Biomarkers for Acute GVHD

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Disclosures

Financial Conflicts of Interest
• none

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• P01-AI33484, “Immunobiology of Tolerance Following Hematopoietic Cell Transplantation”
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Biomarkers in aGVHD

Goals and potential utility

- Improved diagnosis
  - lab based, objective
- Predict outcome
  - identify high risk patients
  - indication for pre-emptive or intensive therapy
- Monitor treatment response
  - guide dose adjustment and duration of IST
- Discovery
  - genes and pathways involved in pathogenesis of aGVHD
  - rationale for developing targeted therapy
Biomarkers in aGVHD

Tissue source

– Blood
  • plasma
  • WBC, PBMC, T cells
– Urine
– Tissue biopsy
  • skin
  • oral mucosa or gut
  • liver
Biomarker Approaches
(and methodologies)

• **Immunophenotyping**
  – enumeration of T cells, B cells, Treg
  – activation markers (expression of HLA-DR, CD25, FAS, etc)

• **Proteomics**
  – mass spec, discovery phase
  – antibody arrays
  – ELISA, bead-based multiplexing (*Luminex*)

• **Genomics**
  – analysis of gene expression (“transcription profile”)
The Graft-vs-Host Reaction and GVHD

Severity = **Strength** [T cell response + inflammation] \( \times \) **Duration**

[Diagram showing the relationship between clonal frequency of anti-host T cells and time post-transplant, with thresholds for different types of GVHD: Progressive GVHD, Therapy Dependent GVHD, Therapy Responsive GVHD, and No Evidence Clinical GVHD.]
Alloreactivity and GVHD

Biology of the Graft-vs-Host Reaction

- initiated by donor T cells
- activation of both adaptive and innate immune systems > acute inflammation
- pre-HCT cytotoxic conditioning therapy > gut injury
  - translocation of bacteria
  - leakage of LPS > liver injury
- all the above,
  \[\text{amplification of inflammation} \rightarrow \text{further tissue injury}\]

Clinical GVHD is a complex multi-system syndrome
Anti-host alloreactivity persists 2-3 years post-HCT

- 25% • median • 75%

- unrelated
- related

CTLp/10^6 CD3+ cells

p<0.01
p>0.1
p<0.01
p>0.1
p>0.1

pre-tx 3 month 1 year 2-3 years 3+ years

Time from transplant
Blood Lymphocytes as Biomarkers for acute GVHD

Activation and Apoptosis of Peripheral Blood Lymphocytes Early after Hematopoietic Cell Transplantation
Lin et al Blood 95:3832, 2000
Correlation between T cell apoptosis after 24-hour culture and HLA-DR expression: 36 patients studied 19-23 days post-HCT


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Apoptosis of Peripheral Blood Lymphocytes Early after Hematopoietic Cell Transplantation

Patients

## Apoptosis of Peripheral Blood Lymphocytes Early after Hematopoietic Cell Transplantation

*Lin et al Blood 95:3832, 2000*

<table>
<thead>
<tr>
<th></th>
<th>% Apoptosis $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD3+</td>
</tr>
<tr>
<td><strong>Patients, 19-23 days</strong></td>
<td>30.4±12.5</td>
</tr>
<tr>
<td>post-HCT (n=51)</td>
<td>(5.1-60.0)</td>
</tr>
<tr>
<td><strong>Normal controls</strong></td>
<td>4.0±1.5</td>
</tr>
<tr>
<td>(n=17)</td>
<td>(1.9-6.9)</td>
</tr>
</tbody>
</table>

$^1$ stained with 7ADD+ after 24-hour culture; apoptosis of CD56+CD3- NK cells, 2.2±1.2
Apoptosis of CD4+ T cells and grade 2-4 acute GVHD (day 19-23 post-HCT, 24-hour in vitro culture)

CD4+ T cell Apoptosis Correlates with grade 2-4 acute GVHD

Summary

- increased apoptosis can be detected in freshly isolated blood
- amplified by short-term culture
- associated with T cell activation
- correlates with lymphopenia
- decreases after initiation of steroids
- recurrence may predict steroid-dependent disease

Data pending replication by independent study
Blood Plasma as a Biomarker for acute GVHD
<table>
<thead>
<tr>
<th>Protein</th>
<th>aGVHD</th>
<th>TRM</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Imamura 1994; Malone 2007</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>Uguccioni 1993; Paczesny 2008</td>
<td>Schots 2003; Paczesny 2008</td>
</tr>
<tr>
<td>IL-10</td>
<td>Liem 1998</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>Nakamura 2000; Mohty 2005</td>
<td></td>
</tr>
<tr>
<td>IL-15</td>
<td>Sakata 2001</td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>Nakamura 2000; Fujimori 2000; Shaiegan 2006; Luft 2007</td>
<td></td>
</tr>
<tr>
<td>CCL8</td>
<td>Hori 2008</td>
<td></td>
</tr>
<tr>
<td>CXCL10</td>
<td>Piper 2007</td>
<td></td>
</tr>
<tr>
<td>HGF</td>
<td>Okamoto 2001; Paczesny 2008</td>
<td>Paczesny 2008</td>
</tr>
<tr>
<td>IFNG</td>
<td>Imamura 1994; Nakamura 2000</td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>Holler 1990; Symington 1990; Imamura 1994</td>
<td></td>
</tr>
<tr>
<td>TNFRI</td>
<td>Or 1996; Kitko 2008; Choi 2008; Paczesny 2008</td>
<td>Paczesny 2008</td>
</tr>
<tr>
<td>Syndecan-1</td>
<td></td>
<td>Seidel 2003</td>
</tr>
</tbody>
</table>
Critique of Published Data

