Transplant Webinar Series: Ep. 5
Post Hematopoietic Stem Cell Transplant  Chimerism Testing & Engraftment Monitoring
Donor Selection for Haematopoietic Stem Cell Transplantation

Featuring
Dr Deborah Sage
NHSBT Tooting
London, UK

24 May 2018

Link to register:
https://immucor.webinato.com/register
Handouts

Continuing Education

- ABHI, ASCLS/P.A.C.E., Florida and California Credits
- 1.0 Contact Hour or 0.15 continuing education credits (CECs) awarded
- Each attendee must register to receive CE credits at:
  
  https://www.surveymonkey.co.uk/r/ImmucorTransplantEp5

- Registration deadline is 4 May 2018
- Certificates will be sent via email only to those who have registered by 18 May 2018
Presentation Recording

• Session will be recorded and posted to Immucor’s LEARN site.
  – Access information will be sent to each registrant when the recording becomes available

• CE credits will be issued to anyone who listens to the recording within one year of the original presentation date (today).

• To access Learn go to: learn.immucor.com
Questions?

- You are all muted
- Q&A following session - Type in questions
• Course content is for information and illustration purposes only. Immucor makes no representation or warranties about the accuracy or reliability of the information presented and this information is not to be used for clinical or maintenance evaluations.

• The opinions contained in this presentation are those of the presenter and do not necessarily reflect those of Immucor.
Post Hematopoietic Stem Cell Transplant Chimerism Testing & Engraftment Monitoring

Anil Handoo, MD
Sr Consultant Hematologist, Director Labs & Transfusion Medicine
Director Academic Affairs, Research & Continuing Education (AARCE)
BLK Super Speciality Hospital, New Delhi
anil.handoo@blkhospital.com
Bone marrow transplantation and or peripheral blood stem cell transplantation

- a well-established treatment procedure for many malignant and non-malignant disorders
History

- Hematopoietic stem cell transplantation in the mouse
  - the radiation protection phenomenon (mid-1950s)
- Hematopoietic stem cell transplantation in the dog
- Hematopoietic stem cell transplantation in human patients
  - 1959–1963: first allogeneic HSCT in humans
  - beginning of the Modern Era of HSCT: the end of 1960
The Nobel Prize, 1990

E. Donnall Thomas

first successful HSCT in treatment of acute leukemias

HSCT - definition

- Definition
  any procedure where hematopoietic stem cells of any donor and any source are given to a recipient with intention of repopulating/replacing the hematopoietic system in total or in part
Milestones in the field of allogeneic transplant.

Transplant Physicians’ Wish list.........
Stem Cell Transplant - Process

SCT

Conditioning

Peripheral blood cell counts

HOST CELLS

DONOR CELLS

Peripheral blood cell counts

Weeks Post Transplant

Non-Myeloablative

Myeloablative
Stem Cell Transplant - Process

Period of anxious waiting!

Weeks Post Transplant

Peripheral blood cell counts

Peripheral blood cell counts

HOST CELLS

DONOR CELLS

Non-Myeloabative

Myeloabative
Tell me u have seen a neutrophil!
Engraftment Monitoring
Engraftment Monitoring

Storek, et al 2004
Transplanted Stem cells

Diseased Cells
Post Transplant Monitoring

Whether the newly developed hematopoietic system is of recipient or donor origin?

The best way to determine the same is by determining the genotypic origin of post-transplant hematopoiesis

CHIMERISM!

&

IMMUNE RECONSTITUTION
‘Chimera’ refers to Greek mythology where Homer described a fire-spitting monster with the head of a lion, the body of a goat and the tail of a serpent terrorizing Lycia, a region in Asia Minor, and which was finally sacrificed by the ancient hero Bellerophon.
The term chimerism

First introduced into medicine by Anderson et al10 in 1951 – ‘chimera is an organism whose cells derive from two or more distinct zygote lineages’

First used in the field of transplantation by Ford in 1956
Chimerism is a dynamic process following allogeneic HSCT. Its kinetics depend on

- The intensity of the conditioning regimen
- Sensitivity of different cell types to chemotherapy and radiation,
- The recipient’s prior therapies,
- Composition of the graft, and
- Other factors.
Chimerism Analysis - Role

