goal to instruct the generation of diagnostic tools and gather information for future immune intervention.

Materials and Methods Derivatives of the full-length HERV-H *env* gene were codon-optimized and synthesized based on annotated sequences. The wild type (WT) sequence was modified regarding the transmembrane-spanning domain including the cytoplasmic tail (TM+CT). For modified membrane tethered variants, the autologous TM+CT sequence was exchanged for a heterologous sequence. In addition, variants lacking the TM+CT or variants deprived of the complete transmembrane subunit were designed, resulting in the expression of a soluble secreted Env trimer or of the monomeric Env (SU). Expression of the HERV-H Env protein was analyzed by western blot and flow cytometry. Groups of 6 Balb/c mice were immunized with DNA vaccine constructs encoding the WT and modified HERV-H Env proteins on day 0 and 14 (prime), respectively, and boosted twice on day 42 and 70 with an adjuvanted formulation of a recombinant trimeric HERV-H Env protein. Antibody responses were monitored by ELISA against various HERV-H Env (trimer, SU and extracellular domain of TM).

Results Western blot and flow cytometry analysis showed proper expression of membrane-bound Env proteins with no significant enhancement of cell surface display for the TM modified protein. Antibody responses against all Env variants could be shown already after two DNA immunizations and were elevated after boosting with the recombinant Env protein. Except for the group immunized with the DNA vaccine encoding the SU, which showed significant higher antibody titers compared to all others, no differences could be seen between the groups following the protein boost.

Conclusions The results prove the antigenicity and immunogenicity of the HERV-H Env protein and its derivatives. All engineered HERV-Env derivatives were able to prime Env specific antibody responses in Balb/c mice. Booster immunization with adjuvanted Env trimer yielded comparable antibody titers, except for SU, which showing superior responses in terms of magnitude. Data and reagents described herein provide a valuable foundation for the development of diagnostic tools for tumor stratification, antibody-based intervention as well as therapeutic vaccination strategies. 1. Zhang, M, Liang, JQ, Zheng, S. Expressional activation and functional roles of human endogenous retroviruses in cancers. Rev Med Virol. 2019; 29:e2025. https://doi.org/10.1002/rmv.2025

Disclosure Information J. Gille: None. **I. Skandorff Pedersen:** None. **C. Thirion:** None. **P.J. Holst:** None. **R. Wagner:** None.

P03.08 NEOANTIGENIC VERSUS MULTI-ANTIGENIC PERSONALIZED B16 MELANOMA VACCINES COMPARISON ACCORDING TO ANTI-TUMOR T CELL RESPONSE INTENSITY

E Ushakova*, A Fedorova, E Lebedeva, A Pichugin, R Ataullakhanov. *NRC Institute of Immunology FMBA of Russia, Moscow, Russian Federation*

10.1136/jitc-2022-ITOC9.36

Background Personalized tumor vaccines based on synthetic peptide neoantigens or those encoded in RNA-vector are successfully studied in phase 1 and 2 clinical trials in melanoma and glioblastoma patients.

Objectives In our study we compare immunogenicity of two personalized tumor vaccine platforms, one based on DNA-vector encoding tumor neoantigens and the other multiantigenic vaccine made of primary tumor tissue with included molecular immunoadjuvants.

Materials and Methods As a neo-antigenic tumor vaccine (Ad-B16) the recombinant adenovirus vector, encoding B16F10 melanoma mutant antigen, was used. A multi-antigenic tumor vaccine (MTV-B16) was made out of the B16F10 tumor tissue. PRR-agonistic molecular immunoadjuvants were included in the multiantigenic B16 tumor tissue-derived vaccine in order to activate antigen-presenting dendritic cells and reprogram myeloid suppressors. The number of antigen-reactive IFNγ-secretory T effector and T effector memory cells was analyzed by ELISPOT. Serum antibodies specific to intracellular antigens of B16F10 melanoma cells were analyzed using ELISA, while FACS was applied to detect B16F10 surface antigens.

Results MTV-B16 vaccine show stronger immunogenicity than Ad-B16 as to generation of tumor-specific IFNγ-secretory T cells in the spleen of mice. After immunization with MTV-B16, up to 6500 IFNγ CD4 and 3500 IFNγ CD8 T-effector cells (per 1 mln T cells) were discovered versus 600 IFNγ CD4 and 650 IFNγ CD8 T-effector cells generated by Ad-B16. Both vaccines induced serum antibodies specifically recognizing B16F10 melanoma's intracellular and cell surface antigens.

