Identification of Novel HLA Class II–Restricted Autoantigens in CD4⁺ T Cell–Driven Autoimmune Diseases Using TargetScan

Olivia Pryor, Catalina Burbano, Tyler Heath, Nathaniel Bagge, Rutuja Kulkarni, Heather F Jones, Candace Perullo, Jinyu Zhu, Ira Jain, Rakshika Balasubramaniyam, Jenny Tadros, Erin Harten, Vivin Karthik, Livio Dukaj, Shoshana Bloom, Jackson Lirette, Prachi Dhanania, Lisa Fleischer, Jenna LaBelle, Chandan Pavuluri, Alexander Cristofaro, Laurie Barefoot, Shrikanta Chattopadhyay, Kimberly M Cirelli, Mollie Jurewicz, Andrew Ferretti, Cagan Gurer

TScan Therapeutics, Waltham, MA

Background: Selective modulation of autoimmune responses through antigen-specific therapies represents a promising direction for improving treatment specificity and safety. Genetic associations with HLA class II alleles, combined with evidence of tissue-infiltrating T cells and T cell–mediated cytokine signatures, highlight the central role of CD4⁺ T cells in driving autoimmune pathology. However, the targets of these pathogenic T cells remain poorly defined, limiting development of antigen-specific therapies. TScan's proprietary TargetScan platform offers an unbiased, high-throughput method to identify natural targets of disease-relevant T cell receptors and support development of antigen-specific tolerizing modalities.

Methods: To identify HLA class II T cell targets for CD4⁺ T cells, the TargetScan platform was adapted to present genetically encoded peptide libraries on ectopically expressed HLA class II molecules. We initially applied this approach to scleroderma. CD4⁺ T cells were isolated from affected skin biopsies of patients and analyzed using 10x Genomics single-cell RNA sequencing. A total of 764 TCRs were identified, with candidates selected for screening based on pathogenic gene expression profiles and CDR3 sequence clustering shared by multiple patients. 61 candidate TCRs were screened for antigen recognition in the context of disease-associated Class II HLA alleles, and identified targets were assessed for disease relevance using follow-up validation assays. Similarly, we used the TargetScan platform to evaluate TCRs from inflamed colon samples from patients with ulcerative colitis.

Results: Screening of the candidate TCRs using the Class II TargetScan platform identified nine novel targets presented by multiple HLA Class II alleles associated with scleroderma (SCD). These reactive TCRs belonged to shared CD3⁺ clusters found in multiple patients and were characterized by high expression of activation and tissue-homing markers, as well as genes associated with Th2 and Th17 profiles. The identified targets exhibited expression patterns consistent with disease relevance and were validated through orthogonal assays, including functional T cell activation assays. In ulcerative colitis, two novel targets were identified with expression patterns consistent with disease pathology.

Conclusion: TScan's Class II TargetScan platform enables unbiased identification of novel autoantigens in CD4⁺ T cell–driven autoimmune diseases where antigenic drivers have remained poorly defined. These discoveries may inform the development of antigen-specific therapeutic strategies, including tolerogenic approaches aimed at restoring immune homeostasis.

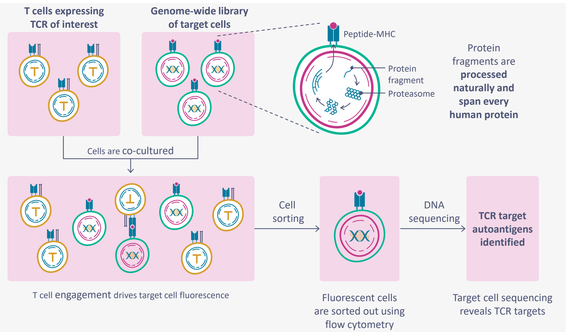
References

1. Huang, H. et al. Nat. Biotechnol. 38, 1194–1202 (2020).
2. Dezfulian, M. H. et al. Cell 186, 5569–5586.e21 (2023).
3. Volkmann, E. R. et al. Lancet 401, 304–318 (2022).
4. Massimino, L. et al. Nat. Comput. Sci. 1, 511–515 (2021).

