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P03 Vaccine Therapy

P03.01 HIGH IMMUNOGENIC VLP-BASED VACCINES ELICIT NEW T CELL SPECIFICITIES AGAINST MELANOMA NEOANTIGENS IN MICE

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Background Neoantigens' (neoAg) identification, which determines T-cell responses against tumors, has fostered the development of personalized vaccines with promising results. While the ranking of the most immunogenic neoAg can be addressed using predictive techniques, their formulation as vaccines needs to be improved. To maximize their therapeutic potential, optimal neoAg-based vaccines should be manufactured in a superb delivery platform that enhances robust new immune responses, able to bypass thymic tolerance and the humoral immunosuppressive microenvironment. These novel T cell responses generated at the periphery will not be exhausted, opposite to TILs. We aim to develop a highly immunogenic vaccine platform, based on engineered HIV-derived Virus-Like Particles (VLP) expressing approximately 2500 copies of each selected neoAg. We tested different neoAgs loaded VLPs (neoVLP) in a melanoma mouse model to evaluate their capability to generate new immunogenic specificities.

Material and Methods Specific non-synonymous mutations from B16F10 cells were identified, selected and used to generate a list of prioritized peptides. NeoAgs were classified as: Tier1, acquiring a mutation that creates an anchor residue to the MHC-I, not present in the WT peptide; Tier2, acquiring a mutation in a position that largely impacts contact with the TCR respect to WT; and Tier3, acquiring a mutation in the TCR contact region but inducing a less drastic change than in Tier2. Frame shift (FS) mutations, expected to be highly immunogenic, were also included. Thirteen to fifteen selected neoAgs from each group were loaded on highly immunogenic neoVLPs. Their immunogenicity was evaluated in C57bl/6 mice by immunization with a neoVLP-coding plasmid DNA (prime) and purified neoVLPs as soluble particles (boost). Spleenocytes were used to evaluate neoAg-specific T cell responses.

Results We have successfully generated and purified neoVLPs, exposing neoAgs from all groups by transient transfection of Expi293 cells. Protein integrity and VLP morphology were confirmed by western blot and cryo-EM. When used for immunization assays, neoVLPs, containing neoAgs from Tier2, Tier3 and FS groups, were capable of generating humoral responses against viral proteins and T cell responses against neoAgs present in the neoVLP. B16F10 inoculated animals, but not vaccinated, did not develop detectable T cell responses against neoAgs present in any tested neoVLP, suggesting that the vaccination with neoVLPs promoted new specificities against selected neoAgs that might contribute to tumor control and eradication.

Conclusion Our data show that the neoVLPs promote the generation of new antitumor-specific immune responses against selected neopeptides, suggesting that neoVLPs vaccination could be an alternative to current therapeutic vaccine approaches and a promising candidate for future personalized immunotherapy.

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P03.02 PROTEIN-BASED CANCER VACCINE COMBINED WITH AN ONCOLYTIC VACCINE PROMOTES POTENT ANTITUMOR IMMUNITY

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Background KISIMATM platform allows the development of protein-based cancer vaccines able to induce a potent, tumor-specific CD8 and CD4 T cells response. While the cell penetrating peptide and peptide agonist for Toll like receptor (TLR)-2 and TLR-4 confer, respectively, the cell delivery and self-adjunctivity properties, the multiantigenic domain allows the targeting of different cancer antigens, resulting in antitumoral efficacy in different murine models. Oncolytic viruses exert their therapeutic effects by a prolonged oncolytic action and the associated intratumoral inflammation as well as general immune activation. Arming oncolytic virus with tumor associated antigens can additionally enhance the tumor-specific T cell portion and therefore positively affect the balance of antitumor versus antiviral immune responses. The protein vaccine KISIMATM and the recombinant oncolytic virus VSV-GP-TAA (vesicular stomatitis virus pseudotyped with LCMV GP expressing tumor-associated antigens) are both promising vaccine candidates that offer a new

cancer vaccination opportunity when combined in heterologous prime-boost regimen.

Materials and Methods Mice were vaccinated with subcutaneous (s.c.) injection of KISIMA-TAA vaccine and/or with intravenous injection of VSV-GP-TAA in different settings. Immunogenicity was assessed by measuring the peripheral antigen-specific response. Anti-tumoral efficacy as well as in depth monitoring of TILs and tumor microenvironment modulation were assessed following therapeutic vaccination in different tumor models. Additionally, transcriptome and immunohistochemistry analyses of the TC-1 tumor have been performed. Combination of heterologous prime-boost with checkpoint blockade PD-1 therapy has been assessed.

