The Role of Plerixafor in the Mobilization of Hematopoietic Stem Cells in Patients With Diffuse Large B-Cell Lymphoma

Objectives
1. Briefly describe the pathophysiology and treatment options for Diffuse Large B-Cell Lymphoma (DLBCL)
2. Understand the benefits of high-dose chemotherapy in DLBCL and the need to support with stem cells
3. Compare the efficacy of agents/regimens used to mobilize stem cells
4. Review the history, mechanism of action and data behind the use of plerixafor for the mobilization of hematopoietic stem cells (HSCs) prior to autologous stem cell transplant (ASCT) in DLBCL
I. Epidemiology

A. Non-Hodgkin Lymphoma (NHL)

1. It is estimated that the incidence of NHL will be 65,980 (35,990 men and 29,990 women) in 2009 and 19,500 (9,830 men and 9,670 women) will die of NHL in the United States (U.S.)

Table 1: Comparison of Cancers in the U.S

<table>
<thead>
<tr>
<th>Estimated New Cases</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Prostate 192,280 (25%)</td>
<td>Breast 192,370 (27%)</td>
</tr>
<tr>
<td>Lung &amp; Bronchus 116,090 (15%)</td>
<td>Lung &amp; Bronchus 103,350 (14%)</td>
</tr>
<tr>
<td>Colon &amp; Rectum 75,590 (10%)</td>
<td>Colon &amp; Rectum 71,380 (10%)</td>
</tr>
<tr>
<td>NHL 35,990 (5%)</td>
<td>NHL 29,990 (4%)</td>
</tr>
</tbody>
</table>

*Excludes basal and squamous cell skin cell cancers and in situ carcinoma except urinary bladder

B. Diffuse Large B-Cell Lymphoma (DLBCL)

1. Most common type of NHL
   a. About 30% to 40% of all lymphoid malignancies
   b. About 30,000 new cases diagnosed annually in the U.S.
2. Median age at diagnosis is 64 years
3. 55% of those affected are male, 45% are female

II. Etiology

A. Genetic abnormalities associated with DLBCL

1. BCL6 (t(3;14))
   a. Most common
   b. Decrease in apoptotic response to DNA damage
   c. 6% to 30% of DLBCL
2. BCL2 (t(14;18))
   a. Leads to cellular resistance to apoptosis
   b. 28% of DLBCL
3. cMYC (t(8;14))
   a. Potent transcriptional activator
   b. 6% to 14% of DLBCL
III. Pathophysiology

A. NHL refers to all peripheral (i.e. nodal, systemic) malignancies of the lymphoid system except Hodgkin’s lymphoma

1. Neoplasm derived from the monoclonal proliferation of malignant B or T lymphocytes and their precursors
2. About 85% of NHL in the U.S. arise from B-cell origin

Figure 1: Hematopoietic Stem Cell Lineages

B. DLBCL arises from normal antigen-exposed B cells that have migrated to or through germinal centers of lymph nodes or secondary lymphoid organs

Figure 2: B-Cell Maturation Stage With Corresponding Malignancy
1. Composed of large, transformed lymphoid cells with nuclei at least twice the size of a small lymphocyte.

2. Typical cell markers:
   a. CD19
   b. CD20
   c. CD22
   d. CD79a
   e. Often have surface immunoglobulin (50% to 75% of cases)
   f. Occasionally CD30
   g. A few may express CD10 or CD5

3. May arise from the transformation of indolent lymphomas, such as follicular lymphoma, chronic lymphocytic leukemia and marginal zone lymphoma.

