Potency & Release Testing for unrelated donor cord blood units

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Pediatric Blood and Marrow Transplant Program
Carolinas Cord Blood Bank
Duke University Medical Center
Selecting CBUs for Transplantation

◆ At the time of Search:
  ◆ Pre-Cryo Total Nucleated Cell (TNC) Count
  ◆ Human Leukocyte Antigen (HLA) Match
  ◆ ?CD34 Pre-Cryo
  ◆ ?CFU Pre-Cryo

◆ At CT: after potency/release testing:
  ◆ Colony Forming Units (CFU)
  ◆ CD34 (+/-) viability
  ◆ ALDHbright
  ◆ TNC +/- Viability
  ◆ High Resolution HLA +/- HLA C
  ◆ ?NIMA
  ◆ ?KIR
Post-Thaw CFU Predicts Engraftment and Survival (p=<0.0001)

Overall Survival by CFU x 10^4/kg Reinfused

- CFU measured on thawed CBU bag
- 2 week assay
- Subjective results
- Difficult to standardize

ALDH bright Assay

- Enzyme activity: Oxidation of aldehydes to carboxylic acids
- Enriched in hematopoietic stem cells
- Clinically relevant for transplantation
- Cells must be viable to score positive in assay
- High correlation with CFUs (nearly 1:1 in fresh cord blood)
Cryopreserved Cord Blood Unit

- HLA CT
- TNC/uL
- CFU
- CD45
- 7-AAD
- Gly-A
- CD34
- ALDH bright
Thaw segment and remove blood with a needle

Dilute with Dextran/Albumin

Wash with HSA/PBS

Centrifugation (2000 g X 30')

30 minute incubation at 37°C with Aldecound Reagent
15 minute incubation on ice for phenotyping

Centrifugation (1200 g X 5')

Perform flow cytometry and analyze

100,000 Cells for HPCA

Activated Aldecound Reagent

CD45, CD34 and Gly A Antibodies

Fluorescent Aldecound Reagent

ALDH^+ cells
CD45^+ cells
RBC and Nonviable cells for exclusion
CD34^+ cells

Spot 2-3 drops of blood on FTA card. Ship overnight to HLA typing lab.
ALDH$^{br}$ Assay: Flow Cytometry
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>W158211491674PH</th>
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<tbody>
<tr>
<td>Sample Type</td>
<td>Segment</td>
</tr>
<tr>
<td>Bank ID</td>
<td>CCBB</td>
</tr>
<tr>
<td>Date of Receipt</td>
<td>03-NOV-2011</td>
</tr>
<tr>
<td>Site</td>
<td>Duke</td>
</tr>
<tr>
<td>Instrument</td>
<td>Accuri C6</td>
</tr>
<tr>
<td>Operator</td>
<td>Pamy Noldner</td>
</tr>
<tr>
<td>Analyst</td>
<td>Pamy Noldner</td>
</tr>
<tr>
<td>Reviewer</td>
<td>Kevin Shoulars</td>
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<tr>
<td>Date of Acquisition</td>
<td>03-NOV-2011</td>
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<tr>
<td>Starting Time</td>
<td>14:18:44:12</td>
</tr>
<tr>
<td>Ending Time</td>
<td>14:21:04:45</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Total CD45 events</th>
<th>101384</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CD34 events</td>
<td>752</td>
</tr>
<tr>
<td>CD34 as a % of CD45</td>
<td>0.74%</td>
</tr>
<tr>
<td>Viable CD34 events</td>
<td>473</td>
</tr>
<tr>
<td>Viable CD34 as a % of Viable CD45</td>
<td>0.58%</td>
</tr>
<tr>
<td>Total ALDHbr Events</td>
<td>568</td>
</tr>
<tr>
<td>ALDHbr % of Total Events</td>
<td>0.2405%</td>
</tr>
<tr>
<td>Viable CD45+ ALDHbr Events</td>
<td>489</td>
</tr>
<tr>
<td>ALDHbr as a percent of Viable CD45</td>
<td>0.5980%</td>
</tr>
<tr>
<td>Total ALDHbr cells (% +CD34/+CD45)</td>
<td>86.91%</td>
</tr>
<tr>
<td>CD34/ul</td>
<td>5.77</td>
</tr>
</tbody>
</table>