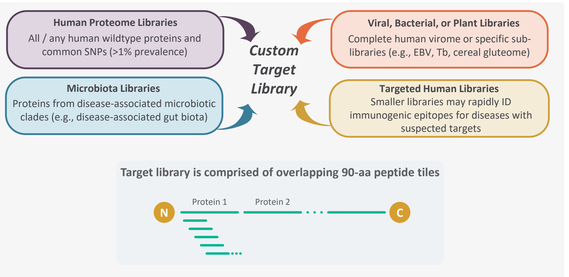
TScan's Proprietary Platform, TargetScan, Enables Identification of the Natural Targets of TCRs

TCR Sampling and Screening Overview

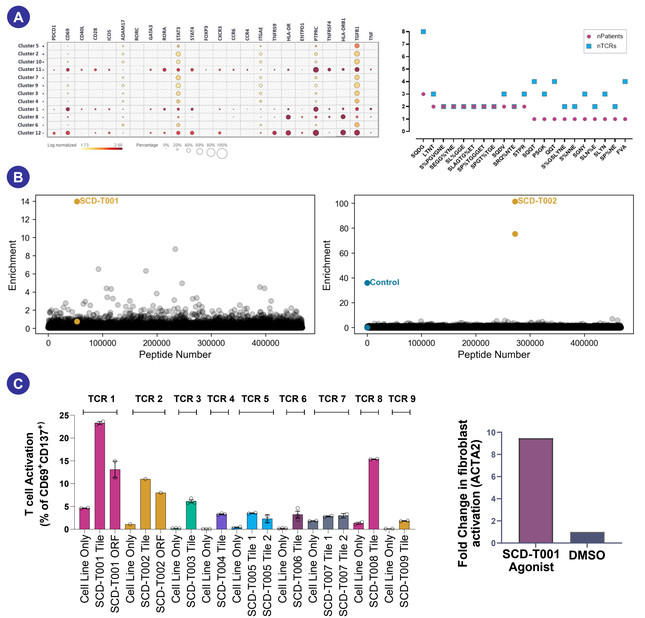
TargetScan Platform



Peptide Library Design



TargetScan Platform Revealed Multiple T cell Targets Associated with Scleroderma

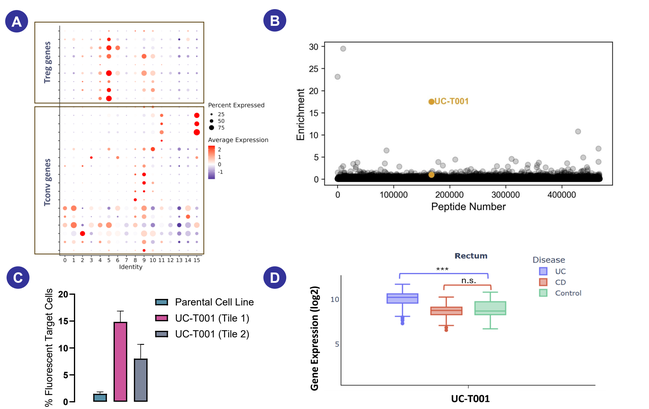


A. **Left Panel:** Patient derived TCRs were selected to screen using SCD-associated gene signatures. Dot size indicates T-cell frequency; color shows normalized expression of the indicated genes. **Right Panel:** GLIPH2 clustering of CD4⁺ TCRs reveals shared motifs across patients, with circles and squares denoting patient and TCR counts, respectively.

B. Example TargetScan screens of SCD-derived TCRs where two candidate HLA class II-presented targets were identified. Enrichment scores for ~500,000 peptides (X-axis) are shown relative to input (Y-axis).

C. **Left Panel:** Nine SCD patient TCRs were validated by T cell co-culture with HLA class II reporter cells presenting enriched peptides or full-length ORFs; activation was assessed by CD69/CD137 expression. **Right Panel:** Agonism of SCD-T001 leads to significant activation of patient derived fibroblasts, a known pathological signature of scleroderma.

TargetScan Platform Identified a Disease-Relevant T cell Target from Ulcerative Colitis Patient Colon Samples



A. Effector CD4⁺ T cells or Tregs were selected using UC-associated gene signatures from colon tissues of patients with ulcerative colitis (UC).

B. TargetScan screen identified putative class II targets of TCRs derived from patients with UC

C. Targets identified with TargetScan were validated by T cell co-culture with HLA class II reporter cells presenting UC-T001 tile 1 or tile 2.

D. Target UC-T001 is expressed at higher levels in disease-relevant tissue in UC patients vs. healthy controls or Crohn's Disease (CD) patients.

Conclusions

- TScan's Class II TargetScan platform enables unbiased identification of novel T cell autoantigens in HLA linked autoimmune diseases.
- TScan has established a broad network of tissue sample providers and has initiated target discovery in scleroderma and ulcerative colitis.
- TargetScan identified nine putative T cell targets in scleroderma and two T cell targets in ulcerative colitis.
- Identified targets were validated through orthogonal assays, and exhibit expression patterns consistent with disease relevance.