Calling All Super Heroes

Research Sample Lifecycle: From blood draw to publication

Stephen Spellman, MBS, Director, Immunobiology Research, CIBMTR

Wael Saber, MD, MS, Associate Professor of Medicine, Division of Hematology and Oncology - Medical College of Wisconsin

Shernan Holtan, MD, Assistant Professor, Blood and Marrow Transplant Program - University of Minnesota

November 9th, 2018
### Disclosures

The following faculty and planning committee staff have the following financial disclosures:

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Disclosure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misty Evans</td>
<td>Vanderbilt University</td>
<td>Jazz Pharmaceuticals, Monetary, Speakers Bureau</td>
</tr>
<tr>
<td>Shernan Holtan, MD</td>
<td>University of Minnesota</td>
<td>Incyte, Consulting Fee, Consulting</td>
</tr>
<tr>
<td>Wael Saber</td>
<td>Medical College of Wisconsin, CIBMTR</td>
<td>None</td>
</tr>
<tr>
<td>Stephen Spellman, MBS</td>
<td>CIBMTR</td>
<td>None</td>
</tr>
<tr>
<td>Stephanie Waldvogel</td>
<td>CIBMTR</td>
<td>None</td>
</tr>
</tbody>
</table>

Grab your cape.
Learning objectives

• At the conclusion of this session, attendees will be able to:
  • Describe the research sample lifecycle
  • Recognize the scientific value of NMDP research samples and their impact on transplant studies
  • Identify recent CIBMTR and BMT CTN studies where research samples contributed to the science
Research Sample Life Cycle: From Blood Draw to Publication

Council Meeting
Stephen Spellman
Director, Immunobiology Research
CIBMTR Research Repository

- **Unrelated Donor Repository (Est. 1987)**
  - >200 Centers Participating
  - >40,500 Adult Recipient/Donor pairs
  - >6,600 Recipient/Cord pairs

- **Related Donor Repository (Est. 2007)**
  - 52 Centers Participating
  - >7,800 Adult Recipient/Donor pairs

- More than 2.6 million aliquots stored
Clinical Trial Support

• CIBMTR Research Biorepository
  – Began supporting clinical sample processing and long-term storage of Blood and Marrow Transplant Clinical Trials Network (BMT CTN) research biospecimen collections in 2007.

• BMT CTN Biospecimen Collections
  – Currently total more than 400,000 biospecimens
  – Clinical samples provided by more than 6,480 subjects, associated with 21 clinical studies.
# CIBMTR Research Biospecimen Processing

## Peripheral Blood Research Specimen Types

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Processing Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>Buffy Coat WBC</td>
</tr>
<tr>
<td>Dried Blood Spots – Filter Cards</td>
<td>PAXgene RNA Lysates</td>
</tr>
<tr>
<td>Serum / Plasma</td>
<td>PAXgene DNA Lysates</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>Viable Peripheral Blood Mononuclear Cells (PBMC)</td>
</tr>
</tbody>
</table>

## Additional Research Specimen Types

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Processing Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal Swabs</td>
<td>PAXgene Marrow Aspirate DNA Lysates</td>
</tr>
<tr>
<td>Viable Bone Marrow Aspirate</td>
<td>Viable PBSC/Bone Marrow Product Mononuclear Cells (BMMC)</td>
</tr>
</tbody>
</table>

## Protocol Development

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Stool</td>
</tr>
</tbody>
</table>
Receiving Samples

Receive and process ~55 samples daily, 6 days/week
Processing

Samples processed, aliquoted and placed in storage per study specific SOPs
Frozen and Room Temperature Dry Storage

Ultralow freezers (-80°C)

Liquid Nitrogen (LN₂)

Room temp/humidity controlled
Research Sample Life Cycle and Impact on Role of AlloHCT for Patients with MDS

Wael Saber, MD, MS
CIBMTR, Medical College of Wisconsin
November, 2018
Why Allogeneic HCT for MDS?

