putative protein product, we further prioritized in-frame fusions (n = 28) and fusions with protein family motifs in both gene partners (n=16). Of the 16 candidates, 14 were private to a single patient and two were recurrent in one sample from one additional patient each. These fusions, *FLNB-SLMAP* and *RAD21-EIF3H*, were lowly expressed concurrent with high expression of the non-fused partner transcripts.

Discussion

Few studies exist on fusion transcripts in multiple samples per HGSC patient. Of the 16 resulting fusion candidates, 5 have been previously reported in cancer, including *RAD21-EIF3H* in HGSC. The low fusion allelic ratio may suggest trans-splicing rather

than gene fusion. Our results indicate that fusion transcripts are recurrently expressed also in HGSCs, although with a low prevalence when requiring expression across tumor sites in each patient.

Targeting Ewing sarcoma with chimeric antigen receptor(CAR) T cell therapy

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Ewing's sarcoma is the second most frequent primary malignant bone tumor with a low survival rate and a high rate of metastasis, mainly affecting children and young adults. Chimeric antigen receptor (CAR) based T-cell immunotherapy as an effective treatment for B cell malignancies in recent years demonstrates that it is a powerful method of hematological malignancies therapy. However, their success in treating solid tumors has been limited due to the limited tumor specific cell surface antigens are found. Six-transmembrane epithelial antigen of prostate-1 (STEAP1), which is expressed in 90% of prostate cancers, and subgroups of other malignancies, like Ewing sarcoma. We devaloped and reported a novel CAR with a 4-1BB co-stimulatory molecule which showed that it has significant efficacy against metastatic prostate cancer both *in vitro* and *in vivo*. Here, we have expended the usage of this CAR T cell therapy to Ewing sarcoma. The *in vitro* and *in vivo* assays results showed similar or even better tumor killing effect targeting a Ewing sarcoma cell line A-673 when compare to the effect targeting the prostate cancer. The mice burden A-673 xenograft tumor showed totally tumor free after 4 weeks treatment of CAR T cell therapy. Furthermore, those mice were re-challenged with A-673 cell without further treatment, and kept tumor free until the end point of the experiment. This result indicates long-lasting therapy effect of this STEAP1 CAR T cells.

Gene expression-based molecular classifier is associated with biochemical recurrence and proves independent of heterogeneity in primary prostate cancer

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Introduction & objectives
Prostate cancer (PCa) is one of the most common cancer types worldwide, and the patients have variable clinical outcomes. Correct treatment stratification and prognostication are complicated by the multifocal nature of this cancer, because of extensive both inter- and intrafocal molecular heterogeneity. There is an urgent need for improved prognostication tools to aid clinical decision-making. The objective of this study was to validate 16 candidate biomarkers and develop a multigene model for prognostication of PCa.

Material & methods
Using transcriptome-wide RNA-sequencing data, we have identified sixteen genes for which the expression levels provide consistent results independently of which malignant tumor focus that is assessed, and that are associated biochemical relapse (BCR) after radical prostatectomy (RP)(Strømme *et al.*, Cancer Gene Therapy 2022). These genes were validated by independent technology, quantitative real-time RT-PCR, in an independent series of one malignant tissue sample from each of 220 patients treated with RP.

Results
With input data from one malignant sample per patient, 7 of the 16 genes were significantly associated with BCR with univariate statistics ($p<0.05$). Using Lasso regression modelling, 6 of the 16 genes have been included in a multi-gene model that calculates a total hazard ratio per patient. This 6-gene classifier, named ProClass, separates the 216 patients into high- and low-risk groups with $p < 0.0001$. ProClass also yields significant association with BCR when analyzing the subcohort with 135 patients with ISUP grade 3 or higher. Principal component analysis demonstrated that tissue samples from different tumor foci within the same prostate had highly similar expression profiles for the 16 genes, in contrast to what is seen for other biomarkers and gene signatures. Interestingly, although the genes were identified from expression data in malignant tissue, the ProClass scores calculated from expression data in benign tissue were also significantly associated with BCR ($p<0.001$).

Conclusion

We have identified 16 candidate prognostic biomarkers, and performed validation with independent technology and in an independent cohort. A 6-gene classifier was developed, ProClass, which is producing stable results despite a highly heterogeneous nature of a multifocal disease. By extending conventional histopathology methods with molecular gene profiling, we demonstrate that molecular pathological assessment of tumor tissue has the potential to facilitate more precise risk stratification and to aid clinical decision-making and prognostication. An additional value is demonstrated by the association between BCR and these genes' expression in benign tissue from the same PCa specimen, which could potentially improve diagnostics.

Normal tissue toxicity in a mouse model following fractionated irradiation of the head and neck with protons or X-rays

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Background: Proton therapy gives less dose to normal tissue compared to conventional X-ray therapy, but systematic comparisons of normal tissue toxicity are lacking. The aim of this study was to investigate early and late normal tissue toxicity in the head and neck of mice following proton- or X-irradiation. Moreover, we aimed at investigating molecular responses by monitoring the cytokine levels in serum and saliva.

Materials and methods: Female C57BL/6JRj mice underwent fractionated irradiation with protons or X-rays to the maximally tolerated acute level. The radiation field covered the oral cavity and the major salivary glands. Early toxicity, such as oral mucositis and dermatitis, were scored through oral cavity examinations at days 7-35 after onset of irradiation. Late toxicity in the salivary glands were investigated by histology on tissues collected at day 105. Saliva and serum were collected before and at different time points after irradiation to assess salivary gland function and cytokine expression.

Results: Oral mucositis appeared earlier, had a higher severity score and was found in a higher percentage of mice after proton- compared to X-irradiation, despite that 30-37% lower total doses were given for protons than X-rays. Dermatitis, on the other hand, had a similar appearance after protons and X-rays. Saliva volume after proton and X-irradiation was significantly lower than for controls and remained reduced at all time points after irradiation. Protons caused reduced saliva production and fewer acinar cells in the submandibular glands compared to X-rays at day 105. X-rays induced a stronger inflammatory cytokine response in saliva compared to protons.

Conclusion: This work supports previous preclinical experiments and indicate that the relative biological effectiveness of protons in normal tissue might be higher than the commonly used value of 1.1. Thus, there is a need for further investigations of the biological effect of protons in normal tissue.

Targeted radionuclides for treating metastatic cancers - dual alpha targeting strategy

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Metastatic cancer is the leading cause of death among cancer patients. New and effective treatments are urgently needed. A novel approach using targeted alpha-emitting radiopharmaceuticals to treat metastatic cancers, particularly affecting bones and soft tissues is presented. The goal is to introduce a treatment that combines two radionuclides, ^{224}Ra and ^{212}Pb, in a single solution to target and destroy cancer cells more effectively. This dual alpha targeting solution contains bone-seeking ^{224}Ra and cell-directed complexes of progeny ^{212}Pb. Cancer cell targeting monoclonal antibodies, their fragments, synthetic proteins or peptides can all be radiolabelled with ^{212}Pb in the ^{224}Ra-solution in transient equilibrium with daughter nuclides. ^{224}Ra targets stromal elements in sclerotic bone metastases and ^{212}Pb-conjugate targets tumour cells of metastatic bone cancer or osteosarcoma. The preliminary preclinical studies provide conceptual evidence that the dual alpha ^{224}Ra-solution with bone or tumour-targeted delivery of ^{212}Pb has potential to inhibit cancer metastases without significant toxicity. In some settings, the use of a booster dose of purified ^{212}Pb-conjugate alone could be required to elevate the effect of this tumour cell directed component, if needed, e.g. in a fractionated treatment regimen, where the dual targeting solution will act as maintenance treatment.

Magnetic resonance imaging with an attention-based vision classifier can be used to depict irradiated regions in a murine model

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Head and neck cancer patients receiving radiation therapy may experience toxicities that appear both early and late in the course of the treatment. Methods for prompt and non-invasive assessment of patients at risk of developing toxicities are greatly needed. To evaluate normal tissue responses and their dependence on treatment factors such radiation field and type, dose, and fractionation scheme, preclinical models with relevant endpoints are required. In this study, fractionated X-irradiation to a total dose of 66 Gy covering the salivary glands, swallowing muscles, and oral cavity was administered to C57BL/6J mice (n=14). This treatment causes significant toxicity both early and late in the animals. After radiotherapy, we acquired T2-weighted MR images three to five days later. The mice that received radiatiotherapy were compared with a control group (n=15) that received the same handling but with no radiation. The aim of this work was to investigate if an attention-based vision classifier applied on the MR images could separate the two treatment groups, since a visual comparison of the MR images did not show any distinct patterns. We re-trained a vision transformer model (ViT Base 16) with a transfer learning approach by using torch vision pretrained weights. With an average accuracy of about 69%, the model was able to largely classify MR images of controls and irradiated animals. Importantly, the attention maps showed a distinct difference between control and irradiated mice in their attention patterns, pointing to the irradiated region in the animals. Thus, attention-based classifiers have the potential for classification and early identification of areas at risk of developing toxicity. In addition, the classifier also showed a strong correlation with late toxicity in terms of reduced saliva production. To our knowledge, this is a novel finding with potential for future clinical applications.

Comprehensive Bioinformatics Services for Bulk, Single-Cell, and Spatial Transcriptomics Data

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The Bioinformatics Core Facility (BCF) is located at the for Cancer Research, Oslo University Hospital (OUH), and is tightly connected to the Oslo node of ELIXIR Norway. We provide high-quality bioinformatics analysis to local, regional, and national researchers. In addition to advanced data analysis, we offer assistance with data management-related topics like data preparation and quality control, data architecture, databases, data integration, metadata management and data transformation. Our bioinformatics expertise covers the analysis of traditional bulk data from DNA (i.e. mutation calling, copy number analysis, structural variations, methylation) and RNA (i.e. differential gene expression, splice variant, gene fusions). In addition, we have established specialized analysis competence for single-cell and spatial transcriptomics data. These fields have undergone significant advancements in recent years, offering valuable insights into complex diseases, immunology, neurology, and more. Single-cell data analysis allows the use of gene expression patterns to cluster cells based on their similarities and to determine the specific cell type or cell state each cluster represents. This information can be coupled with the expression surface proteins and/or TCR and BCR clonotypes to get an even broader understanding of underlying biology. With spatial data, researchers can get high- resolution gene and protein expression information to study cell interactions and tissue microenvironments in their spatial context. Further, it is also possible to integrate single-cell and spatial data to get cell-by-cell resolution to determine specific cell types within a given tissue sample.

Title: Novel approach to improve treatment efficacy by lattice radiotherapy in combination with immunotherapy

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Many patients are not cured with today's radiotherapy (RT) regimen. An emerging and exciting approach to increase cure rate is to combine radiation with immunotherapy, as radiation may cause immunomodulatory effects. Conventional RT applies a homogenous radiation dose to the whole tumor, which largely sterilizes the tumor, including anti-tumor immune cells and lymphatic vessels. This is a major obstacle when combining immunotherapy with radiation, since the presence of anti-tumor immune cells is a prerequisite for the success of this strategy. In this project, we will use lattice RT to irradiate only parts of the tumor in small spots and combine this with immunotherapy. We hypothesize that immune cells outside the irradiated spots within the tumor will survive, providing a better basis for the combination therapy. Pre-clinical testing is needed to demonstrate the potential of this approach prior to a clinical application in a phase I trial.

The aim of this study is to determine whether lattice RT triggers a better anti-tumor immune response and improves treatment efficacy when combined with immunotherapy, compared to whole-tumor irradiation. In collaboration with Yolanda Prezado's lab at the Institute Curie, France, we will utilize advanced technology for lattice irradiation of rodent tumors during a stay in her lab early next year. Ongoing research at OUS includes whole-tumor radiation in collaboration with the core facility and establishment of assays for quantifying immune and treatment responses by digital histopathology. The MOC1 (highly immunogenic) and MOC2 (moderate immunogenic) head and neck cancer syngeneic mouse models are used.

After opening of the pre-clinical proton facility at OUS, we will further evaluate the benefit of using protons, rather than conventional X-rays, for this approach. Proton therapy will be particularly beneficial due to sparing of healthy tissues surrounding the tumor.

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TECPR1 is activated by damage-induced sphingomyelin exposure to mediate noncanonical autophagy

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Membrane damage to endolysosomal compartments is detrimental to cells. Therefore, damage control and homeostatic regulation by various mechanisms are crucial for cellular survival. One such process, conjugation of ATG8 to single membranes (CASM), uses the ATG12-ATG5 containing E3 complex of the core autophagic machinery to signal membrane damage caused by pathogens or toxic compounds. Previously, ATG16L1-containing E3 complex was reported as essential for CASM response to proton gradient loss in endolysosomes. Here, we identified TECPR1 (tectonin beta-propeller repeat containing 1) as an independent sensor for damaged compartments in the presence of clinically relevant nanoparticles, transfection reagents, antihistamines, lysosomotropic compounds, and detergents. Mechanistically, TECPR1 is recruited by damage-induced cytosolic sphingomyelin (SM) exposure using its two DysF domains, resulting in its activation and ATG8 lipidation. In vitro assay using purified human TECPR1-ATG5-ATG12 complexes showed direct activation of TECPR1 E3-activity by SM, whereas the ATG12-ATG5-ATG16L1 complex had no discernible effects upon SM exposure. Interestingly, TECPR1 retains its full E3-activity when S. Typhimurium factor SopF obstructs ATG16L1 CASM-activity, thus providing a stimuli-specific alternative CASM response upon cytosolic SM exposure on damaged endolysosomes.

Fusion genes and heterogeneity in localised prostate cancer

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Prostate cancer is the most frequently diagnosed cancer type in Norway. The patients often have multiple organ-confined tumours at the time of diagnosis, and these tumours are highly heterogeneous with regard to molecular aberrations. Localised prostate cancers generally have few somatic point mutations, and are instead characterised by recurrent fusion genes.

We aimed to characterise the transcriptome of localised prostate cancer, with a particular focus on intrapatient heterogeneity of fusion transcript expression. We utilised both long- and short-read sequencing and analysed multiple tumour samples from each patient. In total, 110 tissue samples from 31 patients were long-read sequenced with an Oxford Nanopore Technologies approach. Short-read sequencing was performed on a total of 159 samples from 45 patients on Illumina platforms.

For technical validation, real-time RT-PCR data were included in the analyses. We found robust correlations between long-read sequencing, short-read sequencing, and real-time RT-PCR results.

With long-read sequencing, and the Genion and LongGF tools, *TMPRSS2-ERG* was found to be the most common fusion transcript (61% of patients). Other ETS fusions were also detected, including *TMPRSS2-ETV1*, *MIPOL1-ETV1*, *SLC45A3-ELK4*, and *RNPEP-ELF3*. Moreover, two non-ETS fusions were identified, namely *AZGP1-GJC3* and the novel ENSG00000284512-*CMC2*. Among these fusions, only *TMPRSS2-ERG* and *TMPRSS2-ETV1* were identified in the short-read sequencing data, utilising the STAR-Fusion tool. In addition, fusion transcripts *FBXO25-SEPTIN14*, *HMGN2P46-AC048338.2* and *TLK2-AC240565.1* were detected exclusively with short-read sequencing.

Distinct *ERG* and *ETV1* transcript variant profiles were observed in samples with *TMPRSS2-ERG* or *TMPRSS2-ETV1*, respectively. Intra- and interfocal heterogeneity were identified in 93% and 92% of patients for *ERG* transcript variants, and in all evaluable patients for *ETV1* variants.

In conclusion, there is a high level of correlation between different methods for gene expression assessment, but our results also highlight key differences in fusion detection between long- and short-read RNA sequencing.

