Novel Phosphopeptide-Specific TCRs for Cancer Cell Therapy

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Summary

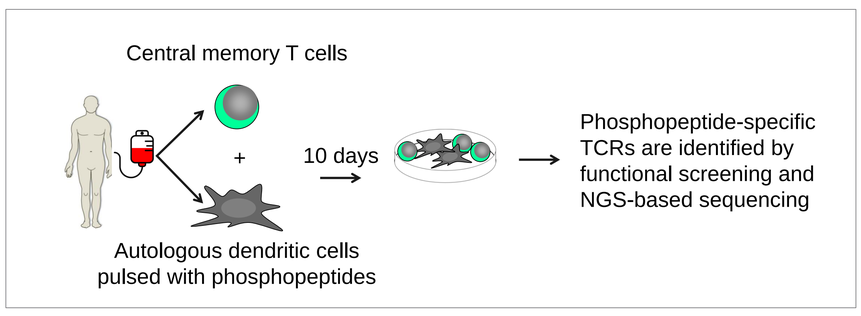
- We report the first discovery and characterization of a fully-human T cell receptor (TCR), AGENt 04002, that is directed against a phosphopeptide tumor target (PTT), a novel class of neo-antigen. Derived from central memory T cells of a healthy donor, AGENt 04002 potently and specifically kills a tumor cell line naturally displaying its cognate mixed lineage leukemia (MLL1) phosphopeptide target. AGENt 04002 mediates T cell activation and target cell killing at low concentrations of the phosphopeptide. Notably, target recognition by AGENt 04002 is highly sequence-specific and depends in particular on the phosphoseryl moiety.
- AGENt 04002 and control TCRs were identified and characterized using a powerful suite of discovery technologies in tandem: a) a primary T cell expansion in which phosphopeptide-specific TCRs were identified by functional screening and NGS-based sequencing; b) a TCR display platform comprising the generation of α and β chain libraries from the donor PBMCs, followed by TCR enrichment for target-specific phosphopeptide binding, activation, and killing; c) a functional testing platform utilizing primary human T cells; and d) a specificity screen to ensure a high degree of targeting precision.
- The discovery of healthy donor, memory T cell-derived, phosphopeptide-specific TCRs provides supportive evidence to the idea that TCRs derived in this manner are expected to possess a preferential safety profile.
- The discovery and characterization of AGENt 04002 showcases the capability of our TCR discovery technologies to identify highly potent and selective phosphopeptide-specific TCRs. Such TCRs constitute prime candidates for clinical development in adoptive cell therapies.

Phosphopeptide Tumor Targets

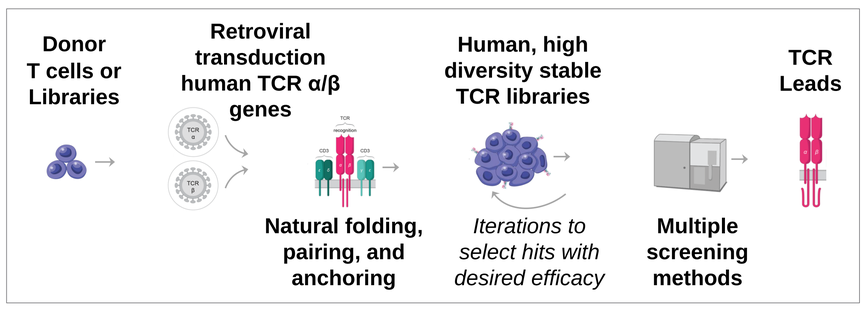
- Deregulated biochemical signaling is a widely recognized hallmark of cancer. It is characterized by aberrant kinase and phosphatase activity, leading to unique, cancer-specific patterns of phosphorylation.
- It has been shown that the immune system of healthy individuals can recognize these phosphorylated antigens, while such immunity is often lacking in cancer patients.
- Such phosphorylated antigens thus represent a novel and compelling class of targets for TCR-directed adoptive cell therapies.

