There are no conflicts of interest to disclose.
Chimera
Why are chimerism studies important?

1) Cell count recovery doesn’t distinguish between autologous or allogeneic recovery.

2) Determines the percent of donor and host cells in a blood or marrow sample following allogeneic HCT.

3) In the case of multiple cord blood units, chimerism studies can distinguish which is the dominant cord.
Case Study #1

• **Recipient**: 30 year old female s/p myeloablative allo HCT for AML in CR2

• **Donor**: HLA-matched brother
Case Study #1

A FISH assay for the XX/XY chromosome can be used to determine chimerism due to sex mismatch
Case Study #1

• Peripheral blood samples were collected on Day +30 & Day +90 to evaluate donor engraftment post-HCT.

  ➢ **Day +30**
  
  500 PBMCs were analyzed by FISH
  400 cells were XY & 100 cells were XX

  ➢ **Day +90**
  
  550 PBMCs were analyzed by FISH
  500 cells were XY & 50 cells were XX
Case Study #1

• F2100 Q99-102
Case Study #1

• Reporting FISH results......
  Day +30

  104. Total cells examined: ___________
  105. Number of donor cells: ___________ - Go to question 108
  106. Were donor cells detected?

  X Yes → 107. Percent donor cells: ___ 80___ %
  □ No

Copy questions 92 – 107 if needed for multiple chimerism studies.

Day +90

  104. Total cells examined: ___________
  105. Number of donor cells: ___________ - Go to question 108
  106. Were donor cells detected?

  X Yes → 107. Percent donor cells: ___ 90___ %
  □ No

Copy questions 92 – 107 if needed for multiple chimerism studies.
Case Study #2

- **Recipient**: 45 year old male s/p non-myeloablative allogeneic HCT for DLBCL (in 1st relapse- sensitive)
- **Donor**: unrelated male donor
Case Study #2

- Peripheral blood samples were sent for CD3+ chimerism by STR at Day +30, +60 & +90 to evaluate donor engraftment.
Case Study #2

- STR report

```
Findings
Specimen type
Blood; enriched for CD3+ cells

STR Enriched Identity Testing
Collection date/time: 6/29/2012 3:01 PM
Received date/time: 7/3/2012 10:18 AM

CD3 cell count and viability: 0.2x10^6 88%
CD15 cell count and viability: 0.08x10^6 25%
Pre CD3: 14   Post CD3: 76   Post CD15: 36
Pre CD15: 66   Post CD3: 3   Post CD15: 1

<table>
<thead>
<tr>
<th>Locus</th>
<th>Result</th>
<th>Post CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>Mixed chimerism</td>
<td>67 % Donor, 33 % Recipient</td>
</tr>
<tr>
<td>VWA</td>
<td>Mixed chimerism</td>
<td>68 % Donor, 32 % Recipient</td>
</tr>
<tr>
<td>FGA</td>
<td>Mixed chimerism</td>
<td></td>
</tr>
<tr>
<td>AMEL</td>
<td>Non-informative</td>
<td></td>
</tr>
<tr>
<td>D8S1179</td>
<td>Mixed chimerism</td>
<td></td>
</tr>
<tr>
<td>D21S11</td>
<td>Mixed chimerism</td>
<td></td>
</tr>
<tr>
<td>D18S51</td>
<td>Mixed chimerism</td>
<td></td>
</tr>
<tr>
<td>D5S818</td>
<td>Mixed chimerism</td>
<td></td>
</tr>
<tr>
<td>D13S317</td>
<td>Mixed chimerism</td>
<td></td>
</tr>
<tr>
<td>D7S820</td>
<td>Non-informative</td>
<td></td>
</tr>
</tbody>
</table>
```
Case Study #2

• STR reporting- Day +30
  ➢ Choose one of the informative alleles to follow
  ➢ In this example, DNA marker D3S1358 was used
Reporting STR/VNTR Results

