The Science Behind HLA Matching
& A Bit More

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Transplant Immunology & Cell Processing Laboratories
Baylor University Medical Center

Clinical Professor, Pathology & Lab Medicine
Texas A&M HSC College of Medicine

November 10, 2018
The following faculty and planning committee staff have the following financial disclosures:

<table>
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<tr>
<th>Name</th>
<th>Institution</th>
<th>Disclosure</th>
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<tr>
<td>Medhat Askar, MD, PhD, FRCPath</td>
<td>Baylor University Medical Center</td>
<td>None</td>
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<tr>
<td></td>
<td>Texas A&amp;M HSC College of Med</td>
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How does all of this work?
Consider:
High Resolution HLA Typing, Antibody Testing, DPB1 Typing

- High resolution HLA typing
- Antibody testing
- DPB1 typing

- Search prognosis tool
- Related Donor Services

Grab your cape.
Immunologic Concepts in Clinical Transplantation
HLA Typing

HLA Ab Testing

Donor Selection
HLA?!

First HLA Antigen, 1953

Jean Dausset (1916-2009)

1980 Nobel Prize Laureate
HLA??!!!
What is Nomenclature?

HLA-C*07:02:01:17N

- Locus Type Silent
- Substitution Expression
- Signifies DNA Subtype
- Differences outside the coding region (introns)
  - Type
  - Silent Substitution
  - Expression
  - Correlates to the serological antigen or family

Corresponds to the serologic antigen or family
B*15:01 = B62
B*15:02 = B71
C*03:03 = Cw9
C*03:04 = Cw10
DRB1*03:01 = DR17
DRB1*03:02 = DR18
DQB1*03:01 = DQ7
DQB2*03:02 = DQ8

The alignment below is a graphical representation to allow comparison of known sequences. Where discrepancies have arisen between reported sequences, the original authors have been contacted where possible, and necessary amendments to published sequences have been incorporated into this alignment. Future sequencing may identify errors in this list and the WHO Nomenclature Committee would welcome any evidence that helps to maintain the accuracy.

Please click here to perform further alignments

### Numbers of Serologically Defined Antigens

<table>
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<tr>
<th>HLA-Locus</th>
<th>A</th>
<th>B</th>
<th>Cw</th>
<th>DR</th>
<th>DQ</th>
<th>DP</th>
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<td>62</td>
<td>10</td>
<td>24</td>
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The alignment below is a graphical representation to allow comparison of known sequences. Where discrepancies have arisen between reported sequences, the original authors have been contacted where possible, and necessary amendments to published sequences have been incorporated into this alignment. Future sequencing may identify errors in this list and the WHO Nomenclature Committee would welcome any evidence that helps to maintain the accuracy.

Please click here to perform further alignments.
<table>
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<tr>
<th>Gene</th>
<th>A</th>
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<tr>
<td>Alleles</td>
<td>4,638</td>
<td>5,590</td>
<td>4,374</td>
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<td>Proteins</td>
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<td>Nulls</td>
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## HLA Class II

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<td>878</td>
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The alignment below is a graphical representation to allow comparison of known sequences. Where discrepancies have arisen between reported sequences, the original authors have been contacted where possible, and necessary amendments to published sequences have been incorporated into this alignment. Future sequencing may identify errors in this list and the WHO Nomenclature Committee would welcome any evidence that helps to maintain the accuracy.

Please click here to perform further alignments.
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<tr>
<td>A*24:02:01:02L</td>
<td>A*31:01:02</td>
<td>Intron 3</td>
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<td>A*31:01:02</td>
<td>Exon 5</td>
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<td>A*24:03:01</td>
<td>A*31:05</td>
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<td>A*24:34</td>
<td>A*31:56</td>
<td>Exon 2</td>
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<td>A*24:57</td>
<td>A*31:29</td>
<td>Exon 3</td>
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<td>A*31:02</td>
<td>Exon 2</td>
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- Ruled out by RSSOP.
- Not on Common list.

**Master Layer**

822 bases between Intron 1 34 and Intron 4 30

Mean BCS: 85
Groove-Domain

Class I

1 Exon 2 Exon 3 Exon 4

1 5 6 7 8

Class II

1 Exon 2 Exon 3

4 5 6
On June 26, 2000 President Clinton, with J. Craig Venter, left, and Francis Collins, announces completion of "the first survey of the entire human genome."

$ 3 Billion (2000)

10 years (1993-2003)
Method of the Year

There are events of the year, persons of the year, images of the year.... We could not resist: why not a Method of the Year?
Initially At Time of Pub

van Dijk et al, 2014
AA6613

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</tr>
<tr>
<td>A*24:02:01:02L</td>
<td>01:02</td>
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</tr>
<tr>
<td>A*24:02:01:03</td>
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</tr>
<tr>
<td>A*24:02:40</td>
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<td>A*24:57</td>
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<td>Exon 3</td>
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<td>A*24:71</td>
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<td>Exon 2</td>
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Ruled out by RSSOP.
Not on Common list.
Ruled out by RSSOP.

