#1066 T Cell Engagers Targeting HLA-A*11:01 KRAS-G12D and KRAS-G12V Mutations for Cancer Immunotherapy

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Abstract

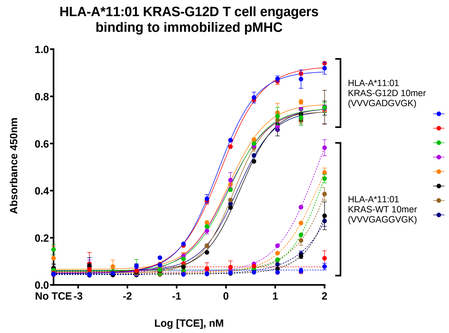
Background: Mutations in the RAS family are highly prevalent in human cancers, including up to 35% of non-small cell lung, 45% of colorectal, and 95% of pancreatic cancers. Kirsten rat sarcoma viral oncogene homologue (KRAS) is the most frequently mutated RAS oncogene and patients with KRAS mutations have poor responses to standard treatment regimens. Leveraging Affini-T's TETHER™ platform, we present the development and characterization of novel bispecific TCR-based T cell engagers (TCEs) targeting HLA-A*11:01 KRAS-G12D and G12V mutations. These TCEs aim to redirect endogenous T cell cytotoxicity against KRAS mutant cancer cells while limiting off-target responses.

Methods: Bispecific TCR T cell engagers were engineered to engage T cells through stimulation of the CD3 complex and target HLA-A*11:01+ cancer cells presenting either KRAS-G12D or KRAS-G12V peptides via a mutant-specific TCR. Yeast display affinity maturation was performed to enhance the specificity and binding affinity of the TCR for its respective KRAS mutant peptide. In vitro pharmacology assays were conducted to evaluate T cell-mediated activation and killing by the TCEs. In parallel, in vitro tolerability studies were performed to evaluate autoantigen cross-reactivity.

Results: The affinity-matured TCRs for both KRAS-G12D and KRAS-G12V exhibited significantly improved binding specificity and affinity, with a greater than 1000-fold affinity enhancement as compared to the parental TCR. Specificity of these affinity-enhanced TCRs was retained, generating TCRs with up to >7000-fold affinity window for the KRAS mutant peptide compared to KRAS wildtype. In vitro pharmacology assays demonstrated that the TCEs effectively mediated potent T cell cytotoxicity against HLA-A*11:01 human tumor cell lines endogenously harboring the corresponding KRAS mutations. Robust T cell activation, indicated by IFNg production, was observed for candidate engagers. Tolerability evaluations reveal minimal off-target effects on normal tissue and favorable off-target profiles in both X-scan mutagenesis scans and an off-target yeast-displayed peptide library.

Conclusions: We report application of the TETHER™ platform to create highly potent and specific T cell engager molecules against multiple mutated oncogenic driver targets. Our approach provides a promising therapeutic strategy for the treatment of cancers driven by KRAS-G12D and KRAS-G12V mutations. The robust preclinical activity and tolerability profiles of these engagers support their potential for application in managing KRAS-mutant malignancies. Additionally, these data support the potential of the TETHER™ platform to produce TCR TCEs against additional mutated oncogenic drivers in solid tumors.

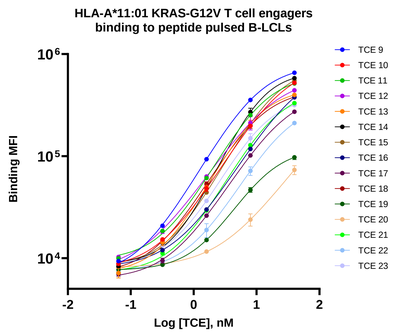
Figure 1: TETHER™ HLA-A*11:01 KRAS-G12D and -G12V T cell engagers (TCEs) demonstrate robust and specific binding to their respective cognate antigens