IV. Presentation
   A. Presentation often dependent on site of involvement
   B. Clinical features
      1. Frequently presents as a rapidly enlarging lymph node
         a. Anatomical location of lymph nodes (see appendix A)
            i. Cervical
            ii. Mediastinal
            iii. Mesenteric
            iv. Inguinal
         b. 40% of patients present with extra-nodal involvement
         c. Sites for extra-nodal involvement (18%)
            i. Gastrointestinal (GI) tract
            ii. Bone marrow
            iii. Spleen
            iv. Central nervous system (CNS)
            v. Liver
      2. One-third have B symptoms
         a. Night sweats
         b. Fever
         c. Weight loss
         d. Fatigue
   C. Abnormal lab values
      1. > 50% present with elevated serum lactate dehydrogenase (LDH)
      2. Complete blood count (CBC) – neutropenia, anemia, thrombocytopenia
      3. Comprehensive metabolic panel (CMP) – creatinine, liver function tests
      4. Uric acid
      5. β-2 microglobulin
V. Diagnosis\textsuperscript{4,5,9}
   A. Nodal architecture
      1. Excisional biopsy essential for definitive diagnosis
         a. Sheets of large, atypical lymphoid cells
         b. Diffuse foci throughout node
      2. Fine needle aspiration or core needle biopsy not recommended
      3. Diagnosed after slides evaluated by hematopathologist
   B. Disease categorized based on morphology, immunophenotype and cytogenetics
   C. Morphology
      1. Heterogeneity results in diverse morphologic features
      2. DLBCL subtypes
         a. Centroblastic
         b. Immunoblastic
         c. Anaplastic
         d. Several uncommon variants
   D. Immunophenotype
      1. Flow cytometry used to identify cell antigens
      2. Markers of B-cell lineage
         a. CD19
         b. CD20
         c. CD79a
      3. Markers seen in subtypes
         a. CD40
         b. CD10
         c. CD5
   E. Cytogenetics
      1. Rearrangement of immunoglobulin heavy and light chain genes
      2. Somatic mutation of the variable region
      3. Gene translocations that result in loss or gain of function mutations
         a. \textit{BCL6} (t(3;14))
         b. \textit{BCL2} (t(14;18))
         c. \textit{cMYC} (t(8;14))

VI. Staging (modified Ann Arbor system)
   A. Originally developed for use in clinical staging of Hodgkin’s lymphoma\textsuperscript{7}

Table 2: Ann Arbor Staging System\textsuperscript{11}

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textbf{I}</td>
<td>Single lymph node region involved</td>
</tr>
<tr>
<td>\textbf{II}</td>
<td>2 or more lymph node regions involved on same side of diaphragm</td>
</tr>
<tr>
<td>\textbf{III}</td>
<td>Lymph nodes on either side of diaphragm involved</td>
</tr>
<tr>
<td>\textbf{IV}</td>
<td>Extra-nodal involvement beyond lymph nodes</td>
</tr>
</tbody>
</table>
B. Subsets
  1. A – No B symptoms
  2. B – Fever, weight loss, night sweats
  3. X – Bulky disease (>10 cm dimension of nodal mass)

VII. Prognosis
  A. International Prognostic Index for aggressive lymphomas in the rituximab era

  Table 3: R-IPI Risk Score and Survival

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>R-IPI Score</th>
<th>CR (%)</th>
<th>Overall Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Good</td>
<td>0</td>
<td>87</td>
<td>94</td>
</tr>
<tr>
<td>Good</td>
<td>1-2</td>
<td>67</td>
<td>79</td>
</tr>
<tr>
<td>Poor</td>
<td>3-5</td>
<td>55</td>
<td>55</td>
</tr>
</tbody>
</table>

B. Adverse prognostic factors
  1. Age > 60 years
  2. ECOG performance status ≥ 2 (see appendix B) One point is given
  3. Elevated LDH
  4. More than two extra-nodal sites for each
  5. Ann Arbor Stage III or IV

C. The overall 5-year relative survival rate for 1999 to 2005 was 67.2%¹

VIII. Treatment of DLBCL
  A. 6 to 8 cycles of RCHOP is the standard of care
    1. RCHOP Regimen (every 3 weeks)¹³,¹⁴
      a. Rituximab 375 mg/m² iv on day 1
      b. Cyclophosphamide 750 mg/m² iv on day 1
      c. Doxorubicin 50 mg/m² iv on day 1
      d. Vincristine 1.4 mg/m² (max 2 mg) iv on day 1
      e. Prednisone 40 mg/m² po daily on days 1 through 5
    2. May need dose adjustments
  B. Complete remission (CR) rate of 75% to 80% with modern regimens¹²
  C. Long term disease-free survival greater than 50%¹²
  D. Relapse/Refractory
    1. Candidate for high-dose therapy (HDT) with autologous stem cell rescue
      a. DHAP (dexamethasone, cisplatin, cytarabine)
      b. ESHAP (etoposide, methylprednisolone, cytarabine, cisplatin)
      c. ICE (ifosfamide, carboplatin, etoposide)
    2. Not a candidate for HDT
      a. Clinical trial
      b. Rituximab
      c. Palliative radiation
      d. Combination chemotherapy +/- rituximab
IX. Advantages of HDT with stem cell rescue

A. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin lymphoma (Philip T et al.)\(^{15}\)
   1. Prospective randomized study
   2. 215 patients with relapsed NHL were treated with two courses of conventional chemotherapy
      a. 109 patients who had a response to chemotherapy were randomly assigned to receive four courses of chemotherapy plus radiotherapy (54 patients) or radiotherapy plus intensive chemotherapy and autologous stem cell transplant (ASCT) (55 patients)
   3. Response rate was 84% after ASCT vs. 44% after chemotherapy alone
   4. 5-year event-free survival was 46% in ASCT group vs. 12% in the chemotherapy alone group (p = 0.001)
   5. Rate of overall survival (OS) was 53% after ASCT vs. 32% in the chemotherapy alone group (p = 0.038)

B. Autologous transplantation for diffuse aggressive non-Hodgkin lymphoma in patients never achieving remission: a report from the Autologous Blood and Marrow Transplant Registry (Vose JM et al.)\(^{16}\)
   1. Autologous Blood and Marrow Transplant Registry records on 184 patients with diffuse aggressive NHL who never achieved a complete remission with conventional chemotherapy and subsequently received ASCT were evaluated
   2. 44% had a complete remission (CR), 19% had a partial remission (PR) and 31% had no response or progressive disease (PD)
   3. Probabilities of progression free survival (PFS) and OS at 5 years after transplantation were 31% (95% confidence interval (CI), 24% to 38%) and 37% (95% CI, 30% to 45%), respectively

C. High-dose chemoradiotherapy and autologous stem cell transplantation for patients with primary refractory aggressive non-Hodgkin lymphoma: an intention-to-treat analysis (Kewalramani et al.)\(^{17}\)
   1. Outcomes of 85 patients with primary refractory aggressive NHL who underwent second-line chemotherapy with ICE (ifosfamide, carboplatin and etoposide) with the intent of administering HDT/ASCT to those patients with chemosensitive disease were reviewed
   2. Among 42 patients who underwent transplantation, the 3-year overall and event-free survival rates were 52.5% and 44.2%, respectively

X. Hematopoietic stem cells

A. Purpose of stem cell rescue\(^{18}\)
   1. Rescue from fatally toxic doses of chemotherapy
   2. Graft vs. malignancy effect
   3. Replace malfunctioning immune system
B. Anatomic location of hematopoietic stem cells (HSCs)\textsuperscript{19}
   1. Bone marrow
   2. Peripheral blood
   3. Umbilical cord blood
C. Benefits of peripheral blood stem cells (PBSC)\textsuperscript{19,21}
   1. Decreased risk of toxicities associated with bone marrow collection
      a. Anesthesia complications
      b. Hemorrhage
      c. Fewer infections
      d. Rarely severe hematomas and neuralgias
      e. Localized pain
      f. More rapid engraftment and restoration of immune function
   2. Disadvantages of PBSC
      a. Exist in circulation in very small numbers (< 0.05% of WBC are CD34+)
      b. PBSCs must be mobilized from the bone marrow
D. Ability to pursue ASCT is dependent on the effectiveness and efficiency of stem cell mobilization
   1. Optimal dose of CD34+ cells has yet to be defined
   2. More than 2 x 10\textsuperscript{6} CD34+ cells/kg is considered the minimum number of cells needed for transplant\textsuperscript{19,21}
   3. Apheresis\textsuperscript{22}
      a. From Greek verb meaning “to take away or withdraw”
      b. Removal of one component of the blood with return of the remaining components to the donor
         i. Leukapheresis (WBC removal)
         ii. Erythrocytapheresis (RBC removal)
         iii. Plateletpheresis (Platelet removal)

XI. Stem cell mobilization
A. Goal is to increase the number of circulating stem cells to facilitate peripheral blood stem cell harvesting (goal range: 2 to 10 x 10\textsuperscript{6}/kg of CD34+ cells)\textsuperscript{19-21}
B. History\textsuperscript{21}
   1. 1960s- recognized that a small number of HSCs circulated in the peripheral blood during steady-state
   2. 1970s- increase in circulating HSC was observed after chemotherapy treatment
   3. 1980s- first autologous stem cell transplants using mobilized PBSCs
C. Factors influencing ability to mobilize\textsuperscript{19}
   1. Tumor infiltration in bone marrow
   2. Fibrotic bone marrow
   3. History of pelvic or abdominal radiation
   4. Marrow hypocellularity
   5. Prior exposure to stem cell toxins (e.g. alkylating agents, purine analogs)
   6. Age > 70 years
7. Baseline platelet count (< 150 x 10^9/L indicates a poor mobilizer)
8. Number of prior regimens
9. Duration of exposure to chemotherapy

