Transitioning from a multi-modular system to a single automated device for TCR-T production

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Medigene’s End-to-End Platform

Multiple Combinable, Exclusive and Proprietary Technologies to Create Best-in-Class TCR-T Therapies for Cancer Patients

Target Screening
- EXPtope-M*
- Allo-HLA TCR Priming*
- CrossTag® Vector System
- JOVI Tag® Enrichment Technology
- Robotic Functional HTS

TCR Generation
- PD1-41BB Switch#
- Precision Pairing*
- Inducible iM-TCR*

TCR-T Therapy Optimization
- SIN-γ- Retroviral Gene Transfer System
- Cell Production Process & Quality Control
- Development Optimization
- Drug Product Immune Assessment*

Manufacturing Scale-up & Process Improvement
- Patient Immune Monitoring*

Clinical Development

Efficacy Enhancements

Safety Enhancements

* Proprietary to MDG
# Exclusive to MDG
^ Proprietary to MDG / HMGU
Introduction: Topics for discussion today

- **Multi-modular system as standard to benchmark transition to automated TCR-T production**
  - Establish benchmarks from Drug Products produced for MDG1011 Trial CD-TCR-001
  - Meet the challenge of using starting leukapheresis materials from heavily pretreated, elderly patients
  - Use enriched CD8+T cells frozen at the start and end of the manufacturing process

- **Characterizing patient Drug Products to establish quality standards**
  - Combine molecular and cellular tools to understand the quality of manufactured TCR-T cell Drug Products at different stages of manufacture

- **Utilizing immune monitoring technologies to assess Drug Product behavior in patients in vivo**
  - Apply fit-for-purpose assays to assess patient immune responses over time

- **Transition to automated closed processing with CliniMACS Prodigy™**
  - Advantages and disadvantages
MDG1011: PRAME VLD / HLA-A2 TCR targeting blood cancers
Phase I study of MDG1011 therapy in AML, MDS and MM

MDG1011: PRAME VLD / HLA-A2-specific TCR-T therapy for blood cancers
- PRAME – well-characterized, broadly expressed cancer-testis antigen
- CD8-enriched TCR-T Drug Products display multi-functionality

Phase I
Patients with refractory/relapsed
- Acute myeloid leukemia (AML)
- Myelodysplastic syndrome (MDS)
- Multiple Myeloma (MM)

Three dose levels tested
- 0.1, 1.0, 5.0 x 10^6 TCR-positive T cells / kg body weight
GMP production of cryopreserved personalized TCR-T cells

1. Leukapheresis
2. Enrichment process
   - CD8+ enrichment
   - Intermediate product: cryopreserved CD8+ enriched cells
3. Activation of T cells
   - Anti-CD3 + anti-CD28
4. Retroviral transduction
   - T cell transduction using retronectin
5. Expansion
6. Freezing
   - G-Rex device
   - Cryo-bags
7. Patient treatment
Manufacturing process based on multi-modular system

Advantages:
- Modular semi-automated system provides flexibility
- Up- and down-scaling possible

Disadvantages:
- High manual interventions with risk of contamination
- Multiple handling steps → highly skilled personnel
- Difficult process to standardize
- Clean room class A in B required
Establishing Benchmarks with Results from CD-TCR-001
Cell numbers with high viability were successfully produced

**MDG1011: Production of required TCR-T cell numbers expressing specific TCR was feasible for Dose Cohorts 1 - 3**

**MDG1011: Drug Products with excellent cell viability before and after freezing and thawing were manufactured for all patients**

0.1 (Cohort 1), 1.0 (Cohort 2), 5.0 x 10^6 (Cohort 3) TCR-positive T cells / kg body weight
Consistent Drug Products from variable starting materials

Consistent CD8^+ TCR-T cell Drug Products were generated
No detectable AML blast contamination in Drug Products

- **CD8+ cells**
- **CD4+ cells**
- **NK cells**
- **B cells**
- **Monocytes**
- **CD34+ cells**

Apheresis

Intermediate

Drug Product

**CD8 enrichment**

**T cell production**
Phenotypic characterization of TCR-T Drug Products
Molecular and cellular tools used for Drug Product study

**Surface Expression**

Double staining of TCR Vβ chain and pHLA-specific multimer measured by flow cytometry

**Antigen-Specific Cytokine Responses**

Intracellular IFN-g-staining of TCR-T cell populations after stimulation with T2 cells + specific peptide; T2 cells + ctrl peptide are IFN-g negative (not shown) Multiple cytokines/ cytotoxins can be studied

**Molecular Expression**

Vector copy number measured in sorted TCR-T cell populations by dPCR
Early T memory cell subsets associated with *in vivo* persistence found in most Drug Products

