Directly Targeting Autoantigen-Specific T cells with Immuno-STATs

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Immuno-STATs are Designed to Restore Immune Balance

**Immuno-STATs:**
- Expand disease-specific Teffs
- Re-activate exhausted Teffs
- Inhibit and/or deplete Tregs

↓ Tregs  
↑ Teffs

↓ Tregs  
↑ Teffs

**Overactive Immune Response**
- Autoimmunity
- Chronic inflammation
- Metabolic inflammation

**Inadequate Immune Response**
- Susceptibility to cancers
- Infectious diseases
- Immune deficiencies

Immuno-STATs:
- Inhibit pathogenic Teffs
- Activate Tregs

**Immune Balance**

Restoration of immune balance is a key pillar of human health

Key: Teffs, effector T cells; Tregs, regulatory T cells
AI Focus

T Cell Mediated Autoimmune Diseases

Antigen-Specific Approach

**Aim:** Deploy class I/II Immuno-STATs to modulate autoreactive T cells and/or generate Ag-specific iTregs

- Focus on diseases with restricted autoreactive antigens
- Focus on earlier stages of breakdown of tolerance prior to antigen/epitope spreading

Pathway-Specific Approach

**Aim:** Deploy engineered regulatory signals to restore immune balance through generation of polyclonal iTregs

- Focus on key signals for Treg induction and expansion (i.e., TGF-β, IL-2)
- Focus on indications and chronic disease stages with diverse self antigens
Target Rationale and Therapeutic Hypothesis

Target rationale:

• Type 1 diabetes (T1D) is a chronic autoimmune disease resulting in severe loss of pancreatic β cells due to the targeting of islet cell autoantigens
  - Imbalances in the number and/or function of antigen-specific (AgS) autoreactive T cells (Teff) and/or regulatory T cells (Tregs) are considered the main effectors of β cell destruction

• ~50% of the genetic risk for T1D derives from specific HLA alleles involved in the presentation of peptide antigens to T cells
  - Allelic associations with disease are known (DR4, DQ8)
  - Critical peptide-HLA class II T cell epitopes are known (e.g., Proinsulin, GAD65, InsB, etc)

• Intervention in T1D may be achieved by:
  - (1) deletion or functional attenuation of AgS pathogenic Teff and/or
  - (2) expansion or functional enhancement of AgS Treg

Therapeutic hypothesis:

• Peptide-HLA class II-based Immuno-STATs (ISTs) can target disease-relevant CD4+ T cells for therapeutic effect in T1D through their deletion-functional attenuation
Design of the Proins$_{76-90}$, K88S/DR4-PDL1 Immuno-STAT (IST)

**Proinsulin$_{76-90}$**
- Major proinsulin epitope, marker of autoreactivity, engineered for enhanced binding affinity/stability

**Class II DRB1*04**
- DR4 allele highly associated with T1D, engineered for enhanced stability

**Fc (effector-less)**
- Engineered to dial out biological and effector functions while maintaining manufacturing and stability advantages

**PI-DR4-PDL1**
- PD-L1: Engineered to provide functional attenuation of targeted cells

**Control molecules**
- MOD-less
  - "PI/DR4"
  - Generated as a control for PD-L1 function
- "GAD/DR4-PDL1"
  - GAD65$_{555-567}$
  - Generated as a specificity control
PI-DR4-PDL1 IST Inhibits Expansion of Proinsulin-Specific T cells In Vitro

D0
PBMC + pathogenic peptide

Non-cognate
PDL1-IST

Cognate
PDL1-IST

D10
Tetramer readout

Expansion of
pathogenic antigen-
specific CD4 T cells

Suppression of pathogenic
antigen-specific CD4 T cell
expansion

PI-DR4-PDL1 IST selectively inhibits antigen-specific CD4+
T cells from T1D donor PBMCs
Potency and Duration of Effect of PI-DR4-PDL1 ISTs In Vitro: In Vitro Assay Format #1

- Assays are based on PBMCs expanded for 14 days with peptide and rhIL-2

**24-hour assay**

Day 0: Thaw, rest 2h and treat with IST O/N

Controls:
- Media
- PI-DR4-modless
- GAD-DR4-PDL1

Day 1: Wash; ELISPOT with PI peptide or HIV peptide (neg control) stimulation

**‘Washout mode’**

Day 1: Wash cells to remove IST

Culture with autologous PBMCs (1:1) + IL-2 (50U/mL) 2 to 6 days

Day 3-7: Wash; ELISPOT with PI peptide or HIV peptide (neg control) stimulation
Overnight Treatment with PI-PDL1 ISTs Inhibits Antigen-Specific CD4+ T Cell IFN-γ-Responses in a PDL1-Dependent, Antigen-Specific Manner

![Graphs showing IFN-γ-SFC responses to various test articles with and without ISTs.](image)
Functional Responses of Proinsulin-Specific CD4+ T cells Remain Suppressed 3-5 Days Post Washout
The Antigen-Specific, PDL1-Dependent Suppression of PI-Specific CD4+ T Cells is Observed Across Multiple Tested Donors
Observed Mechanism is Not Unique to PI-PDL1: Similar Effect is Seen with GAD65-Specific Donors and a GAD65-PDL1 IST
Potency and Duration of Effect of PI-DR4-PDL1 ISTs In Vitro: In Vitro Assay Format #2

