Background T cell engagers (TCEs) are bi-functional biologics that bind a target on tumor cells and the CD3 of the TCR-complex on T cells to induce immune-mediated killing of tumors expressing the epitope of interest. While most TCEs on the market and in development target cell surface antigens with tumor- or lineage-specific expression, this approach cannot exploit the tumor-specific expression of intracellular proteins, which make up the majority of the human proteome. However, peptides derived from virtually all intracellular proteins are presented on the cell surface by Human Leukocyte Antigen (HLA) and can be targeted therapeutically to exploit the tumor-specific expression of intracellular proteins. A key safety concern for therapeutics targeting peptide-HLA complexes is cross-reactivity, as unintended binding of peptides expressed on healthy tissues can lead to lethal off-target toxicity.

Methods Here, we utilize Athebody® DARPin®s (Designed Ankyrin Repeat Proteins), engineered proteins based on an ankyrin repeat scaffold, to generate highly specific binders against an epitope from the cancer-germline antigen MAGE-A4 bound to HLA-A2. Using our proprietary 3T-TRACE™ platform, we perform a global cross-reactivity assessment to identify potential off-target liabilities, many of which are sequence-dissimilar to the intended MAGE-A4 epitope and would therefore be undetectable by traditional methods. The interplay of this cross-reactivity risk evaluation, informing tailored binder selection, allowed us to identify leads of unparalleled efficiency and safety profiles. TCEs armed with these Athebody® DARPin®s induce high potency killing of MAGE-A4-expressing cancer cell lines with no detectable killing of antigen-negative cells. We further tune the format of these molecules to retain high killing activity while minimizing cytokine release, a key determinant of dose-limiting toxicity in the clinic.

Conclusions These optimized TCEs express at high titers and display a favorable developability profile. The work presented here demonstrates a novel approach to develop TCEs with excellent potency and specificity against tumor-restricted intracellular targets.