COMPLEX PRIMARY ORGANOID CULTURES TO DISSECT IMMUNOGENIC EFFECTS OF THERAPY ON MACROPHAGES IN A PRECISION MEDICINE-LIKE APPROACH

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Background Primary co-cultures of colorectal cancer (CRC) organoids with various immune components are emerging as models for probing immunological effects of novel and established cancer treatments. Tumor associated macrophages (TAM) play a central role as regulators, directing responses of other immune cell types in the microenvironment. Cancer associated fibroblasts (CAF) were shown to polarize macrophages. Therefore, we aimed to set up a complex primary co-culture assay consisting of primary organoids, CAFs and TAMs illuminating phenotypic and functional changes of TAM under therapy in CRC patients.

Materials and Methods A living biobank of primary CRC organoids and CAF was established. Organotypic co-cultures of monocytes derived from healthy volunteers and organoids were set up in presence and absence of patient matched CAF. Flow-cytometry-based phagocytosis assays were established to assess functional capacity of monocytes to phagocyte CRC organoid cells. Model treatments included oxaliplatin, 5-FU and two oncolytic influenza A virus prototypes.

Results CAF presence was necessary for monocytes to develop a TAM phenotype upon three days of CRC organoid co-culture, defined by macrophage-like motility within the gel matrix and enhanced expression of TAM associated phenotypic markers CD163 and CD206. Treatment of complex organoids with oxaliplatin, 5-FU or oncolytic virus treatment re-polarized macrophages towards a pro-inflammatory phenotype with respect to marker expression. The magnitude of the potential re-programming was patient dependent. Phagocytosis of cancer cells from intact organoids could be modeled upon treatment. Presence of CAF enhanced phagocytosis of cancer cells. Phagocytosis upon oxaliplatin treatment was abrogated when CRC cell death was inhibited, indicating the observed effect consisted of clearance of dead cells. Phagocytosis under viral treatment was not significantly altered by inhibition of cell death, indicating an immunogenic effect.

Conclusions CAF appear necessary to model the TAM phenotype and their responses to treatment in primary-CRC-organoid-based organotypic assays and functional phagocytosis assays. These systems allow to assess response to therapy on the myeloid compartment in primary organoid cultures using a precision medicine approach.

REFERENCES


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**P09.21** **MODELLING THE SPATIAL DYNAMICS OF ONCOLYTIC VIROTHERAPY IN THE PRESENCE OF VIRUS-RESISTANT TUMOR CELLS**

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**Background** Oncolytic virotherapy is a promising form of cancer treatment that uses native or genetically engineered viruses to target, infect and kill cancer cells. Unfortunately, this form of therapy is not effective in a substantial proportion of cancer patients, partly due to the occurrence of infection-resistant tumor cells.

**Materials and Methods** To shed new light on the mechanisms underlying therapeutic failure and to discover strategies that improve therapeutic efficacy we designed a cell-based model of viral infection. The model allows to investigate the dynamics of infection-sensitive and infection-resistant cells in tumor tissue in presence of the virus. To reflect the importance of the spatial configuration of the tumor on the efficacy of virotherapy, we compare three variants of the model: two 2D models of a monolayer of tumor cells and a 3D model. In all model variants, we systematically investigate how the therapeutic outcome is affected by properties of the virus (e.g. the rate of viral spread), the tumor (e.g. production rate of resistant cells, cost of resistance), the healthy stromal cells (e.g. resistance to virus) and the timing of treatment.

**Results** We find that various therapeutic outcomes are possible when resistant cancer cells arise at low frequency in the tumor. These outcomes depend in an intricate but predictable way on the death rate of infected cells, where faster death leads to rapid virus clearance and cancer persistence. Our simulations reveal three different causes of therapy failure: rapid clearance of the virus, rapid selection of resistant cancer cells, and a low rate of viral spread due to the presence of infection-resistant healthy cells. Our models suggest that improved therapeutic efficacy can be achieved by sensitizing healthy stromal cells to infection, although this remedy has to be weighed against the toxicity induced in the healthy tissue.

**Conclusions** Through a modelling approach, our study provides insight into the dynamics of oncolytic virotherapy and resistance within a spatial framework. We demonstrate that the outcome of virotherapy depends not only on the parameters governing virus replication and the spatial architecture of the tumor but also on the presence of resistant-cancer and stromal cells that act as a barrier to the spread of oncolytic virus. We hope that our computational approach aids in defining the impact of the various factors that may influence resistance and therapeutic efficacy of virotherapy. We are confident that our results, along with experimental observations, can assist the scientific community in improving the design of virotherapy.

Weblink to the preprint and the model: (https://www.biorxiv.org/content/10.1101/2022.04.06.487254v1)

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**P09.22** **TOWARD A RATIONAL DESIGN OF IMMUNONANOTHERAPIES**

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Cancer nanomedicine primarily aim to direct drugs delivery to cancer cells but tumoraccumulation remains suboptimal (Mittelheisser et al. Adv. Mater., 2022). To circumvent thislimitation, activating immune cells with nanoparticles (NPs) is an emerging concept. However, upon engagement, whether NPs change the fate of immune cells that take them up remains unknown and one needs to thoroughly assess such impact to identify optimal NPs. Here, we characterized the response of immune cells to a selection of nanomaterialsclassically used in biomedical applications. Doing so, we aim at rationalizing the selection of specific NPs to undergo novel targeted approaches. Through bulk RNA-sequencing andproteomic analyses, we first investigated the impact of 6 NPs (lipidic, polymeric,organic/inorganic) on negatively isolated CD3 - CD56 + human NK cells and CD3 + human pan T cells. Amongst all the NPs studied, we observed that the oxidated carbon nanotubestriggered wide transcriptome and proteome modifications in both pan-T and NK cells, whereas the other NPs exhibit mild to low impact to both NK and pan T-cells. Interestingly, we observed that only polymeric NPs induced a pre-activated state in NK cells with an overexpression of the CCL5 chemokine and the cathepsin Z. Based on these results, we identified one type of polymeric NPs as potential candidate for further NK cell targeting approaches.

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**P09.23** **IMMUNE CELL INFILTRATION AS A NOVEL MARKER FOR PREDICTING PATIENT OUTCOME IN NEPHROBLASTOMA**

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**Background** Gaining insights into the tumor microenvironment (TME) of adult cancers improved clinical outcome and enabled newlines of therapies for these patients. In pediatric cancers, which are known to have less extensive immune infiltrates, little is known about the TME. In nephroblastoma, with a 5-year survival rate of 80–90%, investigating the TME may hold the key to further improve patient prognosis and risk stratification.

**Materials and Methods** In a retrospective study including 110 patients with renal tumors treated according to the