Activation of unliganded FGFR1 in cancer by inorganic phosphate and oxidative stress

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Fibroblast growth factor receptor 1 (FGFR1) is a receptor tyrosine kinase expressed by many types of cells. FGFR1 has been found deregulated in several cancer types and is an important therapeutic target. Stimulation of the receptor activates intracellular signaling pathways (PI3K/Akt, PLCγ/PKC and Ras/MAPK), leading to cell proliferation, migration or differentiation. Surprisingly, FGFR1 can also be activated by extracelluar phosphate (Pi) without involvement of ligands. However, the role of phosphate-mediated activation of FGFR1 in cancer has not been explored yet, and the mechanism of this unusual activation remains unknown. Our results indicate high extracellular Pi levels can induce growth of FGFR1-dependent osteosarcoma cells. In addition, we found that elevation of blood Pi levels by high phosphate diet leads to increased tumor progression *in vivo*. We also found that phosphate induces mitochondrial production of H_2O_2, leading to oxidation of Cys and Met residues in FGFR1, and subsequent activation of the receptor kinase domain. Therefore, our results suggest oxidative stress can be a ligand-independent activator of FGFR1 with unexplored consequences for cancer cells.

Abstract submission to the Norwegian Cancer Symposium 2024

"Evaluation of the CAIX-binding DOTA conjugate MKV-509 with ^{212}Pb in in vitro Models of Metastatic Human Renal Cell Carcinoma and Pancreatic Adenocarcinomas"

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Carbonic anhydrase IX (CA IX) is a cell-surface glycoprotein which is induced by hypoxia or mutations in the von Hippel-Lindau tumor suppressor protein. It is overexpressed in many solid tumours and is a potential target for cancer therapy and diagnosis (1). ^{177}Lu conjugated to antibodies has shown efficacy, but bone marrow depletion and consequently myelotoxicity has slowed the development of ^{177}Lu-radiotracers (2). A promising alternative is targeted alpha therapy (3), utilizing alpha emitters such as ^{212}Pb and ^{225}Ac and small-molecule CAIX ligands. The novel CAIX targeting ligand MKV-509 is an inhibitor VD11-4-2 (3) conjugated to DOTA (Latvian Institute of Organic Synthesis, Riga, Latvia). In this study, the potential of MKV-509 chelated to ^{212}Pb (radiochemical purity >99%) for the treatment of renal cell carcinoma (RCC) and pancreatic adenocarcinoma was assessed. The RCC cell line SKRC-52 and the pancreatic adenocarcinoma cell line BxPC3 were used as in vitro models. The number of antigens per cell, binding and internalization of ^{212}Pb-MKV-509 were assessed through binding studies. SKRC-52 cells bound 60.8% and internalized 28.6%, whereas BxPC3 cells bound 19.9% and internalized 8.21% of the added activity. Cell cycle distribution, viability, and DNA damage were evaluated by flow cytometry 1-, 3- and 6-days post treatment. ^{212}Pb-CAIX induced a G2-phase arrest and caused DNA damage, but significant induction of apoptosis and cell death was not detected. Multicellular spheroids were used as a model for micrometastases, where growth and viability were assessed by microscopy for up to 21 days. ^{212}Pb-MKV-509 reduced the growth in a dose-dependent manner. These data suggest that this MKV-509 has great potential to bind to CAIX-expressing cells and warrants further studies to conjugate VD11-4-2 to TCMC and evaluate its preclinical therapeutic efficacy *in vivo*.

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Role of mammalian ATG8 proteins in bulk autophagy and PINK1/Parkin-independent mitophagy

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Autophagy-related 8 (ATG8) is a central protein in macroautophagy, having functions in both cargo recruitment and in driving the autophagic pathway. Whereas yeast only have one ATG8 gene, mammalian cells have several ATG8 homologues (mATG8s), segregating into the LC3 (LC3A, LC3B, LC3C) and GABARAP (GABARAP, GABARAPL1, GABARAPL2) subfamilies. It was initially suggested that both subfamilies are essential for general autophagy. However, by using functional, cargo-based autophagy assays, we previously reported that the LC3s are dispensable for bulk autophagy, whereas the GABARAPs are required. Similar results have later been obtained by others.
However, despite that the central role of mATG8s in autophagy has been known for >25 years, it is still not clear at which step in the autophagic pathway the mATG8s predominantly act. Are they predominantly required for autophagosome formation or for autophagosome-lysosome fusion? We have recently performed a series of experiments that carefully and critically address this question. Conclusive results that reveal the specific requirements for LC3 and GABARAP proteins, and the affected step in the autophagic pathway during bulk autophagy and PINK/Parkin-independent mitophagy, will be presented.

Immunological synapse map analysis program (isMap)

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Abstract

We have recently shown that clathrin controls both vesicular release of T cell receptor (TCR) at the immunological synapse by clathrin and ESCRT-mediated ectocytosis (CEME) and internalization of TCR conjugated to peptide-MHC by clathrin-mediated trans-endocytosis (CMTE). These topologically opposite processes are regulated by the temporally dynamic recruitment of clathrin adaptor proteins to TCR microclusters. The ESCRT components HRS and STAM2 initially recruit clathrin there to induce TCR release by CEME, while the endocytic clathrin adaptor epsin-1 subsequently initiates TCR internalization by CMTE. These processes are critical for delivery of T cell help and T cell mediated regulation of pMHC availability on the surface of antigen presenting cells, respectively. However, how they are affected by costimulation and inhibition by signaling pathways modulating TCR activation is not known. Here, we have developed an automated analysis framework termed Immunological Synapse Map Analysis Program (isMap). We are currently applying this to map the average spatiotemporal localization of a panel of costimulatory and inhibitory molecules such as CD80, PD-L1, ICOSL, PVR and CD58, and to analyze how these molecules affect the balance between TCR internalization by CMTE and TCR release by CEME.

Title: A Flexible and Integrated Pipeline for Spatial Biology Data Analysis

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Analyzing spatial biology data requires toggling between disparate image processing tools and statistical libraries. Each has its own limitations: When statistical packages lack functionality, they are not easily extended or modified, while most imaging software is not script-based and difficult to incorporate into a pipeline.

We introduce a pipeline that leverages the Julia programming language to create a common framework for both image processing and statistical analysis.
In Julia, data transformations can be expressed as simple loops over cells and pixels without usual performance concerns. This provides direct and extensible control over both image processing mathematics and cell-based analysis. The unified environment allows for seamless integration between the "cytometry picture" and the "imaging picture."

Our pipeline is effectively applied to single-cell segmented high dimensional Imaging Mass Cytometry (IMC) datasets for mantle cell lymphoma (n = 109). It enables batch correction, rapid multiplex image construction, image alignment and performant spatial analyses. We perform clustering and phenotyping while simultaneously viewing cells in their spatial context. This feedback loop allows us to more accurately assign cell types in the presence of imperfect segmentation. It also helps us instantly understand spatially correlated anomalies and develop strategies for removing outliers. Our integrated approach improves data analysis accuracy, improves the efficiency of our spatial analysis, and enhances the overall reproducibility of our results.

Innate Immunity, Autophagy, and Micronuclei in aggressive cancer progression

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Abstract

Cancer, a chronic disease, now has effective treatments for many types. However, aggressive cancers remain challenging, as cells can spread from the primary tumor and metastasize to other organs, leading to a poor prognosis. Genotoxic treatments such as radiotherapy and chemotherapy often used in clinics can damage DNA, creating abnormal structures called micronuclei. Micronuclei are markers of genomic instability and can drive cancer progression by triggering severe chromosomal rearrangements, known as "chromothripsis." While micronuclei can activate the cGAS-STING pathway, which initiates an innate immune response that may benefit some patients, they also actively contribute to cancer progression. It is now evident that the aggressive cancer cells exploit micronuclei and benefit from cGAS-STING activation for their survival. Therefore, targeting micronuclei or the cGAS-STING pathway presents potential therapeutic strategies for treating aggressive cancers.

Micronuclei indicate cellular damage, which cells typically aim to eliminate. Autophagy, a central process for degrading damaged cellular components, engulfs damaged organelles into autophagosomes, fuses with lysosomes leading to the degradation of the cytosolic cargo. We hypothesize that autophagy could remove micronuclei in aggressive cancer cells. However, in cancer, autophagy can be hijacked by the aggressive cancer cells in their benefit.

Our studies on aggressive triple-negative breast cancer cells reveals a high number of cGAS-positive (ruptured) micronuclei and a constitutively active innate immune response. Micronuclei are sensed by the autophagy machinery, indicated by the presence of autophagy-related proteins such as p62, LC3, and ubiquitin. Micronuclei are also found near lysosomes, suggesting they may be targeted for degradation by autophagy. Live cell imaging over several hours has shown challenges in determining if micronuclei are completely resolved by autophagy. This may suggest that autophagy is either insufficient in fully resolving micronuclei or is involved in piecemeal degradation. Our ongoing studies aim to further explore these consequences in aggressive cancer development.

Keywords: innate immune response, triple-negative breast cancer, micronuclei, cGAS-STING pathway, autophagy.

T-cell-mediated proinflammatory cell death and lysis in cancer

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Abstract (289 words)

The primary goal of most cancer therapies is to induce cancer cell death. Immunotherapy relies on the cytotoxic activity of lymphocytes such as natural killer cells (NKs) and cytotoxic T lymphocytes (CTLs) to achieve this. While a significant amount of research in immunotherapy focuses on the events within cytotoxic lymphocytes or at the interface between cytotoxic lymphocytes and cancer cells, less is known about the molecular mechanisms that orchestrate cancer cell death, particularly in the case of CTL-mediated death.

CTLs are known to induce two types of cell death in cancer: apoptosis and pyroptosis. Apoptosis is generally non-inflammatory, whilst pyroptosis strongly promotes inflammation and potentially intensifies anti-tumor responses. Cell lysis is a common feature of pyroptotic cell death. It is unclear whether lysis can be decoupled from pyroptosis in cancer cells and whether the proinflammatory properties of pyroptosis depend on lysis. Moreover, it remains unknown whether CTLs can induce other forms of cell death in cancer beyond apoptosis and pyroptosis.

We have developed an efficient in vitro killing system to characterise death-related events initiated by CTLs within cancer cells. The system has been validated by selectively disrupting known mediators, resulting in the specific disruption of corresponding death or lysis programmes. In addition, we have evidence suggesting the existence of an undefined, non-apoptotic and non-pyroptotic death programme that can be induced by CTLs. We are currently conducting a genome-wide CRISPR screen to identify mediators regulating this novel form of cancer cell death. Furthermore, we plan to evaluate the impact of various death-related events and associated mediators on in vivo anti-tumour responses. Overall, this research aims to enhance our understanding of the molecular mechanisms of CTL-mediated cancer cell death and potentially reveal new targets to improve the efficacy of cancer immunotherapy.

Establishing PDX-derived organoid models from pseudomyxoma peritonei

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Pseudomyxoma peritonei (PMP) is a rare, slow-growing, abdominal cancer characterised by mucinous tumour spread to the surfaces of the peritoneal cavity. The standard treatment is cytoreductive surgery (CRS) followed by hyperthermic intraperitoneal chemotherapy (HIPEC), which cures half of the patients. However, for patients that are not cured by CRS-HIPEC, there is no efficacious treatment, and our research focuses on development of novel treatment options.

Patient derived tumour organoids are generated by culturing tumour tissue in the form of 3D structures in a gel-based growth medium. Organoid cultures are more complex than conventional 2D models, but less complex than animal models, although potentially more efficient in terms of time and costs. Organoid cultures from individual patients may be utilised to develop patient specific treatment.

PMP is characterised by a low number of slow-growing tumour cells producing large amounts of mucin, making it challenging to culture ex vivo compared to other cancers. We have established several patient-derived xenograft (PDX) mouse models from PMP patients that can provide a regularly available source of PMP tissue. To develop the technology for PMP, organoid cultures have been established from four of the PDX-models. The models have been characterised with respect to mucin and cytokeratin expression using fluorescent confocal microscopy and immunohistochemistry. Results from these analyses show that the organoids have the same characteristics as the tissue of origin, specifically the original patient tumour and tumour from the PDX-model.

Novel drug resistance mechanisms and drug targets in *BRAF*-mutated peritoneal metastasis from colorectal cancer

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Background: Patients with peritoneal metastasis from colorectal cancer (PM-CRC) have inferior prognosis and respond particularly poorly to chemotherapy. This study aims to identify the molecular explanation for the observed clinical behavior and suggest novel treatment strategies in PM-CRC.

Methods: Tumor samples (230) from a Norwegian national cohort undergoing surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) with mitomycin C (MMC) for PM-CRC were subjected to targeted DNA sequencing, and associations with clinical data were analyzed. mRNA sequencing was conducted on a subset of 30 samples to compare gene expression in tumors harboring *BRAF* or *KRAS* mutations and wild-type tumors.

Results: *BRAF* mutations were detected in 27% of the patients, and the BRAF-mutated subgroup had inferior overall survival compared to wild-type cases (median 16 vs 36 months, respectively, $p < 0.001$). *BRAF* mutations were associated with RNF43/RSPO aberrations and low expression of negative Wnt regulators (ligand-dependent Wnt activation). Furthermore, *BRAF* mutations were associated with gene expression changes in transport solute carrier proteins (specifically *SLC7A6*) and drug metabolism enzymes (*CES1* and *CYP3A4*) that could influence the efficacy of MMC and irinotecan, respectively. *BRAF*-mutated tumors additionally exhibited increased expression of members of the novel butyrophilin subfamily of immune checkpoint molecules (*BTN1A1* and *BTNL9*).

Conclusions: *BRAF* mutations were frequently detected and were associated with particularly poor survival in this cohort, possibly related to ligand-dependent Wnt activation and altered drug transport and metabolism that could confer resistance to MMC and irinotecan. Drugs that target ligand-dependent Wnt activation or the BTN immune checkpoints could represent two novel therapy approaches.

Photochemical internalization in combination with the nucleoside analog gemcitabine shows promising cytotoxic effects on cholangiocarcinoma cells

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Cholangiocarcinoma (CCA), commonly known as bile duct cancer, is a rare and extremely lethal epithelial cell malignancy that occurs anywhere along the biliary tree and/or within the hepatic parenchyma. Early stage CCAs are asymptomatic and the majority of cases are diagnosed at an advanced tumor stage with limited treatment options and poor prognosis. Standard first-line palliative treatment of advanced CCA involves systemic chemo-therapy using the nucleoside analog gemcitabine (*Gemzar*) and the platinum-based anticancer drug cisplatin. CCA treatment regimes are not very efficient due to the tumor's high resistance against chemotherapy and radiation, as well as the lack of efficient drug delivery methods.

Photochemical internalization (PCI), developed at the Norwegian Radium Hospital, is a promising drug delivery method that is currently under clinical evaluation.

Here we show, *in vitro*, that gemcitabine in combination with photochemical internalization (PCI) decreases cancer cell viability to a much larger degree than conventional treatments like photodynamic therapy and gemcitabine monotherapy. We further have preliminary data that suggests a positive effect of long-term treatment protocols involving sub-toxic gemcitabine doses.

To measure the cytotoxic effect, we measured metabolic activity and colony formation capacity as well as monitored cell proliferation and cell death events with the Incucyte® Live Cell Analysis System.

Identification of a novel mitophagy regulator in a high-throughput imaging siRNA screen

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Mitochondria can be targeted for lysosomal degradation via the autophagy pathway, denoted as mitophagy. This occurs in all cells under basal conditions and may also be upregulated during development and in response to stressors such as mitochondrial membrane depolarisation and hypoxia. The mechanisms underlying mitophagy are still poorly characterized, but previous work has implicated the existence of specific mitochondrial determinants that are recognized by the autophagy machinery to promote selective degradation of mitochondria.