- Crucial for major therapeutic decisions

These include:

- Dosing and cessation of immunosuppression post-HSCT
- Transfusion of donor lymphocytes or other cellular therapy
- Administration of immunomodulatory cytokines

Consequences - both life-saving and/or life-threatening - relapse and/or GVHD
<table>
<thead>
<tr>
<th>Chimerism state</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete chimerism</td>
<td>100% donor cells detected, suggesting complete hematopoietic replacement</td>
</tr>
<tr>
<td>Mixed chimerism</td>
<td>Host cells are detected in particular cells like lymphocytes. Five to 90% donor cells set the criteria for MC</td>
</tr>
<tr>
<td>Split chimerism</td>
<td>One or more lineages are of host and one or more lineages are of donor, like myeloid cells are 100% host and T-cells are 100% donor</td>
</tr>
<tr>
<td>Micro-chimerism</td>
<td>Less than 1% host cells detected, normally found in solid organ transplants</td>
</tr>
</tbody>
</table>
# Chimerism, definition and implications

<table>
<thead>
<tr>
<th>Chimerism</th>
<th>Dynamic process</th>
<th>Definition</th>
<th>Implication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete chimerism (CC)</td>
<td></td>
<td>Only donor DNA in a given post-transplant sample.</td>
<td>Further follow-up to survey engraftment or to detect recurrence of autologous cells during later periods.</td>
<td>Short intervals are recommended during engraftment in malignant and non-malignant diseases, for example, weekly. This allows timely intervention when chimerism status is changing.</td>
</tr>
</tbody>
</table>
## Chimerism, definition and implications

<table>
<thead>
<tr>
<th>Chimerism</th>
<th>Dynamic process</th>
<th>Definition</th>
<th>Implication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed chimerism (MC)</td>
<td></td>
<td>Both donor and recipient DNA detectable in a post-transplant sample.</td>
<td>Close follow-up to recognize dynamic changes</td>
<td>MC depends on the sensitivity of the used method, for example, STR–PCR about 1–3%</td>
</tr>
<tr>
<td>Decreasing</td>
<td></td>
<td>Recipient DNA immediately post transplant, which spontaneously decreases over time.</td>
<td>Weekly follow-up until CC is established.</td>
<td>-</td>
</tr>
</tbody>
</table>
# Chimerism, definition and implications

<table>
<thead>
<tr>
<th>Chimerism</th>
<th>Dynamic process</th>
<th>Definition</th>
<th>Implication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed chimerism (MC)</td>
<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>Close monitoring during engraftment, thereafter longer intervals, for example, bimonthly to realize late graft rejection.</td>
<td>Weekly investigations during engraftment including T-cell and NK-cell chimerism</td>
</tr>
</tbody>
</table>
**Chimerism, definition and implications**

<table>
<thead>
<tr>
<th>Chimerism</th>
<th>Dynamic process</th>
<th>Definition</th>
<th>Implication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed chimerism (MC)</td>
<td>Increasing</td>
<td>Recipient DNA is increasing compared to the foregoing sample by at least 5%.</td>
<td>Pre-emptive immunotherapy in patients with hematological malignant diseases is recommended.</td>
<td>Protocol based, weekly analyses are performed during the first 200 days because most relapses occur during this period in patients with acute leukemia.</td>
</tr>
</tbody>
</table>

In patients with nonmalignant diseases only when autologous cells exceed 30%.
<table>
<thead>
<tr>
<th>Chimerism</th>
<th>Dynamic process</th>
<th>Definition</th>
<th>Implication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Split chimerism (SC)</td>
<td></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</td>
<td>Analysis of T-cell and NK-cell chimerism can help to guide additional therapy to avoid graft rejection</td>
<td>Analysis of cell subpopulations enables to distinguish between residual malignant cells and nonmalignant hematopoiesis and increases the sensitivity when chimerism is used as indirect MRD marker</td>
</tr>
</tbody>
</table>
## Chimerism, definition and implications

<table>
<thead>
<tr>
<th>Chimerism</th>
<th>Dynamic process</th>
<th>Definition</th>
<th>Implication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous recovery</td>
<td></td>
<td>Only recipient DNA in the posttransplant sample</td>
<td>Reconditioning and second transplant probably necessary</td>
<td></td>
</tr>
</tbody>
</table>


Cell Lineage-Specific Chimerism
Cell Lineage-Specific Chimerism

Low-intensity conditioning (LIC) HSCT leads to Mixed chimerism of donor and recipient cells existing in a state of host and graft tolerance.

