**Background** PRAME is a promising target for immunotherapy due to high prevalence and high expression in multiple tumor indications with high unmet medical need while having limited healthy tissue expression. Therapy with T-cell receptor-modified T-cells (TCR-Ts) targeting PRAME has shown promising early clinical validation. Moreover, accumulating clinical evidence suggests that deep and durable clinical responses in multiple solid tumors require additional T-cell engineering, such as incorporating a CD8 co-receptor (CD8COR) to enable inclusion of CD4 TCR-Ts and engineered receptors to counteract the immunosuppressive tumor microenvironment.

**Methods** We identified PRAME-reactive TCRs using T-knife’s MyT™ platform—a mouse-based human TCR discovery engine with the ability to overcome central tolerance, the natural immune process eliminating high-affinity TCRs for self-antigens in humans. Selected TCR candidates were assessed for reactivity to PRAME-expressing tumor cell lines in vitro. Several of these candidates were co-expressed with CD8COR or combinations of distinct single-chain CD8CORs and switch receptors. Evaluation was based on phenotype and functional activity against target cell lines expressing distinct levels of PRAME.

**Results** PRAME was highly immunogenic in the MyT platform leading to a 100% response rate in mice after immunization. Using a rapid-throughput TCR screening assay, we tested the most-expanded HLA-A*02:01-restricted TCR clonotypes isolated from reactive mouse T-cells and identified close to 100 TCRs with reactivity for PRAME. Most TCRs were specific for the epitope SLL425–433 which is highly abundant on PRAME-expressing tumor cells. We selected 22 of these TCRs for in-depth characterization in vitro. Peptide-dose response assays with these selected TCRs demonstrated a large TCR affinity range for the SLL peptide, translating into differential reactivity to a panel of cell lines with low to high PRAME expression as measured by cytokine secretion and cytotoxicity. Importantly, we identified a number of TCRs of similar or higher reactivity when compared to a clinical-stage PRAME TCR and other publicly disclosed PRAME TCRs. TCR affinity and levels of target expression are expected to influence the requirements of TCR-Ts for co-stimulation. We therefore combined selected TCRs with a variety of CD8COR and switch receptor options available in T-knife’s next-generation toolbox and evaluated the constructs for phenotype and functional activity.

**Conclusions** We demonstrated that the MyT platform can deliver high-affinity, potentially best-in-class PRAME TCR candidates for further clinical evaluation. Combining such high-affinity PRAME TCRs with CD8COR and switch receptor options tailored for TCR, target antigen and targeted indications has the potential to induce deep and durable clinical responses.

**Ethics Approval** Mouse experiments were conducted according to German law under the license number §9 H 0050_21 approved by the Landesamt für Gesundheit und Soziales Berlin.

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