**Current Requirements for release:**

- Viable CD45 >40%
- ALDH<sup>br</sup> % of CD45 ≥0.1%
- CFU Growth

| Viability (7AAD- and GLY-A- cells as a % of CD45) | 80.65% |
| Viability (7AAD- and GLY-A- cells as a % of CD34) | 50.98% |
| Viable CD34 events | 473 |
| Viable CD34 as a % of Viable CD45 | 0.58% |
| Viable ALDHbr Events | 489 |
| ALDHbr as a percent of Viable CD45 | 0.5980% |
| BFU-E (per 1 X 10<sup>5</sup> WBC) | 48.75 |
| CFU-GM (per 1 X 10<sup>5</sup> WBC) | 41.25 |
| CFU-GEMM (per 1 X 10<sup>5</sup> WBC) | 1.25 |
| Total CFU (per 1 X 10<sup>5</sup> WBC) | 91.25 |
Retrospective Study

38 CBUs transplanted at Duke with available segments were selected

21 Rapid Engrafters (<20 days to ANC 500)
17 Non Engrafters

Total ALDH$^{br}$ x10e5 (infused per kg) based on segment data was best predictor of engraftment. CFU also predictive $p=0.006$

Shoulars et. al. ISCT, 2009 and 2012
Segments for Confirmatory Typing

- Since Feb 2010 all segments requested for confirmatory typing (CT) through the Carolinas Cord Blood Bank are being assayed.

- Samples are taken from each segment for HLA typing, CFUs and the ALDH\textsuperscript{br} assay.

- Data from 1625 segments are presented.

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
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<tbody>
<tr>
<td>Segments Assayed</td>
<td>1625</td>
</tr>
<tr>
<td>Cords Transplanted</td>
<td>283</td>
</tr>
<tr>
<td>Outcome Data Received</td>
<td>115</td>
</tr>
</tbody>
</table>
Correlation: ALDH$^{br}$ and CD34 with CFU

$y = 0.2619x$
$R^2 = 0.7151$

$y = 0.0522x + 39.897$
$R^2 = 0.0801$
Correlation of ALDH$^{br}$ with Engraftment

Engraftment rate

- 0.0-0.1% ALDH$^{br}$ = 33.33%
- 0.1-0.2% ALDH$^{br}$ = 88%
- 0.2-0.3% ALDH$^{br}$ = 93%
- 0.3-0.4% ALDH$^{br}$ = 100%

Above 0.4% ALDH$^{br}$ = 100%
ALDHBr and CFUs Correlate with Engraftment
ALDH\textsuperscript{br} Content of Segments Best Predicts Time to Engraftment

- t ratio = -2.55, p value = 0.0122
- t ratio = -1.62, p value = 0.1082
- t ratio = 0.00, p value = 1.0000
Stability vs Potency

• FDA requires a stability protocol and expiry
  – Stability implies lack of loss of potency over time in storage
  – Expiry reflects stability

• Potency, performed prior to CBU release from a TC, better represents the health of the CBU
  – Potency evaluates ability of the UCB unit to confer hematopoietic rescue, regardless of time in storage or other factors.
Stability vs Potency

• There is no data to suggest that cord blood unit potency decreases as a function of time in storage.

• Cord blood units may lose potency due to damage occurring during cryopreservation or thawing.

• To date, this damage cannot be predicted by precryopreservation characteristics of a given cord blood unit.
# Clinical Outcomes