? relative contribution of donor cells to the different T, B and myeloid cell lineages.

Important implications for certain congenital immunodeficiencies :

For disease correction/donor engraftment –

- ?engraftment of donor cells in multiple cell lineage
- ?engraftment in a particular lineage is sufficient for disease correction.
### Examples of Cell Lineage-Specific Chimerism Analyses For Congenital Immunodeficiencies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease</th>
<th>Type of HSCT</th>
<th>PB/WB %</th>
<th>CD3+ %</th>
<th>CD15+ %</th>
<th>CD19+ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>ADA-SCID</td>
<td>NC, MFD</td>
<td>38</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P2</td>
<td>X-SCID</td>
<td>NC, HAPLO</td>
<td>51</td>
<td>100</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>P3</td>
<td>Undefined - SCID</td>
<td>C,MUD</td>
<td>16</td>
<td>44</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>P4</td>
<td>Undefined - SCID</td>
<td>C,MUD</td>
<td>17</td>
<td>77</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>P5</td>
<td>WAS</td>
<td>C,MUD</td>
<td>30</td>
<td>84</td>
<td>4</td>
<td>63</td>
</tr>
<tr>
<td>P6</td>
<td>Neutrophil Disorder</td>
<td>C,MUD</td>
<td>95</td>
<td>22</td>
<td>100</td>
<td>63</td>
</tr>
</tbody>
</table>

**KEY:** NC = no conditioning, C = conditioning, MFD = matched family donor, haplo = haplo-identical donor, MUD = matched unrelated donor.

Excerpt from MACS & more Vol 8 – 2/2004
Lineage-specific chimerism analysis in nonmyeloablative HSCT and its prediction for graft status

<table>
<thead>
<tr>
<th>Lineage-specific chimerism*</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower levels of CD3+, CD8+ donor cell engraftment</td>
<td>Rejection</td>
</tr>
<tr>
<td>Lower levels of CD34+, CD38− donor cell engraftment</td>
<td>Graft failure</td>
</tr>
<tr>
<td>MC in myeloid lineages</td>
<td>Relapse but not lymphoid malignancies</td>
</tr>
<tr>
<td>Mixed chimerism in T-cell subset</td>
<td>Lower risk of acute GVHD</td>
</tr>
</tbody>
</table>

Chimerism Analysis - Methods

Early Methods of Chimerism Analysis
- Cytogenetics
- Blood Group Phenotyping
- Sex Mis-match

PCR-based methods – based on amplification of polymorphic repetitive DNA Sequence
- Short tandem repeats (STR)
- Variable number of tandem repeats (VNTR)
### Chimerism Analysis - Methods

<table>
<thead>
<tr>
<th>Technique</th>
<th>Merits</th>
<th>Problems</th>
<th>Sensitivity (%)</th>
<th>Applicability</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFLP</td>
<td>Highly informative</td>
<td>Time consuming, labor intensive</td>
<td>5-10</td>
<td>High</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td></td>
<td></td>
<td>5</td>
<td>Low</td>
</tr>
<tr>
<td>Red Cell Phenotyping</td>
<td>Simple, accurate</td>
<td>Long latency, lineage specific</td>
<td>1-5</td>
<td>High</td>
</tr>
<tr>
<td>X/Y FISH</td>
<td>Low false positivity, large number of cells, high sensitivity</td>
<td>Restricted to sex-mismatched transplants</td>
<td>0.1-0.001</td>
<td>Low</td>
</tr>
</tbody>
</table>

### Chimerism Analysis - Methods

<table>
<thead>
<tr>
<th>Technique</th>
<th>Merits</th>
<th>Problems</th>
<th>Sensitivity (%)</th>
<th>Applicability</th>
</tr>
</thead>
<tbody>
<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>
</tr>
<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>
</tr>
<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>
</tr>
</tbody>
</table>

Chimerism Analysis - Methods

Most accepted method

STR-based chimerism analysis

Advantages

☑ Predict impending relapse
☑ Prevent overt relapse by pre-emptive immunotherapy

Disadvantages

☑ Not possible to realize impending relapse in all patients
☑ The time interval between the conversion of chimerism and relapse can be very short
What are STRs?

