β-cell targeted Immune Suppressive PD-1 Bispecific Agonists - a Novel Approach to Treat Type 1 Diabetes

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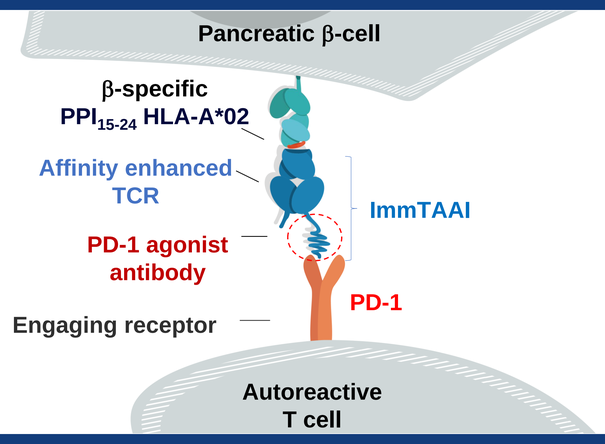
Introduction

- The success of immunosuppressive therapies in protecting pancreatic islets from destruction by autoreactive T cells and treat Type 1 Diabetes (T1D) has, so far, been limited either due to safety concerns or lack of persistent efficacy. More effective therapies with an improved therapeutic window are therefore needed.
- Restricting T cell suppression to the pancreas, thereby avoiding systemic immunosuppression, is an attractive option to treat T1D. One approach to achieve this is to design targeted bispecific T cell inhibitors that bind to pancreatic β-cells and prevent killing by autoreactive T cells.
- The PD-1/PD-L1 pathway negatively regulates T cell activation, mediates tolerance and controls resolution of inflammation [1]. Blocking this pathway in cancer patients can cause diabetes-like symptoms [2] and defects in the PD-1 pathway have been described in patients with T1D [3-4]. Furthermore, PD-1 agonism inhibits T cells in preclinical models [5].
- To preserve insulin production in T1D patients, we have designed a targeted bispecific T cell inhibitor that binds to pancreatic β-cells and suppresses effector functions of autoreactive T cells. As a T cell inhibitor moiety, we chose a PD-1 agonist.
- We refer to this new class of bispecifics as ImmTAAI® (Immune modulating monoclonal TCR Against AutoImmunity) molecules.

Approach



- Targeting moiety:
  - affinity-enhanced TCR specific for PPI_{15-24}-HLA-A2 [6]
- Immune suppressive effector function
  - PD-1 agonist antibody

Results

Figure 1: The PPI TCR, an affinity enhanced β-cell specific targeting moiety

Selective β-cell specific target PPI_{15-24}

- Validated by mass spectrometry
- Average >1000 targets/cell
- Described in the literature [6]

Highly specific

- Affinity-enhanced to 30 pM K_D
- >1,000 fold more selective for specific target over mimetic peptides in biochemical and cellular assays
- Crystal structure: TCR interacts with every sidechain of the PPI_{15-24} peptide

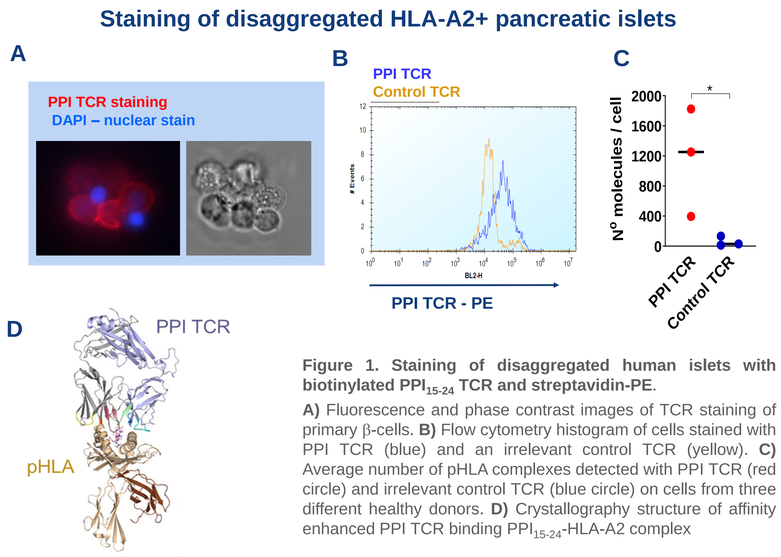


Figure 1. Staining of disaggregated human islets with biotinylated PPI_{15-24} TCR and streptavidin-PE.

