Janet’s Inbox-
A Potpourri of Questions & Answers
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Th. Feb. 18, 2016
## Agenda

1. Autologous boosts/rescues
2. Initial engraftment
3. Reporting FISH and molecular markers
4. Cellular therapy infusions
5. MDS/MPN
6. NHL
7. Multiple Myeloma
Reporting Autologous Boosts/Rescues
Autologous “Boosts/Rescues”

• Performed for engraftment reasons following a HCT
  ➢ No hematopoietic recovery
  ➢ Partial hematopoietic recovery
  ➢ Graft failure

• Autologous boosts/rescues are HCTs
  ➢ Purpose is to restore hematopoiesis

• To reduce the reporting burden on centers forms do not start over
Autologous “Boosts/Rescues”

• Performed for any other reason would be reported as a subsequent HCT & forms would start over

• Indications include-
  - Persistent primary disease
  - Recurrent primary disease
  - Planned second HCT, per protocol
  - New malignancy
  - Stable, mixed chimerism
  - Declining chimerism
Reporting ANC & Platelet Recovery
Initial ANC & Platelet Recovery

- The definitions for ANC & platelet recovery (i.e., engraftment) do not include a time frame as to when “initial” recovery should occur. These data can be reported up to 2 years post HCT.
- There are scenarios that may delay the initial recovery of the ANC and/or platelets:
  - Type of graft (e.g., cord blood)
  - Low CD34+ cell dose in product
  - Persistent disease
Reporting ANC Recovery

• Definition-

Granulopoiesis/neutrophil recovery is defined as an absolute neutrophil count (ANC) of $\geq 0.5 \times 10^9/L$ (500 mm$^3$) for three consecutive laboratory values obtained on different days.
Reporting Platelet Recovery

• Definition-

Platelet recovery is reported when the recipient’s platelet count is $\geq 20 \times 10^9$/L seven days after the last platelet transfusion and maintained for three consecutive lab values on different days.
Reporting Platelet Recovery

- **Scenario 1**
  
  A 55 yo WF with AML in 2\textsuperscript{nd} CR undergoes an allo HCT from a matched sib on 6/1/15.
  
  8/1/15- platelets not yet recovered & now has relapsed AML
  
  8/2-8/5/15- treated with azacitidine & bortezamib
  
  9/5/15- received a DLI 1 x 10\textsuperscript{7} CD3+/kg
  
  10/1/15- Platelet count is 25K without a platelet transfusion in the preceding 7 days. Platelets continue to be \( \geq \)20K on subsequent days.
Reporting Platelet Recovery

• Scenario 1 continued

The date of the initial platelet recovery would be reported as 10/1/15 even though it was 4 months after the HCT.
Reporting Platelet Recovery

• Scenario 2

A 25 yo AAF undergoes an auto HCT for NHL. Platelet counts are shown in the chart below. No platelet transfusions were given. What date would be reported for initial recovery?

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>13</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet Count</td>
<td>45</td>
<td>32</td>
<td>28</td>
<td>20</td>
<td>19</td>
<td>16</td>
<td>ND</td>
<td>18</td>
<td>ND</td>
<td>24</td>
<td>46</td>
<td>88</td>
</tr>
</tbody>
</table>
Reporting FISH & Molecular Results
Molecular Markers

• What are they & why do we care?

  ➢ NCI definition - A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease.

  ➢ The presence of a molecular marker may be associated with favorable or unfavorable survival.

