technologies substantially advanced neoTCR identification in recent years, however, details about single-TCR-determinants for successful therapeutic administration remain to be understood.

Materials and Methods In this study, we combined high-resolution assessment of neoTCR-activation signatures with detailed in vitro and in vivo characterization of these TCRs. Single-cell TCR- and RNA-sequencing were performed from neoepitope-specifically stimulated, $CD137^+$-enriched peripheral-blood derived $CD8^+$ T cells of a metastasized melanoma patient with previously determined reactivity against MS-validated neoantigens. Ex vivo-restimulation prior to analysis enabled the comparison of transcriptomic signatures of activated neoTCR-T cells. In a second step, these neoTCRs were employed for generation of transgenic TCR-T cells from healthy donors for detailed in vitro and in vivo fine-characterization.

Results Beyond confirmation of all previously known neoTCRs, this approach identified two additional clonotypes targeting $KIF2C^{P13L}$ in the patient. Transcriptomic comparison of all activated neoTCR-T cells revealed a spectrum of qualitatively distinct signatures with unexpectedly high heterogeneity even between TCRs sharing MHC-peptide specificity. Employing neoTCR-transgenic T cells, the TCR-intrinsic character of these differences could at least partly be illustrated. Compared to a stronger, burst-like activation pattern requiring strong negative counter-regulation, more moderate stimulation resulted in stable cytotoxicity and coincided with higher frequencies in the patient. In an in vivo xenograft model comparing rejection kinetics of different TCRs upon tumor rechallenge, TCR activity with moderate stimulation strength was associated with superior, sustained tumor control.

Conclusions By single cell-sequencing of specifically expanded, enriched and restimulated $CD8^+$ T cells novel neoTCRs were identified. Together with detailed characterization of TCR-transgenic T cells, we describe a spectrum of qualitatively heterogeneous activation signatures within the neoTCR repertoire of one melanoma patient. Within this spectrum, moderate stimulation was associated with superior in vivo functionality. Altogether, our study provides a sensitive method for detection of neoTCRs and moreover profiling of their activation signatures. Those patterns provide valuable insights for engineering TCR-transgenic T cells for therapeutic application.

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P09.18 TNF INDUCTION IN ESSENTIAL FOR ONCOLYTIC INFLUENZA A VIRUS INDUCED CANCER REGRESSION AND TUMOR ASSOCIATED MACROPHAGE REPOLARIZATION

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Background Oncolytic viruses are becoming an integral part of immunological approaches to cancer treatment. Induction of inflammatory responses can vary drastically between virus families.[1] Understanding the mechanisms of ligand induced immunogenic cell death (ICD) and stimulation of myeloid cells in the immunosuppressive tumor immune microenvironment (TIME) unique to the utilized virus will enable development of specific strategies to optimize different viral prototypes. We therefore sought to characterize involvement of ligands of the tumor necrosis factor (TNF) receptor superfamily (TNFRSF) in influenza A virus induced oncolysis and tumor associated macrophage (TAM) repolarization.

Materials and Methods WM793b melanoma or HT-29 colorectal cancer cell lines were infected with an H5N1 oncolytic virus prototype expressing a truncated NS1 gene, 116 amino acids in length.[2] TNFRSF ligands were inhibited using biotherapeutic molecules. Cell death was assessed via flow cytometry. M2-like macrophages were obtained by *ex vivo* polarization from healthy volunteers, stimulated with supernatants of infected co-cultures of cancer cell lines and primary cancer associated fibroblasts and phenotypic features determined via flow cytometry. Subcutaneous syngeneic CT26 tumors were treated with intratumoral virus injections and intraperitoneal TNF-R2-Fc, and tumors assessed for growth and macrophage immune infiltrate.

Results 24 hours after viral infection, the majority of cell death was due to a bystander effect. This bystander cell death was cooperatively induced by FasL and TNF signals upon oncolytic influenza A virus infection *in vitro*, while TRAIL did not appear necessary. Cell death appeared to be mostly apoptotic in nature. Surprisingly, re-polarization of TAM depended on TNF signaling *ex vivo* and was independent of caspase or RIPK3 based cell death. Treatment response of CT26 tumors to oncolytic influenza virus injections was completely inhibited by TNF-R2-Fc co-treatment. Similarly, TAM extracted from the murine tumors showed a downregulation of inhibitory phenotypic markers CD163 and CD206, the latter being rescued by TNF-R2-Fc co-treatment.

Conclusions Whereas the oncolytic influenza A virus induced bystander effect was dependent on FasL and TNF, TNF alone was essential for repolarization of TAMs and therapeutic efficiency in a murine animal model.

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