By Disease Status for Unrelated Donor

HLA-identical Sib Donor

Early (n=960)

Advanced (n=1,626)

Unrelated Donor

Early (n=1,442)

Advanced (n=2,433)
How Much More Life Might Perfectly Safe Curative Therapy Provide?
# Life Expectancy of Patients with MDS by IPSS, years

<table>
<thead>
<tr>
<th>AGE</th>
<th>IPSS-R Very Low</th>
<th>IPSS-R Low</th>
<th>IPSS-R Intermediate</th>
<th>IPSS-R High</th>
<th>IPSS-R Very High</th>
</tr>
</thead>
<tbody>
<tr>
<td>50y</td>
<td>&gt;13</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>55y</td>
<td>&gt;13</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>60y</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>65y</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>70y</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>75y</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
How Much More Life Might Perfectly Safe Curative Therapy Provide?

- Average life expectancy for:
  - Newborn ~ 76 years
  - 50 year old ~ 30 years (80)
  - 55 year old ~ 25 years (80)
  - 60 year old ~ 22 years (82)
  - 65 year old ~ 18 years (83)
  - 70 year old ~ 14 years (84)
  - 75 year old ~ 10 years (85)
Allogeneic HCT for MDS – Why Not?
Age Distribution of Patients with MDS

- Patients with MDS (N~15,000)
- Patients transplanted for MDS (N~1000)
Transplant-Related Mortality by Age-Standard Intensity Conditioning
TRANSPLANT-RELATED MORTALITY BY AGE
Standard vs Reduced Intensity Conditioning
So, Should Everyone Get a Transplant?

- Need to consider the expected survival with non-transplant therapy

But also

- Need to consider the likelihood of a successful transplant

<table>
<thead>
<tr>
<th></th>
<th>Training Cohort</th>
<th>Validation Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1151</td>
<td>577</td>
</tr>
<tr>
<td>Relapse (3-year)</td>
<td>25 (22-28)%</td>
<td>25 (22-29)%</td>
</tr>
<tr>
<td>TRM (3-year)</td>
<td>34 (31-37)%</td>
<td>31 (27-35)%</td>
</tr>
<tr>
<td>DFS (3-year)</td>
<td>41 (38-44)%</td>
<td>44 (39-48)%</td>
</tr>
<tr>
<td>OS (3-year)</td>
<td>43 (40-46)%</td>
<td>47 (42-51)%</td>
</tr>
<tr>
<td>Median follow-up, months (range)</td>
<td>52 (3-169)</td>
<td>48 (3-145)</td>
</tr>
</tbody>
</table>

Median follow-up, months (range) | 52 (3-169) | 48 (3-145) |
Overall Survival in HLA-matched Validation Cohort

$p < 0.001$
Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation


ABSTRACT
Approach

Cohort: 1514 MDS patients
- Broadly representative: 130 transplant centers
- Uniform diagnosis: MDS
  - No CMML or MDS/MPN
  - Blasts <20%

Analysis
- Samples: pre-HCT whole blood (NMDP biorepository)
- Targeted sequencing: 129 candidate genes
  - Myeloid malignancies
  - Inherited or acquired bone marrow failure
- Clinical annotation: CIBMTR research database
Overview of mutations

3497 mutations in 65 genes, ≥ 1 mutation in 79% of patients
Multivariable Model for Overall Survival

MDS

TP53 mutation

No
TP53 mutation

Age ≥ 40

RAS pathway mutation

JAK2V617F

No RAS pathway mutation

Age < 40

High risk features
1. Therapy-related MDS
2. Platelets < 30 x 10^9/L at HCT
3. Blasts ≥ 15% at diagnosis

≥ 1 High-risk feature

No high-risk features
TP53 mutated MDS
Poor prognosis due to early relapse

MDS

TP53 mutation
Median OS = 8 months

No TP53 mutation

Survival

No TP53 mutation
TP53 mutation
p < 0.0001

Relapse

TP53 mutation
No TP53 mutation
p < 0.0001

Years post-SCT

Probability

Years post-SCT
JAK2 and RAS pathway mutations in patients without *TP53* mutations

- No TP53 mutation
  - Age ≥ 40
  - Age < 40
- RAS pathway mutation*
  - Median OS = 11 months
- No RAS pathway mutation
  - JAK2 V617F
    - Median OS = 6 months
  - No RAS pathway or JAK2 mutation
    - Median OS = 2.3 years