HLA-A*11:01 KRAS-G12D binding kinetics by SPR

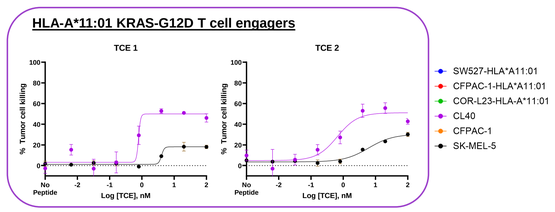
T cell engager	HLA-A*11:01 KRAS-G12D (nM)	HLA-A*11:01 KRAS-WT (nM)
TCE 1	0.15±0.04	1160±328
TCE 2	0.15±0.06	1230±973
TCE 3	<0.1*	278±43
TCE 4	<0.1*	1260±194
TCE 5	<0.1*	1130±205
TCE 6	<0.1*	393±11
TCE 7	<0.1*	1180±96
TCE 8	<0.1*	1360±284

** Off-rate outside of limit of detection*

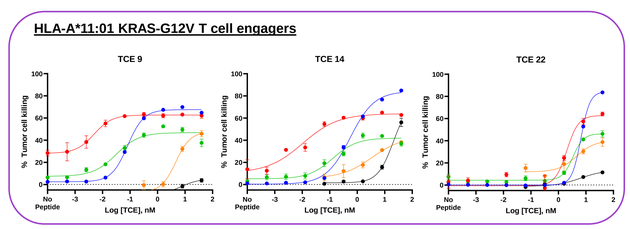


- Affinity-matured TCRs for HLA-A*11:01 KRAS-G12V and –G12D demonstrate robust binding to their respective cognate pMHCs in a dose-dependent manner when formatted as T cell engagers
- Orthogonal methods were used to evaluate TCE binding on immobilized pMHCs complexes and peptide-pulsed B-LCLs
- T cell engagers demonstrate significant binding specificity for the KRAS mutant peptide compared to the wild type peptide, with select engagers exhibiting >7000-fold affinity window

Figure 2: T cell engagers mediate robust and specific tumor cell killing on endogenous and HLA-A*11:01 overexpressing cell lines

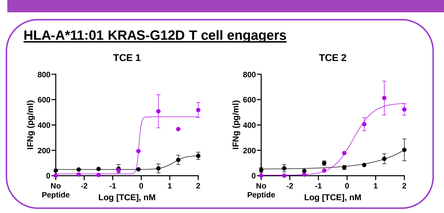


Cell Line	HLA-A*11:01 Status	KRAS Mutation
SW527-HLA-A*11:01	+ (Overexpression)	G12V
CFPAC-1-HLA-A*11:01	+ (Overexpression)	G12V
COR-L23-HLA-A*11:01	+ (Overexpression)	G12V
CL40	+ (Endogenous)	G12D
CFPAC-1	-	G12V
SK-MEL-5	+ (Endogenous)	WT

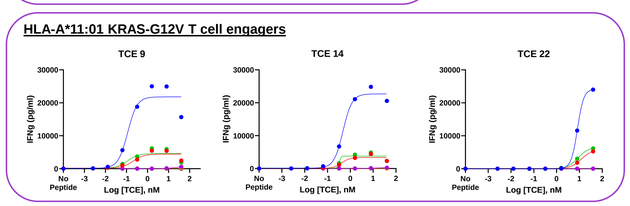


- HLA-A*11:01 KRAS-G12D TCEs demonstrate robust killing of endogenous HLA-A*11:01 KRAS-G12D-expressing CL40, and has specificity over antigen-negative SK-MEL-5 cells
- HLA-A*11:01 KRAS-G12V TCEs demonstrate robust killing of antigen-positive SW527, CFPAC-1, and COR-L23, and demonstrate specificity over antigen-negative CL40, CFPAC-1, and SK-MEL-5

Figure 3: HLA-A*11:01 T cell engagers mediate potent T cell activation by antigen positive tumor cell lines

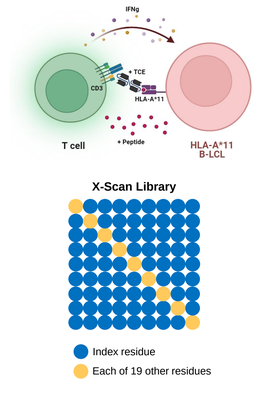


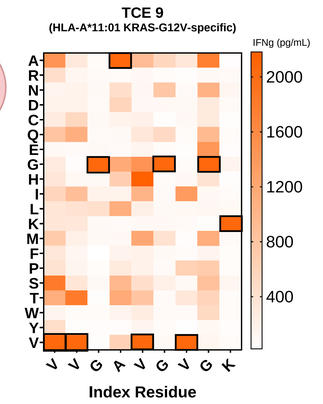
- HLA-A*11:01 KRAS-G12D TCEs mediate potent T cell activation by antigen-positive CL40 cells but not by antigen-negative SK-MEL-5 cells



