TCR-Ts overcoming TME hurdles by switching immunosuppression to T cell activation with integrated Switch Receptor Technology

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“Medigene is developing differentiated, breakthrough cellular therapies to improve the lives of cancer patients”

We develop TCR-T therapies since synergistic signaling mechanisms can be mobilized through multiple natural or synthetic costimulatory pathways.
Our TCR discovery engine provides TCRs that display better **Specificity**, **Sensitivity** and **Safety** for selected target antigens.
One-for-all RV vector system efficiently transfers gene cargo into recipient patient T cells for autologous TCR-T therapy

1. Leukapheresis and T cell isolation
2. GMP: Activation of T cells and RV gene transfer
3. TCR drug product and analytical samples
4. GMP: Expansion, freezing and quality tests
5. Thawing and infusion into patient

One-for-all RV vector system efficiently transfers gene cargo into recipient patient T cells for autologous TCR-T therapy.
TCR-Ts may fail to function in a tumor microenvironment (TME) due to strong inhibition by tumor cells and lack of costimulation. Only inhibition without costimulation. T cells in the TME lack positive signals and face immuno-suppression from the PD1-PD-L1 axis. T cell responses are naturally tuned through positive and negative signals.

**Costimulation and inhibition** in balance.
TCR-Ts need positive costimulation to fully function in a hostile TME. Medigene’s Switch Receptor Technology enable TCR-Ts to receive positive costimulation from tumor cells.

TCR-Ts need costimulation to:
- Override PD-L1-mediated inhibitory signals
- Mediate better anti-tumor effector functions
- Proliferate and survive \textit{in vivo}

Medigene’s Switch Receptor Technology

\textit{Enable TCR-Ts to receive positive costimulation from tumor cells}
Our PD1-41BB Switch Receptor combines the extracellular PD1 domain with the intracellular 41BB-signaling domain for use in TCR-Ts

<table>
<thead>
<tr>
<th>Switch Receptor</th>
<th>Extracellular Domain</th>
<th>Transmembrane Domain</th>
<th>Intracellular Domain</th>
<th>Species</th>
<th>Transgenic expression</th>
<th>Expression with transgenic TCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD1-41BB</td>
<td>PD1 aa 21 – 165</td>
<td>PD1 aa 166 – 191</td>
<td>41BB (CD137) aa 214 – 255</td>
<td>Human-derived</td>
<td>Codon-optimized</td>
<td>Coupled via T2A element</td>
</tr>
</tbody>
</table>

US: US 11,365,237; China: ZL 201780031958.9; AU: 2017236069
PD1-41BB Switch Receptor in TCR-Ts converts PD1 inhibitory signals to positive activation through the 41BB costimulatory pathway

**Costimulation and inhibition in balance**

**Inhibition** w/out costimulation

**Inhibition** switched to costimulation
TCR-Ts co-expressing TCR & PD1-41BB Switch Receptor show enhanced recognition and killing of tumor cells \textit{in vitro}.
TCR-Ts co-expressing TCR & PD1-41BB Switch Receptor show enhanced recognition and killing of tumor cells \textit{in vitro}

Melanoma cell line

![Graph showing target cell count over hours of co-culture](image)

- PD1-41BB_TCR
- TCR
- Mock
- No T cells

Rv vector: 2 in 1 cargo of TCR & PD1-41BB

End
TCR-Ts co-expressing TCR & PD1-41BB show enhanced killing of 3D tumor cell spheroids after repetitive challenge (3X) *in vitro*

Melanoma cell line

Tumor spheroids are added to TCR-Ts in three consecutive challenges
TCR-Ts co-expressing TCR & PD1-41BB show enhanced killing of 3D tumor cell spheroids after repetitive challenge (3X) *in vitro*

Melanoma cell line

TCR alone

PD1:BB_TCR

*Tumor spheroids are added to TCR-Ts in three consecutive challenges*
T cells show better infiltration and faster killing of 3D tumor spheroids when TCR & PD1-41BB are co-expressed in TCR-Ts.