Master Layer
822 bases between Intron 1 34 and Intron 4 30
Mean BCS: 85

<table>
<thead>
<tr>
<th>NGS-ENGINE</th>
<th>Best Match</th>
<th>A<em>24:02:01:01, A</em>31:01:02</th>
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<tr>
<td>Standard Method</td>
<td>Mappability</td>
<td>44188/48644 (90%)</td>
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<tr>
<td>Total Mappability</td>
<td>Reads</td>
<td>223 [35-251]</td>
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<tr>
<td>190645/200000 (95%)</td>
<td>Coverage</td>
<td>(3385, 7611)</td>
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<tr>
<td></td>
<td># Matching Genotypes</td>
<td>1</td>
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HLA Typing

HLA Ab Testing

Donor Selection
Allosensitization

Grab your cape.
HLA Allosensitization

Allosensitization is defined as the development of HLA antibodies following exposure to a sensitizing event such as:

- Pregnancy,
- Blood/product transfusion, and
- Organ transplantation.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Patients (n)</th>
<th>Stem cell source</th>
<th>Conditioning</th>
<th>Anti-HLA%</th>
<th>DSA%</th>
<th>Graft failure with/without DSA</th>
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<td>Spellman et al. (34)</td>
<td>115</td>
<td>Mismatched unrelated</td>
<td>RIC</td>
<td>ND</td>
<td>9</td>
<td>24 versus 1%</td>
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<tr>
<td>Ciurea et al. (36)</td>
<td>592</td>
<td>10/10 and 9/10 unrelated</td>
<td>MACorRIC</td>
<td>19.6</td>
<td>1.4</td>
<td>37.5 versus 2.7%</td>
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<td>Yoshihara et al. (39)</td>
<td>79</td>
<td>Haplo-identical</td>
<td>RIC</td>
<td>20.2</td>
<td>14</td>
<td>27 versus 3%</td>
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<tr>
<td>Ciurea et al. (36)</td>
<td>24</td>
<td>Haplo-identical</td>
<td>RIC</td>
<td>ND</td>
<td>21</td>
<td>60 versus 5%</td>
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<tr>
<td>Chang et al. (40)</td>
<td>345</td>
<td>Haplo-identical</td>
<td>MAC</td>
<td>25.2</td>
<td>11.3</td>
<td>61% (MFI, 10,000) versus 3.2%</td>
</tr>
<tr>
<td>Ciurea et al. (36)</td>
<td>122</td>
<td>Haplo-identical</td>
<td>Non-specified</td>
<td>ND</td>
<td>18</td>
<td>32 versus 4%</td>
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<tr>
<td>Takanashi et al. (41)</td>
<td>386</td>
<td>Single CBU</td>
<td>MAC</td>
<td>23.1</td>
<td>5</td>
<td>83 versus 32%</td>
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<tr>
<td>Cutler et al. (42)</td>
<td>73</td>
<td>Double CBU</td>
<td>MACorRIC</td>
<td>ND</td>
<td>24</td>
<td>57 versus 5.5%</td>
</tr>
<tr>
<td>Ruggeri et al. (43)</td>
<td>294</td>
<td>Single and double CBU</td>
<td>RIC</td>
<td>23</td>
<td>5</td>
<td>81 versus 44%</td>
</tr>
<tr>
<td>Yamamoto et al. (44)</td>
<td>175</td>
<td>Single CBU</td>
<td>MACorRIC</td>
<td>39.4</td>
<td>ND</td>
<td>50% if anti-HLA-C, DP, DQ, DRB1/2/3 versus 16%</td>
</tr>
</tbody>
</table>
Ciurea et al, 2015
EFFECT OF HLA COMPATIBILITY ON ENGRAFTMENT OF BONE MARROW TRANSPLANTS IN PATIENTS WITH LEUKEMIA OR LYMPHOMA

Claudio Anasetti, M.D., Deborah Amos, Patrick G. Beatty, M.D., Ph.D., Frederick R. Appelbaum, M.D., William Bensinger, M.D., C. Dean Buckner, M.D., Reginald Clift, Kristine Doney, M.D., Paul J. Martin, M.D., Eric Mickelson, Brenda Nisperos, John O’Quigley, Ph.D., Robert Ramberg, Jean E. Sanders, M.D., Patricia Stewart, M.D., Rainer Storb, M.D., Keith M. Sullivan, M.D., Robert P. Witherspoon, M.D., E. Donnall Thomas, M.D., and John A. Hansen, M.D.
HLA Mismatches & Donor Source

- Father: 1,8,10, 3,14,17, 10,16,8
- Mother: 2,7,11, 10,16,8
- Brother: 1,8,10, 10,16,8, 2,7,11
- You*: 1,8,10, 2,7,11, 3,14,17
- Sister: 2,7,11, 3,14,17, 10,16,8
- Brother*: 1,8,10
- Sister: 3,14,17

* Identical match
Allogeneic HCT Recipients in the US, by Donor Type

- URD-BM / PB
- HLA-iden Sib
- Other Relative
- URD / UCB

Number of Transplants

Year


CIBMTR
CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH
The European Society for Blood and Marrow Transplantation (EBMT) Consensus Guidelines for the Detection and Treatment of Donor-specific Anti-HLA Antibodies (DSA) in Haploidentical Hematopoietic Cell Transplantation

Stefan O. Ciurea¹ · Kai Cao¹ · Marcelo Fernandez-Vina² · Piyanuch Kongtim³ · Monzr Al Malki⁴ · Ephraim Fuchs⁵ · Leo Luznik⁵ · Xiao-Jun Huang⁶ · Fabio Ciceri⁷ · Franco Locatelli⁸ · Franco Aversa⁹ · Luca Castagna¹⁰ · Andrea Bacigalupo¹¹ · Massimo Martelli¹² · Didier Blaise¹³ · Rupert Handgretinger¹⁴ · Denis-Claude Roy¹⁵ · Paul O’Donnell¹⁶ · Asad Bashey¹⁷ · Hillard M. Lazarus¹⁸ · Karen Ballen¹⁹ · Bipin N. Savani²⁰ · Mohamad Mohty²¹ · Arnon Nagler²²,²³
How To Measure Allosensitization
Parameters / Characteristics

