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. degree of 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 on 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 -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.

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Cancer nanomedicine primarily aim to direct drugs delivery to cancer cells but tumor accumulation remains suboptimal (Mittelheisser et al. Adv. Mater., 2022). To circumvent this limitation, 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 nanomaterials classically used in biomedical applications. Doing so, we aim at rationalizing the selection of specific NPs to undergo novel targeted approaches. Through bulk RNA-sequencing and proteomic analyses, we first investigated the impact of 6 NPs (lipidic, polymeric, organic/inorganic) on negatively isolated CD3 - CD56 + human NK cells and CD3 + human panT cells. Amongst all the NPs studied, we observed that the oxidated carbon nanotubestargeted 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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SIOP therapy protocol, we immunohistochemically stained formalin fixed, paraffin embedded slides of nephroblastoma specimens for T-cell, B-cell, NK-cell and macrophage markers (CD4, CD8, CD20, CD57, CD68). Quantification and comparison between different histologic regions (epithelial, blastemal and mesenchymal) as well as immune cell infiltration analysis was done using HALO AI by Indica labs.

**Results** Out of the three histologic regions of nephroblastoma, the highest concentrations of T-cells and macrophages were found in mesenchymal regions (CD68⁺: 1884.70 cells per mm² and CD8⁺: 58.76 cells per mm²), the least in blastemal regions (CD68⁺: 662.31 cells per mm² and CD8⁺: 4.48 cells per mm²). Concentrations in epithelial regions were found to be in between (CD68⁺: 1560.16 cells per mm² and CD8⁺: 19.96 cells per mm²). Furthermore, overall immune infiltrates of T-cells and macrophages were lowest in patients experiencing tumor relapse (4/85 nephroblastoma patients) or metastasis (9/85 nephroblastoma patients) (p-values for overall relapse vs. no relapse were 0.0004 in CD68⁺ cells and 0.016 in CD8⁺ cells).

**Conclusions** Lowest immune infiltrates of T-cells and macrophages are found in the more stem cell like blastemal regions of nephroblastoma as compared to epithelial and mesenchymal regions. Overall low immune cell infiltrates of T-cells and macrophages are associated with a worse patient prognosis. Taken together this data could provide a tool for risk group stratification and improve therapy outcome.