225 OPTIMAL-AFFINITY MAGE-A1-SPECIFIC T CELL RECEPTORS (TCRS) GENERATED USING THE HUMANIZED TCR-TRANSGENIC MOUSE PLATFORM HUTCR ARE SUPERIOR TO HUMAN DONOR-DERIVED TCRS

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Background As cancer-testis antigens are self-antigens, T cells expressing high-affinity TCRs against such antigens are eliminated via negative selection. Therefore, human-derived TCRs are typically of low affinity and exhibit reduced anti-tumor activity. Affinity maturation by mutagenesis is a common tool to increase affinity but may result in reduced specificity and off-target toxicity. Using our proprietary HuTCR mouse platform, which consists of mouse lines carrying the full human TCR-a/ß loci and human HLA alleles, we have isolated naturally optimized high-affinity TCRs specific for the cancer-testis antigen MAGE-A1 and compared them in vitro and in vivo to human-derived MAGE-A1-specific TCRs that are currently reported to be in clinical development.

Methods MAGE-A1-specific TCRs were isolated from HuTCR mice immunized with the MAGE-1 antigen using scRNAseq or were synthesized based on publicly available databases of human donor-derived MAGE-A1-specific TCRs. All TCRs were re-expressed in primary human T cells as verified using peptide-MHC-multimer staining. Functional activity of the TCRs was analyzed by coculture with T2 target cells loaded with titrated amounts of epitope and measuring cytokine concentration by ELISA. Reactivity of TCRs to endogenously processed MAGE-A1 protein was assessed by coculture with tumor cell lines with variable MAGE-A1 and/or MHC-class-I expression. Tumor rejection potential of TCRs was evaluated in vivo using a syngeneic mouse model (TNA2 mice) expressing MAGE-A1 and HLA-A*02 on syngeneic tumor cells.

Results Immunization of HuTCR mice with the MAGE-A1 antigen resulted in robust CD8+ T cell responses and several TCR clonotypes were identified by scRNAseq, with the majority of clonotypes being specific to the MAGE-A1-derived peptide KVLEYVIKV and TCR functional avidities ranging from 0.3nM to 3nM. In sharp contrast, human-derived TCRs of the same epitope specificity exhibited lower functional avidity with EC50 from 3nM to 60nM. In addition, HuTCR-mouse-derived TCRs were more sensitive in recognition of tumor cells expressing low MAGE-A1 and/or MHC-class-I. Adoptive T-cell transfer to TNA2-mice with established tumors resulted in complete rejection without relapse of tumors only in mice treated with HuTCR-mouse-derived TCR but not with human-derived or control TCRs.

Conclusions The HuTCR mouse platform allows for the generation of high-affinity MAGE-A1-specific human TCRs with increased anti-tumor efficacy as compared to human-derived TCRs against the same cancer antigen. The in vitro and in vivo comparative data presented herein highlight the HuTCR-derived MAGE-A1-specific TCR as the most favorable candidate for clinical translation and a clinical trial evaluating its safety and efficacy in a variety of solid malignancies will be initiated November 2021.

Ethics Approval All animal experiments were performed according to institutional and national guidelines, after approval by the responsible authority (Landesamt für Gesundheit und Soziales, Berlin). Blood collection from healthy human donors was done after prior informed consent and experiments were conducted in accordance with the ethical standards of Declaration of Helsinki.

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