T cell epitopes in HSCT

Katharina Fleischhauer
Institute for Experimental Cellular Therapy
University Hospital Essen, Germany

Teaching Session II:
Epitope Matching in Transplantation:
Cross-linking the solid organ experience with the stem cell world
12.05.2016
Structure of the Presentation

• Mismatched HSCT – Practical Relevance
• Epitopes: Tolerance to Self
• T Cell Epitopes in HSCT: Where are we?
Structure of the Presentation

• Mismatched HSCT – Practical Relevance

• Epitopes: Tolerance to Self

• T Cell Epitopes in HSCT: Where are we?
**HLA Allele Numbers since 1988**

**HLA Typing Methodology for Stem Cell donors**

Low: Serology  
High: IEF (Cl. I), MLR (Cl.II)

Low: SSOP, SSP  
High: SBT, SSP, SSOP

Epitopes  
1996

Amino acids (AA)  
2009

AA + Non-coding polymorphism

10,574

3,658

Class I Alleles  Class II Alleles
T cell epitopes: Mismatched HSCT

HLA Mismatch Frequency

<table>
<thead>
<tr>
<th>HLA Region</th>
<th>Matched</th>
<th>Mismatched</th>
</tr>
</thead>
<tbody>
<tr>
<td>A,B,C,DR</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>DP</td>
<td>85</td>
<td>15</td>
</tr>
</tbody>
</table>

Gragert, NEJM 2014
The need for permissive mismatches

10% decrease in survival by each mismatch

Lee et al., Blood 2007
The need for permissive mismatches

Lodewyck, BBMT 2011

Fürst, Blood 2013

Pidala, Blood 2014
Role of HLA-DPB1 Allele Mismatches

Overall Survival

Relapse

HR 1.09, p=0.07

HR 0.78, p=0.002

N = 5154/5979 (86.2%) DPB1 allele mismatched

Shaw, Blood 2007
Permissive HLA Mismatches

Desirable yet Elusive

Comment on Fleischhauer, p 366

Permissive mismatches for blood and marrow transplantation
Paul Szabolcs


Comment on Spellman, p 740

**THE BOTTOM LINE**

The Ever Elusive Permissive Mismatch
Claudio Anasetti

*BBMT* 2012: 18: 657-9

A closer look at permissive HLA mismatch
Andrea Bacigalupo

*Blood* 2013: 122: 3555-6
The Road to Permissive Mismatches

Working Hypothesis

- HLA Structure
- T Cell Function
- Epitopes

Validation

Experimental
- Ex-vivo

Clinical
- Germany
- Europe
- USA
- Worldwide

Donor Selection Webtools
Permissive HLA Mismatches

A Sysiphus Myth

- Be wary of Dogmas!
- New Stem Cell Sources (BM, PBSC, Cord)
- New Therapies (mAbs, CARs, Cells)
- New Typing Resolution (NGS, outside exons)
- New Gene Variables (LEL, KIR, MIC, Cyt)
- Changing Patient Cohorts (Age, Disease)
- New Immunosuppressive Drugs (Post.Tx Cyt)
Structure of the Presentation

• Mismatched HSCT – Practical Relevance

• Epitopes: Tolerance to Self

• T Cell Epitopes in HSCT: Where are we?
Protective Immunity and Alloreactivity

HLA-restricted peptide recognition

Thymic TCR Selection

Protective Immunity

Bacteria
Virus
Tumor Ag

SNP, Indels

Major Ags

HLA

Minor Ags

Epitopes?

Histocompatibility Antigens
Thymic Selection of the T Cell Repertoire

Alloreactivity depends on the shared self-HLA background!
Humoral and Cellular Allo-Epitopes

Humoral

B Cell

Alloantibodies

Structural Epitopes (Triplets/Eplets)

Unraveled to some extent

Cellular

NK Cell

NK Cell Receptors

Peptide-dependent T Cell Epitopes (TCE)

Largely Elusive!

