VACCINE IMMUNOTHERAPY AGAINST HUMAN ENDOGENOUS RETROVIRUS: A FOCUS ON ANTI-HERV-K ANTIBODIES

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10.1136/jitc-2022-ITOC9.33

Background Immunotherapies have managed to cover the needs of cancer patients that traditional therapies could not treat. Despite the improvement, broadly acting and highly effective therapies capable of eliminating human cancers without mutated antigens still need to be developed. In this regard, virus-like-vaccine (VLV) technology together with the selection of a cancer specific but ubiquitous antigen can be the key to a successful immunotherapy. VLVs combine the benefits of adenoviral vectors and virus-like-particles (VLPs), leading to the activation of both B- and T-cell responses. Human endogenous retroviruses (HERV) are remnants of ancient viral infections that got integrated into our genome millions of years ago, comprising now the 8% of it. HERVs are usually silenced in healthy tissues but are overexpressed in various cancer types, making them good antigen candidates. HERV-K is the most widely studied HERV, and evidence of HERV-K expression is particularly strong in breast cancer. There are currently no curative therapies for advanced breast cancer, thus our research has been focused on the putative effect of our cancer vaccine in advanced breast cancer.

Materials and Methods This project seeks to describe the effects of HERV-K specific humoral immunity on breast cancer progression. Expression of HERV-K protein in a wide range of breast cancer cell lines was initially investigated. We optimized proliferation, cell migration and invasion, and colony formation assays to explore the role of HERV-K in the phenotype of breast cancer cells. These are then subjected to treatment with commercial and in vivo generated antibodies against HERV-K, protease inhibitors, and agents that block putative HERV-K interaction partners.

Results HERV-K was shown to be present in a wide panel of breast cancer cell lines of various HR, ER, and HER2 status. The project is still ongoing, and I will be analyzing data with this cell lines over the next four months, showing the onco-verge, eliciting cross-reacting CD8+ T cell responses which possibly drive the fate of cancer development and progression. An established anti-microorganism T cell memory may turn out to be an anti-cancer T cell memory, able to control the growth of a cancer developed during the lifetime if the expressed TAA is similar to the microorganism-derived epitope. Bioinformatics analyses and ex vivo immunological validations have been performed.

Conclusions The two classes of non-self antigens may converge, eliciting cross-reacting CD8+ T cell responses which possibly drive the fate of cancer development and progression. An established anti-microorganism T cell memory may turn out to be an anti-cancer T cell memory, able to control the growth of a cancer developed during the lifetime if the expressed TAA is similar to the microorganism-derived epitope. This may ultimately represent a relevant selective advantage for cancer patients and may lead to a novel preventive anti-cancer vaccine strategy.

Disclosure Information A. V. Bermejo: A. Employment (full or part-time); Significant; InProTher. None. P. J. Holst: A. Employment (full or part-time); Significant; InProTher.
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 adjuvant 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 significantly 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 showed 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.


Disclosure Information J. Gille: None. C. Thirion: None. P.J. Holst: None. R. Wagner: None.

**Objectives** In our study we compare immunogenicity of two personalized tumor vaccine platforms, one based on DNA-vector encoding tumor neoantigens and the other multi-antigenic 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 multi-antigenic 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 significantly 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).


**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**

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**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 leukemia 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...