If cell sorting was done to isolate a particular cell line (e.g., CD3+ cells) prior to performing a DNA based assay such as STR, do not report the # of cells sorted.
Reporting STR/VNTR Results

• 150,000 CD3+ cells are isolated from blood via cell sorting. The results of the STR assay indicated a mixed chimera-
  • 75% donor & 25% host

• Report the percentage of donor
• Do not report the number of sorted cells.
Reporting multi-donor chimerism

• Testing requires DNA markers (loci) that are informative for both the recipient & each donor

• Short Tandem Repeat (STR) polymorphisms are very useful for this purpose
  ➢ Each loci typically have more than two alleles
Reporting multi-donor chimerism

• What is a “polymorphism”?
  ➢ It’s a variation in the DNA that is too common to be due merely to a new mutation
  ➢ A polymorphism must have a frequency of at least 1% in the population
### Reporting multi-donor chimerism

- **Recipient before HCT**

<table>
<thead>
<tr>
<th>Genetic Marker</th>
<th>Identified Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>16</td>
</tr>
<tr>
<td>TH01</td>
<td>6</td>
</tr>
<tr>
<td>D21S11</td>
<td>30, 33</td>
</tr>
<tr>
<td>D18S51</td>
<td>14, 19</td>
</tr>
<tr>
<td>Penta E</td>
<td>12, 14</td>
</tr>
</tbody>
</table>
## Reporting multi-donor chimerism

- **Donor 1**

<table>
<thead>
<tr>
<th>Genetic Marker</th>
<th>Identified Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>14, 16</td>
</tr>
<tr>
<td>TH01</td>
<td>9, 10</td>
</tr>
<tr>
<td>D21S11</td>
<td>29, 31</td>
</tr>
<tr>
<td>D18S51</td>
<td>14, 15</td>
</tr>
<tr>
<td>Penta E</td>
<td>11</td>
</tr>
</tbody>
</table>
## Reporting multi-donor chimerism

**Donor 2**

<table>
<thead>
<tr>
<th>Genetic Marker</th>
<th>Identified Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>15</td>
</tr>
<tr>
<td>TH01</td>
<td>8, 10</td>
</tr>
<tr>
<td>D21S11</td>
<td>30, 33</td>
</tr>
<tr>
<td>D18S51</td>
<td>15, 18</td>
</tr>
<tr>
<td>Penta E</td>
<td>5, 14</td>
</tr>
</tbody>
</table>
Reporting multi-donor chimerism

**Recipient**
An informative allele is one the recipient has, but the donor(s) does not.

**Donor**
An informative allele is one the donor has, but the recipient does not.
Reporting multi-donor chimerism

• Non-informative alleles
  ➢ Both recipient & donor(s) share the same allele
  ➢ In the case of multiple donors, both donors share the same allele
Reporting multi-donor chimerism

- **Recipient** informative alleles (alleles the donors don’t possess)

<table>
<thead>
<tr>
<th>Genetic Marker</th>
<th>Identified Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH01</td>
<td>6</td>
</tr>
<tr>
<td>D18S51</td>
<td>19</td>
</tr>
<tr>
<td>Penta E</td>
<td>12</td>
</tr>
</tbody>
</table>
## Reporting multi-donor chimerism

- **Donor 1** informative alleles

<table>
<thead>
<tr>
<th>Genetic Marker</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>14</td>
</tr>
<tr>
<td>TH01</td>
<td>9,10</td>
</tr>
<tr>
<td>D21S11</td>
<td>29,31</td>
</tr>
<tr>
<td>D18S51</td>
<td>15</td>
</tr>
<tr>
<td>Penta E</td>
<td>11</td>
</tr>
</tbody>
</table>
### Reporting multi-donor chimerism

- **Donor 2** informative alleles

<table>
<thead>
<tr>
<th>Genetic Marker</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>15</td>
</tr>
<tr>
<td>TH01</td>
<td>8,10</td>
</tr>
<tr>
<td>D21S11</td>
<td>----</td>
</tr>
<tr>
<td>D18S51</td>
<td>15,18</td>
</tr>
<tr>
<td>Penta E</td>
<td>5</td>
</tr>
</tbody>
</table>
Reporting multi-donor chimerism

