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A novel T cell engager targeting HLA-A*02:01 TP53-R175H for cancer immunotherapy

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Abstract

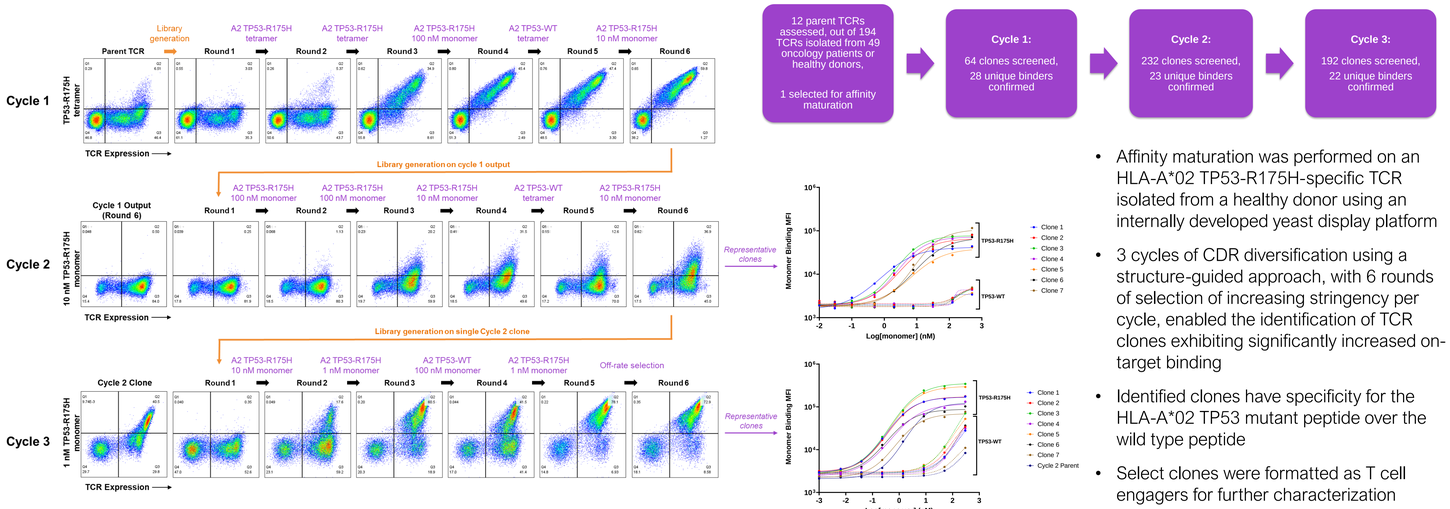
Background: The tumor suppressor protein p53 (TP53) plays a pivotal role in preventing tumor formation by inducing cell cycle arrest and apoptosis in response to DNA damage. However, TP53 mutations are prevalent across various cancer types, and these mutations not only result in loss of tumor suppressive functions but also confer gain-of-function properties that drive oncogenesis. Among these mutations, HLA-A*02:01 TP53-R175H represents the largest TP53 population with >34,000 cases per year in the US and EU across all solid tumor indications, with no therapies explicitly targeting this mutation in the clinic. Leveraging Affini-T's TETHER™ platform, we present the development of a novel TCR-based T cell engager designed to redirect the cytotoxic potential of T cells against mutant TP53-expressing cancer cells.

Methods: A bispecific T cell engager was engineered to simultaneously engage T cells via CD3 and target TP53-R175H-expressing cancer cells by an affinity-matured specific TCR. Affinity maturation of the TCR was performed to increase its specificity and binding affinity towards the mutant peptide. Tumor co-culture assays were conducted to evaluate T cell-mediated cytotoxicity. T cell activation was assessed by measuring cytokine production. The tolerability profile of the engagers was established by X-scan, evaluating T cell activation by normal tissue.

Results: The data demonstrate successful affinity maturation of the TCR, resulting in enhanced recognition of the HLA-A*02:01 TP53-R175H peptide with high specificity. Tumor co-culture assays revealed potent T cell-mediated killing of mutant TP53-R175H-expressing cancer cells by the engager, accompanied by robust T cell activation characterized by cytokine production. Importantly, the engager exhibited favorable tolerability profiles, demonstrated by minimal off-target activation via X-scan and with minimal T cell activation towards normal tissue.