## Distributions

### Days to ANC500

<table>
<thead>
<tr>
<th>Quantiles</th>
<th>Days to Plt 50K</th>
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<tbody>
<tr>
<td>100.0% maximum</td>
<td>104</td>
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<tr>
<td>99.5%</td>
<td>100.75</td>
</tr>
<tr>
<td>97.5%</td>
<td>54.75</td>
</tr>
<tr>
<td>90.0%</td>
<td>37</td>
</tr>
<tr>
<td>75.0% quartile</td>
<td>28</td>
</tr>
<tr>
<td>50.0% median</td>
<td>22</td>
</tr>
<tr>
<td>25.0% quartile</td>
<td>17</td>
</tr>
<tr>
<td>10.0%</td>
<td>14</td>
</tr>
<tr>
<td>2.5%</td>
<td>12</td>
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<tr>
<td>0.5%</td>
<td>7.75</td>
</tr>
<tr>
<td>0.0% minimum</td>
<td>7</td>
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<tr>
<td>Moments</td>
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</tr>
<tr>
<td>Mean</td>
<td>24.722892</td>
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<tr>
<td>Std Dev</td>
<td>12.281621</td>
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<tr>
<td>Std Err Mean</td>
<td>0.7783161</td>
</tr>
<tr>
<td>Upper 95% Mean</td>
<td>26.255844</td>
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<tr>
<td>Lower 95% Mean</td>
<td>23.189939</td>
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### Days to ANC500

<table>
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<tr>
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<tr>
<td>99.5%</td>
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<tr>
<td>97.5%</td>
<td>223.85</td>
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<tr>
<td>50.0% median</td>
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<td>25.0% quartile</td>
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<td>0.0% minimum</td>
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<tr>
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<td>76.650599</td>
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## Summary Statistics

### Days to ANC500

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### Days to ANC500

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<tr>
<td>Upper 95% Mean</td>
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<tr>
<td>Lower 95% Mean</td>
<td>52.399961</td>
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<tr>
<td>N</td>
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</table>
Relationship of Cell Dose and Time in Storage of CBU to Engraftment

- **PreFrz NCC (in 10^7) per Kg**
  - Leverage Plot
  - Days to ANC 500
  - Leverage Residuals
  - PreFrz NCC (in 10^7) per Kg Leverage, P=0.0018

- **DaysFromCollToInfusion**
  - Leverage Plot
  - Days to ANC 500
  - Leverage Residuals
  - DaysFromCollToInfusion Leverage, P=0.0704
Post thaw recoveries of TNCC, CFU and CD34 as a function of time in storage

**Bivariate Fit of Recovery TNCC By DaysFromColleToInfusion**

**Summary of Fit**
- Recovery TNCC = 0.8006416 - 1.7678e-5*DaysFromColleToInfusion
- Rsquare = 0.000576
- R2Adjusted = 0.00051
- Root Mean Square Error = 0.17416
- Mean of Response = 0.77988
- Observations (or Sum Wgts) = 680

**Analysis of Variance**
- Source: Model 1 0.136122 0.136122 4.4878
- Source: Error 678 20.967702 0.94503 0.0345

**Parameter Estimates**
- Term: Intercept 0.8006416 0.012054 66.44 < 0.0001
- Term: DaysFromColleToInfusion -1.7678e-5 8.345e-6 -2.12 0.0345

**Bivariate Fit of Recovery CFU By DaysFromColleToInfusion**

**Summary of Fit**
- Recovery CFU = 0.2806612 - 0.911e-9*DaysFromColleToInfusion
- Rsquare = 0.000551
- R2Adjusted = -0.00867
- Root Mean Square Error = 0.278414
- Mean of Response = 0.275515
- Observations (or Sum Wgts) = 110

**Analysis of Variance**
- Source: Model 1 0.004862 0.004862 0.0028
- Source: Error 108 0.3715284 0.07554 0.0345

**Parameter Estimates**
- Term: Intercept 0.004862 0.004862 0.0028
- Term: DaysFromColleToInfusion -0.911e-9 5.90 < 0.0001

**Bivariate Fit of Recovery CD34+ By DaysFromColleToInfusion**

**Summary of Fit**
- Recovery CD34+ = 0.7265346 + 2.1707e-6*DaysFromColleToInfusion
- Rsquare = 0.000034
- R2Adjusted = -0.00866
- Root Mean Square Error = 0.26511
- Mean of Response = 0.728743
- Observations (or Sum Wgts) = 117

**Analysis of Variance**
- Source: Model 1 0.0002735 0.000275 0.0038
- Source: Error 115 0.0829612 0.070283 0.0002

**Parameter Estimates**
- Term: Intercept 0.7265346 0.042956 16.91 < 0.0001
- Term: DaysFromColleToInfusion 2.1707e-6 3.468e-5 0.06 0.9902
Correlations with ALDHbr, CD34 and CFU Measured on a Segment with Engraftment