Questions and issues

• aGVHD risk and incidence rates vary between Centers
• sample collection and processing is not standardized
• little documentation of assay sensitivity, specificity and reproducibility
• mostly case-control study designs, but selection matching criteria may be variable and/or vague
• usually some degree of missing or excluded data
• studies rarely include 2 phase discovery & validation cohorts, or randomization
PLASMA CYTOKINE LEVELS BEFORE and AFTER THE ONSET OF ACUTE GVHD

George B. McDonald et al, Seattle
(unpublished data)
Experimental design
McDonald et al

Study I

• 146 patients receiving CY/TBI for hematological malignancy (MURD, 139); cyclosporine + methotrexate prophylaxis
• blood drawn weekly to day +56, processed rapidly
• plasma levels of 11 cytokines analyzed by ELISA
### Plasma cytokine level changes over 15 day interval prior to onset of grade III/IV GVHD

<table>
<thead>
<tr>
<th>Rising Levels (slope positive)</th>
<th>No change</th>
<th>Falling Levels (slope negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-1α</strong> +68±18% (p=.004)</td>
<td><strong>IL-1-beta</strong> IL-2 IL-4 IL-10 TNFα IFNγ IL-1RA</td>
<td><strong>TGF-b1</strong> -43±19% (p=.002)</td>
</tr>
<tr>
<td><strong>IL-6</strong> +361±45% (p=.0003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>sTNFRI</strong> +20±6% (p=.002)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Plasma cytokine level changes prior to onset of GVHD

<table>
<thead>
<tr>
<th>GVHD grade</th>
<th>(\Delta) IL-1(\alpha)</th>
<th>(\Delta) IL-6</th>
<th>(\Delta) sTNFRI</th>
<th>(\Delta) TGF-(\beta)1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – I (N = 16)</td>
<td>+22.8 ± 9.2% (p=.03)</td>
<td>-26.9 ± 64.7% (p=.54)</td>
<td>+27.2 ± 12.6% (p=.06)</td>
<td>+18.7±16.3% (p=.27)</td>
</tr>
<tr>
<td>II (N = 61)</td>
<td>+32.2 ±16.7% (p=.08)</td>
<td>+93.0 ±23.3% (p=.003)</td>
<td>+16.1 ± 3.5% (p&lt;.0001)</td>
<td>-43.5±15.8% (p=.0003)</td>
</tr>
<tr>
<td>III – IV (N = 30)</td>
<td>+68.2 ±17.9% (p=.004)</td>
<td>+361.1 ±45% (p=.0003)</td>
<td>+20.2 ± 5.6% (p=.002)</td>
<td>-43.4±18.5% (p=.002)</td>
</tr>
</tbody>
</table>
### Median values for plasma cytokines prior to onset of acute GVHD

McDonald et al

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Normal upper limit (NUL)</th>
<th>GVHD 0/I (N=16)</th>
<th>GVHD II (N=61)</th>
<th>GVHD III/IV (N=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>225 pg/mL</td>
<td>39.8 (.18 x NUL)</td>
<td>43.5 (.19 x NUL)</td>
<td>75.5 (.34 x NUL)</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.7 pg/mL</td>
<td>15.4 (22 x NUL)</td>
<td>20.7 (30 x NUL)</td>
<td>34.9 (50 x NUL)</td>
</tr>
<tr>
<td>TNFα</td>
<td>12 pg/mL</td>
<td>7.7 (.64 x NUL)</td>
<td>6.9 (.58 x NUL)</td>
<td>12.3 (1 x NUL)</td>
</tr>
<tr>
<td>TNFRI (p55)</td>
<td>925 pg/mL</td>
<td>1308 (1.4 x NUL)</td>
<td>1163 (1.3 x NUL)</td>
<td>1161 (1.3 x NUL)</td>
</tr>
<tr>
<td>TGFβ 1</td>
<td>9445 pg/mL</td>
<td>701 (.07 x NUL)</td>
<td>424 (.04 x NUL)</td>
<td>441 (.05 x NUL)</td>
</tr>
</tbody>
</table>
Conclusions – Study I

