Conclusions This HNSCC slice culturing system is a \textit{ex vivo} platform that might complement pre-clinical studies to eventually investigate cancer immune-related drugs and ease the translation to the clinics.

Disclosure Information M. Mayr: A. Employment (full or part-time); Significant; ViraTherapeutics. A. Runge: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; ViraTherapeutics. T. Schwaiger: A. Employment (full or part-time); Significant; ViraTherapeutics. S. Sprung: None. P. Chetta: A. Employment (full or part-time); Significant; Boehringer-Ingelheim. T. Gottfried: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; ViraTherapeutics.

J. Dudas: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; ViraTherapeutics. M. Greier: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; ViraTherapeutics. M. Glatz: A. Employment (full or part-time); Significant; ViraTherapeutics. J. Haybaeck: None. K. Elbers: A. Employment (full or part-time); Significant; ViraTherapeutics.

H. Riechelmann: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; ViraTherapeutics. P. Erllmann: A. Employment (full or part-time); Significant; ViraTherapeutics. M. Petersson: A. Employment (full or part-time); Significant; ViraTherapeutics.

P09.16 MELARV VACCINE WITH MUTATIONS IN THE IMMUNOSUPPRESSIVE DOMAIN ELICITS INCREASED IMMUNITY AND ELIMINATION OF ESTABLISHED TUMORS IN MICE

1,2D Daradoumis*, 1K Pedersen, 1K Nielsen, 1A Vergara, 2E Neukirch, 2A Andersson, 3S Schrodel, 3E Ragonnaud, 3P Holst. University of Copenhagen, Copenhagen, Denmark; 3InProTher, Copenhagen, Denmark; 3Sirion Biotech, Graefelfingen, Germany

Background Endogenous retroviruses (ERVs) account for 8% of our genome and, while being silent in healthy tissues, they become reactivated under pathological conditions such as cancer. Several studies showed a functional role for ERVs in tumor development and progression, primarily mediated by the immunosuppressive domain (ISD) of the ERV envelope (Env) protein. To investigate ERV-targeting in a murine model, we have selected the murine melanoma-associated retrovirus (MelARV) as the target for our vaccine technology named virus-like vaccine (VLV). VLVs are composed of an adenoviral vector (carrier) encoding virus-like particles, in this case, MelARV Gag and Env. This design allows to elicit both cellular and humoral immunity, necessary to target and eliminate ERV-expressing cancers. Additionally, we attempt to improve the immunogenicity and efficacy of the vaccine by introducing a mutation in the ISD (ISDmut) of the MelARV Env.

Materials and Methods The MelARV adenoviral vectors used in the studies are designed in-house and manufactured and quality controlled by Sirion Biotech. Immunogenicity of the vaccine is tested in BALB/c mice at the peak of T cell responses. 17 days after vaccination, mouse splenocytes are restimulated, and CD8+ T cells are stained intracellularly for IFN$\gamma$ and TNF$\alpha$ responses, which are detected by flow cytometry. Therapeutic efficacy is tested against established murine colorectal carcinoma (CT26) tumors in BALB/c mice. Mice are challenged subcutaneously (s.c.) with 5E+05 CT26 cells and vaccinated after 10 days when tumors are palpable (<200 mm$^3$). In addition, 2 mg/mL of anti-PD-1 is administered intra-peritoneally (i.p.) on days 10, 14, 17 and 21. Tumor growth is measured every 2–3 days over 45 days. Mice that cleared CT26 tumors are rechallenged s.c. with a breast cancer cell line (4T1) and are followed over time to show cross-protection (tumor control) against different cancer types.

Results Vaccination with the ISDmut MelARV vaccine generates strong immune responses against MelARV antigen, reaching 8% circulating CD8+ IFN$\gamma$+ T cells. This modified vaccine, in combination with an anti-PD1 checkpoint inhibitor, shows high curative efficacy (80%) against established CT26 tumors, compared to the wild type (WT) (30% survival) and control (22% survival). Furthermore, ISDmut prevents growth of 4T1 cancer cells in almost 40% of the mice that cleared CT26 tumors.