T Cell

T Cell Receptor
HLA Antibody Epitopes

Solid Organ Transplantation

The Matchmaker Concept:
Tolerance to Self-Epitopes

R. Duquesnoy, Tissue Antigens 2011; Transpl Immunol 2014
From HLA-DP Allele to TCE Matching

<table>
<thead>
<tr>
<th>Hypervariable Region (HvR)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPB1*</td>
<td>8</td>
<td>9</td>
<td>11</td>
<td>35</td>
<td>55</td>
<td>56</td>
</tr>
<tr>
<td>09:01</td>
<td>V</td>
<td>H</td>
<td>L</td>
<td>F</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>10:01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17:01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>M</td>
</tr>
<tr>
<td>03:01/0104:01</td>
<td>Y</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>K</td>
<td>-</td>
</tr>
<tr>
<td>14:01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>K</td>
<td>-</td>
</tr>
<tr>
<td>45:01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>E</td>
<td>K</td>
</tr>
<tr>
<td>86:01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>M</td>
<td>G</td>
</tr>
<tr>
<td>01:01</td>
<td>Y</td>
<td>G</td>
<td>Y</td>
<td>A</td>
<td>A</td>
<td>E</td>
</tr>
<tr>
<td>02:01</td>
<td>L</td>
<td>F</td>
<td>G</td>
<td>-</td>
<td>-</td>
<td>E</td>
</tr>
<tr>
<td>02:02</td>
<td>L</td>
<td>F</td>
<td>G</td>
<td>L</td>
<td>-</td>
<td>E</td>
</tr>
<tr>
<td>04:01</td>
<td>L</td>
<td>F</td>
<td>G</td>
<td>-</td>
<td>A</td>
<td>K</td>
</tr>
<tr>
<td>04:02</td>
<td>L</td>
<td>F</td>
<td>G</td>
<td>-</td>
<td>-</td>
<td>K</td>
</tr>
<tr>
<td>05:01</td>
<td>L</td>
<td>F</td>
<td>G</td>
<td>L</td>
<td>E</td>
<td>A</td>
</tr>
<tr>
<td>06:01</td>
<td>-</td>
<td>Y</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>M</td>
</tr>
<tr>
<td>11:01</td>
<td>Y</td>
<td>Y</td>
<td>A</td>
<td>A</td>
<td>E</td>
<td>R</td>
</tr>
<tr>
<td>13:01</td>
<td>-</td>
<td>Y</td>
<td>-</td>
<td>A</td>
<td>A</td>
<td>E</td>
</tr>
<tr>
<td>15:01</td>
<td>-</td>
<td>Y</td>
<td>G</td>
<td>Y</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>16:01</td>
<td>L</td>
<td>F</td>
<td>G</td>
<td>-</td>
<td>-</td>
<td>E</td>
</tr>
<tr>
<td>19:01</td>
<td>L</td>
<td>F</td>
<td>G</td>
<td>-</td>
<td>E</td>
<td>A</td>
</tr>
<tr>
<td>20:01</td>
<td>Y</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>K</td>
<td>M</td>
</tr>
<tr>
<td>23:01</td>
<td>L</td>
<td>F</td>
<td>G</td>
<td>-</td>
<td>A</td>
<td>E</td>
</tr>
<tr>
<td>46:01</td>
<td>L</td>
<td>F</td>
<td>G</td>
<td>-</td>
<td>-</td>
<td>M</td>
</tr>
</tbody>
</table>

TCE Group 1 (complete cross-reactivity)

TCE Group 2 (partial cross-reactivity)

TCE Group 3 (no cross-reactivity)

Immunogenic T Cell Epitope?

Graft Rejection (*Blood 2001*)

Patient DPB1*02:01, 04:01
Donor DPB1*02:01, **09:01**

Alloreactive CD4+ T Cells from the patient
(Non) Permissive DPB1 TCE Group mismatches

Permissive 66%

Non Permissive 33%
Clinical Evidence for TCE Matching

Overall Survival

5428 UD-SCT (10/10) 1993-2007
HR 1.15 (1.05 – 1.25) P=0.002

Fleischhauer Lancet Oncol 2012

3281 UD-SCT (8/8) 1999-2011
HR 1.2 (1.1 – 1.4) P=0.002

Pidala Blood 2014
TCE Matching in Search Algorithms

DPB1 T-Cell Epitope Algorithm

Collaboration with SGH Marsh  
(Anthony Nolan, London)

http://www.ebi.ac.uk/ipd/imgt/hla/dpb.html

Courtesy of CH Müller  
(ZKRD, Ulm)
Biological Rationale for TCE Matching

HLA-DPB1 Allele Mismatched MLR

T cell Repertoire
Thymic Selection

Self-DP TCE3/3

Allo-DP TCE1
Non-Permissive 33%

Allo-DP TCE3
Permissive 66%

Strong Response

Weak Response

Responder T cells
Alloreactive

Arrieta-Bolaños E, et al. EFI 2016
O9 Saturday April 14 (Best Abstracts)
Experimental Evidence for TCE Matching

N = 94 healthy donors

In TCE3/3 Responders, significantly weaker response against allo-TCE3 compared to TCE1/2