Enhanced TCR-T spheroid infiltration
Enhanced killing of 3D tumor spheroids
TCR-Ts co-expressing TCR & PD1-41BB show exquisite specificity and only kill antigen-positive 3D tumor cell spheroids.
TCR-Ts co-expressing TCR & PD1-41BB show exquisite specificity and only kill antigen-positive 3D tumor cell spheroids.
Co-expression of TCR & PD1-41BB strongly enhances TCR-T cell responses to tumor cells in vitro and in vivo

**In vitro response**

Tumor cells: MelA375_PDL1 PRAME_{low} PD-L1_{high}

TCR-T cells:
- UT
- TCR
- TCR_PD1-41BB

P-values were calculated using a two-way ANOVA and Tukey's multiple comparison test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001;

**In vivo efficacy**

T cells expressing a highly potent PRAME-specific T cell receptor in combination with a chimeric PD1-41BB co-stimulatory receptor show a favorable preclinical safety profile and strong anti-tumor reactivity. Sailer et al. Cancers 14, 2022.
IsoPlexis technology measures single cell poly-cytokine release in mixed T cell populations

- **Co-culture and separation of T cells**
- **Single cell analysis on chip**
- **Automated Imaging**
- **Data analysis**

**32 T cell cytokine panel**

- **Polyfunctional Strength Panel, per Cell: PSI**
  - **Single-Cell Multiplexed cytokine intensity**
  - **% Polyfunctional T cells: # of functions per cell**
  - **Polyfunctional Strength index**

- **Regulatory**
  - TCR+ T helper CD4+ T cells, immune response
- **Independent**
  - TCR- activated T helper CD4+ T cells
  - IFN-γ
  - IL-2
  - TNF-α
  - GM-CSF
- **Inflammatory**
  - Activated T helper cytokines
  - IL-1β
  - IL-6
  - IL-8
  - MCP-1
  - IL-17A

- **Cytotoxic**
  - Activated T cells, IFN-γ, TNF-α, IL-2
  - CD8+ T cells

- **Chemokine**
  - Chemokines and cytokines
  - CXCL-10
  - MCP-1
  - MIP-1α
  - MIP-1β
  - Eotaxin

**PSI**

- 5+ cytokine
- 4 cytokine
- 3 cytokine
- 2 cytokine

**Medigene**
TCR-Ts with TCR & PD1-41BB Switch Receptor show superior single cell poly-cytokine signatures compared to TCR alone

T cells expressing a highly potent PRAME-specific T cell receptor in combination with a chimeric PD1-41BB co-stimulatory receptor show a favorable preclinical safety profile and strong anti-tumor reactivity. Sailer1*, et al. Cancers 14, 2022
Unsupervised clustering of transcriptional profiles in UT T cells 14 days after serial stimulation with 3D tumor spheroids.
Unique transcription profiles emerge over time in TCR-Ts with TCR alone vs TCR & PD1-41BB after serial tumor cell stimulation

Unsupervised clustering of transcriptional profiles highlights a strong difference in the transcriptional landscape of T cells expressing the PD1-41BB Switch Receptor at 14 days after serial stimulation with 3D tumor spheroids.
Impact of PD1-41BB Switch Receptor on function of CD8 TCR-Ts

*in vitro* and *in vivo*

- CD8\(^+\) T cells with antigen-specific transgenic TCRs combined with PD1-41BB Switch Receptor showed:
  - Better proliferation
  - Greater poly-functional cytokine secretion profile for strong anti-tumor immunity
  - Enhanced killing of cancer cell lines *in vitro*, especially cells with high PD-L1
  - Improved infiltration of 3D tumor cell spheroids
  - Maintenance of functional activity upon repeated rechallenge with 3D spheroids
  - Superior *in vivo* control of outgrowth of tumors with low antigen and high PD-L1
  - Increased overall survival of animals bearing PD-L1\(^+\) tumors treated with TCR-Ts
TCR-Ts powered through integrated PD1-41BB Switch Receptor offer potential best-in-class treatment strategy for patients with PD-L1+ TME

- Inhibition
- Exhaustion
- Apoptosis

Inhibition

Current therapy options
TCR-Ts in combination with anti-PD1 or anti-PD-L1 antibodies to block inhibitory axis

Future option from Medigene
TCR-Ts with integrated PD1-41BB Switch Receptor for dual impact on inhibition and activation

Improved:
- Effector functions
- Survival
- Longevity
Thank you