Empowering TCR-Ts to infiltrate, proliferate and control solid tumors in a hostile tumor microenvironment

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Empowering T cells to infiltrate, proliferate and control solid tumors in a hostile tumor microenvironment

- Choosing receptor-ligand combinations that have the potential to alter the tumor microenvironment
  - TCR-T production of high IFN-γ
  - Antigen cross-presentation in the TME

- Overcoming inhibitory pathways and tumor-induced exhaustion by strong enhancement of T cell function
  - Altering the PD1-PD-L1 inhibitory axis using a PD1-41BB switch receptor
  - Impacts of PD1-41BB on TCR-Ts metabolic fitness
PRAME mRNA was broadly detected *in silico* and found at varying levels in many different cancer patient specimens.

Analyzed with in-house “Plotty” software, data extracted from TCGA dataset.
PRAME showed a high safety profile with respect to protein expression in healthy tissues based on H-scores in the TMA.

**Analysis by Immunohistochemistry**

*Lung (including bronchioles) (1/3 cores)*

Source: Indivumed GmbH, Germany
PRAME-TCR was isolated from a non-tolerized T cell repertoire.
The TCR Discovery Platform identifies many T cell clones as sources of specific and unique TCRs.

Screening of thousands of T cell clones yields large array of unique TCR sequences for later TCR lead selection.

- **Tens of thousands** of T cell clones are sorted and screened for specificity by testing their responses to HLA-A2+ T2 cells pulsed with relevant or irrelevant peptides.

- **Many hundreds** of individual T cell clones are screened for specificity by testing a panel of antigen-positive and negative tumor cell lines.

- **Unique specific** TCR sequences are analyzed by NGS and are further characterized in Medigene’s “Assay Tree” following transgenic expression.

NGS-based TCR sequence analysis.
Lead PRAME-SLL-specific TCR was selected on the basis of cytokine secretion, tumor cell killing and peptide sensitivity.

<table>
<thead>
<tr>
<th>IFNγ secretion</th>
<th>PRAME positive</th>
<th>PRAME negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>SkMel23</td>
<td>Mel624.38</td>
<td>MelA375</td>
</tr>
<tr>
<td>T23.8-2.1-027-004</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T23.8-2.1-027-085_1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T23.8-2.1-038-038</td>
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<td>+</td>
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<tr>
<td>T23.8-2.1-061-119</td>
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<th>Tumor killing</th>
<th>PRAME positive</th>
<th>PRAME negative</th>
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</tbody>
</table>

EC₅₀ values:
- T23.8-2.1-027-004: 1.04x10⁻⁸ M
- T23.8-2.1-027-085_1: 2.05x10⁻⁸ M
- T23.8-2.1-038-038: 1.13x10⁻⁷ M
- T23.8-2.1-061-119: 9.86x10⁻⁸ M
IFNγ has a strong positive impact against tumor cells in the TME

- Tumor cells can be driven into:
  - Apoptosis
  - Dormancy
  - Senescence
- Angiogenesis can be inhibited
- Metastasis can be inhibited

Jorgovanovic et al. Biomarker Res.8:49, 2020
The two-edged sword: IFNγ can upregulate the expression of PD-L1 on the surface of tumor cells

IFNγ leads to upregulation of PD-L1 surface expression on cancer cells

Jorgovanovic et al. Biomarker Res. 8:49, 2020
Human melanoma lines with different levels of PRAME and PD-L1 vary in growth kinetics in immunodeficient NSG mice *in vivo*

**Mel624.38 cells**
- Growth rate – slow
- HLA-A*02:01 – positive
- PRAME endogenous mRNA – high
- PD-L1 – non-inducible

**MelA375 cells**
- Growth rate – fast
- HLA-A*02:01 – positive
- PRAME endogenous mRNA – low
- PD-L1 – IFNγ inducible
Melanoma control *in vitro* and *in vivo* by TCR-Ts expressing the PRAME-specific TCR in absence of PD1-41BB

For A375, 1 mouse in the control group and 2 mice in the TCR group died due to unknown reasons.
Empowered T cells for hostile solid tumor microenvironment (TME): Co-stimulatory PD1-41BB signal switch receptor

Medigene’s PD1-41BB ‘switch receptor’ converts the inhibitory signal usually observed in the PD-1 / PD-L1 interaction into a stimulatory signal to enable TCR-Ts to function with greater activity and duration in the TME.

Inhibition
- Exhaustion
- Apoptosis

**Hypo-active T cell**

**Inhibition**

**Activation**

**Improved:**
- Effector functions
- Survival
- Longevity

**Current therapy options**
TCR-Ts in combination with anti-PD1 or anti-PD-L1 antibodies

**Future options by Medigene**
TCR-Ts with integrated chimeric PD1-41BB switch receptor
Co-expression of PD1-41BB does not change levels of PRAME TCR cell surface expression
High-avidity PRAME-specific TCR-04 in combination with PD1-41BB signal switch receptor retained specificity, sensitivity and safety profile.