Survival

Percent survival vs. Years post-SCT
JAK2 and RAS pathway mutations in patients without TP53 mutations

- No TP53 mutation
- Age ≥ 40
- Age < 40

RAS pathway mutation*
- Median OS = 11 months

- No RAS pathway mutation

JAK2 V617F
- Median OS = 6 months
- No RAS pathway or JAK2 mutation
- Median OS = 2.3 years

RAS pathway
Poor prognosis driven by relapse

JAK2 V617F
Poor prognosis driven by NRM

*RAS pathway: NRAS, KRAS, CBL, PTPN11, NF1, RIT1, KIT, FLT3
Does dose escalation (in TP53/RAS) of conditioning regimen intensity improve outcome?
TP53 mutation
Myeloablative conditioning does not improve outcome

**TP53 mutation**
**Overall Survival**

**TP53 mutation**
**Relapse**

- MAC
- RIC

$p = 0.20$
RAS pathway mutation

Myeloablative conditioning improves survival and reduces relapse

![Graph showing overall survival and relapse rates for MAC and RIC conditioning with a p-value of 0.01.](image_url)
## Conclusions

### TP53 mutations
- Poor prognosis, independent of age
  - Long-term survivors (20%)
  - No benefit to myeloablative conditioning

### RAS pathway and JAK2 mutations
- Poor prognosis in patients ≥ 40 without TP53 mutations
  - RAS: high early relapse, improved OS and relapse with MAC
  - JAK2: high NRM, no decrease in NRM with RIC
A Personalized Prediction Model for Outcomes after Allogeneic Hematopoietic Stem Cell Transplant in Patients with Myelodysplastic Syndromes (MDS)

There are no conflicts of interest to disclose.
Writing Committee

Aziz Nazha, Zhen-Huan Hu, Wang Tao, Betty Hamilton, Navneet Majhail, Coleman Lindsley, Ronald Sobecks, Uday Popat, Bart Scott, Wael Saber

On behalf of the CIBMTR® Chronic Leukemia Working Committee CIBMTR® is a research collaboration between National Marrow Donor Program®/Be The Match® and Medical College of Wisconsin.
Inclusion criteria:
✓ Pts diagnosed with MDS (WHO 2008) and registered at the CIBMTR database (2005-2014)
✓ Blasts < 20%

• Panel of 129 gene mutations
• Outcomes: OS, Relapse
• RSF algorithm was used to build the new model
• C-index used to evaluate the fit of the proposed model
Methods: Machine Learning Model

Decision-Tree

Random Forest

Random Survival Forest

- Split by log-rank
- Cumulative hazard for pt

- Age
  - <= 65
  - > 65
    - Pt< 50
    - Pt> 50
      - Hb < 8
      - Hb > 8
        - Yes
        - No
Results: New Model Building

Data
- Demographic
- Clinical
- Donor
- Transplant
- Genomics

Random Survival Forest

Training (70%)

Validation (30%)

Important Variables

Important Variables

CIBMTR
CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH
Results: Important Variables
Results: C-index

- OS
  - New Model
  - CIBMTR MDS model
  - IPSS-R

50% 53% 56% 59% 62% 65%
Results: Clinical Application

![Personalized Prediction Model for Myelodysplastic Syndromes](image)

This application will allow estimating a personalized survival probability of MDS patients at different time points. Changing the clinical and/or molecular parameters on the list will change the estimated survival of the patient.

- Survival Probability at 6 months is: **97%**
- Survival Probability at 12 months is: **95%**
- Survival Probability at 24 months is: **79%**
- Survival Probability at 36 months is: **64%**
Myelodysplastic Syndrome

Allogeneic transplantation

Cause of death

Conditioning intensity and outcome

Pasquini MC, Zhu X.
Telomere length in MDS
6 months to 77 years of age

Longest 25%
317 patients

Middle 50%
633 patients

Shortest 25%
317 patients

Slope:
-0.021, p-value < 0.001
-0.004, p-value < 0.001
-0.001, p-value 0.08
Telomere length

Overall survival outcomes based on conditioning regimen

Myeloablative

\(n=582\)

- >75\textsuperscript{th} percentile
- 25-75\textsuperscript{th} percentile
- <25\textsuperscript{th} percentile

