Mobilization of Normal and Leukemic Stem Cells

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Normal Bone Marrow Microenvironment

• Composed of diverse population of stromal cells, osteoblasts, and osteoclasts

• Extracellular matrix rich in fibronectin, collagens, and proteoglycans

• To enter circulation, stem cells must migrate through vascular barriers

• Adhesion molecules (eg, SDF-1 and VCAM-1) tether stem cells to the bone marrow

CXCR4, chemokine receptor 4; HA, hyaluronic acid; HSC, hematopoietic stem cell; KL, kit ligand; SDF-1, stromal cell-derived factor-1; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

AMD3100 (plerixafor)

- A bicyclam molecule
- Reversibly binds to CXCR4 receptor and blocks SDF-1 interaction
- Very water soluble
- Highly charged
- Low molecular weight (MW = 502)
- Rapidly increases mobilization of CD34+ hematopoietic stem cells

Phase III trials in the US, Canada and Germany*

3101: 300 NHL Patients

150 NHL AMD3100 + G-CSF

20% difference

150 NHL G-CSF + placebo

Endpoint:
> 5 million CD34+ cells/kg of patient weight in 4 or fewer apheresis

150 NHL AMD3100 + G-CSF

3102: 300 MM Patients

150 MM AMD3100 + G-CSF

20% difference

150 MM G-CSF + placebo

Endpoint:
> 6 million CD34+ cells/kg of patient weight in 2 or fewer apheresis

DiPersio et al JCO, 2009
DiPersio et al Blood, 2009
NHL Patients (%)\(^a\) Achieving \(\geq 5 \times 10^6\) CD34+ Cells/kg by Apheresis Day – ITT Population

HR = 3.64, 95% CI (2.39, 5.45), \(P < .001\)

Kaplan-Meier Estimate of Proportion of Patients Reaching \(\geq 5 \times 10^6\) CD34+ Cells/kg

CI, confidence interval; HR, hematologic response; ITT, intention-to-treat; NHL, non-Hodgkin’s lymphoma.

\(^a\) Percentage of patients achieving collection goal expressed as Kaplan-Meier estimate.

Secondary Efficacy: Patients Achieving \( \geq 2 \) million CD34+ cells/kg in 4 Days of Apheresis

**Intent-to-Treat Population**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Plerixafor ((n=150))</th>
<th>Placebo ((n=148))</th>
<th>Estimate of Treatment Effect [95% CI]</th>
<th>( p )-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Success</td>
<td>130 (86.7%)</td>
<td>70 (47.3%)</td>
<td>39.4%A [29.7%, 49.1%]</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

A Treatment effect estimated using difference in chance of success

* \( p \)-value of difference in proportions using Pearson’s Chi-Squared test

* *DiPersio et al JCO, 2009*
What about AMD3100 to mobilize allogeneic PBSC?

Questions:

- How will AMD3100 affect homing properties of CD34+ cells?
  - Will engraftment be impaired?
  - What is the half-life of AMD3100 on CXCR4?
  - Will there be downregulation of CXCR4 and dysfunctional homing?
  - How will other cells expressing CXCR4 be affected?
    - T-cells (GvHD, immune reconstitution)
    - Dendritic cells
Murine Competetive Repopulation Studies:

G-CSF Mobilized PBSC

G-CSF treated:
B6 H-2\textsuperscript{b}, Ly5.1
250 PB KLS cells
(Kit\textsuperscript{+}, Lin\textsuperscript{-}, Sca\textsuperscript{+})

BM

BM Donor:
B6 Ly5.1/5.2
1x10\textsuperscript{6} unmanipulated cells

Competitor PBSC

1) G treated
2) AMD treated
3) G+AMD treated
B6 H-2\textsuperscript{b}, Ly5.1
250 KLS cells(Kit\textsuperscript{+}, Lin\textsuperscript{-}, Sca\textsuperscript{+})