D. Regimens of mobilization

1. Myelosuppressive chemotherapy
   a. First clinically useful blood progenitor cell mobilization protocol
   b. During the recovery phase after myelosuppressive chemotherapy a 50-fold increase in peripheral blood colony-forming units of granulocyte-macrophages occurs
   c. Single agent cyclophosphamide is most common agent
   d. Limitations
     i. Neutropenic sepsis
     ii. Bleeding diathesis
     iii. Unpredictable timing of apheresis

2. Hematopoietic growth factors alone
   a. Granulocyte-Colony Stimulating Factor (G-CSF)
      i. Initially developed for chemotherapy-induced neutropenia
      ii. Stimulate release of neutrophil-associated proteases that degrade molecules anchoring PBSCs to the bone marrow
      iii. Side effects
         1. Nausea, vomiting, diarrhea, chills, insomnia, fevers, night sweats and bone pain
   b. Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF)
      i. Does not mobilize as well as G-CSF
      ii. Higher doses are required and sometimes this is associated with unacceptable toxicity
         1. Similar to G-CSF, but increased incidence of fever and length of hospital stay

3. Myelosuppressive chemotherapy plus hematopoietic growth factors
   a. Cyclophosphamide
   b. Paclitaxel
   c. Vinblastine
   d. Combination chemotherapy

### Table 4: Comparison of Stem Cell Mobilization Regimens

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<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>17</td>
<td>22</td>
<td>24</td>
<td>12</td>
<td>12</td>
<td>163</td>
</tr>
<tr>
<td># CD34+ (x10^6) cells/kg</td>
<td>5.3</td>
<td>5.2</td>
<td>2.5</td>
<td>7.2</td>
<td>2.89</td>
<td>6.41</td>
<td>8.4</td>
</tr>
<tr>
<td>p-value</td>
<td>n.s.</td>
<td>p = 0.004</td>
<td>p = 0.009</td>
<td>n/a</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
XII. Plerixafor (Mozobil, AMD3100)\textsuperscript{21, 28}

A. History

1. Found to inhibit HIV-1 and HIV-2
   a. Selectively binds to the CXCR4 chemokine receptor, which is used by T-tropic (X4) HIV in addition to CD4 for membrane fusion and entry into the cell
   b. Target initially thought to be gp120, but turned out to be co-receptor CXCR4 used by T-lymphocyte HIV strains to enter cells
   c. Only effective in patients whose HIV was confirmed to use only CXCR4 and not CCR5 or a dual mechanism
   d. No longer being pursued as an antiretroviral

2. Unexpected increase in CD34\textsuperscript{+} cells in peripheral blood
   a. In phase I trials, before being tested in phase II trials with HIV infected patients, AMD3100 was noted to cause a rapid increase in WBC counts peaking at about 6 hours following IV infusion
   b. WBCs that were mobilized appeared to carry CD34\textsuperscript{+} marker indicating that they were HSCs

B. Mechanism

1. CXCR4 and SDF-1\textsuperscript{21, 28}
   a. Specifically and reversibly blocks SDF-1 binding to CXCR4
   b. Disrupts chemoattractant and adhesion effects that hold HSCs in the bone marrow, thus increasing the number in the peripheral blood

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3.png}
\caption{Mechanism of Action of Plerixafor (AMD3100)\textsuperscript{19}}
\end{figure}
C. Dose finding studies

1. Plerixafor (AMD3100) alone
   a. Single-dose administration of AMD3100 (40-240 µg/kg) caused generalized leukocytosis in healthy human volunteers, with a peak at 6 to 9 hours after drug administration
   b. Single-dose administration of AMD3100 at 80 µg/kg caused a 4-fold increase in circulating CD34+ cells, with a peak value at 6 hours
   c. A peak 10-fold increase in peripheral blood CD34+ cells was seen at 9 hours after subcutaneous injection of AMD3100 at 240 µg/kg
   d. The CD34+ cell response to 240 µg/kg was significantly greater at 9 hours, but not at 6 hours, compared with 160 µg/kg ($P < 0.05$)
   e. The CD34+ cell response induced by 240 µg/kg was significantly greater than the response to 80 µg/kg at 3, 6, and 9 hours ($P < 0.05$)