Arrieta-Bolaños E, et al. EFI 2016
O9 Saturday April 14 (Best Abstracts)
Experimental Evidence for TCE Matching

In TCE2/3 Responders, median response to TCE2 drops to levels similar to TCE3

N = 94 healthy donors

Arrieta-Bolaños E, et al. EFI 2016
O9 Saturday April 14 (Best Abstracts)

Collaboration with JHF Falkenburg
Structure of the Presentation

• Mismatched HSCT – Practical Relevance

• Epitopes: Tolerance to Self

• T Cell Epitopes in HSCT: Where are we?
Alloreactive T Cell Epitopes (TCE) - 1

• Peptide Alloantigens:
  ✓ Minor Histocompatibility Antigen Peptides
  ✓ PIRCHES (Predicted Indirectly Recognizable Peptides)
  ✓ Allo-peptides (non-self HLA restricted self peptides)

  Technically challenging!
• HLA Alloantigens:

  ✓ Acceptable Mismatch Combinations
  ✓ Individual amino acid mismatches
  ✓ “Delta-Functional Distance” of HLA mismatches
  ✓ Relation with serologic epitopes?
Acceptable Mismatch Combinations

High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism

Takakazu Kawase,1 Yasuo Morishima,2 Keitaro Matsuo,3 Koichi Kashiwase,4 Hidetoshi Inoko,5 Hiroh Saji,6 Shunichi Kato,7 Takeo Juji,8 Yoshihisa Kodera,9 and Takehiko Sasazuki,10 for The Japan Marrow Donor Program

Table 2. Nonpermissive allele mismatch combinations for severe aGVHD

<table>
<thead>
<tr>
<th>Mismatch combination, donor-patient</th>
<th>N</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0206-A0201</td>
<td>131</td>
<td>1.78 (1.32-2.41)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>A0206-A0207</td>
<td>27</td>
<td>3.45 (2.09-5.70)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>A2602-A2601</td>
<td>21</td>
<td>3.35 (1.88-5.91)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>A2603-A2601</td>
<td>35</td>
<td>2.17 (1.29-3.64)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>B1501-B1507</td>
<td>19</td>
<td>3.31 (1.85-5.99)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>C0303-C1502</td>
<td>25</td>
<td>3.22 (1.75-5.69)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>C0304-C0601</td>
<td>69</td>
<td>2.34 (1.55-3.52)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>C0401-C0303</td>
<td>42</td>
<td>2.81 (1.72-4.60)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>C0801-C0303</td>
<td>80</td>
<td>2.32 (1.58-3.40)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>C1402-C0304</td>
<td>23</td>
<td>3.66 (2.00-6.68)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>C1502-C0304</td>
<td>27</td>
<td>3.77 (2.20-6.47)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>C1502-C1402</td>
<td>50</td>
<td>4.97 (3.41-7.25)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>DR0405-DR0403</td>
<td>53</td>
<td>2.13 (1.28-3.53)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>(DR1403-DQ0301)-(DR1401-DQ0502)</td>
<td>19</td>
<td>2.81 (1.44-5.51)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>DP0301-DP0501</td>
<td>49</td>
<td>2.41 (1.49-3.89)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>DP0501-DP0901</td>
<td>71</td>
<td>2.03 (1.30-3.16)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

N=5210

15 statistically non-permissive mismatch combinations

No consideration of Self-HLA background
Identification of a permissible HLA mismatch in hematopoietic stem cell transplantation


BLOOD, 20 FEBRUARY 2014

Key Points

• Mismatches in alleles C*03:03/C*03:04 were most frequent (68.7%) among the transplants with a single allele level mismatch in HLA-C.
• The 7/8 C*03:03/C*03:04 mismatch group was not significantly different from the 8/8 HLA matched transplants in any transplant outcome.

Similar Survival to 8/8 matched N=7349

Probability, %

0 20 40 60 80 100

Years

0 1 2 3 4 5

C*03:03/C*03:04 mismatch (n=134)
Other C-allele mismatch (n=61)
C-antigen mismatch (n=700)
HLA-A,B or DRB1 mismatch (n=959)
8/8 match (n=4779)
Individual AA mismatches: pos. 116

Bone marrow transplantation from unrelated donors: the impact of mismatches with substitutions at position 116 of the human leukocyte antigen class I heavy chain

Giovanni B. Ferrara, Andrea Bacigalupo, Teresa Lamparelli, Edoardo Lanino, Laura Dellino, Anna Morabito, Anna M. Parodi, Cinzia Pera, Sarah Pozzi, Maria P. Sormani, Paolo Bruzzi, Domenico Bordo, Martino Bolognesi, Giuseppe Bandini, Andrea Bontadini, Mario Barbanti, and Guido Frumento

TRM in 100 patients

Ferrara 2001

P=0.001
Serologically identical (HLA-B44)

Different IEF Subtypes (B44.1 vs B44.2)

B*44:02
B*44?