![Bar chart showing the percentage of CD3+CD8+ T naïve, TSCM, TCM, TEM, TEMRA, and rest cells in different Drug Products.](chart)

<table>
<thead>
<tr>
<th>Drug Product</th>
<th>T naïve</th>
<th>TSCM</th>
<th>TCM</th>
<th>TEM</th>
<th>TEMRA</th>
<th>Rest</th>
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<td>DP-1</td>
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<td>DP</td>
<td>A</td>
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<td>DP</td>
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</table>

*A: Apheresis  
DP: Drug Product*
All Drug Products showed IFNγ secretion and tumor target killing after antigen-specific stimulation

<table>
<thead>
<tr>
<th>Cells</th>
<th>IFNγ ELISA1</th>
<th>Cytotoxicity2</th>
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<tr>
<td>T2_VLD</td>
<td>1886</td>
<td>4</td>
</tr>
<tr>
<td>K562-A2</td>
<td>2795</td>
<td>63%</td>
</tr>
<tr>
<td>Mel624.38</td>
<td>988</td>
<td>4</td>
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<td>MAR-002</td>
<td>3443</td>
<td>65%</td>
</tr>
<tr>
<td>MAR-004</td>
<td>3420</td>
<td>ND</td>
</tr>
<tr>
<td>MAR-006</td>
<td>1738</td>
<td>12%</td>
</tr>
<tr>
<td>MAR-010</td>
<td>2199</td>
<td>10%</td>
</tr>
<tr>
<td>MAR-012</td>
<td>1771</td>
<td>99%</td>
</tr>
<tr>
<td>MAR-014</td>
<td>771</td>
<td>32%</td>
</tr>
<tr>
<td>MAR-016</td>
<td>1562</td>
<td>99%</td>
</tr>
<tr>
<td>MAR-018</td>
<td>1928</td>
<td>24%</td>
</tr>
<tr>
<td>MAR-022</td>
<td>454</td>
<td>5</td>
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<tr>
<td>MAR-028</td>
<td>1672</td>
<td>24%</td>
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<tr>
<td>MAR-033</td>
<td>195</td>
<td>95%</td>
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<tr>
<td>T2_VLD</td>
<td>2795</td>
<td>4</td>
</tr>
<tr>
<td>K562-A2</td>
<td>988</td>
<td>4</td>
</tr>
<tr>
<td>Mel624.38</td>
<td>971</td>
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</tbody>
</table>

• T2 cells are HLA-A2 positive but PRAME negative. They were pulsed with exogenous PRAME_VLD peptide

• K562 cells are HLA-A2 negative and PRAME positive. They were genetically modified to express HLA-A2

• Mel624.38 cells are endogenously positive for HLA-A2 and PRAME

1 secreted amounts of IFNγ after 24h coculture with indicated target cells in pg/ml
2 normalized killing of indicated target cells after 72h (MAR-004: 16h)
3 normalized killing not calculable due to different method
4 no untransduced control sample available
92% successful Drug Product manufacture (12/13) from heavily pretreated, elderly patients

<table>
<thead>
<tr>
<th>Batch Number</th>
<th>Indication</th>
<th>Manufacturing Date</th>
<th>Cohort Filled</th>
<th>Patient Number</th>
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<tbody>
<tr>
<td>MAR-002</td>
<td>AML</td>
<td>Aug 2018</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>MAR-004</td>
<td>MM</td>
<td>Jan 2019</td>
<td>1</td>
<td>Patient 1</td>
</tr>
<tr>
<td>MAR-006</td>
<td>AML</td>
<td>Aug 2019</td>
<td>1</td>
<td>Patient 2</td>
</tr>
<tr>
<td>MAR-010</td>
<td>AML</td>
<td>Jan 2020</td>
<td>1</td>
<td>Patient 3</td>
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<tr>
<td>MAR-012</td>
<td>AML</td>
<td>Feb 2020</td>
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<tr>
<td>MAR-014</td>
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<td>Apr 2020</td>
<td>2</td>
<td>OOS*</td>
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<td>MAR-016</td>
<td>AML</td>
<td>Jun 2020</td>
<td>2</td>
<td>NA</td>
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<tr>
<td>MAR-018</td>
<td>MM</td>
<td>Jul 2020</td>
<td>2</td>
<td>Patient 2</td>
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<tr>
<td>MAR-022</td>
<td>AML</td>
<td>Sep 2020</td>
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<td>Patient 3</td>
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<td>MAR-024</td>
<td>MDS</td>
<td>Nov 2020</td>
<td>3</td>
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<td>MAR-028</td>
<td>AML</td>
<td>Mar 2021</td>
<td>3</td>
<td>Patient 1</td>
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<td>MAR-030 *</td>
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<td>MAR-033</td>
<td>MDS</td>
<td>Mai 2021</td>
<td>3</td>
<td>Patient 3</td>
</tr>
</tbody>
</table>