- **Short Tandem Repeats (STR)** are repetitive sequences:
  - Tetranucleotide: `AAAG AAAG AAAG AAAG`
  - Trinucleotide: `CTT CTT CTT CTT CTT`
  - Dinucleotide: `AG AG AG AG AG AG AG`

- **Tetra-nucleotides** are favored in HSCTs
  - Good balance of “ease of interpretation” and “variability found in nature”
Short Tandem Repeats

Advantages

- Plentiful
- Small amounts of sample required
- Discrete, easily assigned alleles using allelic ladders
- Digital recording of data
- Rapid DNA purification methods available
- Low molecular weight DNA may be used
- Non-radioactive detection
- Small, defined size ranges allow multiplex detection
- Potential for automation

Disadvantages

- Care required to avoid contamination
- Amplification may produce artifacts
- Less polymorphic than Southern-based VNTR loci
D18S51 “D18”
Chromosomal location FL 18q21.3

1 aattgagcnc aggagtttaa gaccagcctg ggtaacacag tgagacccct gtctctcaca
61 aaaaatacaa aatnagtttg ggcattgtgg cacgtgcctg tagtctcagc taccttgccag 121 gctgaggcag gaggagttct tgagcccaga aggttaaggc tgcagtgagc catgttcatg 181 ccactgcact tcactctgag tgacaaatgg agacctttgtc tcagaaagaag 241 aaagaaagaa agaaaagaaag 301 agaaanagaa aanaaatagtt agcaactgttt attgttaagac atctccacac accagagaag 361 ttaaatttttt aaaaaacatg ttaagaacag agagaagcca acatgtccac cttaggcttaa 421 cggttggggtt atttgtggtg tggctggtag tcggggtttgt tatttttttt aagttttttt 481 caatactttca ttaacaattt ccagttaagttt tttcatcttta caacataaat acgnacaagg 541 attttctttctg gtcaagacca aactaatatt agtcccatagt agagcataat actatcacat 601 ttactaagttt ttctatttttc aatattgactg tagcccatag cttttggtcg gttaagttga 661 gcttaaatgtc gatcgactct agag

The repeat sequence is aaga – this particular individual has 14 repeats
The locus is “where it’s at”

Locus—the physical position of an STR and its associated flanking sequence

Both chromosomes of a homologous pair contain this locus

The allele contained on either chromosome can be the same or different lengths (homozygous or heterozygous)
Chromosome Spread showing the positions of the amplified loci in PowerPlex®16

The PowerPlex® 16 System amplifies 16 loci.

- TPOX
- D3S1358
- FGA
- D5S818
- CSF1PO
- D7S820
- D8S1179
- THO1
- vWA
- Amelogenin
- D13S317
- Penta E
- D16S539
- D18S51
- D21S11
- Amelogenin
- Penta D
Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

Short Tandem Repeats (STRs)

• Repeat region is variable (**polymorphic**)  
  – Each variant is referred to as an **allele**

• Flanking region is constant

**KEY:** Alleles are distinguished by length

Homozygote = both alleles are the same length
Heterozygote = alleles differ and can be resolved from one another
Separating and “Seeing” STR’s

- **Electrophoresis**
  - Separates amplification products based on size
- **Fluorescent detection**
  - Amplification products have a fluorescent “label” attached to the primer
  - Label is seen through excitation via a laser and corresponding emission captured with a camera
Allelic Ladder
Whole Blood Chimerism

Recipient profile

Recipient informative allele

Shared uninformative allele

Donor profile

Donor informative allele

Whole blood chimerism

Recipient and donor alleles both evident
Cell Lineage-Specific Chimerism

- Whole blood chimerism: Recipient and donor alleles visible in whole blood sample.
- CD33 Myeloid fraction: 
- CD3 T-cell fraction: Large proportion of Recipient allele in T-cell population.
- CD19 B-cell fraction.
- CD56 NK fraction.
Example: PS - Post HSCT Chimerism
Example: PS - Post HSCT Chimerism