Conclusions ISDmut increases vaccine immunogenicity by significantly enhancing MelARV-specific T cell responses when compared to the WT vaccine. Moreover, ISDmut in combination with anti-PD1 treatment, can prevent growth of established colorectal carcinoma tumors and protects against rechallenge with 4T1 cancer cells. Therefore, our ISDmut vaccine can be used therapeutically and prophylactically against MelARV expressing tumors, with the prospect of translation into a human ERV-targeting vaccine.

Disclosure Information J. Daradoumis: A. Employment (full or part-time); Significant; InProTher. I. Pedersen: A. Employment (full or part-time); Significant; InProTher. K. Nielsen: A. Employment (full or part-time); Significant; InProTher. A. Vergara: A. Employment (full or part-time); Significant; InProTher. L. Neukirch: A. Employment (full or part-time); Significant; InProTher. A. Andersson: A. Employment (full or part-time); Significant; InProTher. S. Schrodel: A. Employment (full or part-time); Significant; Sirion Biotech. C. Thirion: A. Employment (full or part-time); Significant; Sirion Biotech. E. Ragonnaud: A. Employment (full or part-time); Significant; InProTher. P. Holst: A. Employment (full or part-time); Significant; InProTher.

P09.17 HIGH-RESOLUTION OF NEOANTIGEN-SPECIFIC T CELL RECEPTOR ACTIVATION PATTERNS – MODERATE STIMULATION PREDICTS SUSTAINED ANTI-TUMOR-RESPONSE

1F Füchsl*, 1J Untch, 2V Kavaka, 3S Jarosch, 3C Vogelsang, 4N de Andrade Krätzig, 5D Gossmann, 6H Rad, 6D Busch, 6E Beltran, 4E Bräunlein, 4A Krackhardt. 1Technische Universität München, School of Medicine, Klinik und Poliklinik für Innere Medizin III, Klinikum rechts der Isar, Munich, Germany; 2Ludwig-Maximilians-Universität München, Institute of Clinical Neuroimmunology, Munich, Germany; 3Technische Universität München, Institute for Medical Microbiology, Immunology and Hygiene, München, Germany; 4Technische Universität München, Institute of Molecular Oncology and Functional Genomics, München, Germany

Background Neoantigen-specific T cell receptors (neoTCRs) increasingly receive attention for anti-tumor immunotherapy. Arising from somatic mutations and aberrant post-translational modifications, neoantigens promise safe, highly personalized targets for adoptive cell transfer. Single cell-sequencing
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 neoeptope-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 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.


Background Oncolytic viruses are becoming an integral part of immunological approaches to cancer treatment. Induction of inflammatory responses can vary drastically between virus families. 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. TNFRSF ligands were inhibited using biotechnological 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.

REFERENCES

Disclosure Information J. Homola: None. J. Kabiljo: None. A. Theophil: None. N. Hartman: None. I. Kovacs: None. J. Karall: None. K.E. Lechner: None. C. Klicka: None. J. Laengle: M. Fabits: V.S. Atanasova: B. Dome: H. Dolzlng: G. Egger: H. Walczak: M. Bergmann: Medical University of Vienna, Department of General Surgery, Comprehensive Cancer Center Vienna, Vienna, Austria; 2National Koranyi Institute of Pulmonology, Budapest, Hungary; 3Ludwig Boltzmann Institute Applied Diagnostics, Medical University of Vienna, Vienna, Austria; 4Medical University of Vienna, Department of Thoracic Surgery, Comprehensive Cancer Center Vienna, Vienna, Austria; 5Medical University of Vienna, Center of Pathobiotechnology and Genetics, Institute of Medical Genetics, Vienna, Austria; 6Medical University of Vienna, Department of Pathology, Comprehensive Cancer Center Vienna, Vienna, Austria; 7CECAD Cluster of Excellence, University of Cologne, Cologne, Germany

10.1136/jitc-2022-ITOC9.74

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

J Immunother Cancer 2022;10(Suppl 1):A1–A49 A41