PD1-41BB Switch Receptor Technology Added to TCR-Ts Further Enhances Antitumor Activity in vitro and in vivo compared to TCR alone

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PD1-41BB Switch Receptor
A Next Generation Costimulatory Switch to Enhance T Cell Functionality and Overcome TME

**Unmet need**

Tumor microenvironments (TME) suppress activity of T cells, through PD1-PDL1 axis activation, leading to reduced T cell function, e.g.

- Inhibition
- Exhaustion
- Apoptosis

**Medigene’ solution: PD1-41BB Switch Receptor**

Mechanism of action:

1. Blocks PD1-PD-L1 inhibitory axis between T cells and tumors
2. Activates 4-1BB co-stimulatory pathway in TCR-T cells

Co-stimulation and reduced inhibition mitigate the TME, leading to multiple improvements, e.g.

- Tumor cell killing, effector T cell function and T cell proliferation
PD1-41BB Switch Receptor Strongly Enhances TCR-T Cell Functions

- Enhanced functionality in vitro
- Maintenance of stemness
- In vivo efficacy
- Mitigate inhibitory TME
- Overcome T cell exhaustion
- Sustained proliferation

PD1-41BB
Sustained T cell proliferation
In vivo T cell efficacy
Maintenance of stemness
Mitigate inhibitory TME
Overcome T cell exhaustion
Enhanced T cell functionality

PD1-41BB Switch Receptor Enhances T Cell Functionality
Enhanced TCR-T Cell Functionality
Improved Effector Function of TCR-T cells Co-Expressing Chimeric PD1-41BB Costimulatory Receptor

T-test:
* p>0.01,
** p<0.001

<table>
<thead>
<tr>
<th>Expression</th>
<th>MelA375_PD-L1</th>
<th>Mel624.38_PD-L1</th>
<th>Saos-2</th>
<th>NCI-H1755</th>
<th>NCI-H1703</th>
<th>647-V</th>
<th>MCF7</th>
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<td>+</td>
<td>-</td>
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</table>
Enhanced TCR-T Cell Functionality

IsoPlexis Technology Measures Single Cell Poly-Cytokine Release in Mixed T Cell Populations

Co-culture and separation of T cells

Single cell analysis on a chip

Automated imaging

Data analysis

32 T cell cytokine panel

Polyfunctional Strength Panel, per Cell: PSI

Effectors

Cell lysis intensity on cytokine functions

Cytokines

IL-1β, IL-6, IL-10, IL-15, IFN-γ, TNF-α, IL-23, IL-27, IL-13, IL-22

Regulatory

Chemokines and cytokine immune response

Chemokines

CCL11, CCL5, CXCL1, CXCL5

Inflammatory

IFN-γ, TNF-α, IL-12, IL-6, IL-15

PSI

Single-Cell

Multiplexed cytokine intensity

% Polyfunctional T cells: # of functions per cell

Polyfunctional Strength index

5+ cyt.

4 cyt.

3 cyt.

2 cyt.

PSI
Enhanced TCR-T Cell Functionality
Enhanced Tumor Cell Recognition in Presence with TCR+PD1-41BB vs. Naked TCR

Tumor Cell Line Recognition and T Cell Activation

Superior recognition of PD-L1-positive tumor cell lines with TCR+PD1-41BB vs. "Naked" TCR
Enhanced TCR-T Cell Functionality

Enhanced T Cell Function via Increased Secretion of Multiple Cytokines

Enhanced T cell functionality of TCR+PD1-41BB vs. naked TCR or untransduced (UT) T cells

PD-1-41BB Switch Receptor can be combined with TCRs targeting multiple different antigens

- Similar results observed using PRAME-specific TCR-T cells (data not shown)

**Polyfunctionality** (% of Sample)

- 2 analytes
- 3 analytes
- 4 analytes
- 5+ analytes

**Polyfunctional Strength Index**

Effector: Granzyme B; IFN-γ; MIP-1α; Perforin; TNF-α; TNF-β
Stimulatory: GM-CSF; IL-2; IL-5; IL-6; IL-8; IL-9; IL-12; IL-15; IL-21
Chemoattractive: CCI-11; IP-10; MIP-1β; RANTES
Regulatory: IL-4; IL-10; IL-13; IL-22; TGF β 1; sCD137; sCD40L