Recipient:
B6 H-2\textsuperscript{d}, Ly5.1/5.2

AMD3100
Lineage Specific Chimerism after Competitive Repopulation

Granulocytes

% of Ly5.2+ cells

G-CSF | AMD | AMD+G

B-lymphocytes

% of Ly5.2+ cells

G-CSF | AMD | AMD+G
G-CSF, AMD3100, and G-CSF plus AMD3100 on Engraftment and GVHD

Bone Marrow Donor: B6 H-2b

4 x 10^6 TCD BM (Ly5.2)

Recipient BALB H-2d; Ly5.2

Model A (severe GVHD): 900 cGy irradiated recipients and 2 x 10^6 allogeneic T cells

Model B (moderate GVHD): 900 cGy irradiated recipients and 5 x 10^5 allogeneic T cells

Model C (graft failure): 500 cGy irradiated recipients and 2 x 10^6 allogeneic T cells

T Cell Donor: B6 H-2b

Splenic T cells (Ly5.1)

1. Naïve untreated
2. G-CSF (10μg x 4 days)
3. AMD 3100
4. G+AMD 3100
Conclusions

1. Stem cells mobilized with G vs. G+A vs. A have identical early repopulation capacity

2. T cells mobilized with G vs. G+A vs. A have identical GvHD and engraftment potential
Design: AMD3100 Allogeneic Stem Cell Mobilization Study

Baseline peripheral blood

AMD3100 peripheral blood

G-CSF peripheral blood

Pheresis

7 day rest period

Pheresis

AMD3100 (240 µg/kg)

rhG-CSF (10 µg/kg/day)

Allograft Composition (n = 22)

<table>
<thead>
<tr>
<th></th>
<th>AMD3100</th>
<th>G-CSF*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34 (x10⁶/kg)</td>
<td>3.1 (1.2-6.3)</td>
<td>4.2 (2.5-18.7)</td>
<td>0.006</td>
</tr>
<tr>
<td>CD3 (x10⁸/kg)</td>
<td>4.7 (1.5-7.8)</td>
<td>1.5 (1.2-6.8)</td>
<td>0.006</td>
</tr>
<tr>
<td>CD4 (x10⁸/kg)</td>
<td>3.1 (1.0-5.7)</td>
<td>1.1 (0.7-3.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>CD8 (x10⁸/kg)</td>
<td>1.3 (0.4-3.4)</td>
<td>0.4 (0.3-3.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>CD56 (x10⁷/kg)</td>
<td>2.8 (0.2-4.6)</td>
<td>0.2 (0.1-0.5)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*includes 8 donors mobilized by both AMD3100 and G-CSF

Devine et al Blood, 2008
AMD-ALLO: GVHD (n = 38)
AMD-ALLO: Overall Survival (n = 38)

48%
Optimal Effect of AMD3100: Cmax vs AUC

Donors 22–43

PK/PD

AMD3100 IV over 30 min
80, 160, 240, 320, 400, 480 µg/kg

-5 days

Apheresis

-6 hours

AMD3100 240 µg/kg SC

Transplant with PBSC mobilized with SC AMD3100
Dose Response of CD34$^+$ Cell Mobilization by IV AMD3100

![Graph showing the number of CD34$^+$ cells/μL over time after IV AMD3100 (h) with different dose levels: 320 μg/kg, 240 μg/kg, 160 μg/kg, and 80 μg/kg. The graph displays a time course for 0 to 24 hours, with error bars indicating variability.]
320 µg/kg IV AMD3100: Subset Analysis
Blood cell counts, total CD34 cells, and SRC frequencies after administration of AMD3100 (240\(\mu\)g/kg) or G-CSF (10 \(\mu\)g/kg/day).