Individual AA mismatches: pos. 156

Restriction Map of 7.2Kb Genomic Clone B44.2

Sanger Sequencing B44.2 (B*44:03)
Individual AA mismatches: pos. 156

Greater Peptide Promiscuity of B*44:03
MacDonald J Exp Med 2003
Amino acid substitution at peptide-binding pockets of HLA class I molecules increases risk of severe acute GVHD and mortality


Key Points

- Amino acid substitution at peptide-binding residues of the HLA class I molecule is associated with graft-versus-host disease and mortality.
- Avoidance of donor-recipient combinations that result in amino acid substitution at peptide-binding residues may improve transplant outcomes.

Pos 9, 99, 116 associated with Outcome
Key Amino Acids in HLA-DPB1*09:01

- Site-directed mutagenesis of 12 AA in DPB1*09:01
- Impact on Allorecognition by 17 T cell effectors

Crivello 2015

The Impact of Amino Acid Variability on Alloreactivity Defines a Functional Distance Predictive of Permissive HLA-DPB1 Mismatches in Hematopoietic Stem Cell Transplantation

Pietro Crivello, Laura Zito, Federico Sizzano, Elisabetta Zino, Martin Maiers, Arend Mulder, Cristina Toffalori, Luigi Naldini, Fabio Ciceri, Luca Vago, Katharina Fleischhauer

Received: August 1, 2014; Accepted: October 19, 2014; Published Online: October 23, 2014
Functional Distance from WT-DP9

Allorecognition of DP9 Mutants

Functional Distance (FD)

FD_{aa} = 1 - Median RR_{aa}

Impact of individual Amino Acids (AA)

FD_{aa}: Numerical Score for each AA
- High value: Large Impact
- Low value: Low Impact
Different FD for AAS at pos 35

F35 WT-DP9
Hydrophobic

F35Y
Polar

F35L
Hydrophobic

FD=0
Reference

FD=0.85
High Impact

FD=0.05
Low Impact

AA substitution

High Impact AAS:
• Peptide Binding
• Non-Conservative

P = 0.0025

Crivello P, et al. EFI 2016
O22 Thursday April 12 (HSCT Session)
**Functional Distance Levels**

- **FD<sub>AA</sub>**
  - Median impact of individual AA substitutions at 10 different positions in HLA-DPB1*09:01 on T-cell alloreactivity.

- **FD<sub>Allele</sub>**
  - Sum of FD<sub>AA</sub> scores for any given HLA-DPB1 allele.

- **ΔFD**
  - Absolute difference between the sum of HLA-DPB1 FD<sub>allele</sub> scores in two individuals.

Correlates with TCE groups *(BBMT 2015)*

Clinical Risk Associations in HSCT *(Blood 2016)*
ΔFD is predictive of survival

Patients (UK-Essen)
N = 379

Diagnosis
- AML n = 302 (72.4%)
- MDS n = 57 (13.7%)
- ALL n = 58 (13.9%)

HLA matching status:
- 10/10 matched
- HLA-DPB1 mismatched

Receiver Operator Curves (ROC):
- \( \leq 2.665 \) n=252 (66.5%)
- >2.665 n=127 (33.5%)

79% Overlap with TCE Classification

Kaplan Meier Estimates of Overall Survival

TCE Classification: HR 1.15, 95% CI 0.86 – 1.54, p < .360

Crivello et al., Blood 2016
Take Home Messages

• T Cell Epitopes (TCE) are relevant in mismatched HSCT
  ✓ 10-25% A,B,C,DRB1
  ✓ 85% DPB1

• TCE depend on the self-HLA of donor and recipient (thymic education)

• Major role for AA substitutions at peptide binding positions AND non-conservative
  ✓ Position 9, 99, 116, (156) – HLA class I
  ✓ Position 9, 11, 35, 55, 69, 84 – HLA class II (DPB1)

• Non-Permissive HLA-DPB1 TCE mismatches in clinical search algorithms
  (association with Overall Survival)

• Relationship between Antibody Epitopes and T Cell Epitopes? 17th IHIW...
Thank you for your attention!