PRAME mRNA Expression in AML/MDS and HLA Genotype Analysis: Impact on Population Coverage and Design of TCR-Based Immunotherapies

Richard Addo MD, PhD

62nd ASH Annual Meeting
Dec 5-8 2020
Disclosure: employee of Medigene Immunotherapies GmbH
Introduction

- **Study objectives**
  - To determine how many and which HLA-A-restricted TCRs are needed to ensure optimal coverage of the Caucasian population
  - To determine PRAME expression in AML/MDS patients

- **PRAME as a target for TCR-T immunotherapies**
  - A well described cancer-testis antigen
  - Highly expressed in tumors but scarce or absent in normal tissues
  - Highly expressed in most solid and liquid cancers, including AML/MDS
  - PRAME can stimulate cytotoxic lymphocytes

TCR: T Cell Receptor; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; PRAME: PRAME (PR Referentially expressed Antigen of MElanoma)
### Overview of samples analysed in the study

#### AML/MDS patient cohort (N=165)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Outcome, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease indication</strong></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>133 (80.6%)</td>
</tr>
<tr>
<td>MDS</td>
<td>32 (19.4%)</td>
</tr>
<tr>
<td><strong>Disease status</strong></td>
<td></td>
</tr>
<tr>
<td>First time (initial) diag</td>
<td>96 (58.2%)</td>
</tr>
<tr>
<td>Relapsed Refractory</td>
<td>68 (41.2%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td><strong>Samples</strong></td>
<td></td>
</tr>
<tr>
<td>Paired BM and PB</td>
<td>111 (67.3%)</td>
</tr>
<tr>
<td>Unpaired/Only PB</td>
<td>54 (32.7%)</td>
</tr>
</tbody>
</table>

#### HLA-A distribution analysis (N=141)

<table>
<thead>
<tr>
<th>Healthy donors</th>
<th>Outcome, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>141 (100.0%)</td>
</tr>
</tbody>
</table>

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; BM: bone marrow; PB: peripheral blood
Methods

- **PRAME measurement**
  - via qRT-PCR of cDNA transcribed mRNA isolated from peripheral blood (PB) or bone marrow aspiration (BM)
  - positivity threshold of 99 PRAME mRNA copies at 100% assay specificity was determined using samples from healthy donors
- HLA genotyping was performed using next generation sequencing

BM: bone marrow; PB: peripheral blood
48% of all patients (N= 165) were PRAME positive
Considered positive if expression in PB or BM was ≥ 99 copies

55% of all patients (N= 165) were HLA-A*02:01 positive, Distribution could be skewed due to inclusion of allo-HSCT patients with already known HLA-A*02:01
‘Double’ positivity of AML/ MDS patients

- HLA-A*02:01+ PRAME+ 27%
- HLA-A*02:01 55%
- Others 45%
PRAME expression is similar between first diagnosis and relapsed/refractory AML/MDS patients

<table>
<thead>
<tr>
<th></th>
<th>% of PRAME positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapse/refractory</td>
<td>51.5 (35/68)</td>
</tr>
<tr>
<td>First diagnosis</td>
<td>44.5 (43/96)</td>
</tr>
</tbody>
</table>
PRAME expression can be detected more often in bone marrow compared to peripheral blood

In alignment with disease origin and place of activity, bone marrow is positive more often than peripheral blood

Paired PB and BM samples (111)

- Positive in both BM and PB: 39.6%
- Positive in only BM: 10.8%
- Positive in only PB: 2.7%
- Negative in both PB and BM: 46.8%
Distribution of five most common HLA-A genotypes in the general population (Germany)

- Analysis of 141 healthy blood donors

- HLA-A*02:01: 43.06%
- HLA-A*01:01: 23.39%
- HLA-A*03:01: 25.00%
- HLA-A*24:02: 18.75%
- HLA-A*11:01: 7.64%
TCR products addressing the five most common HLA-A allotypes can lead to coverage of approx. 88% of the population.
Co-authors

**Medigene Immunotherapies GmbH, Germany**
Kathrin Davari, PhD
Silke Raffegerst, PhD
Kai Pinkernell, MD
Dolores Schendel, PhD

**Universitätsklinikums Regensburg, Germany**
Simone Thomas, MD
Thank you