  ➢ Can be used to monitor minimal residual disease (MRD) in post-HCT patients.
Molecular Markers

- **AML**: CEBPA, FLT3, IDH1-2, KIT, NPM1
- **APML**: PML-RARA; t(15;17)
- **ALL**: BCR-ABL, TEL-AML/AML1
- **CML**: BCR-ABL
- **NHL**: BCL-1, t(11;14); BCL-2, t(14;18); BCL-6; IG heavy chain rearrangement; TCR gene rearrangement
Molecular Markers

- Can be determined using a PCR or FISH method
- Molecular markers (e.g., BCR-ABL or PML-RARA) assessed by FISH should not be reported on the Post-TED (F2450) in Q83
- The FISH results would be reported on the Post-TED (F2450) in Q86
Reporting Molecular Markers on F2450

83 Relapse/progression detected by molecular method:
- Yes
- No
- previously reported (answer is only valid on > d100 evaluation)
- Not evaluated

84 Date first seen: __________-____-____

85 Date of Assessment: __________-____-____

86 Relapse/progression detected by cytogenetic/FISH method:
- Yes
- No
- previously reported (answer is only valid on > d100 evaluation)
- Not evaluated

87 Date first seen: __________-____-____

88 Date of Assessment: __________-____-____
Reporting Molecular Markers on F2450

• Patient Scenario

A patient with Ph+ ALL in CR1 (molecular & cytogenetic CR) at time of HCT, is undergoing their 6 month evaluation post-HCT.

A marrow aspirate is sent for BCR-ABL by FISH. Fifty of 500 cells are positive for BCR-ABL.
Reporting Molecular Markers on F2450

Where would the positive BCR-ABL findings be reported on the Post-TED (F2450)?

A. In Q83 Relapse/progression detected by molecular method
B. In Q86 Relapse/progression detected by cytogenetic/FISH method
C. In Q83 & Q86
D. The positive BCR-ABL findings would not be reported in Q83 or Q86
Cellular Therapy (CT)
Cellular therapy (CT)

• Definition-
  A form of therapy in which cellular material is infused into a patient (e.g., T-cells)
Examples of CT

• Donor Cellular Infusion (DCI)
  ➢ Donor T-cells are infused to treat cancer, treat infection, restore immune function, etc.

• CAR T-cell Therapy
  ➢ Autologous T-cells are modified to treat cancer

• Cytotoxic T-lymphocyte (CTL) Therapy
  ➢ CMV specific CTLs
CAR T-cells

- Chimeric Antigen Receptor (CAR) T-cells
  - Comprised of an engineered receptor
  - The receptors are used to graft the specificity of a monoclonal antibody (e.g., CD 19) onto a T-cell
  - Transfer of their coding sequence is facilitated by a retroviral vector
  - The receptors are called chimeric because they are composed of parts from different sources
Structure of a CAR T-cell

• CAR T-cells are fusions of single-chain variable fragments (scFv) derived from monoclonal antibodies, which are fused to the CD3-zeta transmembrane and endodomain.

• CAR T-cells are comprised of three regions
  - Ectodomain
  - Transmembrane domain
  - Endodomain
Structure of a CAR T-cell
Adoptive Cell Transfer Therapy

1) T Cell Collection
2) T Cell Transfection
3) T Cell Adoptive Transfer
4) Patient Monitoring

1. Binding
2. Fusion
3. Integration
4. Transcription and protein expression
5. CAR cell membrane insertion

+/-Lymphodepleting conditioning

a) Disease response
   - CT scans
   - Bone marrow biopsies
   - Peripheral blood flow cytometry
b) CAR-T Cell persistence
   - Immunohistochemistry of bone marrow biopsy
   - RT-PCR and flow cytometry of blood and bone marrow aspirate
Use of CAR T-cell Therapy

- **Malignancies**
  - ALL or CLL (target antigen- CD19)
  - Mantel cell lymphoma (target antigen- CD20)
  - Hodgkin lymphoma (target antigen- CD30)
  - AML (target antigen- CD33)
Cytotoxic T-lymphocyte (CTL) Therapy

• Donor derived CTLs
  ➢ Donor must be seropositive for the particular virus (e.g., CMV) as they will have virus-specific memory T-cells
  ➢ The virus-specific T-cells are expanded in culture using cytokines (e.g., IL-4 & IL-7) before infusion