<table>
<thead>
<tr>
<th>Mobilizing agent</th>
<th>WBC, x10(^3)/mL</th>
<th>Total CD34(^+), Cells/(\mu)L</th>
<th>SRC Frequency, x10(^6) MNC</th>
<th>SRC Frequency x10(^3) CD34(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMD3100</td>
<td>6.5±0.3</td>
<td>2.9±1.0</td>
<td>1 in 8.7</td>
<td>1 in 117</td>
</tr>
<tr>
<td>G-CSF</td>
<td>7.0±0.5</td>
<td>6.2±3.2</td>
<td>1 in 29</td>
<td>1 in 177</td>
</tr>
</tbody>
</table>

ND indicates not determined; Eng., engrafted; SRC, NOD/SCID repopulating cells
Genes Differentially Expressed in Human Peripheral Blood CD34+ Cells Following Mobilization with AMD3100 or G-CSF

GTPase, IMAP family member 8; PIM1 = Pim-1 oncogene; S100A8 = S100 calcium binding protein A8; SOCS3 = Suppressor of cytokine signaling 3; TMEM49 = Transmembrane protein 49; BCL-2 = B-cell CLL/lymphoma 2; CLC = Charcot-Leyden crystal protein; CXCR4 = Chemokine (C-X-C motif) receptor 4; C20orf118 = Chromosome 20 open reading frame 118; DNTT = Deoxynucleotidytransferase, terminal; IRF8 = Interferon regulatory factor 8; PRG2 = Proteoglycan 2; RASD1 = RAS, dexamethasone-induced 1; RNASE6 = Ribonuclease, RNase A family, k6; UHRF1 = Ubiquitin-like, containing PHD and RING finger domains, 1.
Probeset = 204057_at

Expression (Log Scale)

Sample Pair

mobile    A    G    U
AMD3100 Preferentially Mobilizes a CD34\textsuperscript{dim} Subset

Donor No. 2

**AMD3100**

- CD34: 87, Side Scatter: 65.2
- CD34: 13, Side Scatter: 34.8

**G-CSF**

- CD34: 97.7, Side Scatter: 96.5
- CD34: 2.31, Side Scatter: 3.51

Donor No. 3

- CD34: 96.5, Side Scatter: 96.5
- CD34: 3.51, Side Scatter: 3.51

Graph: % CD34\textsuperscript{dim} cells vs. Patient No. for AMD3100 and G-CSF, showing a significant difference (P = 0.02)
Co-expression of CD45RA on CD34\(^+\) cells identifies the CD34\(^{\text{dim}}\) subset
Expression of CD123 (IL-3Rα) and CXCR4 on CD45RA⁺CD34<sup>dim</sup> Cells

Pt. No. D40

Pt. No. D44

Pt. No. D46

CD34

CD45RA

CD45RA

CD123

CXCR4

Expression of CD123 (IL-3Rα) and CXCR4 on CD45RA<sup>+</sup>CD34<sup>dim</sup> Cells
CD45RA

- CD123

AMD3100(SC)

G-CSF

G-CSF + AMD(IV)

CD45RA-CD123⁺/-

CD45RA⁺CD123⁺/-

CD45RA⁺CD123hi

CD45RA-CD123⁺

P = .017

P = .015

P = .017

P = .015

P < .0001

P = .04

P < .0001

P < .0001

P = .026

P = .002

P < .0001

P = .0002

P = .02
**Figure 1** E2-2 function in the plasmacytoid dendritic cell (PDC) developmental pathway. DC development proceeds from hematopoietic stem cells (HSCs) through a common DC progenitor (CDP). Hypothetical precursors for PDCs (pre-PDC) may be specified by the differential expression or activation of transcription factors within CDPs. In the PDC lineage, E2-2 regulates terminal development and interferon production by controlling PDC-related genes.
AMD3100 vs. G-CSF RNA Profiling