**Effector**
- Effector
- Stimulatory
- Chemoattractive
- Regulatory
- Inflammatory

*PD-1-41BB Switch Receptor can be combined with TCRs targeting multiple different antigens

*Similar results observed using PRAME-specific TCR-T cells (data not shown)*
Enhanced TCR-T Cell Functionality

Serial Killing of Tumors using Two Different Cancer-Testis Antigens with TCR+PD1-41BB vs. Naked TCR

Faster killing of 3D tumor spheroids when TCR & PD1-41BB are co-expressed in TCR-T cells
Enhanced TCR-T Cell Functionality

3D Tumor Spheroids Killing Assays in Presence of TCR+PD1-41BB Co-Expressed in TCR-T cells

Better infiltration of T cells and faster killing of 3D tumor spheroids when TCR & PD1-41BB are co-expressed in TCR-T cells

* T cells are labelled

Antigen-positive Tumor Cells
Antigen-specific TCR-T cells* or UT Control
Enhanced TCR-T Cell Functionality

TCR-T Cells Co-expressing TCR+PD1-41BB Show Exquisite Specificity

Specific killing of only antigen-positive 3D tumor cell spheroids
Sustained T cell proliferation

In vivo T cell efficacy

Mitigate inhibitory TME

Overcome T cell exhaustion

Enhanced T cell functionality

Maintenance of stemness

PD1-41BB Switch Receptor Maintenance of Stemness
Maintenance of T Cell Stemness

Serial Antigen Exposure of TCR-T Cells Shows Presence of TCMs and TSCMs after 4 Rechallenges

- TCR and TCR+PD1-41BB show a similar composition of T-mem subgroups
- % T_{SCM} are reduced after challenge 1
- % T_{EMRA} disappeared after challenge 2
- % T_{EM} increased after challenge 1 and stayed comparable after challenge 2 to challenge 4

Decreased T_{SCM}s and expansion of T_{CM}s and T_{EM}s
PD1-41BB Switch Receptor
T Cell Efficacy in vivo
TCR-T Cell Efficacy *in vivo*

Tumor Lines Varying with Levels of Antigen and Endogenous Expression of PD-L1

<table>
<thead>
<tr>
<th>Indication</th>
<th>Melanoma</th>
<th>Melanoma</th>
<th>Breast Cancer</th>
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<tbody>
<tr>
<td>HLA-A2 [Δ MFI]</td>
<td>8078</td>
<td>847</td>
<td>764</td>
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</table>

PRAME expression

PD-L1 expression

neg = PD-L1 negative
TD = PD-L1 transduced
ind = PD-L1 IFNγ inducible
end = PD-L1 endogenously expressed
TCR-T Cell Efficacy \textit{in vivo}

Killing Efficiency of TCR-T cells with Naked TCR Varies with Level of Target Antigen

\textbf{In vitro}

\begin{itemize}
  \item \textbf{Mel624.38 PRAME}\textsuperscript{high}
  \item \textbf{MelA375 PRAME}\textsuperscript{low}
\end{itemize}

\textbf{Tumor growth}

\textbf{Survival}

Antigen level on tumor cells determines killing efficacy of TCR-T cells \textit{in vitro} and \textit{in vivo}.
TCR-T Cell Efficacy *in vivo*

Responses to Low Antigen are Strongly Enhanced upon Co-Expression of TCR with PD1-41BB

*In vivo* efficacy

**Tumor Cell Line:** MelA375_PD-L1  PRAME*low* PD-L1*high*

Antigen level and expression of PD-L1 on tumor cells determines killing efficacy of TCR-T cells in vitro and in vivo
Sustained T cell proliferation

In vivo T cell efficacy

Maintenance of stemness

Mitigate inhibitory TME

Overcome T cell exhaustion

PD1-41BB Switch Receptor
Sustained T Cell Proliferation
Sustained TCR-T Cell Proliferation

