High Fidelity Production of TCR-T Cells from Elderly Hematologically-Challenged Patients With Blood Cancers

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MDG1011: Proof-of-concept phase I study of TCR-T therapy in blood cancer

**MDG1011: PRAME/HLA-A2-specific TCR-T therapy for blood cancers**

- PRAME – well-characterized, broadly expressed cancer-testis antigen
- CD8-enriched TCR-T drug products with multi-functionality

**Phase I part of Phase I/II trial**

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

**Dose escalation study**

- 3+3 clinical trial design
- Data Safety Monitoring Board reviews between cohorts

Single defined dose of CD8⁺ TCR-T cells/kg BW

- 1x10⁵ TCR⁺cells/kg
- 1x10⁶ TCR⁺cells/kg
- 5x10⁶ TCR⁺cells/kg
- up to 1x10⁷ TCR⁺cells/kg (optional)

https://www.clinicaltrialsregister.eu/ctr-search/trial/2017-000440-18/DE
MDG1011: Manufacturing challenges successfully met

Patients with AML, MDS or MM with *relapsed or refractory disease* presented special challenges for manufacture of MDG1011
- Patients were mostly elderly
- Patients had cancer of the blood system
- Patients were heavily pretreated
- AML blasts present at substantial levels in leukapheresis starting materials, in several cases
- Patients had rapidly progressing disease, so time was of essence

We achieved a 92% success rate in production of CD8\(^+\) TCR-Ts
- Drug Products were derived from enriched cryopreserved CD8\(^+\) T cells
- Leukapheresis was repeated for two patients only, with one success and one failure to get starting CD8\(^+\) T cell numbers
- Vein-to-vein time of ~7 weeks: production ~3 weeks; QC ~3 weeks and patient preparation for infusion ~1 week
- Nine patients were successfully dosed, whereas four patients succumbed before IMP administration

The cryopreserved Drug Products showed functional activity *in vitro* and in patients *in vivo*
MDG1011: Background for development of TCR-T therapy for blood cancer and solid tumors – prevalence of PRAME

Scale is given as RSEM. RSEM quantifies gene and isoform abundances from single-end or paired-end RNA-Seq data. Graphic compiled by Medigene, from publicly available TCGA-data.
**MDG1011: Background for development of TCR-T therapy for blood cancer – relevance of PRAME**

<table>
<thead>
<tr>
<th></th>
<th>PRAME</th>
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<tbody>
<tr>
<td>Expression in bulk AML</td>
<td>Cancer-testis antigen</td>
</tr>
<tr>
<td>Expression in LSC</td>
<td>&gt; 65 %</td>
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<tr>
<td>Expression in HSC / other cells</td>
<td>+/-</td>
</tr>
<tr>
<td>Oncogenicity</td>
<td>-</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>++</td>
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<tr>
<td>Clinical efficacy</td>
<td>++</td>
</tr>
<tr>
<td>MRD Monitoring</td>
<td>++</td>
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<tr>
<td>Expression in bulk AML</td>
<td>Established by qPCR</td>
</tr>
</tbody>
</table>

Anguille et al, Leukemia 2012; Lichtenegger et al, Immunotherapy 2013
MDG1011: Background for development of TCR-T therapy for blood cancer – feasibility to recruit PRAME+ HLA-A*02:01+ patients

1. Pre-screening for general eligibility
2. Screening for HLA-A2 and PRAME mRNA in bone marrow or blood
3. Inclusion and treatment

HLA and antigen frequency is critical

- HLA-A*02:01+
- PRAME+
- Other

- 45%
- 55%
- 27%
MDG1011: Establishment of safety profiles of antigen and TCR-T cells

**Target Antigen and HLA-Peptide Ligands at Molecular and Cellular Levels**

- Genetic isotypes, RNA, protein, peptide expression
- Expression in cancer cell lines and tumor specimens
- Expression in cancer databases

**Target safety**

- Expression from databases of healthy tissues
- In vitro models for functional testing

**TCR selectivity**

- Vetting of multiple TCRs to select Lead TCR

**Safety Profile**

- HLA-peptide ligands for lead TCR
- "Go/no go" studies of TCR-T cells in final Drug Product formulation
MDG1011: GMP production of personalized TCR-T therapy

Medigene’s TCRs are delivered via a vector to genetically modify autologous patient T cells to express the desired tumor-specific TCR.

