

T cell receptor-targeting single-domain antibodies as an alternative to CD3-based T cell engagement

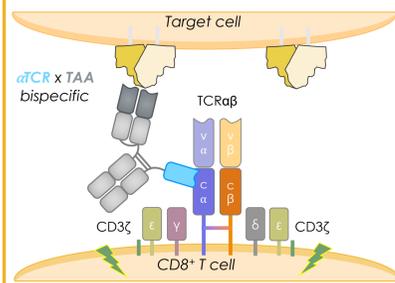
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ADIMAB

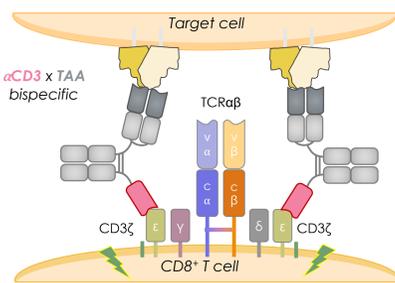
Background – Targeting the TCR constant domain

- T cell engagers (TCE) primarily use CD3 for T cell engagement
- TCR-based redirection is clinically validated (e.g. AZD5492)
- The TCR constant domain presents an alternative engagement node

Anti-TCR-based T Cell Engagers

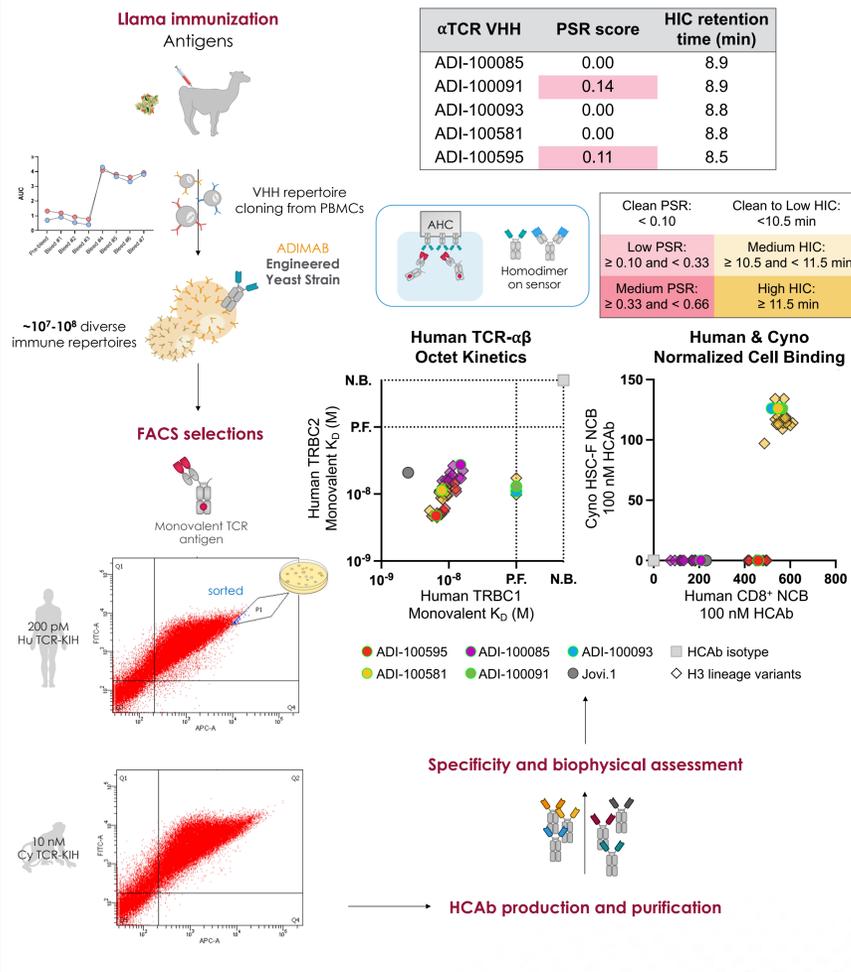


Anti-CD3-based T Cell Engagers



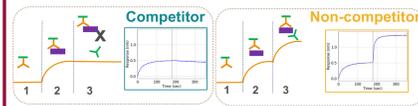
Methods – Discovery and optimization of αTCR VHs

