Unveiling Novel T-cell Receptors (TCRs) for Enhanced Treg Cell Therapy in Type 1 Diabetes (T1D)

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Abstract

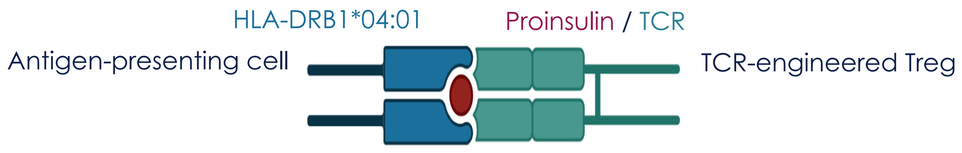
Purpose: Abata Therapeutics is dedicated to developing novel targeted Treg cell therapies for the treatment of tissue-specific autoimmune disorders. Here, we developed a proprietary innovative TCR discovery platform that facilitated the identification of novel MHC class II restricted islet reactive TCRs. These TCRs have the potential to redirect Tregs to the sites of tissue inflammation, provide targeted immune suppression, and promote tissue repair resulting in targeted immune tolerance in patients with T1D.

Methods: Antigen-specific CD4⁺ T cells were enriched *in vitro*, reconstructed *in silico*, and screened in an established set of human cell assays for activation, function, and specificity to enable ranking of candidate TCRs.

Summary of Results: Our robust end-to-end TCR discovery platform allows for the isolation of antigen-specific T cells, *in silico* TCR analysis, functionalization and screening enabling efficient ranking of high functioning TCRs. We narrowed in on two candidate TCRs based on high functional avidity, on-target specificity, and robust IL-10 production. Our lead candidate was identified based on target specificity and high functional suppression.

Conclusions: In summary, we isolated, functionalized, and validated a novel set of candidate TCRs specific for proinsulin, an immunodominant islet autoantigen. Our robust end-to-end TCR discovery platform enabled rapid identification and tiered selection of lead candidate TCRs that exhibited high function, on target specificity, and low cross reactivity. A candidate TCR targeting proinsulin was selected for use in the development of a TCR-engineered Treg cell therapy, for the treatment of T1D.

Targeting Islet Antigens in Patients



- **Disease relevance**: HLA-DRB1*04:01 (DR4) is associated with an increased risk of T1D[1]
- **Penetrance**: HLA-DR3, HLA-DR4 or both present in >90% of T1D subjhects[2]
- **Target specificity**: Proinsulin is a highly enriched immunodominant β–cell target[3-5]

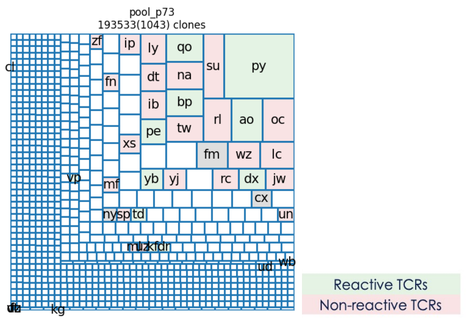
Abata's TCR Discovery Platform

Cell Isolation → Ag-specific T cell enrichment → Single cell profiling → *In silico* analysis → Screening & Validation

- Robust pipeline enables efficient discovery of novel antigen specific TCRs
- TCR activity is validated through a rigorous pipeline of assays grounded in Treg biology
- Pipeline identifies specific TCRs in 3 months; fully validates novel TCRs in 6 months

Expanded Clones Triggered for Screening and Validation

In silico TCR enrichment analysis

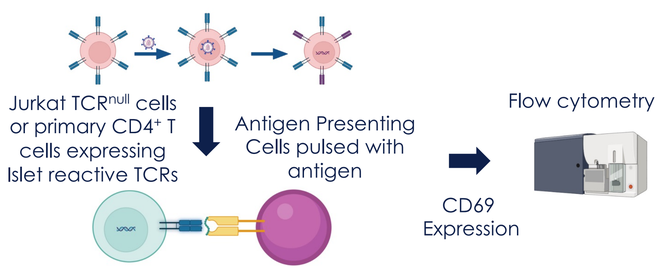


Proinsulin TCRs selected in silico for reactivity screening

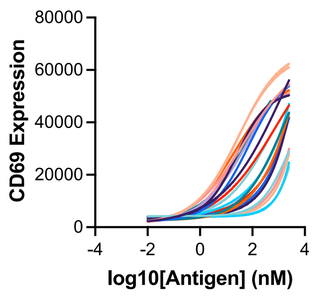
Donor	TCRs Selected for Vectorization
A	43
B	18
C	49
D	1
All	111

Screening Enabled Selection Based on Antigen Specific Activation

T cells transduced with LVV



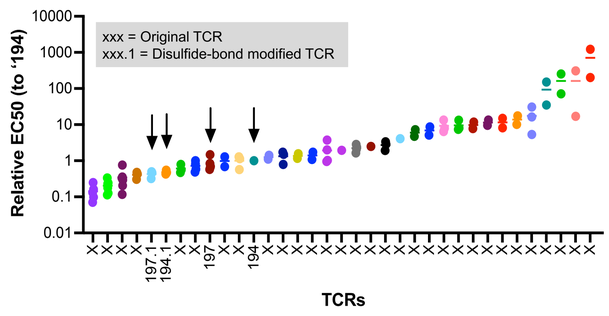
TCRs progressed to primary cell validation