Conclusions A personalized multi-antigen tumor vaccine MTV-B16, made out of tumor tissue and completed with molecular adjuvants, induces siginificantly stronger tumor-specific Th1-type CD4 and CD8 T cell responses than those generated by the neoantigen Ad-B16 vaccine on the adenovirus vector platform.

Funding This study was supported by the Russian Science Foundation (project □20-15-00391).

Disclosure Information E. Ushakova: None. **A. Fedorova:** None. **E. Lebedeva:** None. **A. Pichugin:** None. **R. Ataullakhanov:** None.

P04 Precision medicine meets immunotherapy (immuno-monitoring)

P04.01 VOLATILE PROFILING USING AN ENOSE ALLOWS DIFFERENTIATION OF VOLATILE PHASES DERIVED FROM SERUM, DC, OR MLC CULTURE SUPERNATANTS FROM HEALTHY OR LEUKEMIC SAMPLES

[1]T Baudrexler, [2]T Boeselt, [1]S Bohlscheid, [1]L Li, [3]C Schmid, [3]A Rank, [4]J Schmohl, [1]HM Schmetzer*. [1]*University Hospital Munich LMU, Munich, Germany*; [2]*Phillipps-University Hospital of Marburg, Marburg, Germany*; [3]*University Hospital of Augsburg, Augsburg, Germany*; [4]*Diaconia Hospital Stuttgart, Stuttgart, Germany*

10.1136/jitc-2022-ITOC9.37

Background Volatile organic compounds (VOCs) reflect the metabolism in healthy and pathological conditions. They can be collected easily in a noninvasive matter, directly measured by electronical nose (eNose) and might qualify as a systemic tool to monitor biomarkers related to disease.[1] Myeloid leukemic blasts can be transformed into leukemia derived dendritic cells (DCleu) being able to improve (anti-leukemic) immune responses.[2] To profile the immunological changes in healthy

and acute myeloid leukemic (AML) patients' ex vivo cell cultures, we correlated the cell biological data with the profiles of cell culture supernatants derived VOCs.

Materials and Methods DC/DCleu from leukemic or healthy whole blood (WB) were generated without (Control) or with immunomodulatory Kit M (Granulocyte-macrophage-colony-stimulating-factor (GM-CSF) + prostaglandin E1 (PGE1)) in dendritic cell cultures (DC culture). Kit pretreated/not pretreated WB was used to stimulate T cell enriched immunoreactive cells in mixed lymphocyte cultures (MLC culture). Leukemia-specific adaptive and innate immune cells were detected with degranulation assay (Deg) and an intracellular assay (InCyt). Anti-leukemic cytotoxicity was explored with a cytotoxicity fluorolysis assay (CTX). VOCs collected from DC- and MLC culture supernatants (with vs. without Kit M pretreatment and before vs. after culture) were measured by eNose.

Results Compared to Control (without treatment) a Kit M pretreated leukemia and healthy WB gave rise to higher frequencies of mature (leukemia derived) DC subtypes and activated and memory T cells after MLC. Moreover, antigen (leukemia) specific cells of several lines (innate and adaptive immunity cells) were induced giving rise to blast lysing cells. The eNose could significantly distinguish between healthy and leukemic patients' serum-, DC- and MLC-culture supernatants derived volatile phases and could significantly separate several supernatants (with vs. without Kit M treatment, cultured vs. uncultured) derived VOCs within subgroups (healthy DC or leukemic DC or healthy MLC or leukemic MLC supernatants). Interestingly, the eNose could indicate a Kit M and culture associated effect.

Conclusions The eNose might be a prospective option for a deduction of a VOC based profiling strategy using serum or cell culture supernatants and could be a useful diagnostic tool to recognize AML illness.

REFERENCES

1. Biehl, W., Hattesohl, A., Jorres, R. A., *et al*. VOC pattern recognition of lung cancer: a comparative evaluation of different dog- and eNose-based strategies using different sampling materials. *Acta Oncologica* 2019;**58:** 1216–1224.
2. Amberger, DC, Doraneh-Gard, F, Gunsilius, C., *et al*. PGE1-Containing Protocols Generate Mature (Leukemia-Derived) Dendritic Cells Directly from Leukemic Whole Blood. *International Journal of Molecular Sciences* 2019;**20:**4590.