IRF8

Scaled expression

IRF7

Scaled expression

Spi-B

Scaled expression

E2-2

Scaled expression

Raw signal intensity

BM
AMD3100
G-CSF
AMD-unmatch
G-unmatch

BM
AMD3100
G-CSF
AMD-unmatch
G-unmatch

BM
AMD3100
G-CSF
AMD-unmatch
G-unmatch

BM
AMD3100
G-CSF
AMD-unmatch
G-unmatch

P = .009

P = .009

P = .005

P = .04
AMD3100 vs. G-CSF RNA Profiling

BDCA-2

Scaled expression

P = .001

P = .01

ILT7

Scaled expression

P = .01

P = .008
AMD3100 vs. G-CSF RNA Profiling

**IRF8**
\[ r^2 = 0.115 \]

**IRF7**
\[ r^2 = 0.534 \]

**Spi-B**
\[ r^2 = 0.600 \]

**E2-2**
\[ r^2 = 0.416 \]

**BDCA-2**
\[ r^2 = 0.743 \]

**ILT7**
\[ r^2 = 0.908 \]
In vitro IFNα assay

Control = GpC
TLR9 = CpG

N=46 allografts
N=23 (50%) of patients at risk for CMV
N=5 (21%) developed viremia
N=0 (0%) developed CMV disease
Figure 12. CD62L expression on T lymphocytes. In 5 donors, CD62L expression was assessed on donor T cells before and after treatment with AMD3100 (6 h after 240 µg/kg) or G-CSF (250 µg/kg/d x 5d). Flow cytometric analysis was performed using monoclonal antibodies to CD3, CD4, CD8, and CD62L. The fold change in CD62L mean fluorescence intensity (MFI) was calculated relative to pre-treatment values.
AMD3100 Allogeneic Stem Cell Transplant Trial: Overall Survival

EXP#141 + 143 + 149

Percent survival

Days

EXP#141 + 143 + 149

% weight change

Days

- XRT only (n=6)
- pre-AMD3100 (n=19)
- post-AMD3100 (n=23)
Adhesion Molecules and HSC Mobilization

BIO5192 Blocks Binding of Jurkat Cells to Soluble VCAM-1

% of Max

64.3%

isotype control
vehicle only
BIO5192
Kinetics of Murine Progenitors Mobilization in Response to BIO5192 and Plerixafor

- BIO5192 1 mg/kg IV           n=10
- plerixaflor 5 mg/kg sc         n=10

CFU/mL blood (x10^3) vs. Time after injection (h)
Additive Mobilization of Murine Progenitors After Combination of Plerixaflor SC and BIO5192 IV
Additive Effect on Murine Progenitor
Increased mobilization of progenitors by G-CSF in splenectomized mice

![Graph showing CFU/mL blood (x10^3) over time with p-values for different time points.](image)

- **Spleen**
- **No Spleen**

<table>
<thead>
<tr>
<th>Time after G-CSF injection (h)</th>
<th>CFU/mL blood (x10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>p=NS</td>
</tr>
<tr>
<td>2</td>
<td>p=0.05</td>
</tr>
<tr>
<td>8</td>
<td>p=0.005</td>
</tr>
<tr>
<td>24</td>
<td>p=0.03</td>
</tr>
<tr>
<td>72</td>
<td>p=0.01</td>
</tr>
</tbody>
</table>
Effect of Splenectomy on AMD3100 and BIO5192 Induced HSC mobilization

**AMD3100**

- **spleen**: 13 fold
- **no spleen**: 7 fold

**BIO5192**

- **spleen**: 6 fold
- **no spleen**: 7.7 fold

**AMD3100 + BIO5192**

- **57 fold**
- **21.2 fold**
Long term repopulating assay

Periperal blood mobilized HSC competitors

B6 5.2
Mobilization

Collection of PBMCs

Recipient B6 5.1/5.2

BM competitor

B6 5.1
Bone marrow mononuclear cells

950 cGy
Engraftment of Mobilized HSC Primary Recipients

% donor engraftment

Months after transplantation

- Yellow line: unmobilized (n=3)
- Red line: plerixafor (n=3)
- Green line: BIO5192 (n=3)
- Blue line: plerixafor + BIO5192 (n=3)
- Pink line: G-CSF (n=3)
Environment-Mediated Drug Resistance