2. Plerixafor + G-CSF
   a. Median absolute peripheral blood CD34+ cell count increased from 24 cells/µL to 75 cells/µL 10 to 11 hours after plerixafor administration

3. Plerixafor approved for dosing 240 µg/kg 11 hours prior to apheresis

D. Clinical studies

1. Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin lymphoma (Devine et al, 2004)
   a. Phase I study assessing the safety and clinical effects of AMD3100 in patients with multiple myeloma (MM) and NHL
   b. Methods
      i. Patients
         1. Diagnosis of MM or NHL who were candidates for autologous PBSC transplant
         2. Between 18 and 70 years of age
         3. Received last dose of chemotherapy between 4 and 8 weeks before the study
      ii. Procedures
         1. 13 patients (7 with MM and 6 with NHL) received AMD3100 at a dose of either 160 µg/kg (n = 6) or 240 µg/kg (n = 7)
         2. WBC and peripheral blood CD34+ cell counts were analyzed at predefined intervals up to 6 hours after injection
2. Results

Table 5: Hematologic Effects of Two Different Doses of AMD3100

<table>
<thead>
<tr>
<th>Dose</th>
<th>160 µg/kg</th>
<th>240 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count/µl</td>
<td>4,633 +/- 1,172</td>
<td>4,817 +/- 758</td>
</tr>
<tr>
<td>CD34+, %</td>
<td>0.05 +/- 0.01</td>
<td>0.07 +/- 0.03</td>
</tr>
<tr>
<td>Absolute CD34+ count/µl</td>
<td>2.2 +/- 0.4</td>
<td>3.0 +/- 1.2</td>
</tr>
<tr>
<td><strong>4 hours after injection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count/µl</td>
<td>12,950 +/- 3,330 (p = 0.014)</td>
<td>11,865 +/- 2,167 (p = 0.003)</td>
</tr>
<tr>
<td>CD34+, %</td>
<td>0.10 +/- 0.01 (p = 0.004)</td>
<td>0.16 +/- 0.05 (p = 0.15)</td>
</tr>
<tr>
<td>Absolute CD34+ count/µl</td>
<td>11.3 +/- 2.7 (p = 0.11)</td>
<td>19.3 +/- 6.9 (p = 0.031)</td>
</tr>
<tr>
<td>Fold increase in CD34+</td>
<td>5.1 +/- 0.5</td>
<td>6.5 +/- 1.4</td>
</tr>
<tr>
<td><strong>6 hours after injection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count/µl</td>
<td>14,300 +/- 3,543 (p = 0.012)</td>
<td>12,941 +/- 2,307 (p = 0.003)</td>
</tr>
<tr>
<td>CD34+, %</td>
<td>0.09 +/- 0.01 (p = 0.002)</td>
<td>0.16 +/- 0.05 (p = 0.008)</td>
</tr>
<tr>
<td>Absolute CD34 count/µl</td>
<td>11.3 +/- 2.5 (p = 0.007)</td>
<td>20.4 +/- 7.6 (p = 0.038)</td>
</tr>
<tr>
<td>Fold increase in CD34+ count</td>
<td>5.1 +/- 0.6</td>
<td>7.0 +/- 1.1</td>
</tr>
<tr>
<td><strong>Day 2 after injection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count/µl</td>
<td>11,866 +/- 2,838 (p = 0.010)</td>
<td>11,395 +/- 1,920 (p = 0.011)</td>
</tr>
</tbody>
</table>

3. Toxicity

a. All were grade 1 by the World Health Organization (WHO) scale (see appendix C)

b. Not dose related

Table 6: Toxicities Associated With AMD3100

<table>
<thead>
<tr>
<th>Toxicity</th>
<th># of Affected Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection site erythema or edema</td>
<td>10</td>
</tr>
<tr>
<td>Abdominal bloating or cramping</td>
<td>5</td>
</tr>
<tr>
<td>Flatulence</td>
<td>3</td>
</tr>
<tr>
<td>Diarrhea or soft stools</td>
<td>3</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
</tr>
<tr>
<td>Facial paresthesias</td>
<td>3</td>
</tr>
<tr>
<td>Lightheadedness</td>
<td>2</td>
</tr>
</tbody>
</table>

