2024

# Breakout Session Abstracts

CANCER RESEARCH KI RETREAT 2024

**A one-two-punch approach in cancer therapy. Trying to discover the second punch.**

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Most cancer therapies fail to kill all tumor cells, and the few that remain have often been cataloged as "dormant", "persister" or "senescent". Specifically, senescence, is known to have a dual role in tumorigenesis. Initially, senescence helps to limit the growth or pre-tumoral cells presenting stressors such as oncogene activation or DNA damage. However, when not properly cleared, senescence can derive into a pro-inflammatory state that contributes to tumor propagation and metastasis. In this context, one emerging concept for cancer therapy is that of the "One-two-Punch", whereby the initial chemotherapy (punch) is shortly followed by a second treatment that aims to eradicate cancer cells that resisted the initial one.

To identify such "second punch", we conducted a chemical screen to find drugs that preferentially kill cells with an activated DNA damage response. Interestingly, some of the compounds we found were senolytic, namely, that they are also effective in killing senescent cancer cells. Hence, we explored whether other compounds from our list were novel senolytics. In the seminar, I will present our data with one of the new senolytic drugs that we identified, which we named Seno[1]. Our experiments confirmed that Seno[1] is a potent senolytic in vitro and in vivo. For instance, Seno1 is an efficient "second punch" for cancer cells previously arrested with CDK4/6 inhibitors such as Palbociclib. To understand the mechanism of action of Seno[1], we conducted several genetic screens and transcriptomic analyses that have helped to understand how this molecule exerts its senolytic functions. Our progress so far, and ideas as to how to develop this project in the future, will be presented.

**Keywords:** Senolytics, Senescence

**Astrocyte-expressed FASN as a novel candidate drug target for glioblastoma**

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**Background:** Glioblastoma (GBM) is a disease with an urgent need for deeper understanding and new therapeutic approaches. The tumoral microenvironment guide cancer cell progression. Our earlier studies highlighted astrocytes (ACs) as enhancers of GBM proliferation through analyses of clinical samples and experimental studies.

**Material and Methods**: HTS screening (14000 compounds) was performed on co-cultured GBM cells and astrocytes. Cell proliferation and tumor-sphere formation assay (Luc-GFP coculture system) were used to examine tumor growth and self-renewal ability. Multiplex profiling was used to detect FASN+GFAP+ astrocytes and Ki67 proliferative cancer cells in human GBM specimens. Confocal imaging and FACS were performed to measure mitochondrial transfer from ACs to GBM cells. FASN inhibitors potency screening was used to correlate FASN cellular activity and co-culture activity. Non-FASN inhibitory hits were explored by Proteome Integral Solubility Alteration (PISA) Assay for target identification.

**Results**: A multi-step screening cascade with ACs/GBM co-cultures as primary screen identified around 130 specific hits. Bioinformatics suggested fatty acid synthase (FASN) as an enriched target among hits. Notably, FASN knock-down in astrocytes reduced the ability of astrocytes to support glioblastoma proliferation and self-renewal. Selected hits from screen showed reduced or no activity in co-cultures with FASN knock-down astrocytes. Among FASN inhibitors, there was good correlation between cellular FASN inhibitory potency and growth inhibitory activity. Free fatty acid rescued GBM growth in co-cultures with FASN knock-down astrocytes but had no effect in co-cultures with wt astrocytes. Knockdown of FASN reduced mitochondria transfer from astrocytes to GBM cells. PISA analyses of hits not targeting FASN also indicated effects on fatty acid metabolism. For evaluation of clinical relevance, multiplex staining has been initiated demonstrating FASN-positive astrocytes in human GBM samples.

**Conclusion:** In summary, a novel co-culture-based assay system has identified candidate drugs targeting astrocyte-mediated support of glioblastoma. Collectively, screening results, tissue profiling and knock-down experiments suggest astrocyte-derived FASN and fatty acid metabolism as a critical component of astrocyte-driven glioblastoma growth. These findings are developed in continued mechanistic studies, expanded quantitative profiling of human GBM and through experimental therapy studies, using FASN knockdown astrocytes and FASN inhibitors in orthotopic mouse GBM models.