1. Leukapheresis and T cell isolation
2. Activation of T cells and transfer of TCR
3. Expansion, freezing and quality tests
4. Thawing and infusion into patient

TCR drug product and analytical samples

TCR vector
MDG1011: 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 needed
- Difficult process to standardize
- Clean room class A and B required
MDG1011: Diverse testing was performed for Drug Product release and Drug Product characterization at CMO and MDG

**Release at BioNTech**
- Safety Parameters
- Identity
- Potency
- Quantity / Cell Dose
- Purity / Impurity
- Appearance

**Characterization at BioNTech**
- Cellular Impurities / Cell Composition
- T Cell Subsets
- Viability and Cell Count (during entire process of drug production)

**Characterization at Medigene**
- Process-Related Impurities
- T Cell Subsets
- IFN\(\gamma\) Secretion (ELISA)
- Intracellular Cytokine Staining
- Cytotoxic Activity

CMO for this study was BioNTech IMFS at Idar-Oberstein, Germany. The GMP process for TCR-T Drug Product manufacture was co-developed with the Departments of Translational Medicine and Cell Therapy Process Development at Medigene.
MDG1011: Consistent CD8^+ TCR-T cell Drug Products generated from variable patient leukapheresis starting materials
MDG1011: Production of required TCR-T cell numbers expressing specific TCR was feasible for Dose Cohorts 1 - 3

- Cohort 1: 0.0, 0.1, 0.2, 0.5, 1.0, 1.5
  - 4 batches
  - 6 bags

- Cohort 2: 0.0, 0.1, 0.2, 0.5, 1.0, 1.5
  - 5 batches
  - 10 bags

- Cohort 3: 0.0, 0.1, 0.2, 0.5, 1.0, 1.5
  - 3 batches
  - 6 bags

Transduced cells per kg BW [in $10^6$]
MDG1011: Drug Products with excellent cell viability before and after freezing and thawing were manufactured for all patients.

n = 13 batches, for one batch only one bag was filled.
MDG1011: Drug Product characterization encompassed multiple molecular and cellular technologies

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

**Molecular Expression**
Vector copy number measured in sorted TCR-T cell populations by dPCR

**Antigen-Specific Cytokine Responses**
Intracellular IFN-γ-staining of TCR-T cell populations after stimulation with T2 cells + specific peptide; T2 cells + ctrl peptide are IFN-γ negative (not shown) Multiple cytokines/cytotoxins can be studied
MDG1011: Activation markers and checkpoint receptor expression changed between apheresis starting materials and final Drug Products.
MDG1011: Peptide-specific stimulation triggered poly-functional cytokine release in all Drug Products
MDG1011: Analytical methods to assess T cell pharmacokinetics and PRAME mRNA expression in peripheral blood and bone marrow

- Pharmacokinetics of TCR-T cells in MDG1011 patients was assessed with validated fit-for-purpose assays using:
  - flow cytometry (FC)
    - TCR-expressing cells were identified with a TCRV-beta antibody + pMHC-specific dextramer
    - Double-positive cells were enumerated in the gated population of CD45⁺/CD3⁺/CD8⁺ T cells in the peripheral blood (Limit of quantification (LoQ)= 0.015%)
  - Digital droplet PCR (dPCR)
    - TCR-transduced T cells were identified by the RNA of the viral element Woodchuck Hepatitis Post-transcriptional Regulatory Element (WPRE)
    - Copies of the WPRE element were quantified in bulk RNA of patient peripheral blood samples (Limit of quantification (LoQ) dPCR = 36 copies/100ng RNA)

- Measurement of PRAME mRNA in bone marrow samples and/or peripheral blood and was done using a validated real-time quantitative PCR (qPCR) assay. (Limit of quantification (LoQ) qPCR = 70 copies/25ng RNA)
MDG1011: Biological and clinical activity detected in patients in vivo

CRS as an indicator for biological activity of MDG1011
- Grade I Cytokine Release Syndrome seen in C2P1
- Grade II Cytokine Release Syndrome seen in C3P1

T cell persistence seen in 4 patients treated with the two highest doses of MDG1011

PRAME mRNA was reduced at Week 4 in bone marrow samples of 4 of 5 evaluable patients

PRAME mRNA was reduced at Week 4 in blood of AML/MDS patients treated with highest dose of MDG1011
MDG1011: Benefits of using molecular and cellular Drug Product Immune Assessments and Patient Immune Monitoring

- Complex characterization of TCR-T Drug Products and immune monitoring of TCR-T cells in patients in vivo are critical steps for progressing manufacturing and clinical developments of living immunotherapies.

- Deep characterization of Drug Products and correlation with clinical observations in patients:
  - facilitate deeper understanding of immune responses that may yield clinical benefit
  - provide insight into differences between Drug Products associated with CRS and/or clinical response
  - deliver crucial data on effects of TCR-T variations in Drug Product Immune Assessment in vitro vs. Patient Immune Monitoring in vivo
  - give better insight into parameters needed to define potency of TCR-T cells

- Approaches validated in Phase I study and knowledge gained can be extended in Phase 2 to acquire more data and derive better answers to hypothesis-generated questions from Phase 1.
Thank you