4. Comments

a. AMD3100 was well tolerated and efficacious in rapidly mobilizing WBCs and CD34+ cells in patients with MM and NHL

b. Both doses of AMD3100 were safe and effective
E. Treatment with plerixafor in non-Hodgkin lymphoma and multiple myeloma patients to increase the number of peripheral blood stem cells when given a mobilizing regimen of G-CSF: implications for the heavily pretreated patient (Stiff et al, 2009)\textsuperscript{33}

1. Single arm, multicenter phase II study of the combination of plerixafor and G-CSF to mobilize peripheral blood stem cells (PBSC) in patients with NHL and MM
   a. Exploratory analyses were performed on heavily pretreated patients who are typically considered as poor mobilizers
      i. 10 or more total cycles of chemotherapy
      ii. Received platinum-based chemotherapy either at diagnosis or at the time of relapse
      iii. Received any dose of radiation to bone marrow bearing sites

2. Methods
   a. Patients (n = 49)
      i. Inclusion criteria
         1. Patients with NHL and MM
         2. Age 18 to 70 years
         3. Undergoing a first PBSC mobilization procedure
         4. No more than 3 prior regimens
         5. Had to be 4 weeks from last chemotherapy
         6. ECOG status of 0 or 1
      ii. Exclusion criteria
         1. Central nervous system involvement
         2. Temperature > 38°C
         3. Actual body weight > 175% of ideal body weight
         4. History of ventricular arrhythmias
   b. Procedures
      i. G-CSF at 10 µg/kg SC for up to 9 days, as per local practice
      ii. Plerixafor 240 µg/kg SC in the evening prior to each day of apheresis beginning on the evening of day 4 of G-CSF administration
      iii. Efficacy parameters included: circulating CD34+ cell count before and after the first dose of plerixafor, CD34+ cell yield by apheresis, neutrophil and platelet engraftment, and graft durability
      iv. The primary efficacy endpoint was to determine if patients mobilized by the plerixafor + G-CSF combination had a ≥ 2-fold increase in circulating CD34+ cells after the first dose of plerixafor
3. Results

Table 7: Mobilization data

<table>
<thead>
<tr>
<th>Item</th>
<th>NHL (n = 23)</th>
<th>MM (n = 26)</th>
<th>All (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood CD34+ cells/µl pre-plerixafor</td>
<td>16 (2-57)</td>
<td>41.5 (3.6-209)</td>
<td>24 (2-209)</td>
</tr>
<tr>
<td>Peripheral blood CD34+ cells/µl post-plerixafor</td>
<td>43 (5.3-124)</td>
<td>78.9 (10.8-531)</td>
<td>56 (5.3-531)</td>
</tr>
<tr>
<td>N-fold increase in CD34+ cells/µl</td>
<td>2.5 (1.3-6)</td>
<td>2.6 (1.4-5)</td>
<td>2.5 (1.3-6)</td>
</tr>
<tr>
<td>≥ 2 fold increase (n, %)</td>
<td>20 (87)</td>
<td>17 (65.4)</td>
<td>37 (75.3)</td>
</tr>
<tr>
<td>Apheresis days</td>
<td>3 (1-5)</td>
<td>2 (1-5)</td>
<td>2 (1-5)</td>
</tr>
<tr>
<td>Total CD34+ cells/kg</td>
<td>5.2 (1.5-18.9)</td>
<td>11.1 (4.4-22.5)</td>
<td>5.9 (1.5-22.5)</td>
</tr>
<tr>
<td>Apheresis yield ≥ 5 x 10^6 CD34+ cells/kg (n, %)</td>
<td>13 (56.5)</td>
<td>25 (95.2)</td>
<td>38 (77.6)</td>
</tr>
<tr>
<td>Apheresis yield ≥ 2 x 10^6 CD34+ cells/kg (n, %)</td>
<td>21 (91.3)</td>
<td>26 (100)</td>
<td>47 (95.9)</td>
</tr>
</tbody>
</table>