**Keywords:** Glioblastoma, Microenvironment

**Impact of bacterial type VI secretion system on tumorigenesis**

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During the last decades, a plethora of studies highlights the role of microbial composition in normal host physiology and subsequent disease progression. Changes in microbiota, known as microbiota dysbiosis, have been linked to various inflammatory-associated gastrointestinal diseases, such as colorectal cancer (CRC). It has been previously shown that there is an enrichment of Enterobacteria, including hypervirulent Klebsiella pneumoniae (KP), in the gut microbiota of CRC patients, which is detrimental to the progression of the disease. More specifically, it has been shown that KP accelerates the onset of inflammation, contributing to the progress of inflammatory-associated CRC. However, the ways in which enterobacteria and KP influence tumor development and the underlying mechanisms remain to be demonstrated. In this project, we suggest that the type VI secretion System (T6SS) that promotes bacterial competition and colonization in the gut, can also contribute to inflammation-associated tumorigenesis. ‘
This project aims to decipher the impact of T6SS on gut dysbiosis, chronic inflammation, and tumorigenesis, using KP as a model. Our preliminary data demonstrate that T6SS activity upregulates the inflammatory response in mice. In cellulo studies highlight that the T6SS-dependent inflammatory response occurs in a cell-autonomous way. Moreover, they suggest that intestinal inflammation caused by T6SS impacts tumor progression. Indeed, analysis of mice infected with a T6SS mutant strain showed a decreased number and size of tumors, highlighting the role of T6SS in tumor progression. Overall, these findings indicates that bacteria can influence the host inflammatory response via T6SS activity, potentially opening a new therapeutic avenue against cancer.

**Keywords:** colorectal tumorigenesis, Type VI secretion system

**Title: Gamma Delta T cell recognition and activation potential in Medulloblastoma**

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Medulloblastoma (MB) is a heterogeneous group of tumors developing in the cerebellum and is one of the most common malignant brain tumors in children. Fatal left untreated, the standard therapies for MB involve surgery, chemotherapy, and irradiation (only for >5 years old). Despite an overall good 5-year survival rate around 70%, first line treatment often results in severe neurological and endocrine deficits in the developing brain. Thus, there is a strong need to identify less toxic and more efficient therapeutic strategies. The emergence of cancer immunotherapy has revolutionized cancer treatment, including immune checkpoint blockade, CAR-T cell therapy, and infusion of T cells or NK cells.

Gamma Delta (γδ) T cells, a non-conventional T cell population, are in the spotlight as a novel cancer immunotherapy strategy due to their advantageous combination of non-alloreactivity, a strong tumor cell lysis potential and a broad antigen recognition. In humans, γδ T cells are classified according to their Vδ chain (Vδ1, Vδ2, Vδ3 and Vδ5) where each subpopulation has different functionality and tissue distribution. However, their ability to target and eliminate MB cells is poorly understood.

To explore the possibility of using γδ T cells to recognize and target MB we have ex-vivo expanded different human γδ T cell subpopulations and tested their ability to target a panel of MB cells. In addition, we have characterized the expression of known γδ T cells ligands on both MB cells and in MB patient datasets. We identified Ephrin-A2 receptor and the phosphoantigen/Butyrophilin complex as ligands of interest in triggering respectively Vγ9Vδ1 and Vγ9Vδ2 T cell activation leading to MB cell lysis both in monolayer and spheroid models. Preliminary results have shown that differentiated neurons and neuroepithelial stem cells, generated from IPS cells, are not targeted by γδ T cells, demonstrating the safety of this approach. The optimization of MB cell killing by γδ T cells aim to propose a novel therapeutic strategy for MB patients, with the possibility to expand to others pediatric brain tumors.

**Keywords**: Medulloblastoma – Immunotherapy

**Biological Tumor Traits Predicting Late Recurrence in Premenopausal Breast Cancer Patients – Insights from the STO-5 trial with 20-year follow-up**

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**Background:** Estrogen receptor (ER)-positive breast cancer patients have a long-term risk of distant recurrence, and premenopausal women have an increased risk. The reasons behind long-term risk are not well understood, but tumor dormancy is one suggested mechanism. Here, we aimed to identify key biological traits predicting late risk of distant recurrence.