- HLA-A*11:01 KRAS-G12V TCEs mediate potent T cell activation by antigen-positive HLA-A*11:01 overexpressing tumors SW527, CFPAC-1, and COR-L23 but not by antigen negative CL40, parental CFPAC-1, or SK-MEL-5

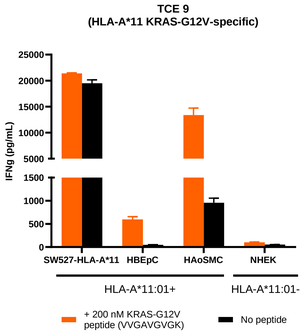
Figure 4: Tolerability of T cell engager 9 is established by X-Scan and T cell activation by selected normal human tissue





*HLA-A*11:01+ B-LCLs were pulsed with 100 nM peptide and incubated with human pan T cells at an E:T ratio of 1.25:1 in the presence of 1 nM of the T cell engager. IFNg secretion was quantified by HTRF 24 hours post-co-culture. Black boxes indicate parent residue.*

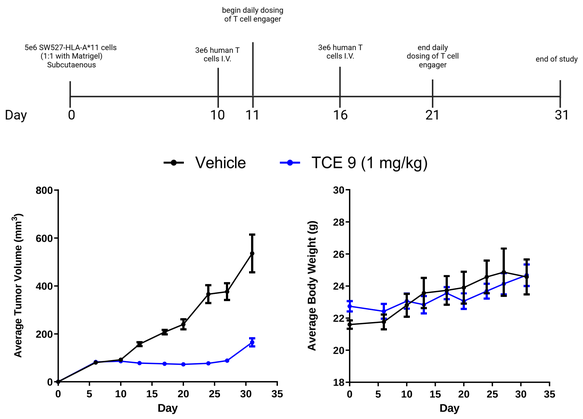
- Permissive mutations in each position is defined as IFNg secretion >20% of the parent peptide (VVGAVGVGK)
- 52 potential off-targets were identified in the human proteome and validation of the off-targets is ongoing



- Co-culture of selected normal tissue with human pan T cells in the presence of the HLA-A*11 KRAS-G12V T cell engager does not lead to T cell activation, as measured by IFNg secretion, suggesting a favorable tolerability profile

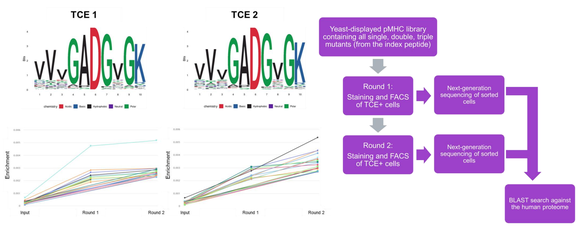
Normal Tissue	HLA-A Alleles
Human Bronchial Epithelial Cells (HBEpC)	A*11:01
Human Aortic Endothelial Cells (HAoEC)	A*02:01/A*11:01
Normal Human Epidermal Keratinocytes (NHEK)	A*02:01

Figure 5: TETHER™ KRAS-G12V T cell engager 9 demonstrates in vivo activity in a SW527 subcutaneous model



- Daily dosing of HLA-A*11:01 KRAS-G12V-specific T cell engager confers tumor growth inhibition and stasis in an HLA-A*11:01 overexpressing SW527 subcutaneous model
- No significant body weight loss was observed in the animals suggesting the engager and dosing regiment is well tolerated

Figure 6: Identification of off-target binders for HLA-A*11:01 KRAS-G12D T cell engagers using a yeast-displayed peptide-MHC library



T cell engager	# of hits in the human proteome
TCE 1	1
TCE 2	0

- MIRO2 was identified as an off-target for TCE 1 and validation of this potential off-target is ongoing
- No off-targets were identified for TCE 2

Conclusion

- Application of Affini-T Therapeutics' TETHER™ platform for the discovery of novel T cell engagers towards oncogenic driver targets
- We report the generation and characterization of highly potent and specific T cell engagers towards HLA-A*11:01 KRAS-G12D and –G12V with in vitro and in vivo activity
- T cell engager molecules have favorable tolerability profiles

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