To unravel mitochondrial determinants important for hypoxia-induced mitophagy, we have carried out an siRNA-based imaging screen targeting mitochondrial and mitochondria-associated genes in U2OS cells expressing a matrix-located mitophagy reporter (NIPSNAP$^{1-53}$-EGFP-mCherry, referred to as iMLS) under hypoxia-mimicking (deferiprone-induced) conditions.

As expected, the depletion of many mitochondrial proteins resulted in increased mitophagy, likely due to perturbed mitochondrial homeostasis. Most interestingly, 39 mitochondrial proteins were required for hypoxia-induced mitophagy, including components of the import machinery, replication complex, the respiratory chain and iron-sulphur cluster biosynthesis. In the latter group is the highly conserved iron-sulphur scaffold protein ISCU that positively regulates hypoxia-induced mitophagy but negatively regulates CCCP-induced Parkin-mediated mitophagy.

Investigating alterations in immune cell composition in response to chemotherapy in peritoneal metastasis

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The peritoneum is a common metastatic site for many different types of cancer including colorectal (CRC) and ovarian cancer (OC) and develops by direct shedding of tumor cells into the peritoneal cavity. Since the surface of the peritoneal cavity is large (1-2 m^2 in adults), extensive tumor burden may be present before symptoms occur, and peritoneal metastasis (PM) are frequently detected at a late stage. Initial small tumor deposits may be difficult to detect, but as peritoneal tumors grow, a range of debilitating symptoms will occur, greatly impairing quality of life, and ultimately causing death of the patient. The patients usually develop resistance to the administrated treatment and it is therefore a need for new treatment options and improved understanding of how the treatment will effect normal and tumor cells.

To improve the outcome for patients with peritoneal metastasis we will investigate how treatment with taxane and platinum based chemotherapy affect the immune cell composition, and if those changes can be exploited with other types of drugs such as immune checkpoint inhibitors.

To achieve this murine cells lines were injected intraperitoneally to establish tumors mimicking peritoneal metastasis and the mice were treated with taxane and platinum based therapy. Mice were sacrificed simultaneously, and tumor, spleen, blood and ascites were harvested and will be analyzed using spectral flow to investigate changes in the immune cell composition in response to the treatment.

Measurement of interferon signalling from cancer cells treated with DNA repair inhibitors and proton or carbon ion radiation as compared to photon radiation.

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Radiotherapy is a main pillar of modern cancer treatment. Conventional radiotherapy employs X-rays to ionise the DNA of tumour cells, leading to DNA damage and subsequent cell death. Nevertheless, X-ray-based radiotherapy also deposits energy to surrounding normal tissue, causing adverse side effects. By replacing photon-based radiotherapy with particles – such as protons or carbon ions – the energy can be more selectively deposited at the actual tumour volume, thus sparing surrounding tissue. Particles traverse the tissue and release most of their energy at a specific depth, yielding local, clustered DNA damage. Particle energy deposition is described by linear energy transfer (LET; energy loss per length), and the higher the LET, the more clustered the damage will be.

We have previously studied various immune signalling, including interferon secretion, from cancer cells exposed to photon radiation (X-rays) and DNA repair inhibitors. If DNA damage remains unrepaired upon mitotic entry, the DNA breakage will lead to the formation of micronuclei. Micronuclear membranes are unstable, rendering harboured DNA to be *de facto* cytosolic. Cytosolic DNA is considered pathogenic, and is detected by the sensor protein cGAS. This initiates production and secretion of interferons, which recruit immune cells. Here we present measurements of interferon-β secretion from cells irradiated with low- and high-LET protons and carbon ions, compared to secretion from photon-irradiated cells, with or without DNA repair inhibition. We found only minor increases in interferon secretion after low- and high-LET particle irradiation alone. The addition of DNA repair inhibitors – particularly of ATR – entailed vastly increased secretion, especially for the higher-LET irradiation.

With the new preclinical proton irradiation facility, we aim towards conducting further studies on immune signalling from particle-irradiated as compared to photon-irradiated, DNA repair-inhibited cancer cells. This signalling will comprise immunogenic cell death, immune checkpoint ligand expression and interferon signalling of micronuclear, mitochondrial or transposon origin.

Title: **Exploring TdT as a therapeutic target in Acute Myeloid Leukemia,**

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Abstract
Acute myeloid leukemia (AML) patients have a high unmet medical need with less than 30% overall 5-year survival. Allogeneic hematopoietic stem cell transplantation is currently the only curative treatment for AML. Relapses are, however, frequent and originate from a rare population of chemotherapy-resistant leukemic stem cells (LSCs).

Recent developments in AML therapeutic strategies, including antibody-based approaches, bispecific T-cell engaging antibodies, and adoptive cell therapy—particularly chimeric antigen receptor T cells—are focused on targeting various cell surface markers aberrantly expressed on LSCs, such as CD33, CD123, CD96, and TIM3. However, on-target off-leukemia toxicities due to shared expression of these markers on normal cells, including hematopoietic stem cells (HSCs), have been observed, posing a major concern that limits the efficacy of these treatment modalities.

T-cell receptor-engineered T cell (TCR-T cell) therapy effectively targets AML-associated intracellular antigens like Wilms tumor 1 (WT1) and PRAME, which are overexpressed on AML blasts and/or LSCs. High-affinity TCRs targeting WT1-derived peptides has been shown to reduce relapse and demonstrated to be safe in high-risk AML patients. Our lab has demonstrated the ability of TCR-T cells to specifically target and eliminate primary patient-derived acute lymphoblastic leukemia (ALL) cells presenting terminal deoxynucleotidyl transferase (TdT) peptides on HLA-A*02:01 in vitro, and in vivo models *(Ali/Giannakopoulou et al, Nat Biotechnol. 2022)*. The confined expression of TdT to a particular developmental stage of normal T and B cells additionally makes it an ideal target for immunotherapy due to expected limited toxicity. This TCR is currently in clinical development.

Here, we characterized TdT expression by flow cytometry in 250 AML patient samples with a specific focus on LSC characterization. More than 50 samples were TdT positive, of which about 20 were HLA-A*02:01 positive. Preliminary in vitro killing data demonstrate that our engineered TCR-T cells specifically target and kill primary AML TdT+ cells.

Norwegian Cancer Symposium Abstract 2024

Generation of immune-evasive allogeneic adaptive NK cell therapies

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Differences in the persistence and anti-tumor responses of various NK cell subsets represent key hurdles to the implementation of NK cell-based cancer immunotherapies. Our lab has recently developed a protocol for the specific expansion of the adaptive NK cell subpopulation. Due to their unique KIR repertoire with dominant expression of one single KIR, adaptive NK cells deliver a strong, predictable missing-self response in an allogeneic, HLA-C/KIR mismatch setting. However, allorejection responses may significanly curtail their efficacy. We have previously shown that knockout of the adhesion ligands ICAM-1 and CD58 protect B2M-knockout iPSC-derived NK cells from host NK cell-mediated rejection. In the present study, we develop a protocol for ICAM-1 and CD58 knockout in *ex vivo*-expanded adaptive NK cells to potentiate their use in an allogeneic setting. We achieved robust expansion of single self-KIR+NKG2C+ adaptive NK cells from donors with our adaptive NK cell expansion protocol. Combining this expansion protocol with multiplexed CRISPR/Cas9 editing at day 7 resulted in knockout efficiencies of over 50% for both ICAM-1 and CD58 in difficult-to-edit, adaptive NK cells. Additionally, we demonstrate that our expanded, multi-edited adaptive NK cells retain their potent targeting of allogeneic tumors. This protocol makes progress towards the development of highly potent, immune-evasive adaptive NK cells for use as off-the-shelf cancer immunotherapies. Future work will test the potency of these cells in models of allorejection.

Optimization of separation methodologies for obtaining high yield-high purity urinary extracellular vesicles

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In 2020, approximately 1.4 million new cases of prostate cancer (PCa) and 375,000 related deaths were recorded worldwide. Although PSA screening is a widely used tool for the diagnosis of PCa, its use leads to overdiagnosis and overtreatment. For this reason, the search for new biomarkers represents an urgent need in the management of PCa. In the last decade, extracellular vesicles (EVs) found in biofluids have become a very promising source of biomarkers. In particular, several studies have focused on urinary EVs because urine is easily collected and is in close contact with the prostate. However, it is important to optimize the methodology for the separation and analysis of urinary EVs to facilitate the use of EV-associated molecules as PCa biomarkers.

In this study, two EV separation methods were optimized and compared in terms of EV yield and purity by analyzing the expression of EV-associated markers such as CD63, syntenin, CD9, CD81, alix and CD13 by immunoblot. By performing differential centrifugation (DC), it was shown that using a 200 nm filtration step largely removed uromodulin from the EV pellet, which is a highly abundant protein in urine that can co-isolate with EVs. On the other hand, concentration of urine samples with a 10 kDa filter before size exclusion chromatography (SEC) also prevented uromodulin contamination with minimal EV loss on the filters. Our results show that SEC provides a slightly higher EV recovery yield with similar purity to DC. Considering that DC is a more laborious and time-consuming technique than SEC, the use of urine ultrafiltration coupled to SEC is a good EV isolation alternative that can help in the implementation of EV-based biomarkers in the clinic.

Process development for clinical manufacturing of a CD8 restricted T cell receptor targeting TdT

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Adoptive T cell therapies using T cell receptors (TCRs) are a promising treatment modality in cancer, and tremendous efforts are underway to identify new TCRs recognizing antigens presented by hematological or solid tumors. In research labs, small numbers of engineered T cells can be successfully generated using *ex vivo* modification and culture systems. For the treatment of patients, it is necessary to establish a suitable GMP-compatible manufacturing process producing a large number of potent engineered T cells.

We have established a manufacturing protocol for a CD8 co-receptor dependent TCR that specifically recognizes a peptide derived from Terminal deoxynucleotidyl Transferase (TdT) presented on HLA-A*02:01 which offers promise in treating ALL and LBL of both, B and T cell lineage (Ali et al 2022; Nature Biotechnology). We utilize a closed, automated manufacturing system, the CliniMACS Prodigy, for our manufacturing process. Here, we addressed the need to optimize the manufacturing process for TCR-T cell therapy. We successfully established a process involving 3 major stages: (1) the activation of CD8 enriched T cells from a cryopreserved apheresis product on Day 0 followed by (2) gamma-retroviral transduction to insert the genetic code of the therapeutic TCR into the T cells' genome on Day 2 and (3) subsequent *ex vivo* expansion before harvest and cryopreservation of the cells. In vitro characterization demonstrated that the drug product is highly functional, potent, and safe. We further assessed the potential risk associated with transducing residual leukemic cells using patient-derived samples. The process is highly reproducible as demonstrated by successful tech transfer to the manufacturing facility. This work represents a step towards advancing TCR-T cell therapy for ALL and LBL treatment produced on a closed automated manufacturing platform.

Genomics Core Facility: High-throughput multi-omics from tissue to single-cells

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The Genomics Core Facility (GCF), located at the Institute for Cancer Research, Oslo University Hospital (OUH), provides local, regional, and national researchers easy access to state-of-the-art competence and technologies in the field of genomics. We are an approved OUH and UiO core facility responsible for the National Infrastructure for Sequencing (NorSeq) cancer-related activities. We offer a vast repertoire of protocols to study the genome, epigenome, and transcriptome using sequencing-, microarray-, or nanoString-based technologies. Over the past 25 years, we have established a broad set of workflows for bulk analysis from a wide range of biological materials (i.e., cell cultures, fresh or frozen tissues, FFPE, and liquid biopsies). In addition, we have pioneered the established single-cell and spatial multi-omics analysis protocols in Norway, enabling researchers to improve the understanding of complex biological processes in health and disease at high resolution and in a spatial perspective.
Today, single-cell sequencing is a cutting-edge technique for cell biology. This has been applied to reveal new cell types, investigate the dynamics of developmental processes, identify new regulatory mechanisms, and reveal cellular heterogeneity in healthy and diseased tissues. Further, studying the expression of genes and proteins and their natural spatial distribution has improved our understanding of cancer, immune response mechanisms, and microenvironment interactions. The spatial technology at the Genomics Core Facility enables the profiling of specific cell types in tissues, revealing functions of distinct and rare cell populations or distinct biological compartments. Further, this technology can identify novel diagnostic, predictive, and prognostic biomarkers for diseases associated with cellular heterogeneity, immune response, and microenvironment. GCF is a certified service provider for two solutions for spatial analysis - the Digital Spatial Profiler (nanoString) and Visium platform (10x Genomics). Visit Oslo.Genomics.no for more information.

SNX10 Regulates Mitochondria Homeostasis

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Abstract

SNX10 is a member of the sorting nexin (SNX) protein family, having a PX domains that interacts with PI(3)P. SNX10 is linked to autosomal recessive osteopetrosis—a genetic disorder marked by heightened bone density and impaired osteoclast function. In the current study we demonstrate that SNX10 localizes to early and late endocytic compartments in a PI(3)P-dependent manner and that it regulates endocytic trafficking. Intriguingly, we find that SNX10 positive vesicles interact dynamically with the mitochondrial network and that SNX10 interacts with mitochondrial proteins. Notably, these vesicles contain mitochondrial material, including COXIV and SAMM50, proteins essential for mitochondrial respiratory chain assembly. Depletion of SNX10 leads to reduced COX4 and SAMM50 levels in vitro and hampers mitochondrial respiration and reduces citrate synthase activity, indicating a role for SNX10 in piece-meal mitophagy for sustaining mitochondrial bioenergetics. Importantly, knockout of Snx10 homologues in zebrafish results in reduced Cox4, elevated cell death, and ROS levels, highlighting the relevance of SNX10 in mitochondrial homeostasis in vivo.

Prognostic molecular factors for liver transplantation in unresectable metastatic colorectal cancer

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Abstract

Patients with unresectable metastatic colorectal cancer have a poor prognosis. Liver transplantation (LT) offers a potential for long-term survival in selected patients with unresectable liver-confined metastasis. Optimal patient selection is crucial to increase the survival benefit from LT and avoid futile transplantations. The only molecular marker included in the current consensus guidelines is the poor-prognostic *BRAF* V600E mutation. We aimed to identify additional molecular prognostic factors and determine whether the favorable survival outcome from LT can be attributed to selection of patients with indolent tumor biology.

We performed mutation profiling of a custom 20-gene panel and transcriptomic profiling of liver metastases from 48 patients included in nonrandomized controlled trials of LT and 99 patients treated by liver resection for metastatic colorectal cancer at Oslo University Hospital, Norway. All patients received neoadjuvant chemotherapy.

Co-occurring mutations of RAS(*NRAS*/*KRAS*) and *TP53* were identified in 38% of patients and associated with a shorter median overall survival after LT (21 versus 60 months; HR 4.0, 95% CI 1.7-9.3, adjusted P = 0.01). Prognostic value of the co-mutation was retained in bivariable analysis with clinicopathological prognostic features, including primary tumor location (HR 2.7, 95% CI 1-6.8, P = 0.04), size of the largest liver metastasis (HR 3.1, 95% CI 1.2-7.6, P = 0.01), and metabolic tumor volume (HR 3.2, 95% CI 1.3-7.9, P = 0.009). There were no significant differences in mutation frequencies between the LT and resection cohorts, and transcriptomic differences were subtle. However, several gene expression pathways involved in cell cycle progression, DNA replication and cell proliferation were enriched in the transplanted tumors, suggesting a proliferative phenotype compared to resected liver metastases.

