Title: Recurrent Non-Normally Processed Transcripts as a Source of Neoantigens for MPNST Immunoprevention

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Neurofibromatosis type 1 (NF1) is an autosomal dominant tumor predisposition syndrome caused by loss-of-function mutations of NF1 gene encoding neurofibromin. Among patients with NF1, loss of the non-mutant allele of NF1 leads to benign NF1-associated plexiform neurofibromas. The main cause of death among NF1 patients is the malignant peripheral nerve sheath tumor (MPNST), a highly aggressive soft tissue sarcoma that most likely develops from benign plexiform neurofibroma (PNF), in particular the so-called atypical PNF. The mutations that govern the development of MPNST (NF1, CDKN2A, and SUZ12 loss) may lead to the expression of recurrent non-normally processed transcripts, such as transcripts undergoing alternative splicing, that could express neoantigens and be used as targets for prophylactic vaccines. Some of these transcripts can have a frameshift downstream of the neojunction, and a premature termination codon (PTC) will be rapidly encountered by the ribosome translating such transcripts. Thus, these non-normally processed transcripts can also express cryptic neoantigens when treated with PTC readthrough drugs. To identify human recurrent non-normally processed transcripts, we analyzed tumor and PDX RNA-seq data from 13 MPNST patients using our pipeline for neojunction discovery. To identify which recurrent transcripts are also tumor-specific, we verified their presence in normal tissue RNA-seq data from GTEx. We have identified 155 recurrent and tumor-specific transcripts. We evaluated the neoantigens and cryptic neoantigens encoded by these transcripts and predicted potential CD8 T cell neoepitopes and cryptic neoepitopes using NetMHCpan algorithm for HLA class I alleles prevalent in the human population. We prioritized the predicted neoepitopes based on binding stability, dissimilarity from human normal proteome and foreignness. We are validating the immunogenicity of the predicted neoepitopes by IFNγ ELISPOT assay. To ensure the predicted neoepitopes are expressed, we are using Ribo-seq of MPNST cell lines to identify actively translated transcripts. To ensure the predicted neoepitopes are presented to the immune system, we are analyzing MHC class I peptides in tumor cell lines by mass spectrometry. The validated immunogenic neoantigens and cryptic neoantigens could be used as a prophylactic vaccine in combination with PTC readthrough drugs to prevent the malignant transformation of benign PNFs into atypical PNFs and to MPNSTs.