Enhanced TCR-T Proliferation with TCR+PD1-41BB vs. Naked TCR

TCR-T Proliferation

Naked TCR

Cell count

Percent Divided: 24.3
Proliferation Index: 1.98
Expansion Index: 2.09
Division Index: 0.48
Replication Index: 5.49
Std. Deviation: 1.36

TCR+PD1-41BB

Cell count

Percent Divided: 42.7
Proliferation Index: 2.91
Expansion Index: 5.05
Division Index: 1.24
Replication Index: 10.5
Std. Deviation: 0.98

Enhanced TCR-T proliferation with TCR+PD1-41BB vs. Naked TCR

Analyzed by FlowJo

Analyzed by FlowJo
Sustained T cell proliferation
In vivo T cell efficacy
Maintenance of stemness
Enhanced T cell functionality
Mitigate inhibitory TME
Overcome T cell exhaustion

PD1-41BB Switch Receptor
Mitigate Inhibitory TME
Mitigate Inhibitory TME
Cytotoxicity and Proliferative Capacity of TCR-Transgenic T cells in Low Glucose Conditions

PD1-41BB expression enhances cytotoxicity and proliferative capacity of TCR-T cells under low glucose
Mitigate Inhibitory TME
Chimeric PD1-41BB Switch Receptor Enhances Metabolic Fitness of TCR-T cells

- Essential role of mitochondria in maintaining the metabolic fitness of TCR-T cells
- Sequentially exposure of TCR-T cells to tumors cells in five restimulations followed by incubated with MitoFM for analysis of mitochondrial content
- TCR-T cells with rTCR without or with PD1-41BB showed the following
  - Equal mitochondria levels after 1 hr (open curves)
  - in absence of PD1-41BB decreased mitochondrial mass at 72 hrs (blue curve)
  - in presence of PD1-41BB increased mitochondrial mass at 72 hrs (red curve)

Presence of PD1-41BB improves metabolic fitness of TCR-T cells by increasing mitochondrial mass
PD1-41BB Switch Receptor
Overcome T Cell Exhaustion
Overcome T Cell Exhaustion
Expression of Transgenic TCR With and Without PD1-41BB Switch Receptor

Naked TCR

<table>
<thead>
<tr>
<th>TCR beta</th>
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Transduced | Untransduced

Enrichment

PD1-41BB+ TCR

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<tr>
<th>TCR beta</th>
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Transduced | Untransduced

Enrichment
Overcome T Cell Exhaustion
PD1-41BB Impacts Sustained 3D Killing Capacity upon Repeated Challenge

Melanoma cell line

![Graph showing integrated intensity over hours of co-culture](image)

- PD1-41BB+TCR
- TCR
- no T cells

PD1-41BB expression elevates killing capacity of tumor spheroids

Tumor spheroids are added to TCR-T cells in three consecutive challenges

This is an embedded video
Overcome T Cell Exhaustion

PD1-41BB Prolonged Killing Activity of TCR-T cells is Dependent on PD-L1 Expression on Tumor Cells
Conclusion

PD1-41BB Switch Receptor Provides Sustainable T Cell Enhancement Through Multiple Mechanisms

- In vivo T cell efficacy
- Sustained T cell proliferation
- Maintenance of stemness
- Mitigate inhibitory TME
- Enhanced T cell functionality
- Overcome T cell exhaustion
The success of TCR-T therapy against solid tumors is dependent on a potent and specific TCR. But the tumor microenvironment is a major challenge for many T cells, including engineered T cells.

The PD1-41BB costimulatory switch receptor significantly:

- Increases the activity and proliferation of tumor antigen-specific TCR-T cells
- Increases T cell efficacy *in vitro* and *in vivo*
- Increases polyfunctionality of T cells, driven by the production of effector, stimulatory and chemoattractive, rather than regulatory and inflammatory cytokines
- Exerts stronger as well as prolonged T cell activity
- Enhances functionality by inverting the inhibitory signals from the TME through PD-L1 by turning them into positive signals, thereby overcoming a major obstacle to the clinical efficacy of adoptive cell therapy against solid tumors.

**Conclusion**

**PD1-41BB Switch Receptor Provides Sustainable T Cell Enhancement Through Multiple Mechanisms**
Thank you for your attention