

in GB and the pathways that determine their functions in primary growth versus post-resection recurrence remain largely unknown. In this project, we characterize the immune landscape of GB before and after surgical resection and explore the role of the inflammasome in its dynamics and regulation.

Materials and Methods GL261-GFP-GLuc mesenchymal-type GB cells were orthotopically injected in WT or inflammasome-deficient (*Ice*^{-/-}) mice. On day 18 post-implantation, tumors and adjacent parenchyma tissue were collected from the unresected group (group 1). In parallel, tumor resection was performed on a second group of mice (group 2). 10 days later, tumors and adjacent parenchyma tissue were collected from group 2. Following tissue dissociation, immune cells were FACS-sorted from GB tumor-bearing mouse brains. Sorted immune cells were multiplexed using barcoded lipid indices into 6 different pools and scRNAseq (10x Genomics) was performed. For the scRNAseq, 30,000 cells/pool, corresponding to 7,500 viable cells/sample were loaded on the 10x chip.

Results Following putative doublet removal and exclusion of stressed or dead cells, we analysed the transcriptomes of ~61,000 single immune cells. Following data integration with Seurat, community detection, non-linear dimension reduction and graph clustering, 23 Louvain clusters were identified, including 15 from the myeloid lineage and 8 from the lymphoid lineage. We observed a significant depletion of microglia (MG)/MG-TAM from the tumor compared to the adjacent non-tumoral parenchyma, which was accompanied by a significant influx of bone-marrow-derived (BM)-TAM and monocytes as already known. Little differences were observed between WT or *Ice*^{-/-} mice before resection. However, post-resection remodelling of the GB TME was regulated by the inflammasome. Notably, monocytes, dendritic cells and regulatory T cells (Treg) subsets increased post resection in the adjacent non-tumoral tissue in WT but not *Ice*^{-/-} mice. Similarly, the intra-tumoral influx of Treg and the compositional changes of BM-TAMs observed in WT mice were blunted in inflammasome-deficient conditions. These TME differences correlated with faster tumor regrowth and decreased survival rates in WT mice compared to inflammasome-deficient mice.

Conclusion Our data reveal a significant impact of GB resection on TME remodeling and implicate the inflammasome in post-resection recurrence.

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P02.09 INTEGRATED SINGLE-CELL PROFILING DISSECTS CELL-STATE-SPECIFIC ENHANCER LANDSCAPES OF HUMAN TUMOR-INFILTRATING T CELLS

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Background Despite extensive studies on the chromatin landscape of exhausted T cells, the transcriptional wiring underlying the heterogeneous functional and dysfunctional states of human tumor-infiltrating lymphocytes (TILs) is incompletely understood.

Materials and Methods We use single-cell chromatin profiling and integrate publicly available single-cell RNA-seq data of TILs from several patients over four cancer entities to study gene-regulation in T cell (dys-)function.

Results We identify gene-regulatory landscapes in a wide breadth of CD8⁺ TIL functional states. Our analysis predicts enhancer-promoter interactions in human TILs and prioritizes key elements by super-enhancer analysis. We define a human common chromatin trajectory to T cell dysfunction and determine involved key enhancers, transcriptional regulators, and deregulated target genes in this process. Finally, we validate enhancer regulation at immunotherapeutically relevant loci by targeting non-coding regulatory elements with potent CRISPR activators and repressors.

Conclusions Our study provides a framework for understanding and manipulating cell-state-specific gene-regulatory cues from human tumor-infiltrating lymphocytes.

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P03 Vaccine therapy

P03.01 DEVELOPMENT OF A MULTI-TUMOUR ANTIGEN VACCINE FOR HARD-TO-TREAT CANCERS

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Background Glioblastoma (GMB), advanced prostate cancer (PCa) and triple negative breast cancer (TNBC) are hard-to-treat cancers with 5-year survival rates of 5%, 12% and 30% respectively in their metastatic stage. With surgery, chemotherapy, and radiotherapy (and hormone therapy for PCa) being the only feasible treatment options, novel therapies are urgently required. Adjuvanted peptide vaccines based on tumour antigens hold promise in prophylactic and therapeutic settings, especially when there is residual disease following treatment. Research suggests that the cancer/testis antigens HAGE and NY-ESO-1 as well as the tumour associated antigen WT1 (antigens of interest) are good candidates for peptide vaccine development, being expressed at various levels in GBM, PCa and TNBC tissues. Additionally, the expression of these antigens can be upregulated by treatment with low-dose DNA methyltransferase inhibitors like decitabine (DAC), offering the possibility to maximise the detection and destruction of residual cancer cells by vaccine-induced T cells.

Materials and Methods A panel of GBM, PCa and TNBC cell lines was treated with 1µM, 5µM and 10µM DAC and tested for antigens of interest using qPCR and western blot, aiming to validate them as immunotherapeutic targets. Peptide sequences derived from these antigens, including a mutated NY-ESO-1-derived sequence, were selected for vaccine development using *in silico* prediction algorithms (SYFPEITHI and IEDB

databases), combined with the novel adjuvant CAF09b and used for the immunisation of HHDII/DR1 and HHDII/DP4 mice. Following splenocyte extraction, peptides-derived vaccines were tested for immunogenicity using IFN γ ELISpot assays and killing of target cells was assessed using the same assay, co-culturing isolated CD3⁺ T cells with antigen-expressing cancer cells.