• All assays are based on CD4+ T cells expanded for 14 days with peptide and rhIL-2

5-day assay

Day 0: Restimulate purified CD4s with peptide loaded autologous DCs in presence of IST

Controls:
- Media
- Proins-modless
- GAD-PDL1

Day 5: Wash; ELISPOT with PI peptide or HIV peptide (neg control) stimulation

‘washout mode’

Day 5: Wash cells to remove IST

Culture with autologous PBMCs (1:1) + IL2 (50U/mL) 2 to 5 days

Day 3-6: Wash; ELISPOT with PI peptide or HIV peptide (neg control) stimulation
The Antigen-Specific, PDL1-Dependent Suppression of PI-Specific CD4+ T Cells is Observed Across Multiple Tested Donors
In Vitro Study Conclusions

- Functional responses of Proinsulin-specific CD4+ T cells are strongly suppressed by overnight PI-PDL1 IST treatment and remain suppressed at 3-5 days post-washout.

- Functional responses of PI-specific CD4+ T cells re-stimulated for 5 days in the presence of PI-PDL1 ISTs are strongly suppressed and remain suppressed at 3 days post washout.

- The observed effects are antigen-specific and PDL1-dependent and are observed across multiple tested donors (both healthy donors and T1D patients).

- Similar results are obtained with GAD65-specific CD4+ T cells treated with a GAD65-PDL1 IST → the mechanism and duration of PDL1-mod-induced suppression is not limited to a single antigen specificity.
Early Intervention with Immuno-STAT Selectively Reduces the Number of Proinsulin (PI) Responsive CD4+ T Cells in Transgenic Mice

Treatment with Proins-DR4-PDL1 IST starting on Day 1 post immunization selectively suppresses expansion of PI-reactive cells without inhibiting expansion of HA-reactive cells.
In Vivo Treatment with Proins$_{76-90, K88S}$/DR4-PDL1 Immuno-STAT Suppresses Cytokine Production by Proins (PI)-Specific CD4+ T Cells

<table>
<thead>
<tr>
<th>Immunization:</th>
<th>PI</th>
<th>PI/HA</th>
<th>PI/HA + IST</th>
</tr>
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<tbody>
<tr>
<td>Restimulation:</td>
<td>PI</td>
<td>PI/HA</td>
<td>PI/HA + IST</td>
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Treatment with Proins-DR4-PDL1 IST suppresses cytokine production of PI-specific CD4+ T cells (IL2, IFNγ, TNFα, IL-17) without affecting HA-reactive cells.
Late Intervention with Immuno-STAT Selectively Reduces the Number of Proins (PI) Responsive CD4+ T Cells In Vivo

Peptide CFA Immunization
Proins76-90, K88S
HA307-319

Day 0

Day 11

Day 11

Day 12

Day 13

Day 12

Day 13

Veh → IST

Proins Response (IL-2)

HA Response (IL-2)

SFC per e6 cells

Days Post Immunization

Vehicle

IST Treated

0.002

0.04

0.05

0.05

0.01

0.06
Intra-Animal ELISpots Confirm Dose-Dependent and Antigen-Specific Suppression of Proins Response Following Single Administration of IST

- Per animal data confirms dose-dependent inhibition of Proins response relative to pre-dose
- Inhibition of HA response was not observed upon IST administration
In Vivo Conclusions

- Treatment with Proins$_{76-90, K88S}$/DR4-PDL1 Immuno-STAT significantly suppressed expansion of Proins-specific CD4+ T cells in transgenic mice.

- The effect of Proins$_{76-90, K88S}$/DR4-PDL1 Immuno-STAT treatment was antigen specific as treatment did not result in a reduction of HA-specific CD4+ T cells.

- A single in vivo administration of Proins$_{76-90, K88S}$/DR4-PDL1 Immuno-STAT at the peak of immunization response significantly reduced the frequency of Proins-responsive T cells in a dose-dependent manner.
Overall Summary

- Successful generation of Immuno-STAT molecules comprised of Class II HLA molecules (DRB1*04) along with PDL1 inhibitory ligand to selectively dampen autoreactive CD4+ T cells
- Proins-DR4-PDL1 IST exhibits favorable in vitro stability and manufacturability
- Proins-DR4-PDL1 IST potently and selectively inhibits Proins-specific responses in expanded human PBMCs
  - Significant suppression is maintained at 96 hours after wash-out, confirming prolonged pharmacodynamic effect from IST exposure
- Proins-DR4-PDL1 IST selectively inhibits Proins-specific responses in vivo within 24 hours of administration
  - Dose-dependent activity was observed
  - Selective inhibition of Proins response was confirmed using 2 assay formats
- ProIns-DR4-PDL1 IST and the Immuno-STAT platform could provide a promising therapeutic option for T1D patients
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