<table>
<thead>
<tr>
<th>Marker</th>
<th>%DCHM</th>
<th>LE</th>
<th>ME</th>
<th>Ignored</th>
<th>%DCHM</th>
</tr>
</thead>
<tbody>
<tr>
<td>D8S1179</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Yes(Auto)</td>
<td></td>
</tr>
<tr>
<td>D21S11</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Yes(Auto)</td>
<td></td>
</tr>
<tr>
<td>D2S820</td>
<td>100.00%</td>
<td>0.41%</td>
<td>29.67%</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>C9I10</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Yes(Auto)</td>
<td></td>
</tr>
<tr>
<td>C9I10E9</td>
<td>95.88%</td>
<td>3.77%</td>
<td>NaN</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

Average Chimerism: 99.59%
St. Dev: 1.30
Coefficient of Variation: 1.31%
MCE: 0.93 (95%)
Number of Informative Loci: 10
Utility

Chimerism analysis serves more likely as a ‘prognostic factor’ due to:

- Provision of information about the alloreactivity and/or tolerance induction of the graft

**IT IS NOT AN INDIRECT MARKER FOR MINIMAL RESIDUAL DISEASE (MRD) – Low sensitivity – about 1%**

Taken together, serial and quantitative analysis of chimerism in the whole peripheral blood by STR–PCR allows the identification of patients at the highest risk for relapse.
The course of post transplant follow-up is given of a 5-year-old boy with c-ALL, who received an haploidentical transplantation in CR2.

The course of a 16-year-old girl with c-ALL, who was transplanted in CR2 from an HLA-identical unrelated donor. This patient received a low dose DLI 1105/kg body weight (BW) when increasing mixed chimerism was developed. This immunotherapy led to a conversion of mixed to complete chimerism and by then MRD was cleared.

Chimerism analysis in myeloablative and nonmyeloablative HSCT

Bone Marrow Transplantation (2004) 34, 1–12
MRD studies are becoming an integral part of the modern management of patients with leukemia.

The selection of the methods to be used in each center depends on the expertise and facilities available and on collaborative links that can be established with laboratories proficient in MRD detection.

The main challenge for expert MRD investigators is to identify new robust markers of leukemia that would allow the simplification and export of the current methods while maintaining or increasing their reliability, thus disseminating the potential benefits of MRD monitoring to all patients.
MRD is predictive of outcome

in first line

in relapsed patients

in the context of transplantation

MRD can detect relapses earlier
Conclusions...

- Chimerism analysis is a valuable tool for monitoring engraftment

- Used as indicator for the recurrence of the underlying disease

- Is a basis for treatment intervention, for example, to avoid graft rejection, to maintain engraftment and to treat imminent relapse by pre-emptive immunotherapy

- Quantitative fluorescence-based STR–PCR with capillary electrophoresis for PCR product resolution is most often used technique

- MRD analyses are performed in bone marrow and peripheral blood samples according to the underlying disease with leukemia clone-specific PCR systems.
Thank You
Questions?

• You are all muted
• Q&A following session - Type in questions
We like you!

Like us on social media!
Questions?

• You are all muted

• Q&A following session - Type in questions

Thank you for joining the webinar today. We will begin shortly.

While the eCurtain is down, you are unable to hear anyone speak, or see any image.
Continuing Education

• ABHI, ASCLS/P.A.C.E., Florida and California Credits
• 1.0 Contact Hour or 0.15 continuing education credits (CECs) awarded
• Each attendee must register to receive CE credits at:
  
  https://www.surveymonkey.co.uk/r/ImmucorTransplantEp5

• Registration deadline is 4 May 2018
• Certificates will be sent via email only to those who have registered by 18 May 2018
Future Webinars

Donor Selection for Haematopoietic Stem Cell Transplantation
Featuring
Dr Deborah Sage
NHSBT Tooting
London, UK
24 May 2018

Link to register:
https://immucor.webinato.com/register
Thank you!