Titers
MFIs

Breadth
PRA
cPRA

Strength

Biology
CDC
C1q, C4d
IgG Subclass

Baylor Scott & White
Baylor University Medical Center
Waco, Texas
Taxonomy Ab Detection Assays

Bray et al 2004
Donor lymphocytes

Pronase

+ Patient serum

+ FITC-anti-IgG

Flow cytometer

[Graphs showing data analysis results]
Taxonomy

Bray et al 2004
Advanced multiplexed analysis with the FlowMetrix™ system

R. Jerrold Fulton,* Ralph L. McDade, Perry L. Smith, Laura J. Kienker,¹ and John R. Kettman Jr.¹
The bead is filled with the special dye mixture.

5.6 microns

100 xMAP microsphere sets
<table>
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<tr>
<th>Test Configuration</th>
<th>Threshold</th>
<th>%SA8</th>
<th>%SA6</th>
<th>%SA4</th>
<th>%SA2</th>
<th>PC</th>
<th>NC</th>
<th>PC/NC</th>
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<td>30</td>
<td>260.266</td>
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<td>B54.Bw6</td>
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</table>
**DSA vs. XM**

- **Positive DSA**: Recipient has HLA antibodies that correspond to the donor mismatched HLA antigens detected by solid phase assay
- **Positive Crossmatch**: Recipient has antibodies that react to antigens on donor lymphocytes (*HLA or Non-HLA including autoantibodies*)
Figure 1: All-cause graft loss, by antibody strength. PCC, positive cytotoxic crossmatch; PFNC, positive flow, negative cytotoxic crossmatch; PLNF, positive Luminex, negative flow crossmatch.

Figure 2: Posttransplant mortality, by antibody strength. PCC, positive cytotoxic crossmatch; PFNC, positive flow, negative cytotoxic crossmatch; PLNF, positive Luminex, negative flow crossmatch.
Detection by PE Conjugated Anti-C1q

1. Add C1q to HI serum sample
2. Add HLA antigen coated beads
3. Add PE conjugated anti-C1q
4. Wash
5. Read with LABScan™100

Legend:
- C1q
- Sample antibody
- Complement binding antibody
- PE conjugated anti-C1q
- HLA antigen coated beads

http://www.onelambda.com
Biology: Biomarkers

Complement-Binding Donor-Specific Anti-HLA Antibodies and Risk of Primary Graft Failure in Hematopoietic Stem Cell Transplantation

Stefan O. Ciurea 1,*, Peter F. Thall 2, Denái R. Milton 2, Titus H. Barnes 3, Piyanuch Kongtim 1, Yudith Carmazzi 3, Asdrúbal A. López 3, Dianne Y. Yap 3, Uday Popat 1, Gabriela Rondon 1, Benjamin Lichtiger 3, Fleur Aung 3, Vahid Afshar-Kharghan 4, Qing Ma 1, Marcelo Fernández-Viña 5, Richard E. Champlin 1, Kai Cao 3
REVIEW

Donor HLA-specific Abs: to BMT or not to BMT?

MS Leffel\textsuperscript{1}, RJ Jones\textsuperscript{2} and DE Gladstone\textsuperscript{2}
<table>
<thead>
<tr>
<th>DSA/XM strength</th>
<th>Preconditioning</th>
<th>Day – 1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Day +1, +2</th>
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<tr>
<td>Low-level DSA+, FCXM –</td>
<td>0</td>
<td>1</td>
<td>If needed, based on day – 1 DSA level&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>CDC XM –, FCXM+</td>
<td>3–4</td>
<td>1</td>
<td>Day +1; day +2 if needed, based on day – 1 DSA level&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>CDC XM titer 1–4</td>
<td>5–6</td>
<td>1</td>
<td>Day +1 and day +2; recommend monitoring DSA level on days +3 and +5&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>CDC XM HLA class I titer ≥ 8</td>
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<td>Consider other options</td>
</tr>
<tr>
<td>CDC XM HLA class II DSA ≥ 1</td>
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<sup>a</sup> DSA/XM strength is determined by the presence or absence of donor-specific antibodies (DSAs) and crossmatch (XM) results.

<sup>b</sup> Day – 1 refers to the day before the transplant.

<sup>c</sup> If needed, based on day – 1 DSA level.

<sup>d</sup> Day +1; day +2 if needed, based on day – 1 DSA level.

<sup>e</sup> Day +1 and day +2; recommend monitoring DSA level on days +3 and +5.