A) Fluorescence and phase contrast images of TCR staining of primary β-cells. **B)** Flow cytometry histogram of cells stained with PPI TCR (blue) and an irrelevant control TCR (yellow). **C)** Average number of pHLA complexes detected with PPI TCR (red circle) and irrelevant control TCR (blue circle) on cells from three different healthy donors. **D)** Crystallography structure of affinity enhanced PPI TCR binding PPI_{15-24}-HLA-A2 complex

Figure 2: β-cell bound PPI PD-1 agonist ImmTAAI accumulates at the immunological synapse

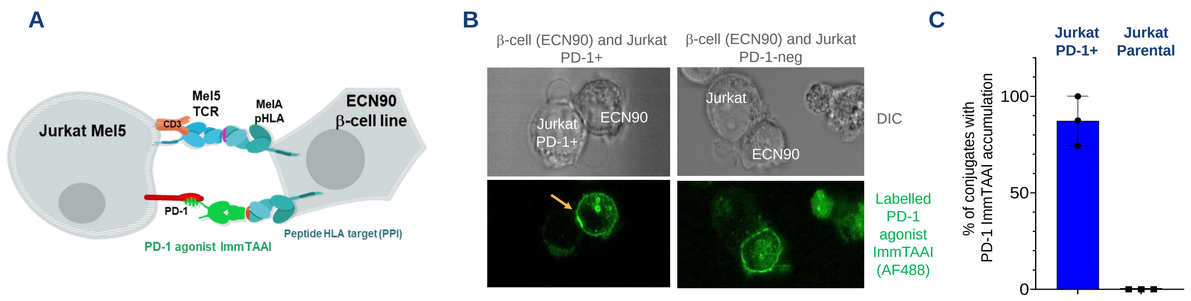
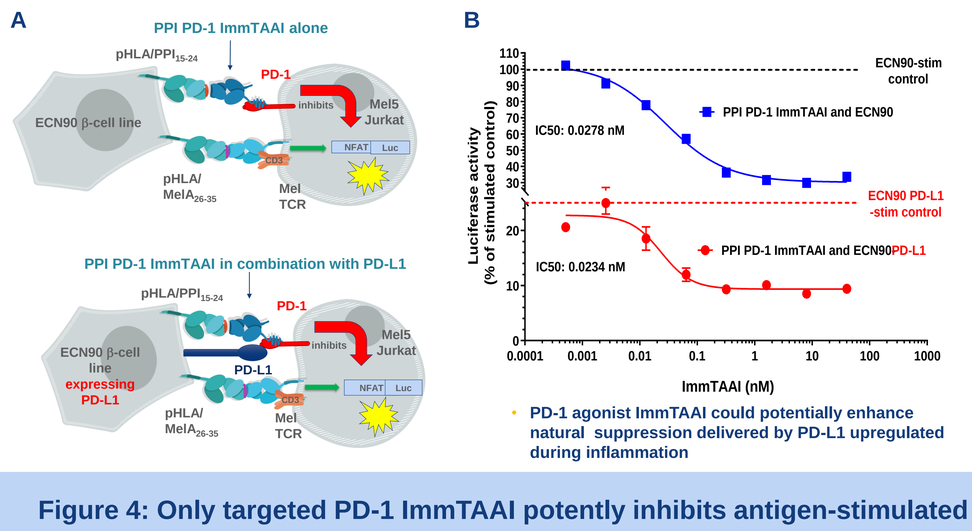


Figure 2. PPI PD-1 agonist ImmTAAI accumulates at the β-cell-T cell contact area upon TCR engagement. A) Schematic of EndoCell ECN90 stained with PD-1 agonist ImmTAAI (AF488), pulsed with 2 μM Melan A_{26-35} peptide and forming contacts with Jurkat cells that express TCR specific for Melan A_{26-35}-HLA-A2 complex. **B)** Images of ImmTAAI-AF488 localisation on target cells co-incubated with parental or PD-1 transduced Jurkat cells. **C)** Quantification of target cell: Jurkat conjugates with ImmTAAI accumulation.

Figure 3: PPI PD-1 agonist ImmTAAI is non-competitive with PD-L1 for PD-1 binding and is additive with PD-L1 in suppressing TCR complex signalling



- **PD-1 agonist ImmTAAI could potentially enhance natural suppression delivered by PD-L1 upregulated during inflammation**

Figure 3: A) Schematic of the reporter assays used. **B)** Parental ECN90 (upper curves) or PD-L1 transduced ECN90 cells (lower curves) were pulsed with Melan A_{26-35} peptide and titrations of PPI PD-1 ImmTAAI molecules added. Jurkat cells, expressing a TCR (Mel5 TCR) specific for Melan A_{26-35}-HLA-A2, PD-1 molecule and a luciferase reporter driven by an NFAT-response element, were incubated with ECN90 cells pulsed with Melan A_{26-35} peptide to trigger TCR signalling and induction of NFAT promoter-mediated luminescence.