McDonald et al, unpublished

1. Plasma levels of IL-6, IL-1a, and sTNFRI (p55) are increasing, and TGFb1 decreasing, prior to onset of clinical GVHD.

2. The slopes of plasma IL-6 levels before GVHD onset are steeper than those of IL-1a and sTNFRI.

3. Plasma IL-6 levels (but not IL-1a and sTNFRI) exceed the normal upper limit for this cytokine in healthy volunteers.

4. Plasma IL-6 level prior to onset of clinical GVHD correlates with severity of GVHD.

1 Study I did not include IL-2Ra, IL-8 or HCF
Experimental design
McDonald et al

Study II

• 160 patients transplanted for hematological malignancy
  • myeloablative and reduced intensity
  • CSP+MTX, FK+MTX, and FK+MMF prophylaxis
• Blood routinely drawn weekly to day +56, and day 80
  • day 14 after starting IST for aGVHD Rx
• Plasma cytokine levels analyzed by Luminex
  • enlarge panel of analytes (IL-2Ra, IL-8, HCF and others)
• Randomly select cases & controls for discovery and replication cohorts
  • correlated with acute GVHD, treatment response, mortality

Status: analysis pending
“A Biomarker Panel for Acute GVHD”


*Blood* 113:273-278, 2009

First GVHD biomarker study to include a separate and independent *discovery* and *validation* phase
Study plan

• **466 subjects** receiving allo HSCT between 2001-2006 at the Univ of Michigan

• **Excluded** patients with VOD, IPS, septic shock (15%)
  – ~70% myeloablative, ~35% unrelated donor
“A biomarker panel for acute GVHD”  

**Study plan**

- **Discovery phase**, 42 patients selected for case-control study
  - 21 patients, grade 3-4 aGVHD
  - 21 patients, grade 0 aGVHD

- **Replication phase**, 424 patients randomly separated into:
  - *training set*, n=282; 166 GVHD (grade 0), 116 GVHD (grade 1-4)
  - *validation set*, n=142; 76 GVHD (grade 0), 66 GVHD (grade 1-4)
A biomarker panel for acute GVHD
Pczesny et al 2009

Results

• Discovery phase:
  – Antibody microarrays identified 35 of 120 plasma proteins significantly associated with severe grade 3-4 aGVHD
  – 23 of the 35 proteins selected for testing in a sequential ELIZA assay (to preserve sample)
  – 8 proteins gave p-value <.01 comparing GVHD+ and GVHD- patients
DISCOVERY PROTEOMICS
POTENTIAL GVHD BIOMARKERS

<table>
<thead>
<tr>
<th>Protein</th>
<th>GVHD-</th>
<th>GVHD+</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2Rα</td>
<td>** ***</td>
<td>*** **</td>
</tr>
<tr>
<td>CRP</td>
<td>** ***</td>
<td>*** **</td>
</tr>
<tr>
<td>IL-8</td>
<td>* ********</td>
<td>***********</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>************</td>
<td>***********</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>************</td>
<td>***********</td>
</tr>
<tr>
<td>TNFR1</td>
<td>************</td>
<td>***********</td>
</tr>
<tr>
<td>HGF</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CA19.9</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VEGF-D</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VACM-1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-10</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Angiostatin</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IGF-1</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>FGF-basic</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>MMP-2</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Angiopoietin</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>TGFβ</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>FasL</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Fold-change from the mean:
- 1/4
- 1/2
- 1
- 2
- 4
A biomarker panel for acute GVHD
Paczesny S et al, 2009

8 proteins selected from the Discovery phase antibody array + ELISA study for Validation

- IL-2Ra
- CRP
- IL-8
- ICAM-1
- TIMP-1
- TNFRI
- HGF
- CA19.9
Study plan

- Replication phase, 424 patients randomly separated into:
  - training set, n=282; 116 GVHD+, 166 GVHD-
  - validation set, n=142; 66 GVHD+, 76 GVHD-
- Sequential ELISA performed for 8 biomarkers
- Median values and individual AUCs determined for the training set

- GVHD+, grade 2-4; GVHD-, grade 0
- median onset, day 30
Results – Training set:

- Linear regression determined that a linear combination of 4 proteins produced the best model to predict acute GVHD

- IL-2Ra
- TNFR1
- HGF
- IL-8

Proteins failing conformation in the training set:

- CRP, ICAM-1, TIM-1, CA19.9
TOP FOUR PROTEINS IN THE TRAINING COHORT


IL-2Rα

TNFR1

HGF

IL-8

GVHD -

GVHD +
ROC Curve of the Training Cohort for Individual Proteins and Composite Panel


(Sensitivity)

True Positive Rate

False Positive Rate

(1 - Specificity)
Survival is predicted independently by GVHD grade and Biomarker Panel


P<0.001

Low, n=286   
High, n=138

Grade 0-I, n=276   
II-IV, n=148

Low, n=286   
High, n=138
Are plasma biomarkers “ready”?

• **Clinical trials**
  – YES, candidate plasma proteins should be incorporated into prospective multi-center trials
    • adjunct or stand alone studies
    • avoid exclusions and missing data
    • evaluate specificity in patients with bacteremia, IPS, VOD, etc
    • determine time-dependent kinetics prior to onset
    • define early changes predictive aGVHD onset & severity

• **Diagnostics, preemptive therapy**
  – pending prospective validation and deeper analyses
Thank you!