TCR Discovery Platforms

Primary T Cell Expansion Platform

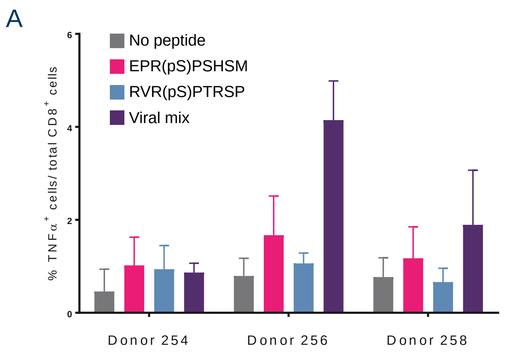


TCR Display Platform: "T-Rx^{TM}"

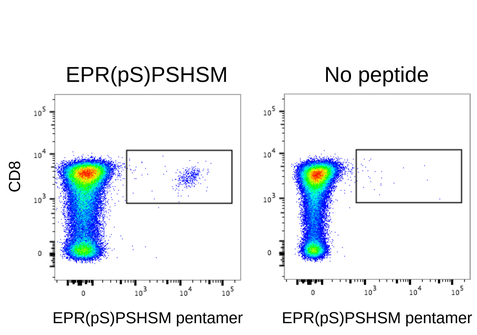


Enrichment of Phosphopeptide-Specific T Cells Using a Primary T Cell Expansion Platform

3 out of 17 HLA*B0702 healthy donors show phosphopeptide-specific cytokine responses



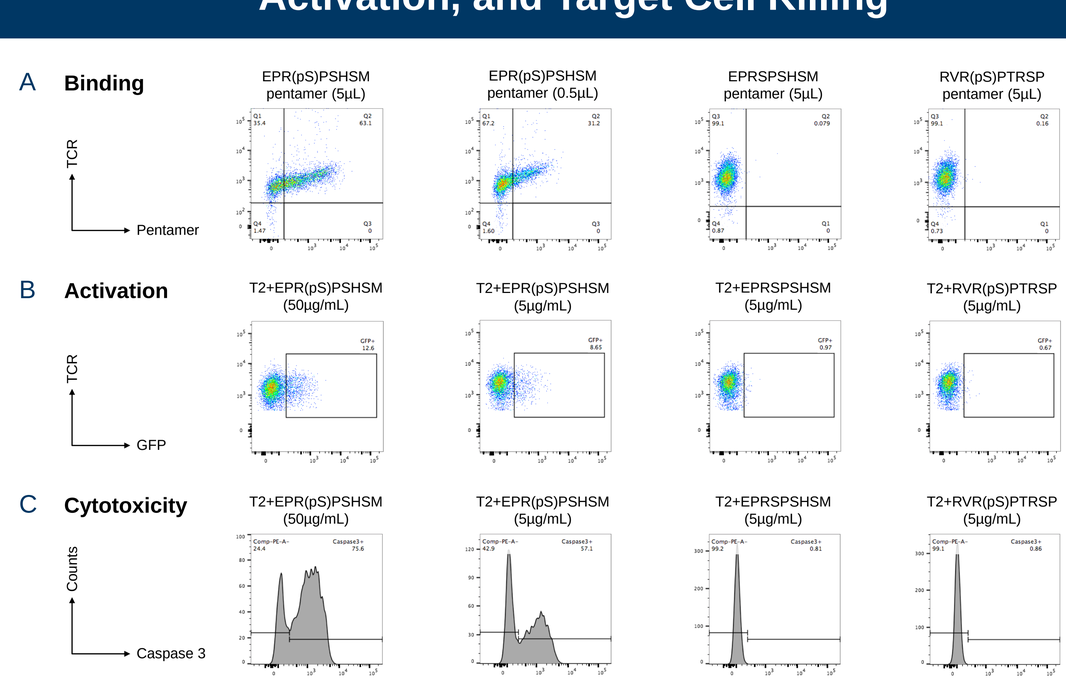
Phosphopeptide-specific T cell population can be expanded from central memory compartment



A. PBMCs from 17 HLA-B*0702 healthy donors were stimulated for 7 days with MLL-specific phosphopeptides EPR(pS)PSHSM or RVR(pS)PTRSP, followed by intracellular cytokine staining for IFNγ and TNFα. A pool of 32 peptides selected from viral T cell epitopes was used as a positive control. No changes in intracellular IFNγ were detected. Results from three donors with increased TNFα production over the no-peptide negative control are shown.