• Third-party CTLs
MDS/MPN Questions
Myeloproliferative Disorders

• Essential Thrombocytosis (ET)
  ➢ A myeloproliferative neoplasm (MPN)
  ➢ Characterized by sustained thrombocytosis (>\(=450 \times 10^9\)/L), increased # of large mature megakaryocytes in the marrow and episodes of thrombosis and/or hemorrhage.
  ➢ After many years, some ET patients may develop BM fibrosis associated with myeloid metaplasia
Myeloproliferative Disorders

- Polycythemia Vera (PV)
  - A myeloproliferative neoplasm (MPN)
  - Characterized by increased production of RBCs independent of mechanisms that normally regulate erythropoiesis
  - Virtually all have the JAK2 mutation
Myeloproliferative Disorders

- Polycythemia vera (PV) continued
- Three phases of PV
  - Pre-polycythemic phase
    Characterized by borderline to mild erythrocytosis
  - Polycythemic phase
    Significantly increased red cell mass
  - Post-polycythemic myelofibrosis phase (post-PV MF)
    Cytopenias (including anemia) are associated with ineffective hematopoiesis, BM fibrosis, extramedullary hematopoiesis & hypersplenism
Myeloproliferative Disorders

• The fibrosis/myelofibrosis that develop in ET & PV patients would **NOT** be considered a transformation to primary myelofibrosis

• The fibrosis/myelofibrosis in ET & PV patients would be considered secondary myelofibrosis

• When ET & PV patients develop marrow fibrosis do **NOT** report “Primary Myelofibrosis” as the primary disease for HCT.
NHL Questions
NHL- unclassifiable

- B-cell lymphoma, unclassifiable, with features intermediate between Burkitt lymphoma & DLBCL
  - Used to classify cases not meeting criteria for Burkitt lymphoma or DLBCL
  - Example of this category includes “double hit” NHLs. They are B-cell lymphomas characterized by a recurrent chromosomal translocation in combination with a 8q24/MYC translocation.
NHL- unclassifiable

- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL & classical Hodgkin lymphoma
  ➢ Synonyms
    - Grey zone lymphoma
    - Large B-cell lymphoma with Hodgkin features
  ➢ Most common in men ages 20-40
  ➢ Usually presents with a large anterior mediastinal mass with or without supraclavicular node involvement
NHL- Concurrent NHLs

• Which lymphoma sub-type to report when two NHLs are diagnosed at the same time?
  
  ➢ Always report the more aggressive lymphoma as the primary disease for HCT.
  
  ➢ For example- If mantel cell & DLBCL are diagnosed concurrently, DLBCL would be reported as the primary disease for HCT.
NHL - Concurrent NHLs

- Pre-TED Questions

585. Is the non-Hodgkin lymphoma histology reported at diagnosis (question 583) a transformation from CLL?
  - Yes (Also complete Disease Classification questions 573 - 576)
  - No ➔ 586. Is the non-Hodgkin lymphoma histology reported (in question 583) a transformation from, or was it diagnosed at the same time as another lymphoma (not CLL)?
    - Yes  
    - No

- Q585 would be answered “no”
- Q586 would be answered “yes”
NHL- Richter’s transformation

• Richter’s transformation (or syndrome)
  ➢ Occurs in about 5-10% of B-cell CLL cases
  ➢ The CLL transforms to a fast growing DLBCL

• Report the primary diagnosis for HCT as DLBCL
NHL- Richter’s transformation

• Reporting DLBCL from a Richter’s transformation on the Pre-TED (F2400)…. 

585. Is the non-Hodgkin lymphoma histology reported at diagnosis (question 583) a transformation from CLL?

☐ Yes (Also complete Disease Classification questions 573 - 576)

☐ No

586. Is the non-Hodgkin lymphoma histology reported (in question 583) a transformation from, or was it diagnosed at the same time as another lymphoma (not CLL)?