Conclusions: These findings highlight the promising activity and tolerability profile of a novel T cell engager targeting HLA-A*02 TP53-R175H for cancer immunotherapy. Harnessing the cytotoxic potential of T cells against mutant TP53-expressing tumors presents a promising approach for the development of innovative cancer treatments.

Figure 1: Identification of high-affinity HLA-A*02:01 TP53-R175H T cell receptors (TCRs) using a yeast display system enables Affini-T's TETHER™ bispecific T cell engager platform



12 parent TCRs assessed, out of 194 TCRs isolated from 49 oncology patients or healthy donors,

1 selected for affinity maturation

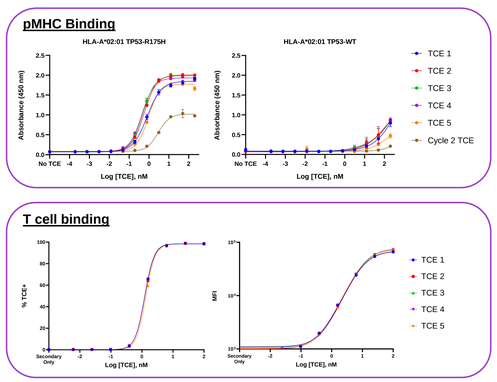
Cycle 1: 64 clones screened, 28 unique binders confirmed

Cycle 2: 232 clones screened, 23 unique binders confirmed

Cycle 3: 192 clones screened, 22 unique binders confirmed

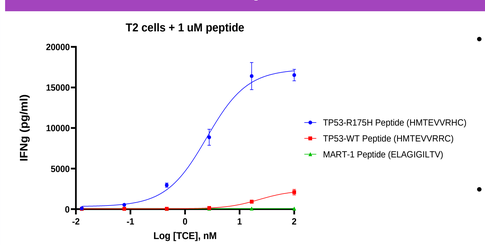
- Affinity maturation was performed on an HLA-A*02 TP53-R175H-specific TCR isolated from a healthy donor using an internally developed yeast display platform
- 3 cycles of CDR diversification using a structure-guided approach, with 6 rounds of selection of increasing stringency per cycle, enabled the identification of TCR clones exhibiting significantly increased on-target binding
- Identified clones have specificity for the HLA-A*02 TP53 mutant peptide over the wild type peptide
- Select clones were formatted as T cell engagers for further characterization

Figure 2: T cell engagers demonstrate robust and specific binding to HLA-A*02:01 TP53-R175H and human T cells



- Dose-dependent binding to immobilized HLA-A*02 TP53-R175H, but not to TP53-WT, suggest affinity-matured TCRs retain their binding specificity when formatted as T cell engagers (TCE)
- T cell engagers demonstrate robust binding to human T cells in a dose-dependent manner and is not impacted by the different TCR binders

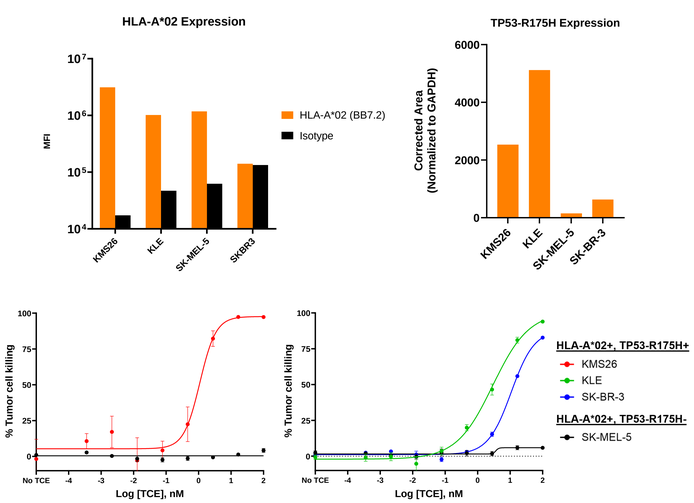
Figure 3: T cell engager 1 mediates robust and specific T cell activation by HLA-A*02 TP53-R175H



T2 cells pulsed with 1 uM peptide were co-cultured with human pan T cells (E:T ratio of 1:1) in the presence of a TP53-R175H specific T cell engager 1. IFNg was quantified by MSD 16 hours post-co-culture.