Table 8: Mobilization data based on prior therapy

<table>
<thead>
<tr>
<th>Item</th>
<th>Heavily Pretreated</th>
<th>Non-heavily Pretreated</th>
</tr>
</thead>
<tbody>
<tr>
<td># of patients</td>
<td>28</td>
<td>21</td>
</tr>
<tr>
<td>N-fold increase in CD34+ cells/µl</td>
<td>2.6 (1.3-6)</td>
<td>2.5 (1.4-5)</td>
</tr>
<tr>
<td>Apheresis days (median, range)</td>
<td>3 (1-5)</td>
<td>2 (1-5)</td>
</tr>
<tr>
<td>Total CD34+ x 10^6 cells/kg collected (median, range)</td>
<td>5.4 (2.9-12.9)</td>
<td>5.2 (1.4-12.5)</td>
</tr>
<tr>
<td>Apheresis yield ≥ 5 x 10^6 CD34+ cells/kg (n, %)</td>
<td>14 (67)</td>
<td>16 (58)</td>
</tr>
</tbody>
</table>

4. Toxicity

Table 9: Adverse events seen during mobilization

<table>
<thead>
<tr>
<th>n (%)</th>
<th>Total</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td>28 (57%)</td>
<td>4 (8.2%)</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>19 (38.8%)</td>
<td>3 (6.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>9 (18.4%)</td>
<td>1 (2.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>3 (6.1%)</td>
<td>1 (2.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Injection site erythema</td>
<td>7 (14.3%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alk Phos elevation</td>
<td>8 (16.3%)</td>
<td>2 (4.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5 (16.2%)</td>
<td>3 (6.1%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>6 (12.2%)</td>
<td>0</td>
<td>6 (12.2%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>3 (6.1%)</td>
<td>1 (2.1%)</td>
<td>0</td>
</tr>
</tbody>
</table>
5. Comments
   a. As seen in other phase I/II trials, plerixafor was safe and efficacious for the mobilization of peripheral blood stem cells
   b. Durable response seen in patients who were classified as heavily pretreated
      i. Prior studies indicated that these patients would have a mobilization failure rate of 20% to 30% and not the 4.1% that was seen in this trial

F. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin lymphoma (DiPersio et al, 2009)34
   1. Purpose was to compare the safety and efficacy of plerixafor and G-CSF with placebo and G-CSF in mobilizing CD34+ cells in patients with NHL
   2. Methods
      a. Patients (150 randomized to plerixafor and 148 to placebo)
         i. Inclusion criteria
            1. Between 18 and 78 years old
            2. Biopsy-confirmed diagnosis of NHL, in first or second complete or partial remission, eligible for autologous HSCT
            3. ≥ 4 weeks since last cycle of chemotherapy
            4. ECOG performance status of 0 or 1
         ii. Exclusion criteria
            1. Co-morbid condition which rendered the patient at high risk from treatment complications
            2. Failed previous HSC collections or collection attempts
            3. ≤ 6 weeks off carmustine before the first dose of G-CSF
            4. Active CNS involvement or infection
            5. Bone marrow involvement higher than 20%
            6. Radiation therapy to the pelvis
            7. Anticipated post-transplantation chemotherapy and/or radiation therapy below the diaphragm
            8. G-CSF, GM-CSF, or pegfilgrastim within 3 weeks
            9. G-CSF within 14 days before the first dose of G-CSF for mobilization
      b. Procedures
         i. G-CSF 10 µg/kg SC daily in the morning for up to 8 days
         ii. On day 4, patients received either plerixafor 240 µg/kg (actual body weight) or placebo SC daily in the evening for up to 4 days
         iii. Apheresis began on day 5 and continued for up to 4 days
c. Results
   i. Significantly more patients in the plerixafor group (59.3%) achieved the primary end point vs. placebo group (19.6%; p < .001)
   ii. Time to reach $\geq 5 \times 10^6$ CD34+ cells was significantly shorter in the plerixafor group (3 days vs. not reached, p < 0.001)
   iii. 90% of plerixafor-treated patients underwent transplantation after initial mobilization vs. 55.4% placebo-treated patients (p < 0.001)

![Graph showing Kaplan-Meier estimate of proportion of patients reaching (A) $\geq 5 \times 10^6$ CD34+ cells/kg or (B) $\geq 2 \times 10^6$ CD34+ cells/kg]

Figure 4: Kaplan-Meier estimate of proportion of patients reaching (A) $\geq 5 \times 10^6$ CD34+ cells/kg or (B) $\geq 2 \times 10^6$ CD34+ cells/kg