☐ Yes ☐ No

• Q585 would be answered “yes”

• Q573 – 576 would also need to be completed for CLL
Multiple Myeloma
Multiple Myeloma Questions

• What is considered measurable disease?
  - Serum M-protein $\geq 1$ g/dL and/or
  - Urine M-protein $\geq 200$ mg/24 hours

Free light chain levels may be used in place of the M-protein, provided the involved chain is $>10$ mg/dL & the $\kappa/\lambda$ ratio is abnormal at diagnosis.
What Baseline to Use When...

Determining disease status at time of HCT

No relapse or progression at any time between diagnosis and 1st HCT:

*Use the disease parameters (DP) from diagnosis as the baseline.*
Patient was treated for a relapse or progression (R/P) in between diagnosis & 1st HCT:

*Use the disease parameters (DP) obtained at the time of relapse or progression (R/P) as the baseline (the baseline is reset to the time of the relapse or progression)*
What Baseline to Use When…

Determining disease response to HCT

HCT planned as part of the initial therapy without a prior disease relapse or progression:

*Use disease parameters (DP) obtained at diagnosis*
What Baseline to Use When…

Determining best response to HCT

Patient had a treated disease progression or relapse (R/P) prior to HCT:

*Use disease parameters (DP) obtained at time of the relapse or progression (R/P)*
What Baseline to Use When…. 

- Patient has not received any therapy within 6 months of HCT or has an untreated relapse or progression (R/P)

Use the disease parameters (DP) obtained prior to the start of prep to determine best response to HCT.
What Baseline to Use When…..

Recipient undergoes a Tandem transplant. Tandem transplants are considered part of “one” treatment plan.

The baseline to use following the 2nd HCT would be the same baseline used for the 1st HCT provided there has not been a disease progression or relapse in between.
### Summary of Which Baseline to Use When Determining Disease Status

<table>
<thead>
<tr>
<th>Has there been a relapse or progression?</th>
<th>Disease Status at Time of HCT</th>
<th>Disease Response to HCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>No R/P</td>
<td>DP at diagnosis</td>
<td>DP at diagnosis</td>
</tr>
<tr>
<td>Yes R/P (treated)</td>
<td>DP at R/P</td>
<td>DP at R/P</td>
</tr>
<tr>
<td>Yes R/P (untreated)</td>
<td>DP prior to the start of prep</td>
<td>DP prior to start of prep</td>
</tr>
</tbody>
</table>

R/P = relapse or progression,  DP = disease parameters
Confirmatory Testing Requirements

- Includes SPEP/UPEP, serum/urine immunofixation & $\kappa/\lambda$ free light chains
- Confirmatory testing does not apply to BM biopsies, skeletal surveys & other radiographic studies
Confirmatory Testing Requirements

- Every disease response (sCR, CR, nCR, VGPR, PR & SD) requires two consecutive assessments (by the same method) made at any time before the initiation of any new therapy.

- Progressive disease (PD) & relapse from CR are a bit different. PD & relapse from CR requires two consecutive assessments (by the same method) before classification, and/or the start of any new therapy.
Confirmatory Testing Requirements

• To report CR, both the serum & urine immunofixation must be negative as well as confirmed!
• Many institutions don’t obtain urine studies on a regular basis.
• CR may be reported as long as there is at least one negative serum & one negative urine immunofixation and one of them is confirmed.
Multiple Myeloma

- Complete Remission (CR) criteria

A treatment response where all of the following criteria are met:

- Negative immunofixation on serum and urine samples
- Disappearance of any soft tissue plasmacytomas
- < 5% plasma cells in the bone marrow (confirmation with repeat bone marrow biopsy not needed)

NOTE: CR Requirements

For recipients with light chain only myeloma, **all** of the following criteria must be met:

- Normal serum free light chain ratio
- Negative immunofixation on urine samples
- Disappearance of any soft tissue plasmacytomas
- < 5% plasma cells in the bone marrow (confirmation with repeat bone marrow biopsy not needed)

For recipients with non-secretory myeloma, **all** of the following criteria must be met:

- Disappearance of all soft tissue plasmacytomas
- < 5% plasma cells in the bone marrow (confirmation with repeat bone marrow biopsy not needed)
Multiple Myeloma

**Question**- If a patient had a BMBx with <5% plasma cells prior to HCT, but did not meet the other CR criteria (e.g., + serum IFE for IgG lambda), can the same BMBx be used post-HCT when evaluating for CR status?