Results Antigen expression of TNBC cell lines as revealed by qPCR suggested that treatment with 5 μ M DAC induced optimal upregulation, with 10 μ M usually leading to a plateau. These observations were confirmed by western blotting. Splenocytes derived from CAF09b-adjuvanted NY-ESO-1/HAGE/WT1 vaccine-immunised mice showed strong IFN γ responses, and the isolated CD3⁺ T cells from these splenocytes could specifically recognise TNBC cells expressing all three antigens in a HLA-A2 restricted manner.

Conclusions We propose the use of mutated NY-ESO-1/HAGE/WT1-derived peptides in combination with CAF09b for the treatment of GBM, PCa and TNBC at a stage where no tumour cells can be detected to prevent or delay relapse in these malignancies.

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P03.02

A MUTATED PROSTATIC ACID PHOSPHATASE (PAP) PEPTIDE-BASED VACCINE INDUCES PAP-SPECIFIC CD8+ T CELLS WITH EX VIVO CYTOTOXIC CAPACITIES IN HHDII/DR1 TRANSGENIC MICE

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Background Prostate cancer (PCa) is the second most frequent cancer in men and the fifth most frequent cause of cancer-related deaths in men worldwide. Current treatments for castrate (hormone)-resistant prostate cancer (CRPC) are limited and not curative, with a median survival from diagnosis of 23 months. Sipuleucel-T is the only FDA approved autologous cellular immunotherapy for PCa targeting prostatic acid phosphatase (PAP), showing a 4.1 month survival benefit for metastatic castration-resistant prostate cancer patients. However, its anti-neoplastic responses remain minimal and is cost prohibitive and while PAP is a good target for future prostate cancer vaccine, new, more affordable therapeutic approaches are therefore needed to treat advanced PCa. We have previously shown that a 15 amino acid (AA) PAP sequence-derived peptide could induce strong immune responses and delay the growth of murine TRAMP-C1 prostate tumours. We have now substituted one amino acid and elongated the sequence to include epitopes predicted to bind to several additional HLA haplotypes. Herein, we present the immunological properties of this 42mer-mutated PAP-derived sequence (MutPAP42mer) and the additional use of another PAP-derived sequence of 15 AA long to increase the CD4⁺ T-cell responses.

Materials and Methods The presence of PAP-135–143 epitope-specific CD8⁺ T cells in the blood of patients with prostate cancer (PCa) was assessed by flow cytometry using Dextramer™ technology. HHDII/DR1 transgenic mice were immunized with mutated and non-mutated PAP-derived 42mer

peptides in the presence of CAF[®]09 or CpG ODN1826 or 2395 (TLR-9 agonist) adjuvants. WT-hPAP-42mer was also used to immunise syngeneic C57Bl/6 mice. Vaccine-induced immune responses were measured by assessing the proportion and functionality of splenic PAP-specific T cells in vitro.

Results PAP-135–143 epitope-specific CD8⁺ T cells were detected in the blood of patients with PCa and stimulation of PBMCs from patients with PCa with mutPAP42mer enhanced their capacity to kill human LNCaP PCa target cells expressing PAP. MutPAP42mer peptide was significantly more immunogenic in HHDII/DR1 mice than the wild type sequence, and immunogenicity was further enhanced when combined with the CAF09b[®] adjuvant. The vaccine induced secretory (IFN γ and TNF α) and cytotoxic CD8⁺ T cells and effector memory splenic T cells.

Conclusions The periphery of patients with PCa exhibits immune responsiveness to the MutPAP42mer peptide and immunization of mice induces/expands T cell-driven, wild-type PAP immunity, and therefore, has the potential to drive protective anti-tumour immunity in patients with PCa.

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P03.03

ACTIVE IMMUNIZATION AGAINST HUMAN ENDOGENOUS RETROVIRUS TYPE K (HERV-K) AS AN IMMUNOTHERAPEUTIC STRATEGY AGAINST SOLID TUMORS

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Background Human endogenous retroviruses constitute 8% of the genome and are distributed among viral families of which HERV-K is the most recently integrated. Endogenous retroviruses are well established, natural targets for immunotherapy. Previously, we observed that encoding an endogenous variant of the murine leukemia virus as a particle-forming transgene in adenoviral vectors, allowed for curative therapy against small established cancers. In addition, immunogenicity could be further improved by point mutations of an immune suppressive domain (ISD) (WO 2019/043127). In humans, HERV-K Gag and Env genes are structurally intact, and while expression is almost absent in healthy tissues, HERV-K proteins are detected in human cancers, including on cell surfaces and exosomes. Functionally, the HERV-K Env genes are implicated in oncogenic signaling pathways, Epithelial Mesenchymal Transition and immune evasion. Consequently, we developed a particle forming HERV-K vaccine incorporating ISD mutations for treatment of cancer with a combined T and B Cell response.

Materials and Methods HERV-K Gag and Env consensus sequences were encoded in human adenovirus type 5 and 19a/64 adenoviral vectors. Expression analyses were performed on human and mouse DCs. Immune responses were analyzed by intracellular cytokine staining and tetramers. Murine colorectal cancer cells were engineered to express the HERV-K Gag and Env antigens. Immunotherapy experiments in tumor-bearing mice were performed by transplantation of selected immune cell populations obtained from vaccinated donor mice.