- Donor 1 & 2
- Non-informative donor alleles include
  - TH01 allele 10
  - D18S51 allele 15
- Since both donors possess these two alleles, they cannot be used to determine the percentage of each donor post HCT.
## Reporting multi-donor chimerism

- **Recipient after HCT**

<table>
<thead>
<tr>
<th>Genetic Marker</th>
<th>Identified Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>14, 15, 16*</td>
</tr>
<tr>
<td>TH01</td>
<td>8, 9, 10</td>
</tr>
<tr>
<td>D21S11</td>
<td>29, 30*, 31, 33*</td>
</tr>
<tr>
<td>D18S51</td>
<td>14*, 15, 18</td>
</tr>
<tr>
<td>Penta E</td>
<td>5, 11, 14*</td>
</tr>
</tbody>
</table>

*An allele shared by one of the donors & the recipient (i.e., not informative)*
Reporting multi-donor chimerism

• Questions to consider……

- Is there evidence of one or two donors post-HCT?
- Is there any evidence of recipient (host) post-HCT?
Reporting multi-donor chimerism

- Chimerism results for a recipient of a double cord HCT must equal 100%

**STR results revealed:**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor 1</td>
<td>5%</td>
</tr>
<tr>
<td>Donor 2</td>
<td>95%</td>
</tr>
<tr>
<td>Host</td>
<td>0%</td>
</tr>
</tbody>
</table>
Reporting multi-donor chimerism

- F2100 Reporting

99. Method
- Karyotyping for XX/XY
- Fluorescent in situ hybridization (FISH) for XX/XY
- Restriction fragment-length polymorphisms (RFLP)
- VNTR or STR, micro or mini satellite (also include AFLP)
- Other → 100. Specify: __________

101. Cell source
- Bone marrow
- Peripheral blood

102. Cell type
- Unsorted / whole - Go to question 104
- Red blood cells - Go to question 106
- Hematopoietic progenitor cells (CD34+ cells) - Go to question 106
- Total mononuclear cells (lymphs & monos) - Go to question 106
Reporting multi-donor chimerism

• Donor 1

104. Total cells examined: ____________
105. Number of donor cells: _______ - Go to question 108
106. Were donor cells detected?
   Yes -> 107. Percent donor cells: ____5____%
   □ No

Copy questions 92 – 107 if needed for multiple chimerism studies.

• Donor 2

104. Total cells examined: ____________
105. Number of donor cells: _______ - Go to question 108
106. Were donor cells detected?
   Yes -> 107. Percent donor cells: ____95____%
   □ No

Copy questions 92 – 107 if needed for multiple chimerism studies.
Reporting multi-donor chimerism

If you get the following STR result:

Donor 1 = 0%
Donor 2 = 100%
Host = 0%

You still must report both donors:

Donor 1 = 0% (Host = 0%)
Donor 2 = 100% (Host = 0%)
Reporting multi-donor chimerism

• Donor 1

104. Total cells examined: __________
105. Number of donor cells: _______  - Go to question 108
106. Were donor cells detected?
   ☒ Yes  →  107. Percent donor cells: _____ 0%  
   ☐ No

Copy questions 92 – 107 if needed for multiple chimerism studies.

• Donor 2

104. Total cells examined: __________
105. Number of donor cells: _______  - Go to question 108
106. Were donor cells detected?
   ☒ Yes  →  107. Percent donor cells: ______ 100%  
   ☐ No

Copy questions 92 – 107 if needed for multiple chimerism studies.
General Guidelines

• No need to report every chimerism result
• Results should be reported on or around
  ➢ Day +30
  ➢ Day +100
  ➢ 6 months
  ➢ Annually
General Guidelines

If a recipient receives an intervention such as a DLI, report:

• The last chimerism result(s) just prior to the intervention
• The first result after the intervention
Questions