Molecular Therapy
Oncolytics
Commentary

Expanding the landscape of TCR gene therapy targeting MAGE

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https://doi.org/10.1016/j.omto.2023.04.004

Adoptive cell therapy (ACT) is a highly promising approach to cancer treatment that is rapidly transforming the clinical landscape of oncology. Two main types of ACT are chimeric antigen receptor T cell (CAR-T) therapy and T cell receptor-modified T cell (TCR-T) therapy.[1] While CAR-T is engineered to recognize cell surface antigens, TCR-T can target a broader spectrum of intracellular proteins that are processed and presented on human leukocyte antigens (HLAs). However, TCR binding is based on epitope recognition in the context of specific HLA alleles and there are over 36,000 unique alleles.[2] Only a small fraction of peptides and cognate TCRs from cancer-associated antigens (165 epitopes and 1,069 TCRs) have been reported in the Immune Epitope Database[3] compared with all other antigens (1,695 epitopes and 170,395 TCRs). Further, the relationship between TCR affinity and quality of TCR signaling for optimal anticancer responses is also ambiguous.[1] The major challenges in developing effective TCR-T immunotherapy for cancer include: (1) the identification of cancer-specific epitopes presented on HLA class I alleles, (2) the isolation of highly specific and potent TCRs, and (3) coverage of a large population across multiple HLA alleles.

de Rooij et al.[4] report systematic studies to expand the repertoire of TCRs targeting melanoma-associated antigen (MAGE) proteins for cancer therapy. The MAGE gene family was initially described as a group of cancer-testis antigens[5] with restricted expression in germ cells, placenta, and tumor cells. Several MAGE-targeted TCR-T therapies have advanced to clinical trials, but previously reported neurological and cardiovascular toxicities[6,7] due to TCR cross-reactivities have raised significant caution. To address this issue, the authors constructed a bioinformatics platform to pre-screen candidate MAGE genes wherein they filtered antigens expressed in benign organs other than the testis and placenta. Only MAGE peptides that share limited similarity with other human proteins were selected. They then applied a multipronged approach to identify epitopes from these candidate MAGE proteins on multiple high-frequency HLA class I alleles using both an *in silico* predictive algorithm and liquid chromatography-mass spectrometry-based immunopeptidomic profiling. This large repertoire of HLA class I alleles covers a vast swath of the ethnic groups represented in the global population. This tour de force approach yielded 38 peptides for potential downstream TCR isolation, greatly expanding the range of established MAGE epitopes for immunotherapeutic development.

T cells bearing high-affinity TCRs targeting MAGE may be extremely rare in peripheral blood mononuclear cells from healthy donors as they may be negatively selected during thymic education.[8] To overcome this, the authors utilized an allogeneic HLA repertoire from 54 healthy donors as HLA mismatch could be leveraged to identify T cells with high-affinity MAGE TCRs using peptide-HLA class I tetramers. Interestingly, the cross-reactivities of TCRs reported all involved other MAGE proteins, likely due to high sequence homology. Another possibility is that TCRs may recognize structurally similar peptide-HLA class I complexes despite differences in peptide sequences. While the efforts by the authors to test a panel of MAGE-negative cell lines is commendable, tractable methods to effectively define a TCR's specificity and predict off-target binding and activation remain elusive in the field. The use of high-throughput libraries with large peptide diversity combined with yeast surface display may facilitate this process.[9]

Seven MAGE-specific TCRs described in this study demonstrated *in vitro* anticancer activity and three showed near-complete tumor eradication *in vivo* indicating significant promise for future clinical translation. Most importantly, the work establishes a foundation for further epitope discovery and the isolation/nomination of TCRs for broad application in TCR gene therapy for cancer.

DECLARATION OF INTERESTS

The authors have intellectual property interests related to T cell immunotherapies.

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