Leffell et al, 2015
HLA Ab Identification

Prediction of Crossmatch results (Virtual XM; vXM)

- Presence of DSA predicts Pos XM
- Absence of DSA Predicts Neg XM
BMT / TRANSPLANT IMMUNOLOGY LAB VIRTUAL CROSSMATCH FORM

To be completed by Transplant Coordinator

Date Completed: _______________       Date Serum Drawn _______________

Patient Name: _______________       DOB: _______________

Donor Selected: ○ Related       ○ Unrelated       ○ Haplo-matched

Donor Name or NMDP ID#: ______________________

DOB (if Applicable): _______________

Planned date of transplantation: __________ / Conditioning Regimen Start Date: __________

Transplant Coordinator: _______________       Physician: _______________

Please review all antibody testing done up to this date. Please make sure the most recent antibody testing has been reported and the virtual crossmatch has been performed on the most recent sample.
To be completed by Transplant Immunology

Donor Selected: HLA-Identical Sibling: ○ YES ○ NO

HLA mismatched alleles/antigens: ____________________________________________

Recipient and selected Donor are matched for all tested loci (A, B, C, DRB1, DRB345, DQB1, DPB1) and VXM and additional HLA antibody testing is not warranted: ○ YES ○ NO

Date Request Received: __________

* Serum Date: _________________ Date Serum Received: ____________

DSA Present: ○ YES ○ NO

DSA (MFI: 1,000-3,999): ______________________________________________________

DSA (MFI: 4,000-10,000): ____________________________________________________

DSA (MFI: >10,000): _________________________________________________________

VXM: T cell crossmatch: __________ B cell crossmatch: _________________

Reviewed By: ______________________ Date: ____________

HLA Antibody Report will include VXM results

Comments: _______________________________________________________________

* Note: VXM may change if blood products received after this date
Patient Testing

- HLA Matching
- KIR
- Engraftment
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Baylor Scott & White
Baylor University Medical Center
Dallas
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<th>DQB1</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DQB1</th>
<th>DQB3</th>
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<td>05:01</td>
<td>06:02</td>
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<td>01:FX</td>
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Genotype Frequency as a Search Prognosis Tool

Patient HLA Type

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<tr>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
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<td>14:01</td>
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+ Patient Ethnicity

Genotype Frequency Tool

Good
• ≥ 3 10/10 donors

Fair
• 1-2 10/10s or
• No 10/10s and
• ≥ 3 9/10 donors

Poor
• No 10/10s and
• < 3 9/10 donors

Wadsworth, K. et. al. Bone Marrow Transplant 2016 Nov;51(11):1476-81

Grab your cape.
<table>
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<th>Pr(n) of 10%</th>
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<th>C</th>
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<th>DQB1</th>
<th>DRB3</th>
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So What? Who Cares?

WE'VE CHANGED OUR MIND. WE DON'T CARE ANY MORE!

THE BANK THAT CARES

Grab your cape.
High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation

Probability of Overall Survival by HLA Matching for Early Disease Stage

Log-rank p-value = < 0.0001

8/8 HLA Matched (n=835)
7/8 HLA Matched (n=379)
6/8 HLA Matched (n=241)

50%
39%
28%

Curtsey of Stephanie Lee
Probability of Overall Survival by HLA Matching for Intermediate Disease Stage

Log-rank p-value < 0.0001

8/8 HLA Matched (n=674)
7/8 HLA Matched (n=412)
6/8 HLA Matched (n=268)

32%
27%
22%

Curtesy of Stephanie Lee
Probability of Overall Survival by HLA Matching for Advanced Disease Stage

Log-rank p-value = 0.02

8/8 HLA Matched (n=327)
7/8 HLA Matched (n=195)
6/8 HLA Matched (n=123)

Curtsey of Stephanie Lee
HLA Match Likelihoods for Hematopoietic Stem-Cell Grafts in the U.S. Registry

Loren Gragert, B.S., B.A., Mary Eapen, M.B., B.S., Eric Williams, Ph.D., John Freeman, B.S., Stephen Spellman, M.B.S., Robert Baitty, M.P.P., Robert Hartzman, M.D., J. Douglas Rizzo, M.D., Mary Horowitz, M.D., Dennis Confer, M.D., and Martin Maiers, B.A.
High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation

Table 2. Single locus mismatches at HLA-A, -B, -C, and -DRB1

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<th>Treatment-related mortality</th>
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<th>Acute graft-versus-host disease</th>
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<td>RR</td>
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Lee et al, 2007
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<td>0.92-1.24</td>
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Refractory Graft-Versus-Host Disease–Free, Relapse-Free Survival as an Accurate and Easy-to-Calculate Endpoint to Assess the Long-Term Transplant Success

Koji Kawamura 1, Hideki Nakasone 1, Saiko Kurosawa 2, Kazuki Yoshimura 1, Yukiko Misaki 1, Ayumi Gomyo 1, Jin Hayakawa 1, Masaharu Tamaki 1, Yu Akahoshi 1, Machiko Kusuda 1, Kazuaki Kameda 1, Hidenori Wada 1, Yoko Ishihara 1, Miki Sato 1, Kiriko Terasako-Saito 1, Misato Kikuchi 1, Shun-ichi Kimura 1, Aki Tanihara 1, Shinichi Kako 1, Heiwa Kanamori 3, Takehiko Mori 4, Satoshi Takahashi 5, Shuichi Taniguchi 6, Yoshiko Atsuta 7,8, Yoshinobu Kanda 1,9,*
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* is the number for survival model. Other models may have had different results.
B*44:02~C*05:01 (Top 3)
B*44:03~C*16:01 (Top 5)
Grab your cape.
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* is the number for survival model. Other models may have had similar results.
Identification of a permissible HLA mismatch in hematopoietic stem cell transplantation

Grade 3-4 aGVHD

TRM
HLA Mismatch Is Associated with Worse Outcomes after Unrelated Donor Reduced-Intensity Conditioning Hematopoietic Cell Transplantation: An Analysis from the Center for International Blood and Marrow Transplant Research