B. Memory CD8 T cell subsets were co-cultured with peptide-pulsed or non-pulsed DCs for 10 days. Next, cells from the co-cultures were stained with EPR(pS)PSHSM pentamer and CD8, followed by binding assessment by flow cytometry.

AGENt 04002* Specifically Mediates T Cell Binding, Activation, and Target Cell Killing

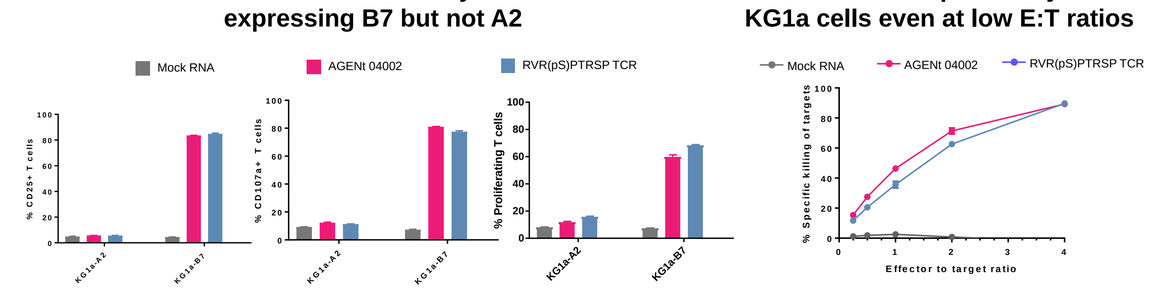


TCRs recovered via NGS-based sequencing were cloned and expressed in TCR display cell line AK-D10R3.
A. Binding of AGENt 04002* -expressing AK-D10R3 cells to peptide-MHC pentamers.
B., C. T2-B7 cells were pulsed with EPR(pS)PSHSM or control peptide EPRSPSHSM. Co-cultures of T2-B7 cells and AGENt 04002*-expressing AK-D10R3 cells were incubated for 16 hours at 37°C. Expression of TCRβ, activation of an IL-2-NFAT$_3$-EGFP reporter, and expression of the apoptosis marker caspase3 were assessed.
*Chimeric form.

AGENt 04002 Expressed on Primary Human T Cells Kills Tumor Cells Presenting its Cognate Phosphopeptide

AGENt 04002 is activated by KG1a cells expressing B7 but not A2

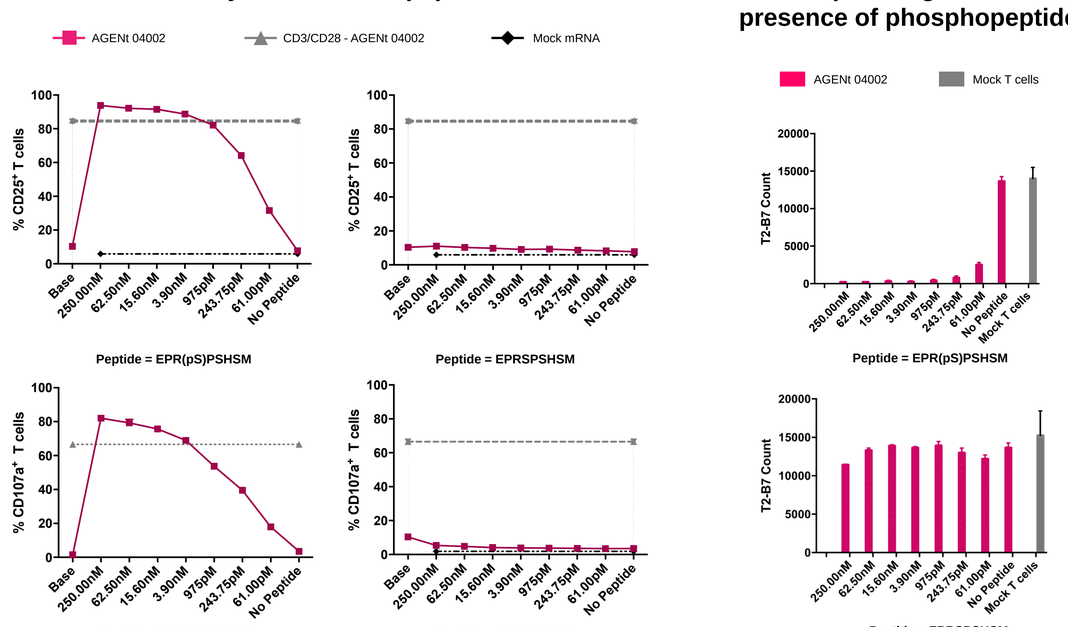
AGENt 04002 specifically kills KG1a cells even at low E:T ratios