*Analytical data from batch MAR-030 are not complete. Due to poor cell growth not all samples could be taken.
* Out of specification (OOS), % CD8+ T cells too low
NA = not applied
Fit-for-Purpose Immune Monitoring assays of patient samples

- **Identification of TCR-T cells**
  - Multimer staining of T cells expressing recombinant TCR
  - Digital droplet PCR for detection of recombinant TCR

- **Characterization of TCR-T cells**
  - Determination of T-memory subset composition
  - Determination of T-activation markers
  - Determination of T-checkpoint status

- **Functional analysis of TCR-T cells**
  - Multiplex assay of TCR-T cytokine secretion
  - Intracellular cytokine staining of TCR-expressing T cells
  - Proliferation of TCR-Ts after stimulation
Detection of MDG1011 \textit{in vivo} persistence without IL-2

T cell persistence seen in 4 patients treated with the two highest doses of TCR-T cells

\textbf{C2P1 (AML)}

\textbf{C2P2 (MM)}

\textbf{C3P1 (AML)}

\textbf{C3P3 (MDS)}

\( \text{nt} = \text{not tested} \)

\( \text{LoQ} = 36 \text{ copies} / 100 \text{ ng RNA} \)
Reduction of PRAME mRNA in blood and bone marrow at 4 weeks

- Reduction of PRAME mRNA expression in patient bone marrow in 1 of 2 MM patients
- Reduction of PRAME mRNA expression in patient bone marrow in 3 of 3 evaluable AML patients
- Reduction of PRAME mRNA expression in patient peripheral blood in 2 patients at the top dose level

C1P2, C1P3, C3P1, C3P3: SCR/SCR2 and/or V05 bone marrow samples not available
Multi-modular process enabled CD-TCR-001 to meet its objectives

- 92% successful manufacturing from heavily pretreated, elderly patients
  - Benchmarks were established for the multi-modular production process
  - Excellent cell viability for all batches before freezing and after thawing

- MDG1011 was well tolerated with no DLT or neurotoxicities

- Signs of biological and/or clinical activity
  - 1x CR
  - 2x CRS
  - 1 MDS patient without progression to AML after > 16 months still under observation; detection of TCR-T cells at EoT visit at 12 months
  - Reduction of PRAME mRNA in blood and bone marrow
Automated fully closed processing of TCR-T cells

Advantages:
• Fully closed system with single-use tubing set
• Can be used in clean room class C
• PIFs and CE-marked reagents available for clinical use
• Reduction of manual handling steps and hands-on time
• Less human error
• Process monitoring in one device

Disadvantages:
• Non-scalable
CliniMACS Prodigy yields high T cell numbers and high viability

X-fold expansion rates increased in the CliniMACS Prodigy compared to multi-modular process
CliniMACS Prodigy T cells show improved transduction rates
CliniMACS Prodigy-derived TCR-T cells display good functionality

IFN-γ ELISA

Prodigy-derived TCR-T cells showed comparable or increased target cell recognition compared to standard

Killing assay

Target: peptide-loaded T2

Target: K562-A2

Target: Mel624.38

Prodigy
Standard
NT control

Prodigy
Standard
NT control

Red count

RCU x µm²/image
CliniMACS Prodigy process met or exceeded benchmarks set by multi-modular process

- Strong Benchmarks were established for the multi-modular production process
  - 92% met release specifications
    - Excellent cell viability for all batches before freezing and after thawing
    - Robust production of CD8-enriched TCR-T drug products
- Results of our first steps in transition to a fully closed system were successful
  - High viability of Drug Products
  - Adequate levels of TCR transduction
  - Improved T cell proliferation
  - High functional capacities of TCR-T Drug Products

Strong Benchmarks guide improved manufacturing options
Acknowledgements

Clinical Trial Centers

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- **Erlangen**: University Hospital - Department of Medicine 5
- **Würzburg**: University Hospital - Internal Medicine II
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- **Freiburg**: University Hospital - Department of Medicine I
- **Heidelberg**: University Hospital - Internal Medicine V
- **Mainz**: University Medical Center (Johannes Gutenberg-University) - Department of Internal Medicine III
- **Frankfurt**: University Hospital - Department of Internal Medicine II
- **Leipzig**: University Hospital – Department of Internal Medicine II

https://clinicaltrials.gov/ct2/show/NCT03503968
Thank you for your attention