Michael R. Verneris¹,*, Stephanie J. Lee², Kwang Woo Ahn³, Hai-Lin Wang⁴, Minoo Battiwalla⁵, Yoshihiro Inamoto², Marcelo A. Fernandez-Vina⁶, James Gajewski⁷, Joseph Pidala⁸, Reinhold Munker⁹, Mahmoud Aljurf¹⁰, Wael Saber⁴, Stephen Spellman¹¹, John Koreth¹²
Non-permissive -DPB1 mismatch among otherwise HLA-matched donor-recipient pairs results in increased overall mortality after myeloablative unrelated allogeneic hematopoietic cell transplantation for hematologic malignancies

The importance of HLA-DPB1 in unrelated donor hematopoietic cell transplantation

Bronwen E. Shaw,1,2 Theodore A. Gooley,3 Mari Malkki,3 J. Alejandro Madrigal,1,4 Ann B. Begovich,5 Mary M. Horowitz,6 Alois Gratwohl,7 Olle Ringdén,8 Steven G. E. Marsh,1,4 and Effie W. Petersdorf3,9

1Anthony Nolan Research Institute, London, United Kingdom; 2Section of Haemato-Oncology, Royal Marsden Hospital/Institute of Cancer Research, Surrey, United Kingdom; 3Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA; 4Royal Free and University College London Medical School, London, United Kingdom; 5Roche Molecular Systems, Alameda, CA; 6Center for International Blood and Marrow Transplant Research, Medical College of Wisconsin, Milwaukee; 7Hematology Department, University Hospital Basel, Basel, Switzerland; 8Division of Clinical Immunology, Karolinska University Hospital Huddinge, Stockholm, Sweden; and 9Department of Medicine, University of Washington School of Medicine, Seattle
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<td>P = .03)</td>
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Shaw et al 2007
Effect of T-cell-epitope matching at HLA-DPB1 in recipients of unrelated-donor haemopoietic-cell transplantation: a retrospective study

Katharina Fleischhauer*, Bronwen E Shaw*, Theodore Gooley, Mari Malkki, Peter Bardy, Jean-Denis Bignon, Valérie Dubois, Mary M Horowitz, J Alejandro Madrigal, Yasuo Morishima, Machteld Oudshoorn, Olle Ringden, Stephen Spellman, Andrea Velardi, Elisabetta Zino, Effie W Petersdorf, on behalf of the International Histocompatibility Working Group in Hematopoietic Cell Transplantation
Figure 1 An algorithm for nonpermissive HLA-DPB1 disparities according to TCE3 or TCE4

A

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B

RECIPIENT DPB1 GROUP

DONOR DPB1 GROUP

Predictions in the Face of Clinical Reality: HistoCheck versus High-Risk HLA Allele Mismatch Combinations Responsible for Severe Acute Graft-versus-Host Disease

Medhat Askar, Ronald Sobecks, Yasuo Morishima, Takakazu Kawase, Amy Nowacki, Hideki Makishima, Jaroslaw Maciejewski
Figure 1. Comparison of SSM in high- versus low-risk mismatch allele combinations. SSM means are not significantly different, and SSM distributions are overlapping among high- and low-risk allele combinations within loci HLA-A, -B, -C, -DRB1, and -DPB1.
Scoring HLA Class I Mismatches by HistoCheck Does Not Predict Clinical Outcome in Unrelated Hematopoietic Stem Cell Transplantation

Stephen Spellman,¹ John Klein,² Michael Haagenson,¹ Medhat Askar,³ Lee Ann Baxter-Lowe,⁴ Jun He,⁵ Susan Hsu,⁶ Rainer Blaszyk,⁷ Carolyn Hurley⁸

Biol Blood Marrow Transplant 18:739-746, 2012
Hickey et al, 2016

Groove-Domain

Class I

1 Exon 2 Exon 3 Exon 4

5 6 7 8

Class II

1 Exon 2 Exon 3

4 5 6

TM Cytoplasmic Tail

Plasma membrane

Peptide Peptide-binding groove

α1, α2, α3, β1, β2, β2m
Non-Coding Sequences
GENOMIC ORGANIZATION OF THE HLA GENES

HLA-A

HLA-B

HLA-C

HLA-DQA1

HLA-DQB1

HLA-DRB1

HLA-DPA1

HLA-DPB1

Courtesy of Marcelo Fernandez-Vina
Differential microRNA regulation of HLA-C expression and its association with HIV control

Smita Kulkarni1,2, Ram Savan3, Ying Qi1,2, Xiaojiang Gao1,2, Yuko Yuki1,2, Sara E. Bass1, Maureen P. Martin1,2, Peter Hunt4, Steven G. Deeks4, Amallo Telenti5, Florencia Pereyra1, David Goldstein6, Steven Wolinsky7, Bruce Walker2, Howard A. Young1 & Mary Carrington1,2

28 April 2011 | Vol 472 | Nature | 495
Polymorphisms in the putative miRNA binding sites in the 3’UTR of HLA-C

Kulkarni et al 2011
Influence of HLA-C Expression Level on HIV Control

Richard Apps et al.
Science 340, 87 (2013);
DOI: 10.1126/science.1232685
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Overall Read Coverage

Diagram 1: C*05:01:01:02 vs C*07:01:01:01

Diagram 2: C*05:01:01:02 vs C*07:01:01:01
HLA-C expression levels define permissible mismatches in hematopoietic cell transplantation