Reduced-intensity

\(n=554\)

- >75\textsuperscript{th} percentile
- 25-75\textsuperscript{th} percentile
- <25\textsuperscript{th} percentile

\begin{tabular}{c|c|c|c|c|c|c}
No. at Risk & >75\textsuperscript{th} percentile & 75-75\textsuperscript{th} percentile & <25\textsuperscript{th} percentile & 75-75\textsuperscript{th} percentile & <25\textsuperscript{th} percentile & <25\textsuperscript{th} percentile \\
\hline
  & 151 & 102 & 87 & 74 & 58 & 46 \\
\hline
  & 291 & 160 & 118 & 97 & 69 & 49 \\
\hline
  & 140 & 62 & 49 & 40 & 27 & 21 \\
\hline
\end{tabular}

\begin{tabular}{c|c|c|c|c|c|c}
No. at Risk & >75\textsuperscript{th} percentile & 75-75\textsuperscript{th} percentile & <25\textsuperscript{th} percentile & 75-75\textsuperscript{th} percentile & <25\textsuperscript{th} percentile & <25\textsuperscript{th} percentile \\
\hline
  & 136 & 93 & 74 & 66 & 49 & 31 \\
\hline
  & 274 & 143 & 120 & 107 & 65 & 44 \\
\hline
  & 145 & 81 & 71 & 53 & 37 & 24 \\
\hline
\end{tabular}

\(P < 0.001\)

\(P = 0.008\)
Shorter telomeres
*Increased NRM in patients receiving MAC*

**Non-relapse mortality**

**Myeloablative**
(n=582)

**Reduced-intensity**
(n=554)

Probability of NRM versus Years after Transplantation.

- **Myeloablative**
  - >75th percentile
  - 25-75th percentile
  - <25th percentile
  - *P = 0.002*

- **Reduced-intensity**
  - >75th percentile
  - 25-75th percentile
  - <25th percentile
  - *P = 0.18*
Shorter telomeres

*No impact on relapse risk*

Relapse

**Myeloablative**

(n=582)

**Reduced-intensity**

(n=554)

- >75<sup>th</sup> percentile
- 25-75<sup>th</sup> percentile
- <25<sup>th</sup> percentile

(P = 0.71)

(P = 0.35)
### Multivariable models

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients</th>
<th>Hazard Ratio [95%]</th>
<th>P value</th>
<th>Death</th>
<th>Death without relapse</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No mutation (reference)</td>
<td>1005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation</td>
<td>262</td>
<td>1.72 [1.46, 2.02]</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPSS-R Risk Category</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (reference)</td>
<td>1133</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very high</td>
<td>134</td>
<td>1.69 [1.37, 2.07]</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recipient Isolation length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longest 25% (reference)</td>
<td>317</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle 50%</td>
<td>633</td>
<td>1.35 [1.13, 1.62]</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortest 25%</td>
<td>317</td>
<td>1.52 [1.24, 1.85]</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matched, Related (reference)</td>
<td>165</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matched, Unrelated</td>
<td>755</td>
<td>1.09 [0.86, 1.39]</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mismatched</td>
<td>242</td>
<td>1.55 [1.19, 2.01]</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost Blood</td>
<td>105</td>
<td>1.76 [1.26, 2.48]</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAS-ryosine kinase pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No mutation (reference)</td>
<td>1118</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation</td>
<td>149</td>
<td>1.35 [1.16, 1.59]</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 35 years old (reference)</td>
<td>755</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 years or older</td>
<td>503</td>
<td>1.25 [1.07, 1.48]</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>9</td>
<td>0.73 [0.56, 1.00]</td>
<td>0.058</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recipient age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 year increase</td>
<td>1287</td>
<td>1.15 [1.04, 1.27]</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year of transplantation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005-2007 (reference)</td>
<td>219</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008-2014</td>
<td>1048</td>
<td>0.78 [0.65, 0.95]</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karnofsky Performance Score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90–100 (reference)</td>
<td>940</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70–80</td>
<td>382</td>
<td>1.23 [1.06, 1.44]</td>
<td>0.008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>245</td>
<td>1.03 [0.86, 1.24]</td>
<td>0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAK2 V617F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No mutation (reference)</td>
<td>1232</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation</td>
<td>35</td>
<td>1.58 [1.09, 2.32]</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Future Direction