Hypothesis: Interruption of Stroma-leukemia cell contact and/or inhibition of stroma-induced signaling in leukemia cells will result in proliferation, apoptosis, differentiation, and sensitization to genotoxic stresses such as chemotherapy.
M2-10B4 Stromal Cell Line Provides In Vitro Chemoprotective to APL Cells

*** P < 0.0001

Effect of stroma on APL proliferation and spontaneous apoptosis

Stroma bound APL

APL no stroma

- Red: apoptosis
- Yellow: CFSE intensity
Reduced Proliferation of APL Cells in the Presence of M2-10B4 Stromal Cells

**Graph:**
- **Y-axis:** Percent
- **X-axis:** Stages of Cell Cycle (G0/G1, S, G2+M)
- **Legend:**
  - No stroma
  - + stroma

**Statistical Significance:**
- P = .01
- P < .001
- P = .002
Effect of stroma on mTOR pathway phosphorylation

Rapamycin

Stroma+  Stroma-

pS6
Total S6
p4E-BP1
Total 4E-BP1
Increased sirolimus-induced APL apoptosis in presence of stromal cells
**APL ENGRAFTMENT**

**APL**

$APL_{luc/GFP}^{10^6}$ IV

129/C57BL6 F1

**Ventral view**

**Dorsal view**

<table>
<thead>
<tr>
<th>day</th>
<th>4</th>
<th>7</th>
<th>11</th>
<th>14</th>
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<tbody>
<tr>
<td>%blasts</td>
<td>0%</td>
<td>2%</td>
<td>10%</td>
<td>40-50%</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%blasts</td>
<td>1-2%</td>
<td>10%</td>
<td>40%</td>
<td>80-90%</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%blasts</td>
<td>1-2%</td>
<td>20%</td>
<td>40%</td>
<td>90%</td>
</tr>
<tr>
<td>BM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%blasts</td>
<td>5,000</td>
<td>8,000</td>
<td>15,000</td>
<td>75,000</td>
</tr>
<tr>
<td>WBC/ul</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%blasts</td>
<td>50 mg</td>
<td>150 mg</td>
<td>750 mg</td>
<td>1000 mg</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
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</tr>
</tbody>
</table>
AMD3100 Mobilization of mAPL

Mobilization of leukemia cells increases the efficacy of anti-leukemic chemotherapy.

<table>
<thead>
<tr>
<th></th>
<th>Day 12</th>
<th>Day 13</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AraC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AraC + AMD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Doxo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Doxo + AMD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AMD d5-6</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10^6 APL IV

129/C57BL6 F1

n=6 mice/group

**AraC** (500mg/kg/sc); **Doxo** (10mg/kg/sc); **AMD** (5mg/kg/sc)
AMD3100 enhances effect of chemotherapy

AMD3100 enhances effect of chemotherapy

Phase I/II Study of AMD3100 + MEC in Relapsed or Refractory AML

Eligibility Criteria

1. Diagnosis of AML and either 1st refractory or relapsed disease (For pts in 1st relapse, must have CR1 duration < 12mo)
2. Age 18-65, ECOG PS < 2
3. Blast count < 30,000/mm^3
4. No previous MEC salvage

AMD3100
- Dose level 1: 80 mcg/kg SQ on d0-5
- Dose level 2: 160 mcg/kg SQ on d0-5
- Dose level 3: 240 mcg/kg SQ on d0-5

Mitoxantrone
- 8 mg/m^2 IV and 1-5

Etoposide
- 100 mg/m^2 IV and 1-5

Day 0
- Mobilization studies

Day 1
- MEC

Day 2
- MEC

Day 3
- MEC

Day 4
- MEC

Day 5
- MEC
### Patient Demographics (n=40)