**Methods:** Secondary analysis of the Stockholm tamoxifen (STO-5) randomized trial including premenopausal patients from 1990 to 1996, with 20-year complete follow-up. Gene expression data (Agilent Technologies with ~21.5K unique genes) was used to analyze significant (p.adj.BH< 0.05) enriched MSigDB cancer hallmarks, gene ontology terms and Consensus$^{TME}$ immune cells, in patients with late distant recurrence (between 10 and 20 years of primary diagnosis) compared to early recurrence (within 5 years) and no recurrence at 20 years.

**Results.** Besides decreased proliferation-related hallmarks (NES < -2.0), EMT was notably enriched in patients with late recurrence compared to patients with early (NES=2.51) and no recurrence (NES=2.42), suggesting the importance of cellular plasticity to leave the primary tumor and enter dormancy. Additionally, hypoxia emerged as a prominent process among late recurrence patients (NES >1.5), strongly correlating with EMT (p-cor = 0.72), indicating its potential role as an EMT driver. Moreover, late recurrence patients showed negative enrichment
of immune related hallmarks and gene ontology terms (NES < -1.5), as decreased immune cell enrichment suggesting long-term immune evasion.

**Conclusions:** Our findings indicate that premenopausal patients with late recurrence have heightened cellular plasticity and low proliferation enabling prolonged dormancy. Increased EMT suggest enhanced potential for distant tumor cell infiltration from the primary tumor due to unfavorable conditions, such as hypoxia. Further, decreased immune activation and infiltration both indicate immune evasion, facilitating sustained quiescence. Insights into the biology driving late metastatic disease is vital to improve management of premenopausal patients with ER-positive breast cancer.

**Keywords**: Breast cancer, Late metastatic recurrence

**Investigating interval breast cancer with statistical tumour growth models**

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**Introduction:** In Nordic countries and across Europe, breast cancer screening participation is high. Despite this, a considerable number of breast cancer cases are diagnosed due to symptoms between scheduled screenings, termed "interval cancers." Radiologists use the proportion of interval cancers as a proxy of the screening's false negative rate (i.e., 1-sensitivity).

**Method:** Our objective is to enhance the understanding of interval cancers by applying statistical tumour growth models to data from a register-based population study involving incident invasive breast cancer cases. We develop an analytical expression for the proportion of interval breast cancer cases among regularly screened women. We rely on some parametric assumptions and theorems about the stationary distribution of tumour volume at detection and growth rate, but not on prior estimated background cancer rates. This analytical expression is then incorporated into a methodology for fitting tumour growth models for incident case data. We fit the model on 3493 cases diagnosed in Sweden between 2001 and 2008 using a likelihood-based approach.

**Results:** Our methodology allows us to estimate the distribution of tumour sizes at the most recent (negative) screen for interval cancers. These estimates indicate that around 6% of interval cancers do not have a tumour of diameter 0.5 mm or larger at their previous negative screen, and that around 45% have an undiagnosed tumour greater than 7.5 mm at their previous negative screen. We estimate a tumour Mean Doubling Time of 226 days, and a Mean Sojourn Time (i.e., time between tumour onset and symptomatic diagnosis) of 9.9 years in the general population. Additionally, we evaluate the association between screening interval length and the interval cancer proportion, finding that in a population screened every 1-2-3 years the proportion of interval cases would be 13%, 28.5%, 42.5%, respectively. Importantly, we find that our model-based expected incidence of interval breast cancers in the months between consecutive screens aligns closely with observed patterns in our study and in a large Norwegian screening cohort.

**Conclusions**: Our analytical expression serves as a valuable tool for gaining insights into the performance of population-based breast cancer screening programmes. For future work, we plan to utilize the proportion of interval cancers to explore differences in tumour growth rates among various breast cancer subtypes.

**Keywords**: Breast cancer screening, Interval cancer