Effie W. Petersdorf,1,2 Theodore A. Gooley,1 Mari Malkki,3 Andrea P. Bacigalupo,3 Anne Cesbron,4 Ernette Du Toit,5 Gerhard Ehninger,6 Torstein Egeland,7 Gottfried F. Fischer,8 Thibaut Gervais,9 Michael D. Haagenson,10 Mary M. Horowitz,11 Katharine Hsu,12 Pavel Jindra,13 Alejandro Madrigal,14 Machteld Oudshoorn,15 Olle Ringdén,16 Marlis L. Schroeder,17 Stephen R. Spellman,10 Jean-Marie Tiercy,18 Andrea Velardi,19 Campbell S. Witt,20 Colm O’Huigin,21 Richard Apps,21,22 and Mary Carrington,21,22 for the International Histocompatibility Working Group in Hematopoietic Cell Transplantation

Key Points

• The expression level of patient HLA-C allotypes affects GVHD and mortality after HCT from HLA-C-mismatched unrelated donors.
• Transplant outcome can be improved by avoiding high-risk HLA-C-mismatched donors when no matched stem cell source is available.
High HLA-DP Expression and Graft-versus-Host Disease

Effie W. Petersdorf, M.D., Mari Malkki, Ph.D., Colm O'hUigin, Ph.D., Mary Carrington, Ph.D., Ted Gooley, Ph.D., Michael D. Haagenson, M.S., Mary M. Horowitz, M.D., Stephen R. Spellman, M.B.S., Tao Wang, Ph.D., and Philip Stevenson, M.S.
Patient Testing

- HLA Matching
- KIR
- Engraftment
What elephant?
The Bottom Line

The Killer Immunoglobulin-Like Receptor Dilemma: How Do We Harness the Power of Killer Immunoglobulin-like Receptors?

Medhat Askar*

Department of Pathology and Laboratory Medicine, Baylor University Medical Center, Dallas, Texas
Applications of KIR Genotyping

Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants

Loredana Ruggeri,¹ Marusca Capanni,¹ Elena Urbani,¹ Katia Perruccio,¹ Warren D. Shlomchik,² Antonella Tosti,¹ Sabrina Posati,¹ Daniela Rogaia,¹ Francesco Frassoni,³ Franco Aversa,¹ Massimo F. Martelli,¹ Andrea Velardi¹*

www.sciencemag.org  SCIENCE  VOL 295  15 MARCH 2002
HLA-C–Dependent Prevention of Leukemia Relapse by Donor Activating KIR2DS1

Jeffrey M. Venstrom, M.D., Gianfranco Pittari, M.D., Ted A. Gooley, Ph.D., Joseph H. Chewning, M.D., Stephen Spellman, M.S., Michael Haagenson, M.S., Meighan M. Gallagher, B.A., Mari Malkki, Ph.D., Effie Petersdorf, M.D., Bo Dupont, M.D., D.Sc., and Katharine C. Hsu, M.D., Ph.D.
KIR and HLA Genotypes Predictive of Low-Affinity Interactions Are Associated with Lower Relapse in Autologous Hematopoietic Cell Transplantation for Acute Myeloid Leukemia

John Marra,* Justin Greene,* Jimmy Hwang,† Juan Du,* Lloyd Damon,* Tom Martin,* and Jeffrey M. Venstrom*

The Journal of Immunology, 2015, 194: 4222–4230
KIR Nomenclature

- Receptors: 3DL1, 3DL2
- Extracellular region
- Cytoplasmic tail: ITIM, ITIM

Rajalingam, 2002
KIR Nomenclature

Receptors
- 3DL1
- 3DL2
- 3DS1

Extracellular region

Cytoplasmic tail
- ITIM
- ITIM

Rajalingam, 2002
KIR Nomenclature

Receptors
- 3DL1
- 3DL2
- 3DS1
- 2DL1
- 2DL2
- 2DL3
- 2DL4
- 2DL5

Extracellular region

Cytoplasmic tail
- ITIM
- ITIM

Rajalingam, 2002
KIR Nomenclature

Receptors
- 3DL1
- 3DL2
- 3DS1
- 2DL1
- 2DL2
- 2DL3
- 2DL4
- 2DL5
- 2DS1
- 2DS2
- 2DS3
- 2DS4
- 2DS5

Extracellular region

Cytoplasmic tail
- ITIM
- ITIM

Rajalingam, 2002
KIR Ligands

Kulkarni et al, 2008
KIR Ligands

Kulkarni et al, 2008
KIR Ligands

Kulkarni et al, 2008
### KIR Alleles

<table>
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<th>Gene</th>
<th>2DL1</th>
<th>2DL2</th>
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HLA class I and KIR: functionally related gene clusters exhibiting extreme polymorphism

**MHC**
- MICB
- MICA
- HLA-B
- HLA-C
- HLA-E
- HLA-J
- HLA-H
- HLA-G
- HLA-F

**LRC**
- ILT
- LAIR
- ILT
- KIR
- FcR
- Nkp46
- GPV

**chr. 6p21.3**

**chr. 19q13.4**

**Haplotype A**
- 3DL3 2DL3 2DP1 2DL1 3DP1
- 2DL4 3DL1 2DS4 3DL2

**Haplotype B**
- 3DL3 2DS2 2DL2 3DP1
- 2DL4 3DS1 2DL5 2DS5 2DS1 3DL2

- inhibitory
- activating
- pseudogene
Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia

Sarah Cooley,1 Daniel J. Weisdorf,1 Lisbeth A. Guethlein,2 John P. Klein,3 Tao Wang,3 Chap T. Le,4 Steven G. E. Marsh,5 Daniel Geraghty,6 Stephen Spellman,7 Michael D. Haagenson,8 Martha Ladner,9 Elizabeth Trachtenberg,9 Peter Parham,2 and Jeffrey S. Miller1
### Table: Centromeric and Telomeric Part Motifs