Fit Y by X Group
- Bivariate Fit of Days to ANC 500 By ALDHbr (% of viable CD45)
- Bivariate Fit of Days to ANC 500 By CD34 (% of viable CD45)
- Bivariate Fit of Days to ANC 500 By CFU (Total Post Seg Thaw in 10^5)

Linear Fit
Days to ANC 500 = 22.082797 - 2.563832*ALDHbr (% of viable CD45)

Summary of Fit
- RSquare: 0.002585
- RSquare Adj: -0.00616
- Root Mean Square Error: 10.69188
- Mean of Response: 21.19966
- Observations (or Sum Wgts): 115

Analysis of Variance
- Source: DF Squares Mean Square F Ratio
  - Model: 1 33.776 33.776 0.2955
  - Error: 114 13032.062 114.316 Prob > F
  - C. Total: 115 13065.828

Parameter Estimates
- Term: Estimate Std Error t Ratio Prob>|t|
  - Intercept: 22.082797 1.919728 11.59 <0.001*
  - ALDHbr (% of viable CD45): -2.563832 4.716724 -0.54 0.5878

Linear Fit
Days to ANC 500 = 20.936154 + 0.295284*CD34 (% of viable CD45)

Summary of Fit
- RSquare: 0.000234
- RSquare Adj: -0.009854
- Root Mean Square Error: 10.70447
- Mean of Response: 21.19966
- Observations (or Sum Wgts): 116

Analysis of Variance
- Source: DF Squares Mean Square F Ratio
  - Model: 1 3.051 3.051 0.0266
  - Error: 114 13098.755 114.586 Prob > F
  - C. Total: 115 13065.828

Parameter Estimates
- Term: Estimate Std Error t Ratio Prob>|t|
  - Intercept: 20.936154 1.841465 11.35 <0.001*
  - CD34 (% of viable CD45): 0.2952849 0.090474 3.28 0.0012

Linear Fit
Days to ANC 500 = 21.841496 - 0.0095764*CFU (Total Post Seg Thaw in 10^5)

Summary of Fit
- RSquare: 0.001787
- RSquare Adj: -0.00967
- Root Mean Square Error: 10.69165
- Mean of Response: 21.19966
- Observations (or Sum Wgts): 116

Analysis of Variance
- Source: DF Squares Mean Square F Ratio
  - Model: 1 23.347 23.347 0.2041
  - Error: 114 13042.241 114.408 Prob > F
  - C. Total: 115 13065.828

Parameter Estimates
- Term: Estimate Std Error t Ratio Prob>|t|
  - Intercept: 21.841496 1.730291 12.81 <0.001*
  - CFU (Total Post Seg Thaw in 10^5): -0.0095766 0.021199 -0.45 0.6523
CCBB Proposed Stability Plan

- Examine results post thaw from “duke to duke” units
- Supplement with lab thaws if 3 units per year of banking were not transplanted for any given year.

- Follow segment potency testing with a plan to augment the protocol with segment testing over time
  - More samples to study
  - Faster release criteria
Conclusions

- The ALDH\textsuperscript{br} content of segments attached to cryo-preserved cold blood units correlates with engraftment after UCBT.

- ALDH\textsuperscript{br} correlates with CFUs and the cells are metabolically active and viable.

- Further prospective studies to validate these findings must be performed.
Conclusions

- Loss of Stability for cryopreserved cord blood units has not yet been demonstrated.
- Expiry has not been established.
- Potency is a superior measure of the ability of a cord blood unit to rescue hematopoiesis.
- Potency is affected by factors not associated with time in storage.
- Potency should be utilized for CBU release, regardless of time in storage.
What is the ultimate benchmark: Engraftment in the patient or Technical results in the laboratory?
Spencer, Age 11
ALD
9 years post
UCBT

Maddy, Age 13
Hurler Syndrome
12 years post
UCBT

Madison, Age 7
Krabbe Disease
7 years post
UCBT
Acknowledgements

Kevin Shoulars, PhD
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CIBMTR
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NMDP
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