In conclusion, RAS/*TP53* co-mutations might be associated with a poor survival benefit from LT, and we suggest that the co-mutation is evaluated as a contraindication for LT in patients with favorable clinicopathological features. The extensive disease burden of patients selected for LT might be attributed to proliferative tumor biology.

Combined treatment with radiation and ATM inhibition induces interferon immune signaling from NSCLC-derived cancer-associated fibroblasts

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Radiotherapy combined with immune checkpoint inhibitors holds great potential for treating non-small cell lung cancer (NSCLC). However, its efficacy is limited to only a fraction of the patients, likely because radiation induces both immunostimulatory and immunosuppressive effects. Our research aims to find new strategies that can increase the immunostimulatory effects of radiotherapy in NSCLC.

We have recently demonstrated that inhibitors of cell cycle checkpoints can increase type 1 interferon (IFN-1) signaling from tumor cells after irradiation, likely through immune sensing of cytosolic DNA from ruptured micronuclei. Here, we hypothesized that cell cycle checkpoint inhibition would also enhance IFN-1 signaling from cancer associated fibroblasts (CAFs). CAFs are stromal cells that can make up a large part of a tumor, and they secrete factors that promote immunosuppression and cancer cell survival. In this study, we treated patient-derived CAFs from NSCLC with radiation (X-ray) and an ATM kinase inhibitor, and measured the IFN-1 response. We found that this combination treatment induces IFN-1 release from CAFs, with the response magnitude varying in a donor-dependent manner. Moreover, co-culturing CAFs with lung cancer cell lines significantly amplifies the IFN-1 response. We further show that the ATM inhibitor impedes the radiation-induced G1 checkpoint, causing CAFs to aberrantly enter the cell cycle post-radiation. Furthermore, our results indicate that the IFN-1 response after the combination treatment partially depends on the cytosolic DNA sensor cGAS and the RNA sensor RIG-I.

Since IFN-1 is increasingly recognized as essential for radiation-induced antitumor immunity, e.g. by activating dendritic cells and bridging innate and adaptive immunity, our findings suggest that combining radiotherapy with ATM inhibition may reprogram NSCLC-CAFs towards an immune-stimulating phenotype.

Blocking S100A9-signaling is detrimental to the initiation of anti-tumor immunity

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Abstract

S100A9, a multifunctional protein mainly expressed by neutrophils and monocytes, poses an immunological paradox. In virus infections or sterile inflammation, it is known as an alarmin with chemotactic effect attracting innate immune cells, as well as mediating proinflammatory effects through TLR4 signaling. However, in cancer it has shown potential as a biomarker of poor prognosis and lack of response to immunotherapy. Its expression by myeloid cells has been related to an immune suppressive phenotype, the so-called myeloid derived suppressor cells (MDSCs). Targeting S100A9 in cancer has therefore been proposed as a potential way to relieve myeloid-mediated immune suppression.

Surprisingly, we found that blocking the extracellular signaling from S100A9 to TLR4 using the inhibitor Paquinimod resulted in increased tumor growth and a detrimental effect on anti-PD-L1 efficacy in the CT26 tumor model. This effect was caused by a reduction in the tumor immune infiltration to about half of untreated controls, and the reduction was made up of a 5-fold decrease in $Ly6C^{high}$ monocytic cells. Suppressive $Ly6G^{+}$ myeloid cells in spleen were not reduced by Paquinimod treatment, contradictory to our expectation, indicating that the mechanisms by which S100A9 contributes to myeloid-mediated suppression are far from understood.

Finally, we found that intratumoral injection of recombinant S100A9 could provide anti-tumor effect in the CT26 model. These findings indicate a previously understudied role of S100A9 as an alarmin and immune stimulatory signal in cancer and highlight the potential to exploit such signals to promote beneficial anti-tumor responses.

The diagnostic efficacy of Nanopore sequencing for global DNA methylation analysis

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In 2018, the German Cancer Research Center (DKFZ) published a tumour classification tool based on array-based DNA methylation analysis of CNS tumour samples. The 2021 WHO classification of CNS tumours acknowledged the potential of methylome profiling in reliably identifying most CNS tumour types and subtypes, despite challenges in methodological approaches, regulatory issues, and limited availability of the technology.

Since September 2021, the Department of Pathology at Oslo University Hospital, in collaboration with the Genomics Core Facility, has utilized array-based DNA methylation analysis for brain tumour diagnostics, analysing almost 300 diagnostic neuropathological specimens. Recently, our group successfully completed the "DNA Methylation Array 935K / Brain Tumor Classifier (MolPath) 2023" proficiency test.

Despite these advancements, array-based analysis presents challenges. The DKFZ classifier considers a calibrated score ≥ 0.9 as a match, but our interim analysis shows only around 50% of cases reach this threshold, compared to a 12% failure rate reported by DKFZ in 2018. Additionally, the smallest array is designed for 8 samples, leading to delays in pathological reports due to the need for batch processing. Data upload requirements to the DKFZ server also raise technical and data protection concerns.

To address these issues, we are exploring the diagnostic efficacy of Nanopore sequencing. Our first subproject will compare DNA methylation classification of CNS tumours using Nanopore sequencing versus the EPIC 935k array-based analysis. We will analyse DNA from 50 patient samples, extracted from both fresh frozen and formalin-fixed tissue, using Oxford Nanopore sequencing. The data will be assessed with classifiers provided by DKFZ and analysed using ROBIN (Rapid nanopOre Brain intraoperatIve classificatioN), a real-time analysis tool developed by Matt Loose. The project aims to evaluate the success rate and concordance of disease entity matches between Nanopore and EPIC array results, enhancing the diagnostic landscape for CNS tumours.

Spatial transcriptomics of ductal carcinoma in situ reveal subtype specific differences in tumoral and stromal cell compartments

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Up to 50% of patients diagnosed with ductal carcinoma in situ (DCIS) never experience progression to invasive disease, even if left untreated. Current knowledge of what makes DCIS become invasive is limited and we lack diagnostic tools to predict which patients can be spared treatment. Escape of tumor cells from the breast ducts is influenced by characteristics of the tumor cells, the microenvironment surrounding the ducts, and the interplay between the two. Intraductal tumor cells in DCIS are not physically in contact with extraductal stromal cells. Until now, analyses of DCIS tissue have mainly been performed on bulk tissue, however, this approach does not take into consideration the unique morphology of DCIS. We used the Nanostring GeoMX® digital spatial profiling platform to determine the transcriptome of DCIS tumor cells and the surrounding stromal cells separately. From a large cohort of >500 DCIS cases from Akershus and Oslo University Hospitals, we selected 23 pure DCIS cases, grade 3, of different molecular subtypes: triple negative (TN), Luminal A (LumA) and HER2-enriched. At least four tumor-stroma pairs were selected from each case. Bioinformatic analyses were used to explore both tumor and stroma expression data and the interplay between the two cellular compartments. There were distinct gene expression differences between DCIS of different subtypes. Intertumoral heterogeneity was larger than intratumoral heterogeneity in all subtypes. This was also apparent in stromal cell compartments, although less pronounced. Gene ontology analyses of DCIS tumor cells from each of the three subtypes showed several overlapping biological processes, suggesting common mechanisms for regulating tumor cell growth in DCIS. The immune microenvironment differed between the subtypes: LumA DCIS were characterized by lower immune cell infiltration than TN and HER2-enriched, however, there was variation between and within the cases. Using in silico cell deconvolution, we found that B-cells were more abundant in HER2-enriched tumors, while T-cells were more common in TN and LumA. T- regulatory cells were found in all subtypes. The GeoMX® digital spatial profiling platform is well suited for exploring the transcriptome of DCIS samples. We found biologically relevant differences between tumor cells of the different molecular subtypes already at the DCIS stage in breast cancer progression. The immune cell composition surrounding DCIS lesions also differed between the subtypes. Further studies of the tumor-stroma interplay are required to understand the processes involved in progression of DCIS to invasive disease.

Co-occurring mutations in microsatellite stable colorectal cancer identify prognostic subgroups

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Abstract

Background: Co-occurring mutations in pairs of genes can pinpoint clinically relevant subgroups of cancer. The majority of colorectal cancers (CRCs) are microsatellite stable (MSS) with few prevalent mutations. Large patient cohorts and broad genomic coverage are needed for comprehensive co-mutation profiling.

Methods: Co-mutations were identified in a population-based Swedish cohort analyzed by whole-genome sequencing (n = 819 stage I-IV MSS CRCs), and validated in a publicly available dataset of clinically sequenced metastatic CRCs (MSK-IMPACT; n = 934 MSS). Multivariable Cox proportional hazards analyses with clinicopathological parameters were performed for locoregional (stage I-III) and metastatic (stage IV and recurrent) cancers separately.

Results: Prevalent co-mutations (frequency above 5%) were detected in 23 unique gene pairs, 20 of which included *APC*, *TP53*, *KRAS* and/or *PIK3CA*. Several co-mutations involving *APC* were associated with a good overall survival among patients with locoregional CRC, including *APC*-*TCF7L2* (multivariable HR: 0.49, 95% CI 0.27-0.89). This co-mutation had similar prognostic associations also among metastatic cancers in both cohorts, although restricted to patients not treated by metastasectomy in MSK-IMPACT (multivariable HR: 0.29, 95% CI 0.11-0.78). Furthermore, *APC*-*SOX9* co-mutations were mutually exclusive with *APC*-*TCF7L2*, and both had stronger prognostic associations than *APC* alone. Among patients treated by metastasectomy, co-mutations of *FBXW7* with either *PIK3CA* and/or RAS hotspots were associated with a worse overall survival (multivariable HR: 10.64 and 4.94, 95% CI 0.3.21-35.29 and 1.69-14.45, respectively). Co-mutated *BRAF* and *RNF43* was associated with worse overall survival (multivariable HR: 4.13, 95% CI: 1.78-9.54) among the locoregional patients.

Conclusions: We report a genome-wide evaluation of co-occurring mutations in MSS CRCs, and suggest strong prognostic relevance in particular for *APC*-*SOX9*/*TCF7L2* co-mutations among patients not treated by metastasectomy.

InPreD – providing advanced molecular diagnostics for cancer patients in the sphere between cancer research and the clinic

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For the implementation of precision cancer medicine (PCM), it is essential to have access to advanced molecular diagnostics and expert support frameworks. With an increasing number of targeted drugs and biomarker driven clinical studies, there is an unmet need of broad molecular diagnostics for Norwegian cancer patients. Infrastructure for Precision Diagnostics (InPreD) was a core initiative for moving PCM forward in Norway.

In 2020, the CEOs of the four Norwegian health regions allocated funding to the six university hospitals to form InPreD, a collaborative network with dedicated environments (nodes) at each site for implementing next-generation cancer diagnostics.

InPreD at Oslo University Hospital (OUH), initiated as the first InPreD node, built a transdisciplinary environment and implemented a complete pipeline for comprehensive gene profiling (CGP) testing of patients eligible for experimental cancer treatment and trial inclusion (TSO500 gene panel). InPreD-OUH also established and organize weekly national Molecular Multidisciplinary Tumor Board (Mol-MDT) meetings where the clinical implication of CGP test results is conveyed to the patient's treating oncologist. There are multiple challenges related to the establishment of an optimal CGP pipeline beyond the next-generation sequencing itself, including efforts to provide scalable solutions at the various steps *e.g*. tissue handling, library prep, data processing, molecular interpretation and reporting. Molecular diagnostics tests/assays beyond TSO500 are under evaluation to assess novel complex biomarkers, underscoring development as a major task for InPreD-OUH. All workflows established at InPreD-OUH are shared with other InPreD nodes to harmonize procedures and the molecular data interpretation presented at Mol-MDTs. We will present sample logistics and statistics of cases that have completed CGP profiling at InPreD-OUH.

InPreD-OUH holds a unique position as it operates in the sphere between cancer research and the clinic, fostering transdisciplinary interactions. In addition, the environment provides novel career opportunities and collaborations with scientists at Institute for Cancer Research.

ATPase activity of DFCP1 controls selective autophagy

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Abstract

Cellular homeostasis is governed by removal of damaged organelles and protein aggregates by selective autophagy mediated by cargo adaptors such as p62/SQSTM1. Autophagosomes can assemble in specialized cup-shaped regions of the endoplasmic reticulum (ER) known as omegasomes, which are characterized by the presence of the ER protein DFCP1/ZFYVE1. The function of DFCP1 is unknown, as are the mechanisms of omegasome formation and constriction. Here, we demonstrate that DFCP1 is an ATPase that is activated by membrane binding and dimerizes in an ATP-dependent fashion. Whereas depletion of DFCP1 has a minor effect on bulk autophagic flux, DFCP1 is required to maintain the autophagic flux of p62 under both fed and starved conditions, and this is dependent on its ability to bind and hydrolyse ATP. While DFCP1 mutants defective in ATP binding or hydrolysis localize to forming omegasomes, these omegasomes fail to constrict properly in a size-dependent manner. Consequently, the release of nascent autophagosomes from large omegasomes is markedly delayed. While knockout of DFCP1 does not affect bulk autophagy, it inhibits selective autophagy, including aggrephagy, mitophagy and micronucleophagy. We conclude that DFCP1 mediates ATPase-driven constriction of large omegasomes to release autophagosomes for selective autophagy.

Poster abstract

A urine DNA methylation test for diagnosis and surveillance of bladder cancer

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Cystoscopy is the gold standard for both diagnosis and surveillance of non-muscle invasive bladder cancer (NMIBC), but is costly, invasive and has suboptimal accuracy. We have developed BladMetrix; a urine test consisting of eight DNA methylation biomarkers, and have assessed its performance for 1) diagnosis among patients with gross hematuria, and 2) surveillance of patients with NMIBC.

In a large prospective and blinded series of patients with gross hematuria (n=273), BladMetrix detected bladder cancer with 92% sensitivity, 93% specificity, a negative predictive value of 98%, and showed potential to reduce 56% of the cystoscopies[1]. This level of accuracy is rare among competing commercial urine tests.

In a unique longitudinal series of patients (n=47) followed for bladder cancer recurrence for 2 years, BladMetrix achieved 91% sensitivity, had less than 1% false negative test results, and could have reduced the number of cystoscopies by 55%[2]. Interestingly, BladMetrix also showed potential to detect recurrences earlier than cystoscopy, and to indicate minimal residual disease and field effect. A national multicenter study is ongoing, where we follow around 500 NMIBC patients for recurrence for two years, and aim to demonstrate clinical utility.

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[2] Vedeld, H.M., Pharo, H., Sørbø, A.K. *et al.* Distinct longitudinal patterns of urine tumor DNA in patients undergoing surveillance for bladder cancer. *Submitted to Molecular Oncology 6th of September 2023.*

The IMPRESS-Norway trial: - Safety analysis of the first 186 patient included in the trial.

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Background

In IMPRESS-Norway patients with advanced malignancies with no further treatment options are included and treated in multiple treatment cohorts, defined by tumor type, molecular alteration and study drug. Toxicity profiles of the drugs used in IMPRESS-Norway are well described, however new safety signals could be detected when drugs are used in new indications.

Methods

At the time of the analysis 16 different treatment or treatment combinations were used and data on clinically relevant adverse events (AEs) and serious adverse events (SAEs) were collected. AEs grade ≥ 3 and SAEs were recorded for all drugs, and AEs grad 2 for relatively new drugs as alpelisib and pemigatinib.

Results

The trial started recruitment in April 2021, and by September 2023, 186 patients were included in treatment cohorts. Patients were treated with: trametinib (n=41); pertuzumab+trastuzumab (n=35); atezolizumab (n=26); vemurafenib+cobimetinib (n=19); alpelisib+/-fulvestrant (n=20); trametinib+dabrafenib (n=14); atezolizumab+bevacizumab (n=8); pemigatinib (n=7); vismodegib (n=5); olaparib (n=3); imatinib (n=3); alectinib (n=2); bortezomib (n=1), melphalan (n=1) and entrectinib (n=1).