Disclosure Information T. Baudrexler: None. **T. Boeselt:** None. **S. Bohlscheid:** None. **L. Li:** None. **C. Schmid:** None. **A. Rank:** None. **J. Schmohl:** None. **H.M. Schmetzer:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Modiblast Pharma GmbH, Oberhaching, European Patent 15 801 987.7–1118.

P04.02 A HIGH SENSITIVITY, TUMOR-INFORMED LIQUID BIOPSY PLATFORM, DESIGNED TO DETECT MINIMUM RESIDUAL DISEASE AT PART PER MILLION RESOLUTION

M Pruess*, E Newburn, SM Boyle, D Norton, RM Pyke, F Navarro, J West, R Chen. *Personalis, Inc., Menlo Park, CA, USA*

10.1136/jitc-2022-ITOC9.38

Background While circulating tumor-derived DNA (ctDNA) is an emerging biomarker for many cancers, the limited sensitivity of current detection methods reduces its utility for diagnosing Molecular Residual Disease (MRD) across a variety of clinical applications. Sensitive detection and quantification of MRD remains a key challenge, particularly in early-stage cancers, where timely detection of small micrometastatic lesions may enable treatment that prevents progression to advanced metastatic, incurable disease. To address these challenges, NeXT Personal was developed to deliver industry-leading MRD sensitivity in the range of 1–3 parts per million (PPM) representing a 10–100X increase over other available methods, while requiring only a single tube of blood (4 mL plasma/15ng cfDNA), and 1mm^3 of FFPE tumor tissue.

Materials and Methods NeXT Personal leverages tumor/normal whole genome sequencing (WGS) to design personalized, targeted MRD liquid biopsy panels for each patient. The MRD portion of the panel is composed of up to 1800 somatic tumor variants, enabling higher sensitivity MRD detection in plasma through tracking of high quality and lower noise variants. Additionally, further content included in the panel design enables simultaneous tracking of clinically-relevant, individual variants longitudinally to enable a deeper understanding of tumor biology and its dynamic response to therapy.

Results Performance characterization using cell line dilution series and clinical samples revealed the linearity of tumor signal down to the 1–3 PPM range. Orthogonal confirmation using droplet digital PCR (ddPCR) demonstrated near-perfect concordance down to ddPCR LOD. Further, an input titration curve showed consistent performance using 5 to 50 ng of input cfDNA material from patient samples. Additionally, NeXT Personal achieved 100% MRD specificity when tested on healthy donor plasma samples.

Conclusions NeXT Personal achieved highly sensitive and specific MRD detection, reproducibly demonstrating a LOD down to 1 PPM in different cancer indications, patient sample dilutions, and cell line dilutions. The high sensitivity of NeXT Personal potentially enables MRD detection across a broad variety of cancers and stages, including typically challenging early stage, low mutational burden, and low shedding cancers. In addition to MRD, NeXT Personal tracks and annotates individual variants to advance knowledge of tumor biology and observe emergence of known clinical and resistance hotspots.

Disclosure Information M. Pruess: A. Employment (full or part-time); Significant; Personalis, Inc. **E. Newburn:** A. Employment (full or part-time); Significant; Personalis, Inc. **S. M. Boyle:** A. Employment (full or part-time); Significant; Personalis, Inc. **D. Norton:** A. Employment (full or part-time); Significant; Personalis, Inc. **R.M. Pyke:** A. Employment (full or part-time); Significant; Personalis, Inc. **F. Navarro:** A. Employment (full or part-time); Significant; Personalis, Inc. **J. West:** A. Employment (full or part-time); Significant; Personalis, Inc.. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Personalis, Inc. **R. Chen:** A. Employment (full or part-time); Significant; Personalis, Inc.. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Personalis, Inc..

P04.03 CANCER IMMUNOTHERAPIES, COMPANION DIAGNOSTICS AND PRECISION MEDICINE

N Cascante-Estepa*, S Mayrhofer, H Enzmann. *Federal Institute for Drugs and Medical Devices (BAM), Bonn, Germany*

10.1136/jitc-2022-ITOC9.39