1350 NEO-OPEN READING FRAME PEPTIDES AS A SOURCE OF HIGHLY IMMUNOGENIC NEOANTIGENS FOR MRNA THERAPEUTIC CANCER VACCINES

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Background Neoantigens expressed and presented by tumor cells can be recognized by the immune system and facilitate destruction of tumors by cytotoxic $CD8^+$ T cells. The main source of neoantigens has been single nucleotide variations with only one amino acid difference from a wildtype protein. We have instead focused on a class of neoantigens that arise from insertions and deletions (indels) or other gene re-arrangements, leading to formation of neo-open reading frame peptides (NOPs). NOPs are stretches of novel amino acid sequences containing numerous potentially highly immunogenic epitopes and therefore provide a valuable source of neoantigens with the capability to improve efficacy of cancer vaccines.

Methods The analysis of The Cancer Genome Atlas (TCGA) database and in-house sequenced human tumor samples was used to predict expression of shared neoantigens resulting from indels and other gene re-arrangements. Several selected candidates underwent thorough *in vitro* experimental validation to confirm tumor cell presentation and immunogenicity of NOPs. Additionally, we tested immunogenicity of mouse surrogate NOPs identified via the same pipeline in the melanoma cell line B16F10 by encoding ten different NOP sequences in an mRNA vaccine.

Results We identified several different NOP sequences with predicted expression in human tumor samples that were selected for further validation. From each tested NOP, one or multiple epitopes were binding *in vitro* to either HLA-A*02:01, HLA-A*01:01, or HLA-A*24:02 allele. Presentation of epitopes on the tumor cell surface was confirmed by eluting the epitopes from HLA alleles followed by mass spectrometry analysis. We also detected presence of antigen specific $CD8^+$ T cells in blood of healthy donors by tetramer staining. *In vitro* expanded NOP specific $CD8^+$ T cell clones were able to recognize target positive tumor cell lines as measured by increased activation and degranulation markers. *In vivo* immunogenicity assessment after vaccination of mice with a mRNA vaccine encoding B16F10-derived NOPs showed large number (up to 60%) of polyfunctional $CD8^+$ T cells, and to lower extent also $CD4^+$ T cells after *in vitro* re-stimulation of splenocytes with NOP peptide pools. Deconvolution confirmed that 3/10 of NOPs induced $CD8^+$ T cell responses and 2/10 NOPs mounted $CD4^+$ T cells responses.

Conclusions The neo-open reading frame peptides represent a class of experimentally validated, highly immunogenic neoantigens, suitable to induce robust *in vivo* immune responses when encoded in an mRNA vaccine format.

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