- T cell engager 1 mediates specific T cell activation in a dose-dependent manner by the TP53-R175H peptide but not by the irrelevant HLA-A*02-restricted MART-1 peptide
- Minimal T cell activation is observed in the context of HLA-A*02 TP53-WT peptide

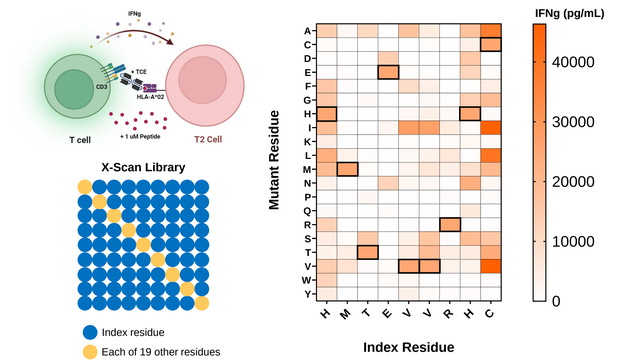
Figure 4: T cell engager 1 demonstrate potent and specific killing of endogenous HLA-A*02:01 TP53-R175H-expressing tumor cells



*Tumor cells were co-cultured with human pan T cells at an E:T ratio of 10:1 in the presence of the T cell engager. Tumor cell killing was quantified by luciferase 72 hours post-co-culture and normalized to a no T cell engager control condition. HLA-A*02 expression of the tumor lines was confirmed by flow cytometry and TP53-R175H expression was quantified by western blot.*

- T cell engager 1 redirects T cells to mediate specific tumor cell killing against HLA-A*02 TP53-R175H-expressing tumor cells in a dose-dependent manner
- T cell engager 1 mediates T cell mediated tumor cell killing against the SK-BR-3 cell line with notably low expression of HLA-A*02

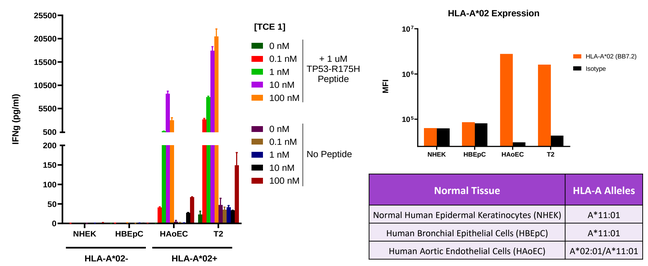
Figure 5: X-Scan analysis suggests no potential off-targets for T cell engager 1 in the human proteome



T2 cells pulsed with 1 uM peptide were incubated with human pan T cells at an E:T ratio of 1:1 in the presence of 10 nM of the T cell engager. IFNg secretion was quantified by MSD 16 hours post-co-culture. Black boxes indicate parent residue.

- Permissive mutations in each position is defined as IFNg secretion >10% of the index peptide (HMTEVVRHC)
- No potential off-targets were identified in the human proteome suggesting a favorable tolerability profile of T cell engager 1

Figure 6: T cell engager 1 does not mediate T cell activation towards selected human normal tissue



Normal Tissue	HLA-A Alleles
Normal Human Epidermal Keratinocytes (NHEK)	A*11:01
Human Bronchial Epithelial Cells (HBEpC)	A*11:01
Human Aortic Endothelial Cells (HAoEC)	A*02:01/A*11:01

*Normal tissue or T2 cells pulsed with or without 1 uM TP53-R175H peptide were incubated with human pan T cells at an E:T ratio of 1:1 in the presence of the T cell engager. IFNg secretion was quantified by MSD 16 hours post-co-culture. HLA-A*02 expression was confirmed by flow cytometry.*

- Co-culture of selected normal tissue with human pan T cells in the presence of T cell engager 1 does not lead to T cell activation, as measured by IFNg secretion, suggesting a favorable tolerability profile

Conclusion

- Identification of high-affinity HLA-A*02 TP53-R175H-specific TCRs using an internally developed yeast display platform that retain their binding potency and specificity when formatted as a T cell engager
- HLA-A*02 TP53-R175H T cell engagers from Affini-T's TETHER™ bispecific T cell engager platform demonstrate potent and specific T cell-mediated tumor cell killing and T cell activation
- HLA-A*02 TP53-R175H T cell engagers demonstrate a favorable tolerability profile with no potential off-targets identified by X-Scan and no T cell activation by normal tissue

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