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<th>2DL3</th>
<th>2DL5B</th>
<th>2DS3/5</th>
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<th>2DL1</th>
<th>3DP1</th>
<th>2DL4</th>
<th>3DL1</th>
<th>3DS1</th>
<th>2DL5A</th>
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### Table B

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<th>Centromeric (2DS2, 2DL2, 2DL3)</th>
<th>N (%)</th>
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<tr>
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<td>AML</td>
<td>ALL</td>
</tr>
<tr>
<td>Cen-A/A 2DL3 only</td>
<td>532 (49)</td>
<td>155 (48)</td>
</tr>
<tr>
<td>Cen-A/B 2DL3 with 2DS2 and/or 2DL2</td>
<td>439 (40)</td>
<td>140 (43)</td>
</tr>
<tr>
<td>Cen-B/B 2DS2 and/or 2DL2; no 2DL3</td>
<td>115 (11)</td>
<td>28 (9)</td>
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</table>

<table>
<thead>
<tr>
<th>Telomeric (3DL1, 3DS1, 2DS1, 2DS4)</th>
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<tr>
<td>Tel-A/A 3DL1 and 2DS4 only</td>
<td>659 (61)</td>
<td>185 (57)</td>
</tr>
<tr>
<td>Tel-A/B 3DL1 and 2DS4 with 3DS1 and/or 2DS1</td>
<td>382 (35)</td>
<td>119 (37)</td>
</tr>
<tr>
<td>Tel-B/B lacking 3DL1 and/or 2DS4</td>
<td>45 (4)</td>
<td>19 (6)</td>
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### Table C

<table>
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<tr>
<th>Donor KIR Genotype Score</th>
<th>Donor KIR Genotype</th>
<th>B content</th>
<th>N (%)</th>
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<td>B/B</td>
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<tr>
<td>A/B</td>
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<td>A/B</td>
</tr>
<tr>
<td>B/B</td>
<td>2</td>
<td></td>
<td>A/B</td>
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<td>A/B</td>
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Cooley et al, 2010
Cooley et al, 2010

<table>
<thead>
<tr>
<th>AML</th>
<th>Relapse</th>
<th>Disease-free survival (DFS)</th>
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<tr>
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<td><img src="image" alt="Relapse Graph" /></td>
<td><img src="image" alt="Disease-free Survival Graph" /></td>
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<tr>
<td></td>
<td>Cen-A/A N=515</td>
<td>Cen-B/B N=114</td>
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<tr>
<td></td>
<td>Cen-A/B N=425</td>
<td>Cen-A/A N=515</td>
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<tr>
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<td>Cen-B/B N=114</td>
<td>Cen-A/B N=425</td>
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<table>
<thead>
<tr>
<th>ALL</th>
<th>Relapse</th>
<th>Disease-free survival (DFS)</th>
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<td><img src="image" alt="Relapse Graph" /></td>
<td><img src="image" alt="Disease-free Survival Graph" /></td>
</tr>
<tr>
<td></td>
<td>Cen-A/A N=154</td>
<td>Cen-B/B N=28</td>
</tr>
<tr>
<td></td>
<td>Cen-A/B N=136</td>
<td>Cen-A/A N=154</td>
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<tr>
<td></td>
<td>Cen-A/B N=136</td>
<td>Cen-B/B N=28</td>
</tr>
</tbody>
</table>

Time since transplantation (years)
Engraftment Monitoring
Clinical Utility of Quantitative PCR for Chimerism and Engraftment Monitoring after Allogeneic Stem Cell Transplantation for Hematologic Malignancies

Müberra Ahci ¹, Karin Stempelmann ¹, Ulrike Buttkereit ², Pietro Crivello ¹, Mirko Trilling ³, Andreas Heinold ⁴, Nina Kristin Steckel ², Michael Koldehoff ², Peter A. Horn ⁴, Dietrich W. Beelen ², Katharina Fleischhauer ¹,⁵,*
TRANSPLANTATION

Engraftment Kinetics After Nonmyeloablative Allogeneic Peripheral Blood Stem Cell Transplantation: Full Donor T-Cell Chimerism Precedes Alloimmune Responses


<table>
<thead>
<tr>
<th>Chimerism</th>
<th>Dynamic process</th>
<th>Definition</th>
<th>Implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete chimerism (CC)</td>
<td>Only donor DNA in a given post-transplant sample.</td>
<td></td>
<td>Further follow-up to survey engraftment or to detect recurrence of autologous cells during later periods.</td>
</tr>
<tr>
<td>Mixed chimerism (MC)</td>
<td>Both donor and recipient DNA detectable in a post-transplant sample.</td>
<td></td>
<td>Close follow-up to recognize dynamic changes.</td>
</tr>
<tr>
<td>Decreasing</td>
<td>Recipient DNA immediately post transplant, which spontaneously decreases over time.</td>
<td></td>
<td>Weekly follow-up until CC is established.</td>
</tr>
<tr>
<td>Stable</td>
<td>Both donor and recipient DNA. Relation does not significantly change over time, for example, in patients with SCID or often in patients with nonmalignant disease after reduced conditioning regimens.</td>
<td></td>
<td>Close monitoring during engraftment, thereafter longer intervals, for example, bimonthly to realize late graft rejection.</td>
</tr>
<tr>
<td>Increasing</td>
<td>Recipient DNA is increasing compared to the foregoing sample by at least 5%.</td>
<td></td>
<td>Pre-emptive immunotherapy in patients with hematological malignant diseases is recommended. In patients with nonmalignant diseases only when autologous cells exceed 30%. Analysis of T-cell and NK-cell chimerism can help to guide additional therapy to avoid graft rejection.</td>
</tr>
<tr>
<td>Split chimerism</td>
<td>Recipient DNA is not detectable in all cell lines, for example, only in T cells, whereas the other cell lines are complete donors as in SCID patients or often in patients after reduced intensity conditioning transplants. Only recipient DNA in the post-transplant sample.</td>
<td></td>
<td>Reconditioning and second transplant probably necessary.</td>
</tr>
</tbody>
</table>
Short Tandem Repeats (STR)

