1395 TARGETING INTRACELLULAR TUMOR ANTIGENS TO FIGUREHT CANCER: DISCOVERY AND DEVELOPMENT OF FUNCTIONAL AND SPECIFIC T-CELL ENGAGERS AGAINST A MAGE-A4 PMHC

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Background Bispecific T-cell engagers (TCEs) activate the immune system to Figureht cancer. TCEs that target peptides displayed on major histocompatibility complexes (pMHC) have shown promise for unlocking previously inaccessible intracellular tumor-specific antigens. Bispecific antibodies have the potential to overcome challenges associated with other pMHC-targeting modalities, such as soluble T cell receptors, by eliminating the need for extensive engineering of endogenous TCRs to produce molecules with the requisite affinity and specificity.[1]

Peptides derived from melanoma-associated antigen 4 (MAGE-A4) are presented by MHC class I (MHC-I) in many solid tumors, but rarely in healthy tissues. However, developing TCE therapies against MAGE-A4 pMHCs presents complex and unique challenges. The tumor-binding arm must bind specifically to small (~10 amino acid) MAGE-A4 peptides that are highly homologous to other proteins in the MAGE family, avoid substantial interactions with the MHC complex, bind tumor cells expressing very low levels of the target, and work in concert with the CD3-binding arm to activate T cells.

To address these challenges, we have developed a technology platform to discover optimal TCEs that combines hundreds of diverse, fully human CD3-binding antibodies with antibody discovery, engineering, functional screening, and development capabilities. We discovered diverse and developable human antibodies that showed high specificity and affinity to a human MAGE-A4 peptide presented on MHC-I (HLA:02*01).We strategically selected and paired a panel of these MAGE-A4-pMHC-binding antibodies with diverse CD3-binders and used our high-throughput characterization platform to identify TCEs with optimal specificity and functional profiles.

Methods We used our OrthoMab™ multispecifics platform and high-throughput expression to generate more than 400 MAGE-A4-pMHCxCD3 TCEs in bispecific and multispecific formats, and measured purity by mass spectrometry, aSEC, and CE-SDS. We assessed the functional activity of these TCEs in high-throughput T cell cytotoxicity and cytokine release assays. We investigated potential off-target cross-reactivity in MAGE-A4-pMHC-binding parental antibodies with a rationally designed screening library based on X-scan binding data. In-depth binding structural and kinetic assessments were also performed to map antibody-epitope interactions.

Results We identified a panel of MAGE-A4-pMHC-specific TCEs that induce T-cell dependent cellular cytotoxicity while maintaining low cytokine release in MAGE-A4-positive tumor cell lines. We mapped multiple binding parameters including target specificity, kinetics, and affinity to the functional properties of TCEs in different structural formats to identify candidates for further development.

Conclusions We have identified a panel of functional and specific MAGE-A4-pMHCxCD3 TCEs as potential immunotherapies against solid tumors.

REFERENCE

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