On Day 0, primary human T cells were electroporated with AGENt 04002 mRNA, a control TCR recognizing RVR(pS)PTRSP, or mock mRNA. On Day 1, KG1a cells expressing the MLL phosphopeptides and HLA-B*0702 ("KG1a-B7") or HLA-A*0201 ("KG1a-A2") were labeled with CFSE and co-cultured with Celltrace Violet-labeled T cells. On Day 2, the cells were evaluated for CD25 expression, CD107a expression, T cell proliferation, and specific killing of target cells using flow cytometry.

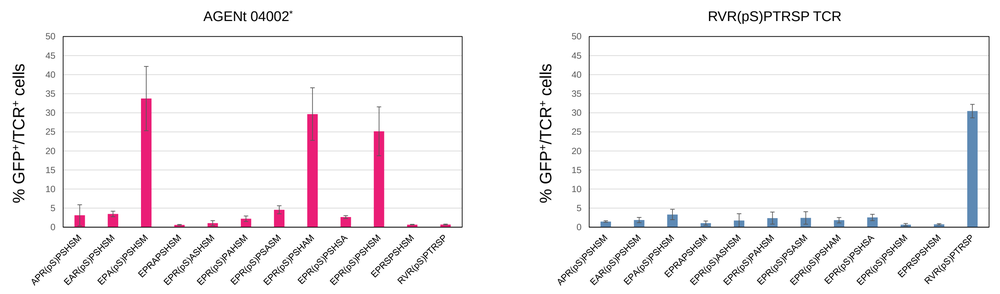
AGENt 04002 is activated by very low doses of peptide

T2-B7 cells are only killed by TCR-expressing cells in the presence of phosphopeptide



On Day 0, primary human T cells were electroporated with AGENt 04002 mRNA or mock mRNA. On Day 1, T2 cells expressing HLA-B*0702 ("T2-B7") were labeled with Celltrace Violet, pulsed with a dose titration of EPR[pS]PSHSM or the non-phosphorylated control peptide EPRSPSHSM, and then co-cultured with CFSE-labeled T cells. On Day 2, the cells were evaluated for CD25 expression, CD107a expression, and specific killing of target cells using flow cytometry.

AGENt 04002* Exhibits a High Degree of Target Specificity



To assess peptide binding specificity, AK-D10R3 cells expressing an IL-2-NFAT$_3$-EGFP reporter construct and AGENt 04002* or a control TCR recognizing RVR(pS)PTRSP were co-cultured with T2-B7 cells pulsed with alanine modified variants of EPR(pS)PSHSM peptide. TCRβ expression and activation of the IL-2-NFAT$_3$-EGFP reporter were assessed by flow cytometry. Cells were gated for TCR expression (APC+) versus T cell activation (GFP+). Using the FlowJo software dot plots were generated and the percentage (%) of APC+ GFP+ cells determined. Data were copied into Microsoft Excel for background correction by subtraction of activation values determined for co-cultures containing T2-B7 cells not pulsed with peptides for graph generation.

*Chimeric form.