**Answer**- Yes! A repeat BMBx is not needed since the plasma cells were already <5%.
MYELOMA CASE STUDY

- Malignant B cell
- Apoptosis
- CD19 Antigens
- Anti CD19 monoclonal antibody
- ADCC Effector Cell
- Complement dependent cytotoxicity (CDC)
Myeloma Case Study

A 55 year old AA male is diagnosed with IgG lambda myeloma. Results of the initial work-up include:

- Serum M-spike = 4 g/dL (or 4000 mg/dL)
- 24-hr urine M-protein = 1000 mg/24 hr
- Bone marrow aspirate = 60% plasma cells

Patient receives 2 cycles of Revlimid & Dex, then re-evaluated:

- Serum M-spike = 2000 mg/dL
- 24-hr urine M-protein = 195 mg/24 hr
Myeloma Case Study

What is the patient’s disease response after two cycles of Rev/Dex?

A) Very Good Partial Remission (VGPR)
B) Partial Remission (PR)
C) Stable Disease (SD)
Myeloma Case Study

The patient’s PR status was confirmed with a 2\textsuperscript{nd} measurement. The patient received two additional cycles of Rev/Dex & re-evaluated for disease response.

- Serum M-spike = 2900 mg/dL
- 24-hr urine M-protein = 600 mg/24 hr
- Bone marrow aspirate = 30\% plasma cells
Myeloma Case Study

What is the patient’s disease response after a total of 4 cycles of Rev/Dex?

A) Very Good Partial Response (VGPR)
B) Partial Response (PR)
C) Stable Disease (SD)
D) Progressive Disease (PD)
Myeloma Case Study

Patient is switched to Vincristine, Adriamycin & Decadron (VAD) and is re-evaluated after two cycles.

- Serum M-spike = 1400 mg/dL
- 24-hr urine M-protein = 190 mg/24 hr
- Bone marrow aspirate = 15% plasma cells

The plan is to give IV Cytoxan mobilization. What is the patient’s disease response to the 2 cycles of VAD?
Myeloma Case Study

The patient achieved a PR after two cycles of VAD. What studies were used as a baseline to make that determination?

A) The studies obtained at diagnosis
B) The studies obtained after first two cycles of Rev/Dex
C) The studies obtained at time of progression
Myeloma Case Study

The patient has undergone their autologous PBSC HSCT & has been evaluated monthly for the 1st three months post HSCT.

• Day +30 evaluation:
  ▪ Serum M-spike = 1000 mg/dL
  ▪ Serum immunofixation (+) for IgG lambda
  ▪ 24-hr urine M-protein = 190 mg/24 hrs
  ▪ Bone marrow biopsy = 7% plasma cells
Myeloma Case Study

Day +60 evaluation:

- SPEP/UPEP - no monoclonal band
- Serum/Urine immunofixation (+) for IgG lambda
- 24-hr urine for M-protein = 90 mg/24 hrs
Myeloma Case Study

Day +100 evaluation:

- SPEP/UPEP - no monoclonal band
- Serum/Urine immunofixation (+) for IgG lambda
- 24-hr urine for M-protein = 0 mg/24 hrs
- Bone marrow aspirate <5% plasma cells
Myeloma Case Study

What is the best disease response to HCT that you would report at Day +100 for this patient?

A) Stable Disease (SD)
B) Partial Remission (PR)
C) Very Good Partial Remission (VGPR)
D) Near Complete Remission (nCR)
E) Complete Remission (CR)
Questions