In total, 309 AEs were registered, and of those 216 were SAEs leading in majority of cases to initial or prolonged hospitalization. Most of the reported AEs were grade 2 or 3 (278 of 309). Majority of the AEs and SAEs were considered as not related or unlikely to be related with treatment, 55% and 63% respectively. 26% of all AEs were considered as possibly related and 19% as related to treatment. Conversely, 26% of all SAEs were possibly related and 11% as related to treatment. Eleven deaths were registered during the treatment period, 2 considered to be treatment related, and 6 suspected unexpected serious adverse reactions (SUSARs) were reported.

Conclusion

Current data indicates safety profile consistent with the one reported in earlier trials.

Development and evaluation of a lymph node invasion prediction model in intermediate- and high-risk prostate cancer patients

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Background: International guidelines recommend to use nomograms to predict pre-operative risk of lymph node metastasis in prostate cancer patients. But still more than 75% of all patients that undergo a pelvic lymph node dissection (PLND) are staged as lymph node negative.

Objective: Develop a prediction model utilizing readily available clinical, histopathological, and MRI variables. Evaluate Briganti 2012, Memorial Sloan Kettering Cancer Center (MSKCC) and Briganti 2019, some of the most frequently used prediction models.

Design, setting, and participants: A retrospective cohort with 903 prostate cancer patients undergone robot-assisted radical prostatectomy and extended PLND between January 2015 and December 2022 at Oslo University Hospital (OUS). Temporal validation was assessed using at cohort of 151 patients from 2023 to May 2024.

Outcome measurements and statistical analysis: Bayesian logistic regression was applied and overall performance was evaluated using R^2 and Brier score. Discrimination was assessed by area under the receiver operating characteristic curve

(AUC), calibration plots to determine reliability and clinical benefits using decision curve analyses.

Results and limitations: The Briganti 2019 model (AUC 0.74, 95% CI: 0.70-0.78) outperformed both the MSKCC (AUC 0.65, 95% CI: 0.61-0.70) and Briganti 2012 (AUC 0.68, 95% CI: 0.64-0.72) models. All three models overestimated the predicted probabilities of the OUS cohort, which had a lymph node involvement prevalence of 26%. The Oslo model had an AUC of 0.77 (95%: 0.76-0.78) and R^2 of 0.23, while Briganti 2019 had a R^2 of 0.00. Temporal validation of the Oslo model indicates that it has high reliability as demonstrated by the calibration plot and overall performance based on the R^2 of 0.29 and AUC of 0.79 (95% CI: 0.72-0.88). Limitations are the size of the temporal validation cohort and lack of an external validation.

Conclusions: The Oslo model improved predictive performance in intermediate and high-risk contemporary patients. Accessible variables and Bayesian probability will increase the clinical usability of the presented Oslo model.

The Flow Cytometry Core Facility at Institute for Cancer Research

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The Flow Cytometry Core Facility (FCCF) at OUS and UiO offers advanced flow cytometry instruments and assistance. The FCCF node at Institute for Cancer Research has been operational since 1995, and currently has 30 years of combined staff competence. We offer full-service and do-it-your-self cell sorting on 3 cell sorters, including the latest generation of full-spectrum Aurora CS. On the Analyzer side, we have 4 high-parameter fluorescence instruments including a full-spectrum 5-laser Aurora. We also have a mass cytometry system for analyzing cell suspensions or imaging tissue slide with 40-60 markers. We offer user training and extensive assistance in instrumentation, FCM theory, robust experiment design and advanced data analysis. We have broad experience with a diverse range of applications; cell cycle, apoptosis, quantitative measurements, immunophenotyping, proliferation, bacterial analyses, and functional fluorescent probes to measure e.g. Ca^{2+} flux and ROS.

The invention of mass cytometry caused a rapid development of fluorescence-based cytometry to catch up with the number of markers being possible in the same tube. Full-spectrum flow cytometers have advantages over both conventional flow cytometry and mass cytometry. Signal spreading error limits conventional high-parameter flow, while sample throughput and running costs are limiting for mass cytometry. Full-spectrum flow cytometry uses a higher number of detectors compared to markers to better identify the contribution of each dye, i.e. oversampling. Mathematical unmixing models quantify the abundance of each dye from the signal measured in all detectors, from all lasers. Additionally, the modeling can define and subtract the autofluorescence of the cell in question, significantly increasing the resolution in many cases. Full-spectrum flow cytometry is the future for most high-parameter applications, but is also helpful for the analysis of highly autofluorescent samples.

Prognostic value of elevated Plk1 levels in prostate cancer assessed by deep learning-based immunohistochemistry measurement

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Background: Polo-like kinase 1 (Plk1) is a serine/threonine kinase that regulates key biological processes, including mitosis, apoptosis, metabolism, RNA processing, vesicle transport, and DNA damage response. Elevated Plk1 levels are frequently observed in various cancers including prostate cancer (PCa) and correlate with poor survival rates. This study investigates Plk1's prognostic value in PCa.

Materials and methods: Plk1 levels were measured in tissue sections from radical prostatectomy specimens using immunohistochemistry. A previously developed deep learning model quantified Plk1 levels, with a threshold set in a discovery cohort (n=253) and validated in an independent cohort (n=327). One tumor tissue block per patient was analyzed in the discovery cohort, whereas three were analyzed in the validation cohort, encompassing a total of 1178 tumor areas. The endpoint for the discovery cohort was clinical recurrence (locoregional recurrence, distant metastasis or death from PCa), while biochemical recurrence (a single prostate-specific antigen (PSA) ≥ 0.4 ng/ml) was the endpoint for the validation cohort. The marker was analyzed in univariable and multivariable survival analyses, incorporating age, Gleason grade groups, PSA levels, and status of seminal vesicle infiltration, extraprostatic extension, surgical margins, and lymph node involvement.

Results: Plk1 positivity in over 1.2% of cancer cells significantly increased the risk of clinical recurrence in both univariable (hazard ratio [HR] 2.47, 95% confidence interval [CI] 1.52 to 3.99; p<0.001) and multivariable (HR 2.6, 95% CI 1.52 to 4.44; p<0.001) analyses in the discovery cohort. Similarly, in the validation cohort, Plk1 positivity was significantly associated with increased risk of biochemical recurrence in both univariable (HR 3.65, 95% CI 2.1 to 6.34; p<0.001) and multivariable (HR 2.2, 95% CI 1.11 to 4.22; p=0.024) analyses.

Conclusion: Elevated Plk1 levels are associated with a higher risk of recurrence in PCa, suggesting its potential as a prognostic marker for the disease across different clinical endpoints.

Proteasome inhibition overcomes resistance to targeted therapies in B-cell malignancy models and in an index patient

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Treatment of B-cell malignancies with the PI3K inhibitor (PI3Ki) idelalisib is frequently associated with high toxicity and development of resistance. However, the resistance mechanisms are poorly understood, and treatment options in the relapsed/refractory (R/R) setting are often missing since idelalisib administration is recommended as a late or last line therapy. To elucidate resistance mechanisms and identify subsequent treatment options, we studied functional phenotypes of idelalisib-resistant or -intolerant B-cell malignancy models. While the idelalisib-resistant KARPAS1718 model remained sensitive to Bcl-2 inhibitors (Bcl-2i), Bcl-2i sensitivity was reduced in the idelalisib-resistant VL51 model, relative to parental counterparts. Phosphorylation or expression of the Bcl-2 family members Bcl-2 and Bim correlated with the sensitivity. Target addiction scoring revealed high dependence on the proteasome, and proteasome inhibitors (PIs) were effective in the two models and in primary chronic lymphocytic leukemia (CLL) cells, independently of their PI3Ki- or Bcl-2i-sensitivities. Bim and Mcl-1 were consistently upregulated in response to PI treatment, while Bcl-2 was upregulated in KARPAS1718 and CLL cells only. Bcl-2i plus PI combinations were synergistic in these models. To study the clinical relevance of the combination, a multi-refractory CLL patient was treated with Bcl-2i plus PI in the IMPRESS-Norway trial (NCT04817956). The patient showed an initially good response with improved quality of life, but relapsed within four months. Upregulation of Bim and Mcl-1 in response to PI treatment was confirmed, and reduced cytotoxic $CD8^+$ T-cell and $CD56^{dim}$ NK-cell populations off treatment was observed. Together, our findings suggest that proteasome inhibition may overcome resistance to targeted therapies in B-cell malignancies. However, the response duration may be short and additional measures may be needed.

Exercise-induced extracellular vesicles in breast cancer

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Introduction: Studies demonstrate that physical activity reduces tumor incidence, however, the molecular mechanisms behind these effects are poorly understood. The beneficial effects of physical activity are also mediated by factors secreted into the circulation during exercise, not only as soluble molecules, but also associated to extracellular vesicles (EVs). The aim of this study is to explore the interplay between exercise, EVs, and breast cancer (BC). This may offer insights into EV-based novel therapeutic strategies and preventive measures for cancer.

Methods: Plasma EVs were isolated by size exclusion chromatography (SEC) from both human and mice running-trained healthy females. EVs were characterized using electron microscopy, nanoparticle tracking analysis (NTA), and western blot. Mass spectrometry was used to analyze EV protein composition. Tumor-bearing mice were administered with exercise-induced EVs and the immune response was assed using flow cytometry.

Results: Mass spectrometry analysis on female running-trained human plasma-EVs showed that thioredoxin, a key antioxidant enzyme, was the protein with the highest fold change after exercise. NTA results showed that plasma EV concentration is higher in running-trained Balb/c mice. Moreover, administration of exercise-induced plasma EVs into a triple-negative BC syngenic mice model delayed tumor growth by 19-57%. Finally, we observed differences in tumor-infiltrating immune cells, with a substantial influx of CD8+ T lymphocytes in the exercise-induced EV treated groups.

Conclusion: These results reveal a role for exercise in modulating the presence of antioxidant molecules in EVs, which could protect tissues from oxidative stress and provide a novel mechanism to explain the beneficial effects of physical activity. Moreover, our study demonstrates that treatment with exercise-induced EVs have immunomodulatory effects that may promote an antitumor immune response. These findings support the incorporation of physical activities in the treatment plans of BC patients and provide a rationale for further investigations of EVs as potential exercise mimetics.

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Therapeutic targeting of the cyclic-AMP/Prostaglandin E2 pathway in ovarian cancer.

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Prostaglandin E_2 (PGE_2), the most abundant prostanoid in the human body, plays a crucial role in sustaining immune homeostasis. In ovarian cancer (OvCa) however, PGE_2 signaling promotes an immunosuppressive microenvironment through its receptors EP2 and EP4, primarily via the cyclic AMP (cAMP) signaling pathway in effector T cells (Teffs). Therefore, we aimed to abrogate the PGE2/cAMP-mediated suppression of metastatic OvCa Teffs and restore immunity in these cells by inhibiting the PGE_2-EP2/EP4 receptors.

We have found that PGE_2 levels are significantly higher in the plasma and ascites of metastatic OvCa patients compared to age- and gender-matched healthy donors. In addition, the activated T cell subsets from ascites and peripheral T cells of these cancer patients produce low levels of IFN-γ, IL-2, and TNF-α, while expressing high levels of T cell exhaustion markers PD-1, TIM-3, HLA-DR, and CD39. These findings could be associated with the observed increase in PGE_2 levels.

TPST-1495 is a dual EP2-EP4 antagonist currently undergoing a phase 1a/1b clinical trial for several cancer types (NCT04344795). Antagonism of the EP2/EP4 receptors by TPST-1495 countered PGE_2's immunosuppressive effects on activated Teffs. TPST-1495 not only induced IFNγ, IL-2, and TNFα production and decreased the expression of both PD-1 and TIM-3, but also, countered the PGE_2 effect on these exhausted immune cells by enhancing their proliferation. Furthermore, inhibition of COX1/2 or PKA type 1 all significantly increased immune responses, implicating the cAMP-PKA pathway in immune regulation.

Moreover, PGE_2 promotes regulatory T cells (Tregs) suppressive function. We could demonstrate that Tregs inhibit anti-tumour immune responses in patients with OvCa, and depletion of $CD25^+$ T cells dramatically augmented both IFNγ and IL-2 expressions in T cells from these cancer patients.

This study highlights the potential of blocking the EP2/EP4 receptors to improve anti-tumor responses, particularly in cancers with an incomplete response to immune checkpoint inhibitors.

Immunoprofiling of Chemo Resistant Breast Cancer Patient-Derived Xenografts (PDXs) by using FLOW Cytometry

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Triple negative breast cancer (TNBC) is the most aggressive subtype among all BC with limited treatment options once resistance towards chemotherapy is obtained. The last decade of immunotherapy revolutionized cancer treatment. Even though BC has long been considered a "cold" tumor, TNBC exhibits a greater presence of infiltrating lymphocytes, thereby establishing a favorable immune microenvironment for the potential utilization of immunotherapies. The aim of this study is to identify immune cell composition and their phenotypic appearance in chemo resistant TNBC and its potential usage as actionable targets to sensitize resistant TNBC.

To this end we have established an isogenic pair of Mas98.12 PDXs, consisting of paclitaxel-sensitive and resistant variants. In addition, a protocol for multiparameter FLOW cytometry including 19 markers has been optimized allowing identification of both tumor and various immune cells. Thus, by using FLOW cytometry, we identified a higher number of newly arrived and MHC-II positive macrophages, inflammatory monocytes, dendritic cells and eosinophils in the paclitaxel resistant PDX variant compared to the sensitive one. We have also observed similar results in another TNBC isogenic Enhertu (HER2-topoisomerase inhibitor conjugate) sensitive and resistant Hbc x 39 PDXs model.

To investigate treatment-induced/associated differences, both sensitive and resistant Mas98.12 PDXs models were subjected to various chemo- and targeted therapies. Both variants reduced tumor size upon treatments compared to control. The preliminary FLOW cytometry results indicate that various treatments had similar effects on sensitive Mas98.12 PDX where numbers of newly arrived macrophages (in most cases), inflammatory monocytes and dendritic cells were increased. Such immune cell composition is similar to what was observed in the paclitaxel resistant Mas98.12 variant. We also observed similar results when the resistant Mas98.12 variant was treated with other chemotherapies than paclitaxel. What exact role these cells have in relation to treatment response and resistance is still to be addressed.

ShinySpatial: Leveraging Spatial Transcriptomics Data with ShinyApplication for Advanced Biological Insights

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Abstract:

Spatial transcriptomics merges gene expression data with precise spatial locations in tissue samples, offering unique insights into tissue dynamics. The Shiny web application framework supports this by enabling interactive analysis and visualization of such data, catering to both experts and novices. The ShinySpatial particularly addresses challenges in data interpretation by allowing dynamic sample selection, and interactive UMAP and spatial plotting for effective gene expression exploration within tissues. It aids in comparing spatial gene expression across tissues to identify differential expression and potential therapeutic targets, with functionalities to export plots and data for further research or publication. ShinySpatial is designed to overcome analytical hurdles through an interactive web interface for data formatted in Seurat version5 as RDS file. Key features include: 1) Dynamic Sample Selection: Users can select specific samples via a dropdown for comparative studies. 2) Interactive Spatial Plots and UMAP: These tools aid in visualizing clustering in spatial contexts and as well as UMAPS. 3) Gene Expression: A Feature of Interest Tab interface facilitates querying and visualizing expression levels of specific genes, highlighting expression hotspots.