No change in functional avidity

No difference in HLA-A*02 sub-type specificity

No change in off-target toxicity in panel of lymphoblastoid cell lines (LCL)

* LCL express low levels of PRAME, determined by qPCR
Expression of PD1-41BB enhances specific release of IFNγ in response to recognition of cancer cell lines expressing high PD-L1.

* 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

**RPKM data derived from TRON database**

neg = PD-L1 negative
TD = PD-L1 transduced
ind = PD-L1 IFN-γ inducible
end = PD-L1 enogenously expressed
Expression of PD1-41BB in TCR-Ts leads to improved repetitive killing capacity

- TCR-T cells expressing PD1-41BB – improved killing of PD-L1-positive tumor cells.
- PD1-41BB overcomes the inhibitory signal delivered via the PD-1/PD-L1 checkpoint pathway.
Expression of PD1-41BB enhances the fitness of TCR-Ts after repeated exposure (6x) to 3D tumor cell spheroids

- PD-L1

- + PD-L1

P-values were calculated using a one-way ANOVA and Tukey’s multiple comparison test.

*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001

Mean of 3 donors from same experimental set-up

Triplicates per donor and tumor cell line

Similar results from previous datasets
PD1-41BB co-stimulatory switch to overcome inhibitory signals in the tumor milieu for persistence in solid tumor indications

**Enhanced T cell expansion**

<table>
<thead>
<tr>
<th>Glucose [mM]</th>
<th>PRAME_PD1-41BB_TCR-T</th>
<th>PRAME_TCR-T (&quot;naked&quot;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
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</table>

**Enhanced TCR-T spheroid infiltration**

- Mel624.38_PD-L1
- PRAME<sup>pos</sup>(+++)
- PD-L<sup>1pos</sup>(+++)

**Enhanced killing of 3D tumor spheroids**

- Count of infiltrated T cells
- Spheroid volume x10<sup>6</sup> [μm<sup>3</sup>]

*Graphs showing cellular counts and spheroid volumes over time.*
Expression of biomarkers in MelA375_PD-L1 melanoma cells with stable PD-L1 expression used for *in vivo* experiments

**Flow cytometry**

- **HLA-A2 staining**
  - stained
  - Isotype ctrl
  - MelA375
  - MelA375_PD-L1

- **PD-L1 staining**
  - stained
  - Isotype ctrl
  - MelA375
  - MelA375_PD-L1

**qPCR**

- PRAME expression normalized to GUSB (copies/ng RNA)

- **PRAME levels**
  - Mel624.38
  - MelA375
  - MelA375_PD-L1
  - 647-V

- **HLA-A2 staining**
  - Median FI
  - unstained
  - Isotype ctrl
  - MelA375
  - MelA375_PD-L1

- **PD-L1 staining**
  - Median FI
  - unstained
  - Isotype ctrl
  - MelA375
  - MelA375_PD-L1
Co-expression of TCR-4 with PD1-41BB strongly enhances *in vitro* and *in vivo* responses

**In vitro response**

- **Tumor cells:**
  - MelA375
  - MelA375_PD-L1
  - PRAME$_{low}$ PD-L1$_{high}$

- **TCR-T cells:**
  - UT
  - TCR
  - TCR_PDL1-41BB

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**

- **Tumor cells:**
  - T cell injection
  - UT
  - TCR
  - TCR_PD1-41BB

- **Tumor volume [mm$^3$]**

- **Days after tumor injection**
  - 0 7 14 21 28 35 42

- **IFN-$\gamma$ (pg/ml)**
  - ns ✱✱✱✱

- **IFN-$\gamma$ (pg/ml)**
  - ns ✱✱✱✱

- **Individual mice (6 per group):**
  - 10 mio PRAME-TCR$^+$ T cells
The IsoPlexis 32 T cell cytokine panel showed enhanced polyfunctionality and PSI in TCR-Ts expressing TCR with PD1-41BB.
Polyfunctionality heat map revealed major differences in the cytokine signatures of PRAME-specific TCR-T cells +/- PD1-41BB.
Summary of impact of the PD1-41BB switch receptor on function of CD8\(^+\) T cells expressing the PRAME-specific TCR *in vitro* and *in vivo*

- CD8\(^+\) T cells with PRAME TCR with PD1-41BB switch receptor showed enhanced:
  - proliferation
  - polyfunctional cytokine secretion, profile for strong anti-tumor immunity
  - killing of cancer cell lines *in vitro*, especially with high PD-L1
  - tumor infiltration into 3D spheroids
  - function upon repeated exposure to 3D tumor spheroids (6X)
  - metabolic fitness in presence of low glucose and TCGFβ
  - *in vivo* control of tumor outgrowth and increased overall survival of tumors with low antigen and high PD-L1
Thank you