Figure 4: Only targeted PD-1 ImmTAAI potently inhibits antigen-stimulated primary CD4+ T cells

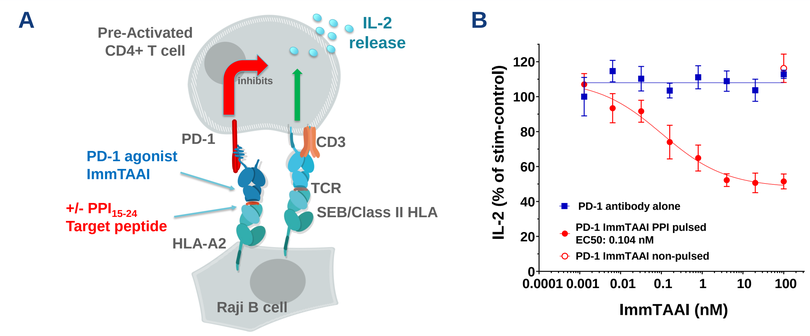


Figure 4: ImmTAAI inhibits IL-2 release from CD4+ T cells only in the presence of peptide-pulsed target cells. A) Schematic of assay performed to CD4+ SEB assay. Raji cells were untreated or pulsed with target peptide, loaded with SEB, and irradiated. ImmTAAI molecules were then added, followed by addition of SEB-pretreated CD4+ T cells. After co-incubation for 48 hrs supernatants were collected and IL-2 levels measured by ELISA **B)** Dose response of ImmTAAI molecules or PD-1 agonist antibody domain alone in CD4+ SEB assay using either pulsed or unpulsed target cells.

- **Unbound ImmTAAI is inactive**
- **Potential for large therapeutic window**

Figure 5: PD-1 agonist ImmTAAI suppresses CD8+ autoreactive T cell clone

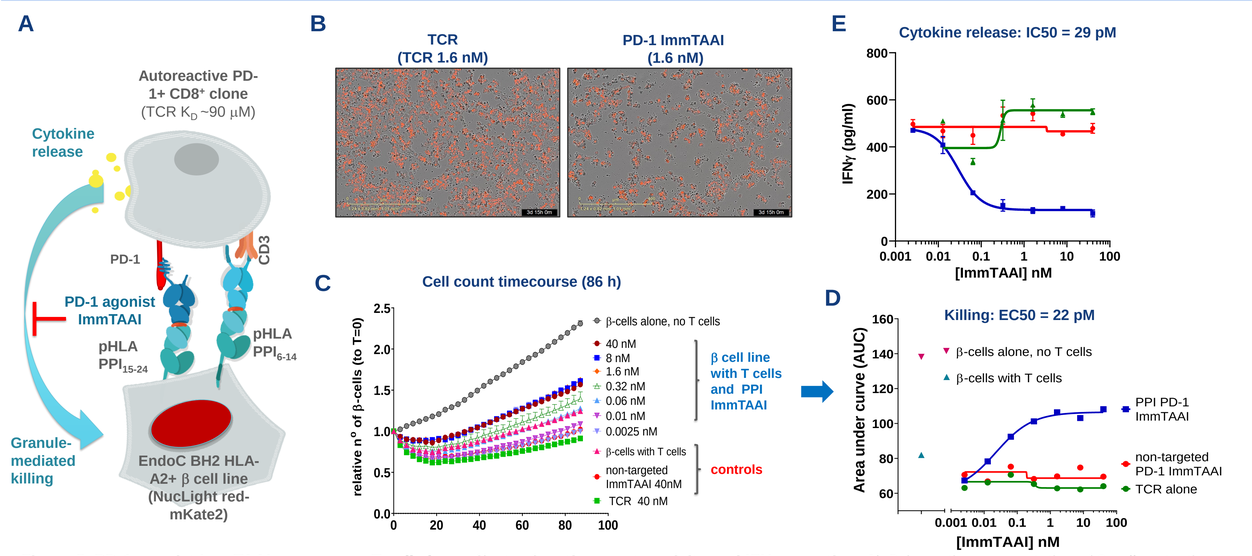


Figure 5. PD-1 agonist ImmTAAI suppresses T cell clone effector functions: cytotoxicity and IFNγ secretion. A) Schematic representation of β-cell protection assay. Cytotoxicity assays were done with CD8+ T cell clones recognizing the PPI_{6-14} peptide-HLA and BH2 target cells transduced with HLA-A2/β2m and NucLight red. Target cells were pre-incubated with 1uM PPI_{6-14} peptide and ImmTAAI molecule for 2 hrs, followed by addition of T cells and continuous monitoring of cell number (IncuCyte). **B)** Images of NucLight red nucleus labelled β-cells after incubation with TCR alone or PPI PD-1 ImmTAAI and T cell clone at 1.6 nM. **C)** β-cell number tracked over time for each ImmTAAI concentration as indicated and **(D)** AUC value for each curve calculated and plotted. **E)** IFNγ level in culture supernatants measured by MSD ELISA after 24 h.

Conclusions

- PPI PD-1 ImmTAAI designed as an effective targeted therapy for T1D
  - Affinity-enhanced TCR against PPI_{15-24} pHLA targets PD-1 agonist to human primary β-cells
- β-cell targeted PD-1 agonist ImmTAAI is active at the immunological synapse
  - Accumulates at cell:cell interface and inhibits TCR signalling
- PD-1 agonist ImmTAAI suppresses CD4+ T cell response and protects β-cells from CD8+ T cell mediated cytotoxicity
  - Preserves β-cells and suppresses inflammation with picomolar potency
- Promise for an improved safety profile versus systemic treatments
  - Non-competitive / additive with PD-L1
  - Enhances natural mechanism of suppression
- Lack of activity in solution is an exciting feature
- These features make PD-1 ImmTAAI molecule an attractive and novel platform to treat T1D

References

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