Antigen-specific TCRs Confirmed and Ranked

Relative EC50 in transduced CD4⁺ T cells (readout CD69)

Range of Proinsulin-Responsive TCRs



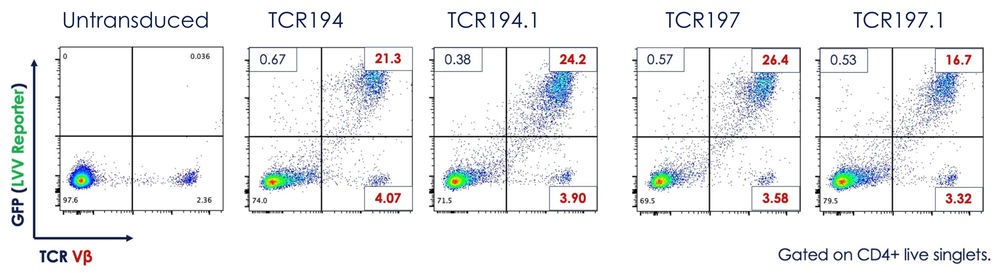
Narrowed in on 4 high function TCRs

TCR ID	EC50 Mean (nM)	EC50 SD (nM)	Ratio of WT/DS EC50 > 1 = positive impact on functional avidity
215.1	0.6265	0.4777	1.55 (+)
199.1	1.695	1.361	0.96 (-)
197.1	2.184	1.71	2.02 (+)
194.1	2.774	2.016	2.21 (+)

DS = disulfide modified

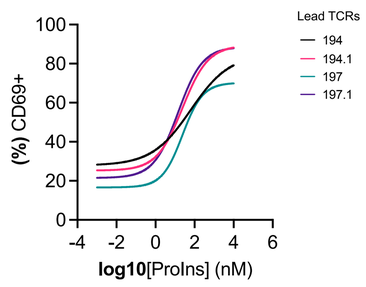
2 Candidate TCRs Had Available Validated Detection Reagents

TCR expression detection reagents validated for TCR 194.1 and TCR 197.1

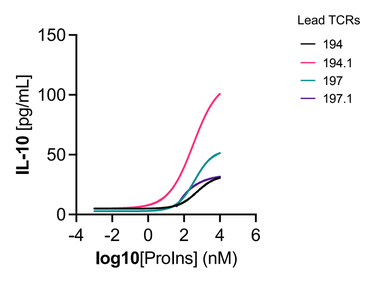


High Avidity TCRs Functionally Validated in Human Tregs

Antigen-specific activation from TCR-Tregs

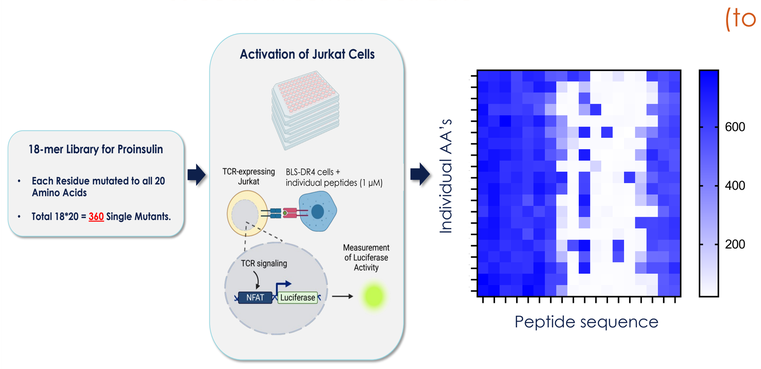


Production of IL-10 from TCR-Tregs in Response to Antigen



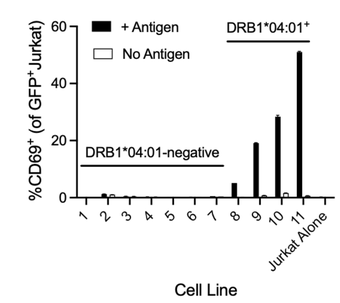
Lead Candidate TCR Demonstrates On-target Specificity

X-Scan in Jurkat Cell Line



- Core recognition motif identified

Alloreactivity
(to achieve ~90% HLA coverage)



- Minimal alloreactivity observed
- On-target specificity observed with DR4 allele

Summary

- Abata Therapeutics has developed a robust TCR discovery platform that resulted in oligoclonal expansion of CD4⁺ T cells expressing islet reactive TCRs.
- Through our screening and validation pipeline in TCR^{null} Jurkats and primary conventional CD4⁺ T cells, we validated over a dozen novel proinsulin reactive TCRs.
- Through further characterization in a Treg chassis, we validated and ranked clear therapeutic candidates that met defined criteria and functional metrics enabling identification of a lead TCR for the T1D program.
- Taken together, selection of our lead candidate enables the development of a Treg cell therapy for the treatment of T1D.
- Program moving towards IND enabling studies.

References

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3. Kent SC, Chen Y, Bregoli L et al. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. Nature. 2005 May 12;435(7039):224-8.
4. Durinovic-Bello I, O Boehm B, Ziegler A-G. J Autoimmun. Predominantly recognized proinsulin T helper cell epitopes in individuals with or without islet cell autoimmunity, Feb; 18(1)55-66
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