Conclusion: ShinySpatial enhances the accessibility and interpretability of complex spatial datasets. It offers tools for interactive exploration, empowering researchers to discover detailed spatial gene expression patterns. Future enhancements may include integrating advanced analytical tools like machine learning for predictive modeling and deeper tissue analysis using deconvolutional methods.

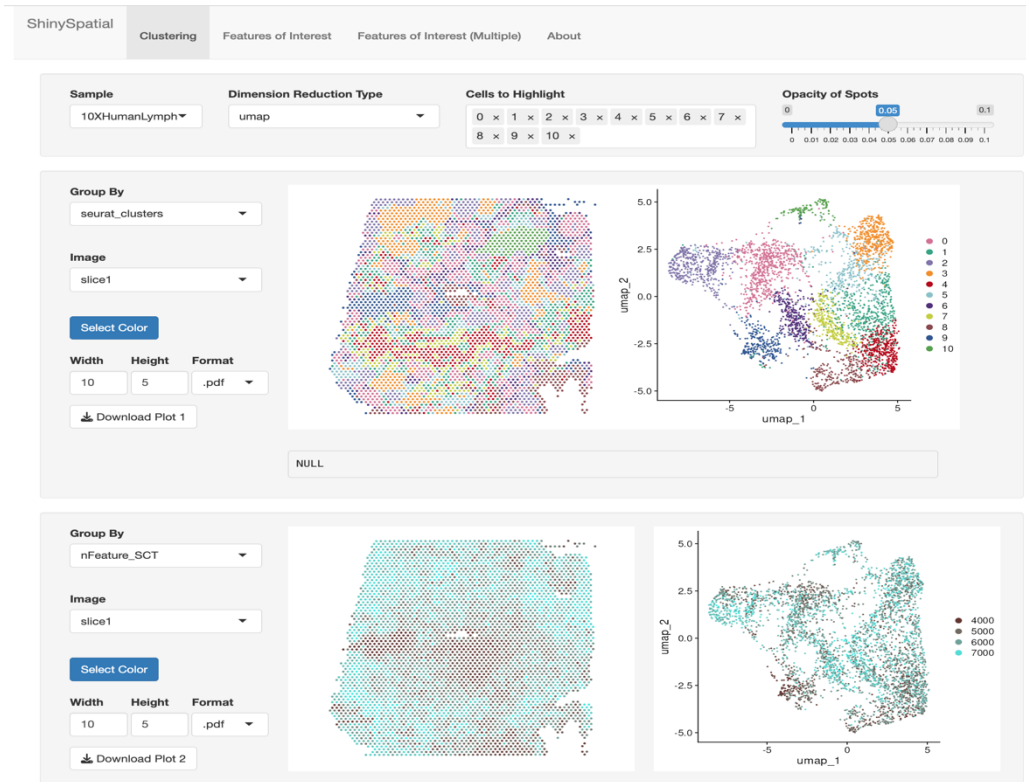

Figure 1: Showing Demo ShinySpatial interface visualizing Human Lymph Node data from10X Genomics Human analyzed with Seurat Version 5. ShinySpatial is available at https://myklebust.medisin.uio.no/spatial/Demo_ShinySpatial/

Identification of novel mitophagy regulators through a high-throughput imaging siRNA screen

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Mitophagy is a catabolic process responsible for selectively degrading dysfunctional mitochondria through autophagy. Dysregulation of this pathway has been linked to a wide spectrum of diseases, encompassing neurodegenerative disorders, cancer, and metabolic disorders. Mitophagy can be triggered by various stimuli, including mitochondrial depolarization, reactive oxygen species (ROS), hypoxia, and nutrient deprivation.

In this study, we aimed to identify novel regulators of mitophagy using a high-throughput imaging siRNA screen targeting 1536 mitochondrial and mitochondrial-associated proteins. The screen was performed in U2OS cells expressing an EGFP-mCherry-tagged mitochondrial matrix reporter, both under normal basal conditions and conditions simulating hypoxia-induced mitophagy (induced by DFP).

As expected, the depletion of several mitochondrial proteins resulted in an increase in mitophagy, likely due to the disruption of mitochondrial homeostasis. Most interestingly, 39 mitochondrial proteins were found to be required for DFP-induced mitochondrial turnover, including components of the mitochondrial import machinery, mitochondrial replication complex, iron-sulfur cluster and mitochondrial respiratory chain complexes.

Subsequently, 20 of the candidates required for DFP-induced mitophagy were further analyzed for a role in mitophagy upon hypoxia and membrane de-polarization (CCCP-induced) in cells with and without Parkin co-expression. Data from our current characterization of the precise mechanisms involved in the regulation of mitophagy by some of these candidates will be presented. In conclusion, we have identified novel regulators of mitophagy that could be potential therapeutic targets for mitochondrial dysfunction-associated diseases.

Spatial analysis of miRNA regulation at defined tumor hypoxia levels reveals biological traits of aggressive prostate cancer

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Tumor hypoxia is associated with aggressive disease and radiotherapy resistance in prostate cancer. Cancer cells adapt to hypoxia by altering gene expression through regulatory mechanisms dependent on hypoxia level, which may range from mild to moderate and severe within tumors. Knowledge of these mechanisms may facilitate development of more efficient drugs to be combined with radiation, but remain unexplored in patient tumors due to challenges in assessing hypoxia levels. We aimed to determine miRNA regulation of gene expression at defined hypoxia levels in prostate cancer. Biopsies from 95 patients were used, of which 83 received the hypoxia marker pimonidazole before prostatectomy. Hypoxia levels were extracted from pimonidazole-stained sections by a previously established histopathology method and correlated with miRNA and gene expression profiles from RNA-sequencing and Illumina beadarrays. This analysis identified miRNAs associated with moderate (n=7) and severe (n=28) hypoxia and predicted their target genes. miRNA and target gene scores showed prognostic significance, as validated in an external cohort of 417 patients. Target genes showed enrichment for cell proliferation and MYC activation across all hypoxia levels and PTEN inactivation at severe hypoxia, as confirmed by RT-qPCR of *MYC* and *PTEN,* Ki67-immunohistochemistry, and gene set analysis in external cohort. To assess whether miRNA regulation occurred within the predicted hypoxic regions, a method quantifying co-localization of histopathology parameters at defined hypoxia levels was developed. High Ki67-proliferation indices significantly co-localized with hypoxia at all levels and the co-localization was strongly associated with poor prognosis. Absence of PTEN-staining significantly co-localized with severe hypoxia, and expression of the miRNA candidate miR-210-3p was confirmed to occur within severe hypoxia by *in situ* hybridization. In conclusion, cancer cells within hypoxic regions of aggressive prostate tumors exhibit distinct proliferative gene expression programs regulated by miRNAs. This program and its regulation show intratumor heterogeneity, depending on hypoxia level.

Breast cancer with different chemosensitivity has distinct influence on the immune microenvironment

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Triple Negative Breast Cancer (TNBC) is a BC subgroup with the least favorable outcome and limited therapeutic options. Chemotherapy has long been the standard treatment, but resistance often develops. In recent years, various immunotherapy approaches have diversified, including combinations of chemo and immunotherapies. Increased understanding on how the difference in chemosensitivity affects the immune cells is of importance for further development of chemo-immunotherapies for TNBC.

In this project we investigate how TNBC with distinct chemosensitivity modulate the immune microenvironment. Specifically, we explore the influence of the secretome, derived from the chemoresistant versus the sensitive BC cells, on monocytes and the derived macrophages (MΦs) as well as on T cells. We have disclosed the chemoresistance-dependent differences in the secretome composition, which resulted in potentiated monocyte recruitment, skewed MΦ polarization and reduced MΦ response to classical activation.

To study the influence of the secretomes on T cells, we employed T cell receptor (TCR)-engineered T cells that become activated in the presence of target cells. We showed that activation of such T cells was attenuated in the presence of the “resistant” secretome, which reduced expression of activation markers (CD137 and CD69) as well as T cell proliferation. Collectively, it suggested immunosuppressive influence of the resistant secretome.

Taken together, upon chemoresistance, tumor cells enrich their inflammatory secretome and influence both innate and adaptive immune cell activation state, highlighting their distinct communication with the immune microenvironment.

A multi-functional prognostic model predicts progression free survival for chronic lymphocytic leukemia patients treated with ibrutinib plus venetoclax in the HO141/VISION trial

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Background:
Targeted therapies have made a breakthrough in treatment of chronic lymphocytic leukemia (CLL), but many patients develop resistance, experience side effects, or relapse during therapy. Since traditional biomarkers for chemoimmunotherapy have failed to predict clinical outcomes on time-limited combination targeted therapies, new clinicobiological features are warranted. HO141/VISION (NCT03226301) is a randomized phase II trial that evaluates the efficacy of minimal residual disease (MRD) guided combination treatment with the Bcl-2 inhibitor (Bcl-2i) venetoclax and the BTK inhibitor (BTKi) ibrutinib in relapsed/refractory (R/R) CLL patients.

Aims:
i) Identify associations between functional, genetic, and clinical characteristics in CLL
ii) Define biomarkers that predict progression free survival (PFS) on the HO141/VISION trial

Methods:
Peripheral blood mononuclear cells (PBMCs) were collected from CLL patients (n = 177) enrolled in the HO141/VISION trial prior to treatment. The samples were subjected to functional analyses:

immunophenotyping (6 surface markers for B-, T- and NK-cell subpopulations) and (phospho)protein profiling (31 markers) by flow cytometry, and drug sensitivity screening (95 single agents, 87 combinations) using the CellTiter-Glo assay. Association analyses between functional, genetic (IGVH, *TP53* aberration, karyotype), and clinical (Binet stage, MRD, PFS) features were investigated using the Wilcoxon rank sum test. SHAP values were calculated from random forest to understand the directionality and importance of each feature in predicting drug sensitivity scores. To identify a biomarker predictive of PFS (20 events), the patient samples were divided into a training set (n=80) and a test set (n=77) stratified on IGVH and *TP53* mutational status, and an independent validation set (n=20). The proteins detected by flow cytometry were paired and used as binary patient-specific protein-pair features. Univariate Cox regression and permutation testing in random survival forest identified the most predictive features using 10-CV in the training set ($p<0.1$), and the overlapping features from the two selection methods were utilized as a multi-modal signature to predict PFS with various machine learning models.

Results:
Genetic subgroups of CLL showed distinct ex vivo drug sensitivities. We confirmed that *TP53* aberrant CLL is less sensitive to chemotherapy and nutlin 3a, and identified a trend towards higher sensitivity to BTKi and PI3Ki single agents or combinations in high-risk (*TP53* aberrant, IGVH unmutated) CLL, and to Bcl-2i/combinations in low-risk (IGVH mutated) CLL. We found that phosphorylation of PLCγ2 (pY759), which is downstream of BTK, was significantly higher in low-risk CLL. This may explain the reduced ex vivo sensitivity to BTKi in this group. Association studies of functional, genetic, and clinical features showed that protein profiles had the highest predictive power on ex vivo drug sensitivity. In our model of PFS, predictive features included ex vivo sensitivity to vandetanib, ruxolitinib+venetoclax, and acalabrutinib+ZSTK474; relative expression of Bcl-2|BTK (pY223) and p90RSK (pS380)|PLCγ2 (pY759); and a high proportion of $CD8^+$ T cells. The prognostic score demonstrated increased performance when compared to CLL-IPI.

Summary/conclusion:
i) Genetic subgroups of CLL show distinct ex vivo drug sensitivities, and protein profiles have the highest predictive power on ex vivo drug sensitivity
ii) Functional features can predict PFS in R/R CLL patients treated with ibrutinib + venetoclax

Abstract Title

The NAPEER trial: Optimized neoadjuvant endocrine and chemotherapy in an open label phase II trial using molecular biomarkers and on-treatment endocrine efficacy evaluation in patients treated with letrozole with capivasertib, and chemotherapy with bevacizumab (if ViRP signature positive).

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Abstract

NAPEER (I-BCT-2), is a randomized phase 2 study, where the treatment outline is adapted to the most recent results of neoadjuvant treatment sequence in hormone receptor positive breast cancer [1, 2]. In addition, patients that are candidates for chemotherapy treatment will be tested with ViRP score, previously demonstrated to identify responders with benefit from bevacizumab, and randomized to receive such optimized therapy in combination with chemotherapy [3].

The patients will be evaluated at study entry with tumor tissue sampling and clinical evaluation, and patients with high tumor burden, low estrogen receptor level or high risk will be guided to receive chemotherapy. However, the majority of patients will be treated with neoadjuvant endocrine therapy and randomized to treatment with or without capivasertib. After 15 days the endocrine treatment response will be assessed by Ki67-expression, to decide if the patient is a responder or not, and the results of the molecular evaluation of the sample taken at study entry will be available for guiding further patient treatment. If the patient is not responding to the endocrine therapy (Ki67 > 10% of

the tumor cells), or has a molecular intermediate or high risk in the pretreatment sample, the patient will continue with chemotherapy and be assessed for ViRP signature for inclusion into the study of effects of bevacizumab in combination with chemotherapy. Otherwise, patients responding to the endocrine treatment with or without capivasertib at day 15 (the primary endpoint) will be continued with such therapy until week 12, where a new evaluation will be done. If the patient continues to respond, endocrine therapy duration can be prolonged up to 6 months.

In the ViRP positive patients treated with chemotherapy with or without bevacizumab, the primary endpoint is the percentage of complete pathological response in breast and lymph nodes at surgery. In both the endocrine and chemotherapy parts of the study, the invasive disease-free survival in the different treatment groups are secondary endpoints.

The study is ongoing and has recruited 52 patients.

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High-throughput phosphoflow cytometry assay models T cell signaling in response to PD-1 inhibition *in vitro*

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Immune checkpoint blockade (ICB) has revolutionized cancer therapy. Still, a significant portion of patients do not respond to this therapy and our complete understanding of the working mechanism of ICB is still lacking. Thus, there is a need to better understand the molecular underpinnings of PD-1-mediated inhibition and to develop methods to better predict what patient will respond to the therapy and not. Here, we developed a high-throughput phosphoflow cytometry assay to evaluate PD-1 signaling in T cells from human peripheral blood and lymphoid tissue (tonsils). We showed that, with simultaneous T cell receptor (TCR)- and CD28 co-stimulatory activation (anti-CD3/CD2/CD28), the PD-1 ligands PD-L1 and PD-L2 effectively inhibited phosphorylation of key TCR- and co-stimulatory related signaling proteins. This inhibition was reversed by anti-PD-(L)1 therapy, but to varying degree in different PD-1 subsets; the PD-1^{low} subset showed greater reversal potential than their PD-1^{high} counterparts. We also explored whether this assay could be used to retrospectively stratify non-small cell lung cancer (NSCLC) patient responses to anti-PD-(L)1 therapy in a preliminary sample set (n=4 patients). This assay will be used to further unravel the cellular signaling mechanisms of PD-1-mediated inhibition and to investigate its potential uses in stratifying patients considered for anti-PD-1 therapy.

Beyond Helpers: Unraveling the Potential of CD4$^+$ Cytotoxic T Cells in B-cell lymphoma

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CD4$^+$ T cells exhibit remarkable heterogeneity and consist of several subsets. While most CD4$^+$ T cells exhibit either helper or immunoregulatory function, emerging evidence points to the existence of a unique subset of CD4$^+$ T cells with cytotoxic potential. Recently, there has been growing interest in these CD4$^+$ cytotoxic T lymphocytes (CTLs) due to their potential involvement in anti-tumor immune responses and relevance in immunotherapy.