<table>
<thead>
<tr>
<th>Age, median yrs (range)</th>
<th>49 (19-71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td></td>
</tr>
<tr>
<td>Core Binding Factor</td>
<td>6</td>
</tr>
<tr>
<td>M LL translocation</td>
<td>4</td>
</tr>
<tr>
<td>Normal</td>
<td>24</td>
</tr>
<tr>
<td>Complex (&gt;3 abn)</td>
<td>6</td>
</tr>
<tr>
<td>Treatment indication</td>
<td></td>
</tr>
<tr>
<td>1st relapse, 1st salvage</td>
<td>30 (median CR 1224 days)</td>
</tr>
<tr>
<td>1st refractory</td>
<td>8</td>
</tr>
<tr>
<td>1st relapse, 2nd salvage</td>
<td>2</td>
</tr>
</tbody>
</table>
Toxicities from Phase I

- No dose limiting toxicities have been observed, no hyperleukocytosis
- Neutrophil recovery at median 29.5 days (range 19-37)
- Platelet recovery at median of 26 days (range 21-40)
- Nonhematologic toxicities
  - 12/12 Febrile neutropenia
  - 1 pt with Grade 4 opportunistic infection (Aspergillus sinusitis) requiring debridement
  - 1 pt Grade 2 bradycardia
  - Grade 3 hypotension, diarrhea, colitis, confusion, back pain observed in 1 each and thought to be unrelated to administration of AMD3100
  - Grade 3 hypokalemia (<3) observed in 2 patients
## Response Evaluation

<table>
<thead>
<tr>
<th>All doses</th>
<th>240 mcg/kg</th>
<th>(n = 43)</th>
<th>(n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>17</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>CRi</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total Responses</td>
<td>20</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Persistent Disease</td>
<td>21</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Early Death</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Failures</td>
<td>23</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

Overall Response Rate: 46% 49%
## Historical RR with MEC

<table>
<thead>
<tr>
<th>Patients</th>
<th>CR Rate</th>
<th>OS</th>
<th>MEC Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tallman</td>
<td>56/114/2</td>
<td>24%</td>
<td>2.5-3.5 mos</td>
</tr>
<tr>
<td>Greenberg</td>
<td>63 vs 66 pts</td>
<td>4.6 vs 5.4 mos</td>
<td>8 mg/m2 x 5</td>
</tr>
<tr>
<td>Tallman</td>
<td>17 vs 25%</td>
<td>150 vs 50%</td>
<td>100 mg/m2 x 5</td>
</tr>
<tr>
<td>Greenberg</td>
<td>177 vs 86 pts</td>
<td>128 vs 176 days</td>
<td>180 mg/m2 x 5</td>
</tr>
<tr>
<td>Liles</td>
<td>35 vs 10%</td>
<td>156d vs 5.5 mos</td>
<td>Laromustine 600 mg/m2 on day 2</td>
</tr>
<tr>
<td>Uy/DiPersio</td>
<td>50-50 vs 60; 47</td>
<td>49%</td>
<td>HDA C 1.5 gm/m2 days 1-3 CVI</td>
</tr>
<tr>
<td>Tallman</td>
<td>47</td>
<td>NR</td>
<td>8 mg/m2 x 5</td>
</tr>
<tr>
<td>Tallman</td>
<td>100 mg/m2 x 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Effect of SC AMD3100 (240 mcg/kg) on AML Blast Mobilization
Surface CXCR4 Expression & Response

CD184(12G5)

P = 0.006

P = 0.02
Peripheral Blood FISH post AMD3100 Mobilization

<table>
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<tr>
<th>Patient #</th>
<th>Probe</th>
<th>Time Post AMD3100 (# FISH+ cells / total)</th>
<th>0 HRS</th>
<th>6 HRS</th>
<th>24 HRS</th>
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<tbody>
<tr>
<td>#1</td>
<td>MLL</td>
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<td>130/200</td>
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<tr>
<td>#2</td>
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<td>128/200</td>
<td>106/200</td>
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<tr>
<td>#3</td>
<td>CBF B</td>
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<td>12/200</td>
<td>15/200</td>
<td>11/200</td>
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<td>AML - ETO</td>
<td></td>
<td>47/200</td>
<td>33/200</td>
<td>41/200</td>
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</table>
Increased Surface CXCR4 Expression after AMD3100
Phase I/II Study of G-CSF + AMD3100 + MEC in Relapsed or Refractory AML