“Precision Medicine Initiative to optimize HCT outcomes in MDS”
Multivariable Model for Overall Survival

MDS

TP53 mutation

No TP53 mutation

Age ≥ 40

RAS pathway mutation

No RAS pathway mutation

JAK2V617F

≥ 1 High-risk feature

No high-risk features

Age < 40

No RAS pathway mutation

≥ 1 High-risk feature

No high-risk features

High risk features
1. Therapy-related MDS
2. Platelets < 30 x 10⁹/L at HCT
3. Blasts ≥ 15% at diagnosis

CIBMTR
CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH
Conclusions

- High quality centrally processed samples linked to clinically annotated database is an extremely valuable resource.

- Big data, hypothesis free projects are expected to become the forefront and we need to position ourselves in order to lead.

- A PMI TF is currently being assembled to provide recommendations to CIBMTR Advisory board so that the CIBMTR is well positioned.
Research Sample Life Cycle:
Acute Graft-versus-Host Disease

Shernan Holtan, MD
University of Minnesota

November 9, 2018
Outline

- Acute GVHD: models vs the human condition
- Patient story
- Using stored BMT CTN samples to test novel hypotheses
  - Epidermal growth factor (EGF)
  - Amphiregulin (AREG)
- New diagnostic and therapeutic options based on BMT CTN samples
  - Human chorionic gonadotropin/epidermal growth factor (off-label)
- Future directions
Acute GVHD Overview

Target organs:
• Skin
• GI tract
• Liver

- Common complication of allogeneic hematopoietic cell transplantation
- Essentially all prophylaxis and treatment of GVHD targets the GRAFT
  - ATG
  - Campath
  - CD34 selection
  - CNI, sirolimus, etc...

Grab your cape.
GVHD models vs the clinical syndrome

He and Holtan (2018) Current Hematol Malig Reports
Meet “Steve”

- 60 year-old male
- Day +163 post-matched sibling BMT
- Low-grade fevers
- Subtle skin rash
- Feels nauseated
- Can’t eat
- Having 8-10 diarrhea episodes/day
- Diagnosed and treated for C diff
• Serum albumin drops down to 1.4
• Does not get better with PO vancomycin
• Started on TPN and methylprednisolone for probable GVHD, undergoes endoscopy
Endoscopy with biopsy shows mild acute GVHD.

Does he really have “mild” acute GVHD?

How can we tell?

Grab your cape.

SPECIMEN(S):
A: Duodenal biopsy
B: Stomach biopsy

FINAL DIAGNOSIS:
A. DUODENAL BIOPSY:
- Mild active duodenitis
- Apoptotic bodies identified, consistent with mild graft versus host disease (GVHD)

B. STOMACH BIOPSY:
- Mild chronic gastritis
- Apoptotic bodies identified, consistent with mild graft versus host disease (GVHD)
- No H. pylori like organisms identified on routine staining
- Negative for intestinal metaplasia or dysplasia

COMMENT:
CMV IHC is in progress and the results will be submitted in an addendum report

I have personally reviewed all specimens and/or slides, including the listed special stains, and used them with my medical judgement to determine or confirm the final diagnosis.

Electronically signed out by:

Mahmoud Khalifa, M.D., PhD, UMPhysicians
Addition of biomarkers to clinical risk

- Ann Arbor (AA) biomarkers **ST2 (inflammation and damage)** and **REG3a (GI damage)** tested at GVHD onset:
  - AA1 and AA2 = less severe disease, but candidates for BMT CTN 1501
  - AA3 = more severe disease, excluded from BMT CTN 1501

- University of Minnesota approach in development:
  - Imbalance of circulating tissue repair factors
  - Prognostic information comes from:
    - Severity of damage
    - Likelihood that host can recover from the damage (regenerative capacity)
Using samples from BMT CTN 0302/0802 to test novel hypotheses about recovery from GVHD
Biomarkers of tissue repair in acute GVHD

- **Epidermal growth factor (EGF)**
  - Strong growth stimulator
  - Normally ~25-75 pg/ml in plasma