Recently, we employed single-cell RNA sequencing (scRNAseq) and T-cell receptor (TCR) sequencing to explore the landscape of CD4$^+$ T cells in lymphoma. We included diagnostic biopsies of diffuse large B-cell lymphoma (DLBCL, *n*=3) and follicular lymphoma (FL, *n*=3), alongside non-malignant tonsils (*n*=3). Among the CD4$^+$ T cell clusters, one was characterized by high expression of a cytotoxic gene signature (*GZMK*, *NKG7*, *CST7*, *GZMA*, *GZMB*) and high expression of checkpoint receptors (*LAG3*, *HAVCR2*, *TNFSF9*). The CD4$^+$ CTLs accounted for over 20% of CD4$^+$ T cells within DLBCL and over 10% in FL tumors. Strikingly, the CD4$^+$ CTLs were almost absent in non-malignant tonsils. Their tumor-specific nature was further highlighted by TCR-clonality analysis as CD4$^+$ CTLs had the highest clonal expansion among CD4$^+$ T-cell subsets.
We further validated the presence of CD4$^+$ CTLs within an independent series of DLBCL tumors using flow cytometry by identifying CD4$^+$ CTLs as Granzyme B$^+$ Perforin$^+$ cells within the CD4$^+$CD8$^-$FOXP3$^-$ CD56$^-$ population, excluding CD8$^+$ T cells, regulatory T cells and NK cells. CD4$^+$ CTLs ranged from 1-23% of intratumoral CD4$^+$ T cells in DLBCL and consistently with the scRNAseq findings, these cells were absent in tonsils.

In conclusion, by employing state-of-the-art technologies we have characterized CD4$^+$ CTLs in lymphoma and demonstrated substantial clonal expansion in DLBCL. Our findings might have implications for therapeutic strategies that leverage the yet untapped potential of this cytotoxic CD4$^+$ T-cell subset in cancer immunotherapy.

Implications of genomic alterations in *RNF43* and *RSPO3* for the response of $BRAF^{V600E}$ metastatic colorectal cancer to anti-EGFR/BRAF combinatory therapy

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The $BRAF^{V600E}$ mutation, present in 8 – 10% of colorectal cancers (CRCs), is associated with poor patient outcome and has limited treatment options. Recent studies have shown that mutations in the E3 ubiquitin ligase RNF43 predicts positive response to anti-EGFR/BRAF combinatory therapy in patients with $BRAF^{V600E}$ metastatic CRC. This implies an unknown but clinically significant molecular relationship between *RNF43* loss-of-function and the MAPK signaling pathway. RNF43 attenuates WNT signaling by promoting the endocytosis and lysosomal degradation of Frizzled receptors to desensitize the cells to WNT ligands. RNF43 itself is in turn negatively regulated by R-spondins (RSPOs), and both inactivating mutations in *RNF43* and gene fusions involving *RSPO2* or *-3*, encoding for R-spondin2 and -3, are frequently found in CRC. The aim of the present project is to obtain a better understanding of how loss of the R-spondin/RNF43 signaling module mediates a positive response to EGFR/BRAF inhibitor combination treatment.

Our lab has established a living biobank of more than 370 tumor organoids derived from liver metastases of CRC patients. We are currently in the process of characterizing signaling dynamics governing patient outcome to BRAF/EGFR co-targeting by utilizing patient-derived tumor organoids that harbor clinically relevant genomic alterations in *BRAF*, *RNF43*, and *RSPO3*. This includes various omics approaches such as mass spectrometry, RNA sequencing, and kinome profiling of these organoids treated with EGFR and BRAF inhibitors alone or in combination. Here, we present preliminary results from this study, showing how these treatments differentially affect MAPK and WNT signaling depending on the mutational profile of the organoids.

Cancer Treatment Gets Logical

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Abstract

What if there is a fast, cheap way to find synergistic drug combinations? Finding faster and more efficient solutions is a major goal in modern medicine, especially when it comes to the best drug combinations. Testing all possible combinations in the lab is expensive and time-consuming. In this project, we simulate and predict in-silico the effect of drug combinations on a cancerous system. The goal is to implement a new Python-based pipeline to simplify this process by improving an existing automated system that predicts which drug combinations will benefit a patient in a given disease context.

How is it carried out? Let's see the power of Logical Models and Modeling. Logical Models are simplified representations of biological systems, where Boolean algebra rules represent the interactions between the system's components, e.g. genes or proteins. Logical Modeling helps simulate and understand regulatory and signaling processes in biological systems. Our pipeline has two main components: Gitsbe that finds the best models, and Drabme that predicts synergistic drug combinations. One module finds a set of Boolean models compliant with observations (e.g. a patient's gene expression profile) using an automated genetic algorithm and one module performs the combinatorial drug response analysis.

Benchmarking results show that the Python updated version is significantly faster compared to the old implementation (up to x5 speedup) without sacrificing performance.

These effective simulations underscore the power of combining computational biology and effective optimization algorithms. This synergy is paving the way for new approaches in cancer treatment. This automated pipeline now could predict drug synergies more effectively without the need for extensive experimental data, providing a scalable solution for exploring numerous drug combinations.

Core Facility for Advanced Light Micrsocopy

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Abstract

The Core Facility for Advanced Light Microscopy at The Radium Hospital provides services within a range of light microscopy imaging techniques. We provide access to state-of-the-art light microscopes for researchers for performing a variety of imaging techniques including live-cell imaging, widefield imaging, confocal microscopy (point scanning or spinning disk), and also high content imaging and a range of superresolution techniques such as 3D-SIM, Airyscan, SoRa, STORM and TIRF. CLEM can be performed in collaboration with the Advanced Electron Microscopy Core Facility. Furthermore, we provide access to advanced image analysis software and training or assistance in microscopy as well as in image analysis. The imaging technologies and methodologies developed at the Core Facility are being used to study various model systems including cultured cells, organoids, tissue, and model organisms such as fruitflies and zebrafish, and are routinely applied in studies of intracellular structures and processes (e.g. endocytosis, autophagy, mitochondria) and in studies of cell behavior (e.g. migration, division, cell-cell-interactions).

Non-selective beta-blockers prolong response to androgen deprivation therapy in hormone sensitive prostate cancer models

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The use of beta-blockers (BB), beta-adrenergic receptor antagonists, has been shown to enhance the efficacy of prostate cancer therapy. In this study, we demonstrated that treatment with non-selective BBs delayed the onset of castration-resistant prostate cancer (CRPC) and reduced tumor burden in both subcutaneous CWR22 and orthotopic LNCaP cells in castrated nude Balb/c mice. Furthermore, inhibiting $beta_2$- and $beta_3$-adrenergic receptors reduced ^{3}H-thymidine incorporation in prostate cancer cell lines cultured under androgen deprivated therapy (ADT)-mimicking conditions and reduced the volume of LNCaP cell colonies grown in soft agar.

The BB treatment induced a metabolic shift by up-regulating the expression of oxidative phosphorylation-related transcripts and down-regulating those encoding components of fatty acid synthesis and the PI3K/AKT/mTOR pathway. Additionally, BB-treatment reduced androgen receptor signaling and elevated serum levels of proinflammatory cytokines, including components of the IL23/IL17 axis, and increased infiltration of intra-tumoral CD68+ immune cells.

In summary, we propose that non-selective BBs prolong the ADT response in prostate cancer patients through the alteration of energy metabolism and promoting a pro-inflammatory environment.

Precision EV Forum

Cambridge, UK 23-24 October 2024

Title:

A Transcriptomic Biomarker for Prostate Cancer Active Surveillance in Extracellular Vesicles from Liquid Biopsies

Authors:

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Abstract Text:

Prostate cancer (PCa) is the most common cancer among men worldwide. Many patients have non-aggressive tumors and do not require radical treatments. Low-risk PCa patients often undergo active surveillance (AS) with regular check ups to monitor disease progression. However, reliable biomarkers are needed to differentiate between aggressive and non-aggressive PCa types to identify patients suitable for AS.

A robust liquid biopsy test would enable more frequent monitoring of patients under AS. Liquid biopsies represent a non-invasive approach that generally has low risk and causes less discomfort for patients compared to traditional tissue biopsies. For PCa, semen is a particularly valuable biofluid, as a significant portion of the seminal fluid is produced by the prostate gland. This prostate proximity offers a high potential to identify robust biomarkers that can accurately reflect PCa biology.

Recently, extracellular vesicles (EVs) have emerged as promising tools for the discovery of biomarkers in liquid biopsies. Their ability to reflect the molecular composition of their cells of origin provides insight into disease states and progression. Therefore, the isolation of seminal extracellular vesicles from semen samples will offer a new avenue for PCa classification.

Our goal is to identify prostate cancer biomarkers in seminar fluid. In order to do that, we are optimizing protocols for isolating seminal extracellular vesicles. Using samples from healthy donors, we demonstrated that both sequential centrifugation and size exclusion chromatography effectively yield EVs with similar levels of CD63, syntenin, CD9, and CD81. We obtained approximately 140 ug protein per ml of seminal fluid in the EV pellets. Next, we plan to isolate RNA from seminal EVs of 20 PCa patients for transcriptomic analysis.

Dissecting intratumoral heterogeneity in gastrointestinal stromal tumors

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In the era of precision medicine, imatinib treatment has been very successful in gastrointestinal stromal tumors (GIST). The tyrosine kinase inhibitor imatinib is used to treat patients at high risk of relapse and metastasis. Imatinib works by blocking overactive KIT or PDGFRA pathways, which are the oncogenic drivers in GIST. In a localized setting, adjuvant treatment is recommended for high-risk patients. However, 40% of the patients relapse within three years after stopping adjuvant therapy, and from historical data, approximately half are cured by surgery alone. Thus, there is a need to understand the progression and development of resistance better. Using spatial transcriptomics, we aim to characterize the inter- and intra-tumor heterogeneity of treatment naïve cancer with different clinical behaviors. We improved the Seurat classification by applying deconvolution methods to identify areas characterized by specific cell types and histological structures. In samples with normal gastric mucosa, tissue structures like the crypts also showed specific transcriptomics patterns, validating our analysis. Across tumor samples, we have identified spatial heterogeneity at the transcriptional level involving pathways critical for cancer development, such as proliferation, stemness, and metabolism. Despite the same driver mutation, malignant cells show high transcriptomic variability, including marker genes associated with different fibroblast types. In addition, we observed inferred inter- and intra-tumoral heterogeneity in DNA copy number, defining regions with unique chromosomal characteristics. Moreover, we determined cancer margins with increased chromosomal unbalance that could be associated with acquired malignant characteristics by normal cells or tumor infiltration.

In conclusion, the heterogeneity observed may drive the different transcriptional profiles within the tumor. We are expanding the number of samples, including KIT and PDGFRA mutated tumors, and correlating intra-tumoral heterogeneity to clinical behavior to identify novel dependencies for progression and resistance. This will be essential to improve the stratification, management, and treatment of GIST patients.

Role of the NEDD4 family of E3 ubiquitin ligases in mediating the loss of intercellular communication via gap junctions during cancer development

Max Zachrisson Totland[1], Nikoline Lander Rasmussen[7], Mathias Larsen[1], Vilde C. Wivestad Elster[1], Dina Glømmi Bergmann[1], Jakob Mørkved Stenersen[1], Zeremariam Yohannes[1], Lars Mørland Knudsen[1], Joachim Jordal Moe[1], Kushtrim Kryeziu[1], Vigdis Sørensen[2,3,5], Andreas Brech[2,3,5], Yasufumi Omori[8], Trond Aasen[9], Ragnhild A. Lothe[1,4,6] and Edward Leithe[#1]

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Intercellular communication via gap junctions has important roles in maintaining tissue homeostasis, and is frequently lost during cancer development. Such loss may contribute to increased cancer cell growth and affect their response to radio- and chemotherapy. Connexin43 (Cx43) is the most ubiquitously expressed gap junction channel protein in human tissues. Cx43 has a relatively high turnover rate, and by modulating the rate at which Cx43 undergoes endocytosis and degradation, cells can rapidly adjust the level of functional gap junctions in response to intracellular or extracellular cues. Many oncogenes and tumor promoters, such as the protein kinase C activator TPA induce loss of gap junctions by increasing the Cx43 endocytosis and degradation rate. In this project, we investigate the post-translational mechanisms involved in mediating the loss of Cx43-based gap junctions during cancer pathogenesis. The NEDD4 family of E3 ubiquitin ligases comprises nine members, several of which have been shown to display oncogenic or tumor suppressor functions. Here, we show that in cervical cancer cells, three members of this protein family - NEDD4, ITCH, and SMURF2 - act in concert to regulate Cx43 ubiquitination, degradation and gap junction levels. Simultaneous depletion of NEDD4, ITCH, and SMURF2 by siRNA resulted in a significantly lower Cx43 ubiquitination level and reduced Cx43 degradation rate compared with their single depletion. The combined knockdown of these E3 ubiquitin ligases also caused an additive increase in the cellular level of Cx43 and gap junction size. In addition, the triple knockdown strongly counteracted the TPA-induced degradation of Cx43. Collectively, these data suggest that NEDD4, ITCH, and SMURF2 act together to mediate the basal and TPA-induced degradation of Cx43. To our knowledge, this study represents the first evidence that Cx43 ubiquitination, degradation and, consequently, the size of gap junctions, is controlled by the concurrent participation of multiple E3 ubiquitin ligases.

Poster abstract

DNA methylation biomarkers analyzed in liquid biopsies to detect cholangiocarcinoma among high-risk PSC patients

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Patients with primary sclerosing cholangitis (PSC) have up to 20% lifetime risk of developing cholangiocarcinoma (CCA). The survival of patients with CCA is poor, but an early and accurate diagnosis has the potential to increase survival. Identifying CCA in patients with PSC is, however, challenging and current strategies are suffering from low sensitivity. Consequently, the majority of patients are diagnosed at an advanced, incurable stage of disease. In the current study, we aim to establish robust DNA methylation biomarkers in liquid biopsies (bile and blood) for improved detection of CCA in patients with PSC. From Reduced Representation Bisulfite Sequencing (RRBS) of tissue samples from CCA and PSC patients we identified a number of candidate biomarkers. These were analyzed with droplet digital PCR (ddPCR) in tissue samples (quality control) and ~300 bile samples from CCA and PSC patients. In bile, a combination of the biomarkers gave a sensitivity and specificity >90% for differentiating between patients with CCA and PSC. The biomarkers have been multiplexed and will be further analyzed in blood samples from a subset of the patients (n=128), including 27 with CCA and 201 with PSC.

Cell Biology of Cancer Cachexia

Katharina Vestre[1,2,3,4] , Min Deng[1,2,3,4] , Amrinder Singh[1,2,3] , Gareth Sullivan[1,4], and Tor Erik Rusten [1,2,3]

Cancer cachexia is a severe health problem that affects the majority of late-stage cancer patients and contributes to 20% of all cancer deaths. This complex multi-organ syndrome involves systemic effects induced by the tumor, which result in muscle and adipose tissue wasting, liver fat accumulation, inflammation, and metabolic reprogramming. Currently, there are no effective treatments for cancer cachexia, and our understanding of the cell biological mechanisms underlying the disease are limited.

In this project, we aim to build a human *in vitro* model of cancer cachexia using induced pluripotent stem cell-derived organoids. We will employ a combination of multi-omics analysis, advanced microscopy, and biochemical techniques to investigate the tumor-induced effects on liver, fat, and muscle organoids. By co-culturing multiple organoids, our goal is to reveal causal multi-organ interactions that can be targeted to treat cancer cachexia, and to build a novel platform for organ interaction studies.

