A VACCINE TARGETING THE RECURRENT DRIVER MUTATION H3K27M INDUCES MUTATION SPECIFIC T- AND B-CELL RESPONSES IN PATIENTS WITH DIFFUSE MIDLINE GLIOMAS

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Background A recurrent point mutation at position 27 in the histone-3 gene (H3K27M) defines a distinct subtype of highly aggressive diffuse midline gliomas (DMG) characterized by high mortality and morbidity rates. Despite the high clinical need and several clinical trials focusing on novel treatments, effective treatments remain limited. A vaccine targeting the neoepitope H3K27M has been shown to induce a mutation-specific CD4+ T-cell response and to control H3K27M-mutated syngeneic tumors in an MHC humanized mouse model.

Methods We have developed a protocol to expand neoantigen-reactive T cells from patients with H3K27M-mutated diffuse midline gliomas not eligible for the currently ongoing Phase 1 clinical trial (ClinicalTrials.gov Identifier: NCT04808245) and vaccinated with a mutant long peptide vaccine (H3-vac) to recover T-cell receptors (TCR) using combined single cell RNA and VDJ sequencing. We established a pipeline to clone TCRs from expanded populations of T cells and test their neoepitope specificity. Applying a novel DNA assembly process, we significantly decreased costs, allowing us to test more than 200 TCRs, derived from three different patients, for neoepitope specific reactivity using a co-culture assay with immortalized patient-derived B cell lines.

Results Employing a panel of different healthy-donor-derived immortalized B cell lines and by establishing CRISPR/Cas9-mediated HLA knock-out lines we determined the HLA-restrictions of all identified H3.3K27M reactive TCRs. We demonstrated that the H3K27M peptide vaccine induces an immune response across diverse HLA alleleotypes: the deconvoluted HLA restrictions of cognate CD4+ TCRs demonstrate restrictions to members of multiple class II HLA loci.

Additionally, we confirmed the presence of vaccine-induced mutation-specific CD4+ TCRs in a cerebrospinal fluid (CSF) sample from a vaccinated patient. The functionality of the CD4+ T cell clones identified in this study is further underlined by their central memory phenotype and the prominent cluster of activated B cells expressing H3K27M-specific BCRs in the CSF. We validated these B cells to encode H3K27M-specific antibodies. Cloning and testing two B cell receptors (BCRs) recovered from single B cells revealed mutation-specific binding of H3K27M peptide as well as full length protein.

Conclusions Taken together, our data show that a neoepitope vaccine targeting H3K27M not only induces a CD4+ specific T-cell response across various HLA alleles but also induces a mutation-specific B-cell response with development of H3K27M-targeting antibodies.