- **Amphiregulin (AREG)**
  - 10x weaker ligand than EGF
  - Normal high expression in GI tract
  - Should not be in circulation

Plasma EGF is low, AREG is high in severe acute GVHD

- Elevated AREG/EGF ratio at GVHD diagnosis is associated with a 9.4-fold increased risk of death.
- Our patient’s baseline tissue repair biomarkers:
  - AREG = 182.3 pg/ml
  - EGF = 5.3 pg/ml

Holtan et al (2015) BBMT
AREG can be tested in serum or plasma (BMT CTN 0302/0802)

**AREG cutoff by 2-fold cross-validation**

- **AREG < 33 pg/ml = “low AREG”**
- **AREG ≥ 33 pg/ml = “high AREG”**

OS by AREG and MN Risk

AREG-modified Minnesota Acute GVHD Risk Score

Eligible for high risk clinical trial

Our patient

Proportion of plasma AREG to EGF

Healthy tissue homeostasis

Unresolved tissue damage

How can we use this for therapy?

Grab your cape.
Regenerative therapy based upon our tissue repair biomarkers?

- High AREG reflects unresolved damage, little value in inhibiting it
- Increase available EGF, but how?
  - Parenteral recombinant EGF not available
### Circulating EGF increases by 20-fold in normal pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Pregnant Median (N=16)</th>
<th>Control Median (N=11)</th>
<th>Fold difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Higher in pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GROa</td>
<td>1018.6</td>
<td>341.6</td>
<td>3.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PDGF-AA</td>
<td>10740.6</td>
<td>563.8</td>
<td>19.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TGFα</td>
<td>11.7</td>
<td>1.8</td>
<td>6.5</td>
<td>0.002</td>
</tr>
<tr>
<td>EGF</td>
<td>489.4</td>
<td>24.8</td>
<td>19.7</td>
<td>0.003</td>
</tr>
<tr>
<td>PDGF-AB/BB</td>
<td>10760.7</td>
<td>3410.4</td>
<td>3.2</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Holtan, Chen, Kaimal et al. (2015) *J of Immunol Research*
EGF is concentrated in urinary-derived hCG preparations

EGF median 50,833 pg/ml

Holtan and Panoskaltsis-Mortari (2015) BMT
Holtan (unpublished)
MT2014-12

Phase I/II Study of Human Chorionic Gonadotropin and Epidermal Growth Factor Supplementation (Pregnyl®) to Support Tolerance and Repair As Adjunct Therapy in High-Risk or Refractory Acute Graft-Versus-Host Disease

**ARM 1**  
(High Risk)  
Standard of care  
Steroids  
+  
Pregnyl supplementation  
QOD x 1 week

**ARM 2**  
(Steroid Dependent/Refractory)  
Standard of care  
Steroids (increased if dependent)  
+  
Second line agent (if refractory)  
+  
Pregnyl supplementation  
QOD x 2 weeks
Our patient took >6 months to normalize serum albumin, AREG 21.8 pg/ml and EGF 5.9 pg/ml at end of treatment.

Resolution of N/V/D, but intermittent pain and residual inflammation on CT enterography.

Steroids + hCG/EGF
Future Directions

Grab your cape.
Concept of tissue repair in GVHD and inflammatory diseases gaining traction

• Mucosal healing more difficult to ascertain than skin
  • Inflammatory bowel disease: reassessed no sooner than 6 weeks
  • Might be monitored by blood biomarkers: MONITr (IBD), AREG (GVHD, ?IBD)

• Current goal = clinical complete response

• Future goal 1 = clinical complete response + complete tissue repair?
  • Resolve subclinical damage to prevent late effects

• Figure goal 2 = prediction and prevention of tissue damage in the first place?
Summary

- Tissue repair factors are altered at GVHD onset
  - Validated with 0302/0802 samples
  - EGF is low at acute GVHD onset, very low in steroid refractory GVHD
  - AREG is high, 33+ pg/ml indicates high risk

- Prospective trial for high-risk/refractory acute GVHD developed based upon results

- Future samples may help us know when we can taper/stop immunosuppression

Thank you!
sgholtan@umn.edu
@sghmd

Grab your cape.
Questions?
Thank you!