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Targeting the Desert: Spatial Mapping and Precision Targeting of Adult Soft Tissue Sarcoma

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Abstract theme: Spatial Cancer Biology

Soft tissue sarcomas (STS) are mesenchyme-derived tumours with heterogeneous prognosis and few approved disease-specific treatment options. While typically categorized as immunological “deserts”, infiltration of immune cells and particularly of natural killer (NK) cells has been suggested to have positive prognostic value. This notion is supported in our pan-cancer NK cell reference atlas, showing a beneficial role of tumor infiltrating NK (TiNK) cells in several tumor types. Here, we establish a 43-color Imaging Mass Cytometry (IMC) panel to investigate the immune tumor microenvironment (TME) of myxofibrosarcoma (MFS) and undifferentiated pleomorphic sarcoma (UPS) samples from Oslo University Hospital biobank. Production of a tissue micro-array containing selected regions of interest (ROIs) from primary tumors and metastasis in a cohort of 100+ patients is ongoing. Following pathologist-guided selection of ROIs, samples from 8 patients have been acquired in a pilot study and are currently being analysed. Preliminary analysis has revealed immune infiltrates in peri-vascular and peri-tumoral regions even in samples otherwise classified as immunological “deserts”.

In parallel with the spatial mapping of the TME, we explored the potential of a novel NK cell immunotherapy platform based on selectively expanded NKG2C+ adaptive NK cells (ADAPT-NK). FACS-based functional assays and long-term 2D/3D killing assays in the IncucyteS3 platform show that ADAPT-NK cells effectively kill patient-derived STS cells. Combination with a tri-specific NK cell engager (TriKE) aCD16/IL-15/a-B7H3 demonstrated potent degranulation against STS cells in the HLA-C/KIR mismatched setting, whereas ADAPT-NK cells in HLA-C/KIR matched settings were still largely inhibited despite directed targeting. Spheroid models were sectioned and imaged using IMC to investigate NK cell infiltration and killing in a 3D setting. Analysis of these models, combined with spatial mapping of the TME in a large clinical cohort will deepen our understanding of disease and open new avenues for the development of NK cell-based precision immunotherapy of sarcoma.

300words

TSOPPI suite: TSO500 Post-Processing in InPreD

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Infrastructure for **Pre**cision **D**iagnostics (InPreD) is a national collaboration that weekly performs molecular profiling of advanced cancer patients with the aim to identify biomarkers relevant for diagnostics and/or inclusion into Norwegian drug trials. To provide broad genomic profiling of tumors, we implemented the **T**ru**S**ight **O**ncology **500** (TSO500) pan-cancer targeted panel from Illumina, which offers assaying selected loci in both DNA (~520 genes) and RNA (~50 genes) samples utilizing hybrid-capture chemistry. The initial sequencing data analysis is enabled by locally-deployable Illumina software, offering customized pre-processing (including read alignment, FFPE artifact correction and de-duplication) together with basic variant calling and annotation (small variant and copy number variant [CNV] calling, assessment of tumor mutational burden [TMB] and microsatellite instability [MSI], as well as fusion variant calling).

Since adoption of the assay in 2019, InPreD has developed a set of tools for TSO500 sample post-processing. The TSOPPI suite (**TSO**500 **P**ost-**P**rocessing in **I**nPreD) aims to build on the initial sample analysis by 1) offering custom-developed functionality necessary for quality control routines, variant interpretation work and tumor board meeting needs within InPreD, and 2) employing external open-source tools (e.g., PCGR and PureCN) for expanding the variant calling and annotation capabilities of the sample analysis in an effort to better utilize the assay's potential. TSOPPI provides joint processing of matched patient samples (tumor DNA, tumor RNA, normal DNA), annotation-based variant prioritization, variant and quality metrics visualization, assistance to interactive variant inspection and utilization of in-house controls for tumor-only analyses, enabling easier recognition of rare germline variants and artifact calls commonly reported with the assay. Current development is focused on comparisons of variant sets across samples, improved calling of CNVs and medium-sized variants (e.g., tandem repeats or deletions spanning multiple exons) and post-processing of samples prepared with TSO500 ctDNA and HRD-add-on assays.

Targeting protein homeostasis in LTK-positive multiple myeloma by repurposing ALK-inhibitors

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Multiple myeloma (MM) is an incurable malignancy of plasma cell within the bone marrow characterised by excessive immunoglobulin secretion. MM cells are therefore addicted to mechanisms that maintain proteome homeostasis, hence the success of the proteosome inhibitor bortezomib. However, patients still relapse, and new treatment options are still needed.

Leukocyte receptor Tyrosine Kinase (LTK) is the first reported receptor tyrosine kinase that regulates export from the endoplasmic reticulum (ER). The ER is the site for proteostasis (protein homeostasis) and is where the cell regulates the proteome. Inhibition of this ER-resident tyrosine kinase is a possible new target for myeloma therapy. Here we show that targeting LTK sensitize hypersecretory MM and normal plasma cells to ER-stress and apoptosis. LTK has high homology to Anaplastic Lymphoma Kinase (ALK). We find that the ALK targeting drugs, ceritinib, crizotinib, alectinib, brigatinib, ensartinib and entrectinib induces cell death in $ALK^{Negative}$ plasma cells and MM cells. When using various ALK-inhibitors in 3 h inhibition assays, a decrease in secreted immunoglobulins is detected by ELISA, and an accumulation of intracellular immunoglobulins is detected by flow cytometry, suggesting a rapid block of ER-secretion by LTK-inhibitors. A decreased viability was seen in 24-72 h assays of myeloma cell lines and in primary myeloma cells by CellTiterGlo drug screens, and flow cytometry based assays. In a pilot in vivo study, healthy donor PBMCs were injected in NSG mice and treated for five days with entrectinib (20 mg/kg/day). A flow analysis of the bone marrow of treated mice versus control, showed reduced immunoglobulin producing cells and decreased bone marrow resident plasma cells and plasmablasts (1.3 % vs 4.3 %, p = .012).

We find that ALK-inhibitors may be repurposed to treat $ALK^{Negative}$, $LTK^{-Positive}$ malignancies with high secretory load and dependency on proteostasis. This has now been included in IMPRESS with its own study, where MM patients are screened for LTK expression in the MM cells combined with a high serum M-component as inclusion criteria.

Identification of small molecules targeting FoxP3 for anti tumor treatment: high-throughput drug screening

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FoxP3$^+$ regulatory T cells (Tregs) regulate immune homeostasis by suppressing excessive anti-self immunity of effector T cells (Teffs) and thereby inhibit anti-tumor immune responses. Thus, targeting Tregs is a popular therapeutic strategy in drug discovery for the treatment of cancer. However, most current efforts targeting surface markers or signaling pathways in Tregs may result in immune-related adverse effects due to overlapping expression profiles between T cell subsets. FoxP3 is the key lineage-defining transcription factor in Tregs, which regulates the expression of genes essential for Treg activation and suppressive functions, thus becoming a promising target for drug discovery with low side effects.

To identify small molecules specifically targeting FoxP3 in Tregs, we have developed both phenotypic and biochemical drug screening methods. First, we established a high-throughput flow cytometry-based phenotypic screen by measuring the percentage of FoxP3 in CD4$^+$ T cells. CD3$^+$ T cells isolated from healthy human donors were treated with drugs in 384-well plates, followed by antibody staining and high-throughput flow cytometry analysis in an automated pipeline. We first screened a library of 1522 approved drugs and then successfully expanded to screen a large diversity-oriented library of 28,500 compounds. As a secondary screen, we developed a high-throughput AlphaScreen assay to identify compounds affecting FoxP3 and DNA binding activity. Identified FoxP3-DNA binding regulators were further validated by SPR analysis to determine their affinity kinetics.

Next, the original hit and its more efficient analogs searched by *in-silico* predictions were further examined in well-established Treg functional assays by measuring their effects on regulating Treg suppressive function on Teffs. As an example, one candidate from the screen and its effective analogs were validated with down-regulation effects on FoxP3 through direct binding and interference with FoxP3-DNA affinity, showing anti-tumor immunity both in cancer patient samples *ex vivo and* in a mouse tumor model *in vivo*.

Intercellular transfer of cancer cell invasiveness via endosome-mediated protease shedding

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Abstract

Overexpression of the transmembrane matrix metalloproteinase MT1-MMP/MMP14 promotes cancer cell invasion. Here we show that MT1-MMP-positive cancer cells turn MT1-MMP-negative cells invasive by transferring a soluble catalytic ectodomain of MT1-MMP. Surprisingly, this effect depends on the presence of TKS4 and TKS5 in the donor cell, adaptor proteins previously implicated in invadopodia formation. In endosomes of the donor cell, TKS4/5 promote ADAM-mediated cleavage of MT1-MMP by bridging the two proteases, and cleavage is stimulated by the low intraluminal pH of endosomes. The bridging depends on the PX domains of TKS4/5, which coincidently interact with the cytosolic tail of MT1-MMP and endosomal phosphatidylinositol 3-phosphate. MT1-MMP recruits TKS4/5 into multivesicular endosomes for their subsequent co-secretion in extracellular vesicles, together with the enzymatically active ectodomain. The shed ectodomain converts non-invasive recipient cells into an invasive phenotype. Thus, TKS4/5 promote intercellular transfer of cancer cell invasiveness by facilitating ADAM-mediated shedding of MT1-MMP in acidic endosomes.

Protein profiles predict treatment responses to the PI3K inhibitor umbralisib in patients with chronic lymphocytic leukemia

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The management of chronic lymphocytic leukemia (CLL) has significantly improved with targeted therapies. However, many patients experience a suboptimal response. To optimally select the best therapy, predictive biomarkers are necessary. Here, we used the PI3K inhibitor umbralisib as a model to (i) understand how targeted treatment affects cell signaling and immunophenotypes in responders and non-responders; (ii) identify molecular features that predict individual treatment responses; and (iii) suggest alternative treatment options for the non-responders. We performed functional phenotyping of CLL cells from patients enrolled in two clinical trials with umbralisib, administered either as a monotherapy (NCT02742090, n=55) or in combination with the BTK inhibitor acalabrutinib (NCT04624633, n=12). We found that umbralisib monotherapy led to significant changes in (phospho)protein levels, including AKT (pS473), in responders but not in non-responders. Furthermore, the proportion of cytotoxic natural killer cells increased at the end of study, but only in responders, suggesting a role in the anti-tumor response. To identify molecular predictors of response, we used the baseline levels of 30 (phospho)proteins in the monotherapy cohort as input features for a machine learning model, which achieved a significant prediction accuracy in cross-validation, and maintained its predictive power in the combination cohort. Drug sensitivity profiling of the CLL cells at baseline suggested that PI3K + Bcl-2 inhibitors are effective in umbralisib non-responders. Together, our findings show that functional phenotyping reveals differential cellular responses to umbralisib treatment in responders and non-responders; predicts treatment response of individual CLL patients; and suggests alternative treatment options for the non-responders.

Targeting IRE1α reprograms the tumor microenvironment and enhances anti-tumor immunity in prostate cancer

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Abstract

Unfolded protein response (UPR) is a central intracellular stress response pathway that is hijacked by tumor cells for their survival. However, how activation of UPR in cancer cells may shape the tumor microenvironment (TME) remains largely unexplored. Here, we investigated the potential role of IRE1α signaling on modulation of TME dynamics in prostate cancer (PCa). We found that IRE1α is increased in PCa patient tumors and genetic inhibition of IRE1α in syngeneic mouse PCa models, as well as in an orthotopic model, dramatically reduced tumor growth. Multiomics analysis suggested that IRE1α ablation in cancer cells significantly potentiated interferon (IFN) response and activation of immune system related pathways in the TME. Single-cell RNA-sequencing (scRNA-seq) revealed that the abundance of immunosuppressive cells, such as tumor-associated macrophages (TAMs), were markedly reduced in the IRE1α deficient tumors. Geneset Enrichment Analysis demonstrated that IFN response was significantly enriched in TAMs, cancer cells, and dendritic cells. Notably, the small molecule IRE1α inhibitor MKC8866 (ORIN1001), currently in clinical trials, reprogrammed the TME and enhanced the response to anti-PD-1 blockade therapy in syngeneic PCa mouse models. This is significant because PCa is considered an immunologically 'cold' tumor that does not respond to checkpoint inhibitor therapies. Furthermore, a novel scRNA-seq-derived TAM gene signature is strongly associated with poor PCa survival, which is reduced by the MKC8866 + anti-PD-1 combination therapy. Our findings indicate that activation of IRE1α signaling not only promotes cancer cell growth and survival, but it also interferes with anti-tumor immunity in the TME. Thus, targeting IRE1α could present a novel approach for enhancing anti-PD-1 immunotherapy in PCa and potentially in other cancer types that are resistant to checkpoint inhibitors.

A Pareto-Driven Ensemble Feature Selection Approach Optimizes Biomarker Discovery in Multi-omics Pancreatic Cancer Studies

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Abstract

To address the pressing clinical demands of today, it is crucial to implement models that select minimal, cost-effective features. Feature selection in machine learning aims to fulfill this need by identifying the most predictive biomarkers with minimal redundancy. We have developed a **multi-omics ensemble feature selection (EFS) approach** that identifies the most significant biomarkers for a given cohort of patients. Our approach leverages multiple machine learning algorithms to discover optimal features for classification, regression, and survival analysis tasks.

The EFS method ranks features using **voting theory**, ensuring that all ensemble model perspectives are considered. The optimal number of selected features is determined through a **Pareto-based knee-point identification method**, providing a trade-off between sparsity and performance. When applied to multi-omics datasets from pancreatic cancer studies, our approach successfully identifies minimal biomarkers relevant to both the clinical outcome and the underlying biology of the disease. Overall, EFS offers a reliable and clinically valuable tool for biomarker discovery in cancer research.

Significantly poorer survival in irradiated breast cancer patients with estrogen receptor negative and low tumor-infiltrating lymphocytes (TILs) tumors, a DBCG study

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Background:

Previous studies of a large national Danish cohort of breast cancer (BC) patients revealed that high levels of tumor-infiltrating lymphocytes (TILs) in treatment-naïve tumor tissue predicts improved overall survival (OS) after adjuvant radiotherapy (RT), especially in estrogen receptor (ER) negative tumors (ER-). The association has later been shown to be mediated through distant tumor control. We aimed to validate these findings in modern treated irradiated BC patients with varying risk profiles.

Methods and Materials:

Treatment-naïve tumor tissue from 1329 irradiated BC patients from two Danish Breast Cancer Group (DBCG) cohorts; high-risk BC patients (DBCG-IMN2) and low-risk BC patients (DBCG-HYPO) were analyzed for stromal TILs using international guidelines. Endpoints included loco-regional recurrence (LRR), distant metastasis (DM), and OS.

Results:

Patients were categorized into "low" and "high" TILs groups using a 30% cutoff. In the high-risk DBCG-IMN2 cohort, ER-/low TILs tumors showed significantly worse OS after RT compared to ER-/high TILs (Hazard ratio (HR) 0.35 (95% CI: 0.21-0.58)) with an absolute decrease in OS at 10-years of 29.4% for ER-/low TILs tumors. No significant difference in OS was observed in ER+ tumors (HR 1.02 (0.68-1.55)). Interaction test between ER-status and TILs was significant ($p<0.001$). A similar association was found for DM (HR 0.36 (0.20-0.63)), where ER-/low TILs tumors had a higher risk (10-year increase of 24.1%) compared to ER-/high TILs. TILs did not impact loco-regional control in ER- tumors (HR=0.87 (0.28-2.67)). Similar trends were observed in the low-risk DBCG-HYPO cohort.

Conclusions:

The study validates previous findings, indicating a robust association between TILs and ER-status in irradiated BC patients with diverse risk profiles. Importantly, it suggests that superior OS in irradiated patients with high TILs is likely mediated through distant tumor control rather than improved local control.