Results Priming with KISIMA-TAA followed by VSV-GP-TAA boost induced a large pool of polyfunctional and persistent antigen-specific cytotoxic T cells in the periphery as well as within the tumor in several tumor models. Frequencies of antigen specific T cells are significantly higher than the respective homologous vaccinations. Additionally, transcriptome analysis of a cold tumor model revealed profound changes in the tumor microenvironment upon heterologous vaccination, including a strong upregulation of gene signatures of several pro-inflammatory cytokines and chemokines required for anti-tumor immunity along with dendritic and T cell trafficking and activation. This was corroborated by flow-cytometric analysis of tumor-infiltrating leukocytes showing massive CD8⁺ and CD4⁺ T cell infiltration as well as repolarization of M2-like macrophages towards M1-phenotype. The presence of the CD8⁺ T cells within the tumor core was confirmed by immunohistochemistry analysis. Moreover, combining heterologous vaccination with checkpoint blockade further improved its therapeutic efficacy and the number of long-term survivors.

Conclusions The KISIMA/VSV-GP heterologous prime-boost approach holds great promise for patients with primary or acquired resistance to checkpoint blockade due to its ability to induce tumor-specific T cell, improve T cell infiltration and increase tumor inflammation, even in tumors with limited permissivity for the oncolytic virus.

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P03.03

NOVEL MACHINE-LEARNING TOOLS IMPROVE COST-EFFECTIVE DEVELOPMENT OF PERSONALISED IMMUNOTHERAPIES: LOWERING FALSE POSITIVE RATES IN THE SEARCH FOR ACTIONABLE IMMUNOGENIC NEOANTIGENS

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Background Personalised immunotherapy approaches rely on the ability of tumour-derived neoantigens to elicit a T-cell

immune reaction able to recognise and kill the tumour cells expressing them. Clinical attempts to leverage the power of neoantigens have however yielded mixed results. This can mostly be attributed to the difficulty of finding truly immunogenic peptides from the set of novel peptides generated by mutations in a given cancer patient. In silico approaches can help alleviate this heavy cost by reducing the neoantigen search space, prioritising epitopes based on various parameters such as epitope expression or MHC binding likelihood. Here we present a suite of tools aimed at further assisting clinicians in selecting the most actionable peptides from a set of potential candidates.

Materials and Methods We developed neoMS, a neural network algorithm able to predict epitope presentation at the cell surface with unparalleled performance. Furthermore, the neoIM algorithm is able to discriminate, in an HLA-agnostic fashion, which of the presented peptides will elicit a T-cell immune reaction. This first-in-class algorithm is a random forest classifier specifically trained to classify short peptides of length 9-11 amino acids as immunogenic or non-immunogenic.

Results The neoMS model achieves up to 0.61 precision at recall 0.4 on its test set, vastly outperforming the current industry standards. In addition, due to its sequence-based comparison method, neoMS exhibits extrapolation capabilities, achieving non-zero predictive power when evaluated on ground truth ligandome data derived from an HLA allele completely absent from the training set. In some cancer indications we showed, moreover, that the neoMS-predicted rate of neoantigen presentation can be used in combination with tumour mutational burden as a high-specificity predictor of response to immune checkpoint inhibitor treatment. neoIM vastly outperforms the currently available methods and can predict peptide immunogenicity with high accuracy (AUC=0.88). Interestingly, neoIM confirmed ELISPOT data obtained by Dillon et al. (2017) showing a response in 4 out of 11 breast cancer patients to a vaccine consisting of 9 MHC class-I restricted breast cancer-associated peptides. The 2 antigens that resulted in a CD8⁺ T-cell specific response were predicted by neoIM as the highest scoring showing its potential in finding the truly immunogenic neoantigens.

Conclusions Taken together, these tools decrease false positive rates significantly as they enable improved identification of immunogenic peptides and the predictions correlate with intensity of immune response and clinical benefits. As such, these tools represent a cost-efficient preliminary step in the search for actionable, immunogenic neoantigens.

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P03.04

NOVEL ALGORITHM DISCOVERS UP TO 35 PERCENT MORE EPITOPES TRANSLATED FROM NON-CODING REGIONS IN COLD TUMOURS

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