---

d. Toxicity

Table 10: Most Common Adverse Events

<table>
<thead>
<tr>
<th>Adverse Events</th>
<th>Plerixafor (n = 150)</th>
<th>Placebo (n = 145)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any related</td>
<td>98 (65.3%)</td>
<td>60 (41.4%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>57 (38%)</td>
<td>9 (6.2%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>26 (17.3%)</td>
<td>8 (5.5%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>9 (6%)</td>
<td>2 (1.4%)</td>
</tr>
<tr>
<td>Injection site</td>
<td>44 (29.3%)</td>
<td>9 (6.2%)</td>
</tr>
<tr>
<td>Injection site pruritis</td>
<td>12 (8%)</td>
<td>0</td>
</tr>
<tr>
<td>Bone pain</td>
<td>16 (10.7%)</td>
<td>10 (6.9%)</td>
</tr>
<tr>
<td>Headache</td>
<td>17 (11.3%)</td>
<td>9 (6.2%)</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>10 (6.7%)</td>
<td>4 (2.8%)</td>
</tr>
</tbody>
</table>
e. Comments
   i. Plerixafor + G-CSF mobilization regimen was well tolerated and resulted in a statistically significant higher proportion of patients collecting both the optimal and minimal CD34+ cell dose for transplantation compared with placebo + G-CSF
   ii. The addition of plerixafor to G-CSF allowed collection of \( \geq 2 \times 10^6 \) CD34+ cells/kg in fewer apheresis days (56.5% of plerixafor-treated patients collected this minimum target after only 1 apheresis day compared with 59.8% of placebo-treated patients after 4 apheresis days)
   iii. The failure rate of 80.4% in the placebo and G-CSF group was surprisingly higher than those reported in the literature
      1. This difference may be because of the use of \( \geq 5 \times 10^6 \) CD34+ cells/kg as the target versus \( \geq 2 \times 10^6 \) CD34+ cells/kg

XIII. Conclusions
   A. Plerixafor (AMD3100, Mozobil®) was recently FDA approved for use in combination with G-CSF to mobilize hematopoietic stem cells to the peripheral blood for collection and subsequent ASCT in patients with NHL and MM
   B. DLBCL is very responsive to treatment with RCHOP in most cases, but when patients relapse or have refractory disease, the prognosis is much worse and HDT with ASCT provides the best option for these patients
   C. Plerixafor’s novel mechanism of action, interfering with the adhesive interactions between SDF-1 and CXCR4, allows it to work synergistically with G-CSF to enhance the number of CD34+ mobilized to the peripheral blood
   D. Plerixafor’s place in clinical practice is still controversial, as it has yet to be compared head-to-head in clinical trials with G-CSF + chemotherapy, the standard of care in most institutions
   E. Based on current data, plerixafor may best be utilized in combination with G-CSF for patients who meet predictive criteria to be potentially poor mobilizers and in whom any additional increase in CD34+ cell count in the peripheral blood might increase the probability for a successful transplantation
   F. Chemotherapy + G-CSF should still be used first line whenever possible, as it has the best data and clinical experience, and plerixafor should only be added to G-CSF to increase the likelihood of achieving sufficient CD34+ yields in patients whom are likely to be poor mobilizers.
   G. Plerixafor + G-CSF should not be used ubiquitously until there is data to support its efficacy in improving outcomes for patients undergoing ASCT compared to chemotherapy + G-CSF
References


Appendix A

Figure 5: Anatomical location of lymph nodes

Appendix B

Table 11: ECOG Performance Status

<table>
<thead>
<tr>
<th>Grade</th>
<th>ECOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fully active, able to carry on all pre-disease performance without restriction.</td>
</tr>
<tr>
<td>1</td>
<td>Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.</td>
</tr>
<tr>
<td>2</td>
<td>Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.</td>
</tr>
<tr>
<td>3</td>
<td>Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.</td>
</tr>
<tr>
<td>4</td>
<td>Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.</td>
</tr>
<tr>
<td>5</td>
<td>Dead</td>
</tr>
</tbody>
</table>

Adapted from: http://www.ecog.org/general/perf_stat.html
### Appendix C

Table 12: Modified WHO Toxicity Grading Scale

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – Mild</td>
<td>No interference with activity</td>
</tr>
<tr>
<td>2 – Moderate</td>
<td>Some interference with activity not requiring medical intervention</td>
</tr>
<tr>
<td>3 – Severe</td>
<td>Prevents daily activity and requires medical intervention</td>
</tr>
<tr>
<td>4 – Potentially Life-Threatening</td>
<td>ER visit or hospitalization</td>
</tr>
</tbody>
</table>