- Five lead lineages were identified via FACS selection
- Three of the five lineages are cross reactive, binding to both recombinant cynomolgus TCR and cynomolgus T cells



Results - The five αTCR VHH lineages are in a shared bin

- Matrix binning reveals that the five identified αTCR VHH lineages are in a shared bin, distinct from Jovi.1 and AZD5492 αTCR antibodies

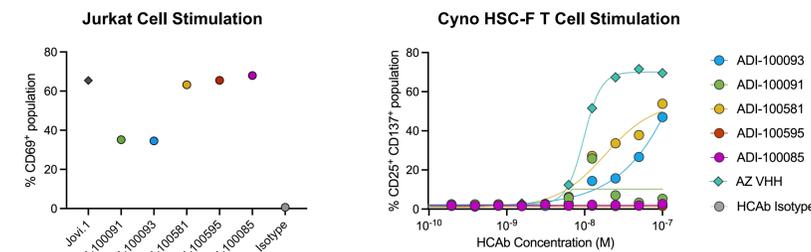
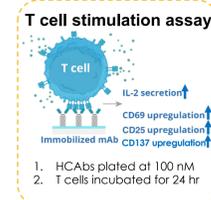


- Octet competition assay:
- Ab on sensor
 - Antigen loading (Hu TRBC1 TCR-Fc)
 - Comparison antibody

Competitor	Jovi.1	ADI-100085	ADI-100093	ADI-100595	ADI-100091	ADI-100581	AZ VHH
Jovi.1	Yes	No	No	No	No	No	No
ADI-100085	No	Yes	Yes	Yes	Yes	Yes	No
ADI-100093	No	Yes	Yes	Yes	Yes	Yes	No
ADI-100595	No	Yes	Yes	Yes	Yes	Yes	No
ADI-100091	No	Yes	Yes	Yes	Yes	Yes	No
ADI-100581	No	Yes	Yes	Yes	Yes	Yes	No
AZ VHH	No	No	No	No	No	No	Yes

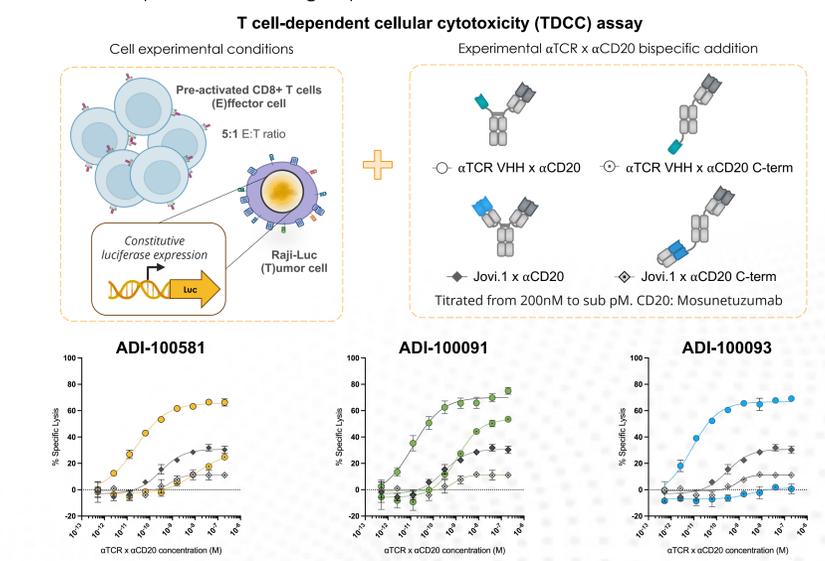
Results - ADI αTCR VHs stimulate human and cyno T cells

- αTCR VHH lineages were immobilized on plates for T cell stimulation assays
- All five lineages stimulate human Jurkat cells at 100 nM HCAb concentration, as measured by CD69 upregulation
- Three lineages stimulate cyno HSC-F cells as measured by double CD25 and CD137 upregulation