13 CODIS Core STR Loci with Chromosomal Positions

- TPOX
- D3S1358
- D5S818
- FGA
- CSF1PO
- D8S1179
- TH01
- VWA
- D7S820
- AMEL
- D13S317
- D16S539
- D18S51
- D21S11
- AMEL
- 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Engraftment Analysis (STR)

Figure 5.5, J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition* © 2005 Elsevier Academic Press
## Method Comparison

**Table 2** Survey of methods for chimerism analysis

<table>
<thead>
<tr>
<th>Technique</th>
<th>Merits</th>
<th>Problems</th>
<th>Sensitivity (%)</th>
<th>Applicability</th>
<th>References</th>
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<tr>
<td>RFLP</td>
<td>High informativity</td>
<td>Time consuming, labor intensive</td>
<td>5–10</td>
<td>High</td>
<td>24–27</td>
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<tr>
<td>Cytogenetics</td>
<td></td>
<td>Time consuming</td>
<td>5</td>
<td>Low</td>
<td>20, 21</td>
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<tr>
<td>Red cell phenotyping</td>
<td>Simple, accurate</td>
<td>Long latency, lineage specific</td>
<td>1–5</td>
<td>High</td>
<td>22–23</td>
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<tr>
<td>X/Y FISH</td>
<td>Low false positivity, large number of cells, high sensitivity transplants</td>
<td>Restricted to sex-mismatched transplants</td>
<td>0.1–0.001</td>
<td>Low</td>
<td>28–33</td>
</tr>
<tr>
<td>Fluorescence-based STR–PCR</td>
<td>Robust, fast, high quantitative accuracy</td>
<td>Moderate sensitivity</td>
<td>1–5</td>
<td>Very high</td>
<td>44–47</td>
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<tr>
<td>STR in subpopulation</td>
<td>Very high sensitivity</td>
<td>Labor intensive, expensive</td>
<td>0.1–0.001</td>
<td>Very high</td>
<td>17, 59</td>
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<tr>
<td>Real-time PCR</td>
<td>High sensitivity, rapid</td>
<td>False-positive results in SNP-based assays, high specificity in Y-chromosome-specific PCRs</td>
<td>0.001–0.0001</td>
<td>Medium–high</td>
<td>49–50, 54–56</td>
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</tbody>
</table>

FISH = fluorescent in situ hybridization; RFLP = restriction fragment length polymorphism; STR = short tandem repeat markers.

Bader et al., 2005
OR27: Novel Next Generation Sequencing Based Chimerism Assay for Engraftment Monitoring in Hematopoietic Cell Transplantation
Artificial Mixes

• Samples of known chimeric status, ranging from 0%-100% recipient DNA

\[ y = 0.9989x + 0.2047 \]

\[ R^2 = 0.9977 \]
<table>
<thead>
<tr>
<th>Value</th>
<th>Patient %</th>
<th>95% CI (Lower)</th>
<th>95% CI (Upper)</th>
<th># Markers</th>
<th>Limit of Detection</th>
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<td>28</td>
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# Precision

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<td>3.1</td>
<td>3.08</td>
<td>3.73</td>
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<td>4.02</td>
<td>3.58</td>
<td>4.19</td>
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<tr>
<td>5.65</td>
<td>5.41</td>
<td>5.01</td>
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</tbody>
</table>
Project: Haplotypes in Families

Haplotypes in Families by NGS

Project leaders: Medhat Askar & Kazutoyo Osoegawa

Anthropological study of families by NGS of full-length HLA genes to determine haplotype segregation in multiple populations. Samples from family quartets consisting of two parents and at least two non-HLA identical children or family trios consisting of one parent and at least two non-HLA identical children are required. The families should be previously HLA typed at any level (serology, DNA).
Investigators may submit samples only or samples and NGS data.
The HLA Allele Cataloguing Project

Project leaders: Faviel Gonzalez, Derek Middleton, Kazutoyo Osoegawa & Medhat Askar

In this project participants are invited to submit data of samples of HLA alleles that are novel, classified as rare or with no enough information in the Allele Frequency Net Database (AFND, http://www.allelefrequencies.net). The project has 2 components:

1. Submission of data to AFND confirming alleles that fulfill the criteria set above. These data will also be shared with the CWD alleles working group to be considered in generating future CWD alleles are equivalent/alternate lists.
Summary

• Matching at HLA-A, -B, -C, and DRB1 remains to have the highest impact on clinical outcomes

• There is accumulating evidence of clinical relevance of DP, DQ and non-coding sequences

• Advances in sequencing technology will continue to reduce cost, increase HLA typing resolution, precision of engraftment monitoring

• Presence of donor HLA specific antibodies are associated with failure of engraftment and can be managed proactively