Eligibility Criteria:
1. Dx of AML and either refractory or relapsed disease
2. Age 18 - 65, ECOG PS < 2
3. Blast count < 30,000/mm
4. No previous MEC salvage

AMD3100: 240-480 mcg/kg IV qd/bid on d3-8
G-CSF: 10 mcg/kg SQ on d1-8
Mitoxantrone: 8 mg/m² IV on d4-8
Etoposide: 100 mg/m² IV on d4-8
Cytarabine: 1000 mg/m² IV on d4-8
MDS Protocol Design

• Phase I dose-escalating trial
  – AMD3100 (A) + G-CSF (10mcg/kg) + azacitidine (V) (75mg/m²)

Repeat for minimum 2 cycles
Expression of CXCR4, VLA-4, CD44, and E-Selectin Ligand on APL 18-4c clone
Figure 21. BIO5192 induces a rapid and transient mobilization of APL blasts into the peripheral blood. Syngeneic B6129F1 recipient mice were injected with $10^6$ APL cells. Twelve days after APL injection, mice were treated with a single IV dose of 1 mg/kg BIO5192. Peripheral blood samples were collected immediately before and 0.5, 1, 3 and 6 hours after BIO5192 administration. (A) Representative FACS profiles showing mobilization of Gr1$^+$CD34$^+$ APL blast cells. (B) APL blast cell counts.
APL Chemosensitization by G-CSF & BIO5192

1. APL\textsuperscript{Luc} = 10^6 cells/mouse IV
2. G-CSF = 250 μg/kg/day SC
3. BIO5192 = 1 mg/kg IV
4. Ara-C = 500 mg/kg SC at 4h after G-CSF/AMD injection
BIO5192 and G-CSF Enhance the Effect of Chemotherapy
Effect of 3rd Generation CXCR4 Inhibitors

![Graph showing the effect of 3rd generation CXCR4 inhibitors on CFU/μL over time (h). The graph compares different inhibitors to vehicle only.]

- **Vehicle only**
- AMD3100 (SC)
- ALT1128 (SC)
- ALT1187 (SC)
- ALT1188 (SC)
- ALT1228 (SC)
The Hematopoietic Inductive Microenvironment (HIM) niches include osteoblasts, stromal / mesenchymal cells, endothelial cells and extracellular matrix components. AML blast quiescence, proliferation and apoptosis are influenced by receptor kinases, adhesive receptors and signaling via matrix mediated / bound chemokines and cytokines. AMD3100 and AMD3465 CXCR4 antagonists +/- sorafenib or inhibitors of VLA-4/VCAM-1 interactions chemosensitize AML blasts within the HIM niches. Shh: sonic hedgehog, Notch, vascular endothelial factor and other adhesion receptors signals promote leukemic stem cell survival and expansion and can be targeted also to overcome AML chemoresistance.
CONCLUSIONS

1. AMD3100 is a safe and effective and rapidly mobilizes stem cells from mouse and man.

2. AMD3100 mobilized stem cells and T cells function normally in mouse competitive repopulation and GvHD models.

3. Infusion of AMD3100 mobilized human stem cell products into HLA matched sibling recipients can be performed in the majority of recipients after a single collection and is associated with outcomes, kinetics of engraftment and GvHD that are not dissimilar to those seen with G-CSF mobilized PBSC products.

4. APL cells were mobilized from the BM into PB after plerixafor administration.

5. Blockade of CXCR4 (via AMD3100 or G-CSF) and VLA-4 in in vivo sensitizes APL cells to chemotherapy.

6. We hypothesize that we can overcome resistance to chemotherapeutic agents by mobilizing AML from the BM into the PB. Mouse models and human clinical trials will test this hypothesis.

7. Interruption of the VLA-4/VCAM axis synergizes with CXCR4 blockade resulting in enhanced HSC and leukemic cell mobilization.
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