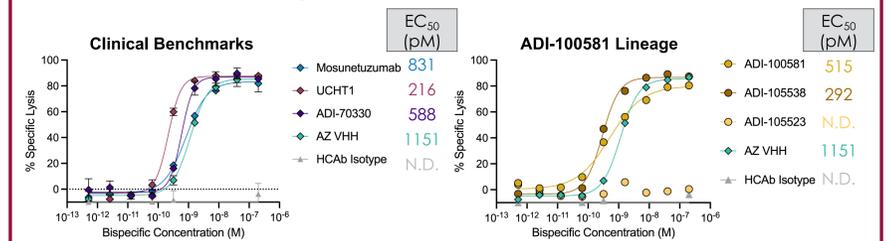
Results – Geometry of T cell-engaging domain is important for potency of αTCR bispecifics

- We investigated N-terminal and C-terminal fusions for CD20 bispecifics that include an αTCR arm as a T cell engager
- N-terminal fusions had higher potency than C-terminal fusions and were used in all subsequent studies using bispecific constructs



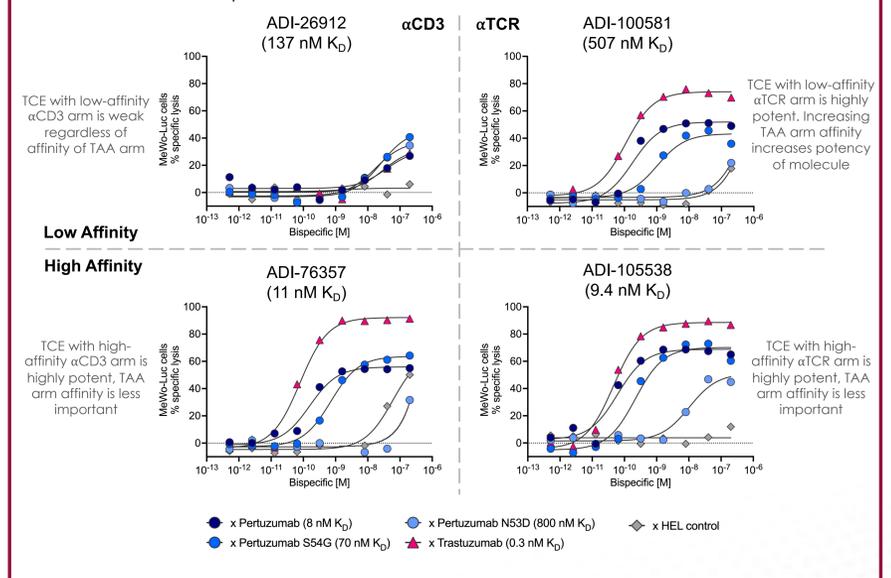
Results – ADI-100581 lineage drives potent cytotoxicity

- The five αTCR VHH lineages were optimized, and higher- and lower-affinity progeny were selected for functional characterization as CD20 bispecifics
- Higher-affinity ADI-100581 progeny (ADI-105538) has improved cytotoxicity compared to clinical-stage αCD3 and αTCR benchmarks



Results – Affinity of T cell-engaging arm is less important for potency in αTCR bispecifics than CD3 bispecifics

- We paired affinity variants of αHER2 (Pertuzumab, 8 nM- 800 nM; Trastuzumab, 0.3 nM) with high- and low-affinity TCEs
- TAA-arm affinity drives potency of αCD3 and αTCR TCEs
- TCE-arm affinity strongly influences potency for αCD3 bispecifics but has limited effect for αTCR bispecifics



Conclusions

- The VHHs described here target the TCR constant domain and occupy a distinct epitope bin, engaging both TRBC1 and TRBC2 for pan-αβ T cell redirection.
- Bispecific constructs containing αTCR VHHs drive potent cytotoxicity, with potency governed by engagement geometry and TAA-arm affinity. This data supports a broad therapeutic window.
- Cross-species reactivity with cynomolgus TCR was confirmed in biochemical and functional studies. This establishes TCR constant domain targeting as an alternative to CD3ε engagement with translatability to non-human primate studies.

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