Molecules and mechanisms of the graft versus leukemia (GVL) effect
Back row (from left to right):
David Brown
Arnold Naimark
Robert Hoeschen
Colin Sinclair
Earl Herschfield
Gordon Watters
Monty Hart
Tom Casey
Larry Kusin

Front Row:
Sherman Herschfield
George Yee
Martin Hollenberg
Gerald Goldenberg
Rod McPherson

University of Manitoba Six-Man Football Champions 1956

University of Manitoba Faculty of Medicine Archives
The “Goldenberg” Rules

• We are fortunate to have the opportunity to participate in research. Do not squander the opportunity -- commit yourself to a lifetime of learning.

2. Patients and professors can teach us much, if we pay attention.

3. Treasure the time you have for research, it is special.

4. Use correct grammar!!
Immunologic Non Identity Contributes To The Efficacy Of Allogeneic HCT

CML > CLL, low grade lymphoma, myeloma > AML, ALL
Myeloablative Conditioning (Age <50)

- Full donor chimerism
- No circulating malignant cells

Toxicity
- GVHD
- Relapse

Success rate ~ 30 - 60% in AML/ALL/MDS, 80% CML

Nonmyeloablative Conditioning (Age up to 70)

- Mixed hematopoietic chimerism
- Circulating malignant cells
- CSA/MMF immunosuppression

GVHD
- Relapse

Success rate ? (~50% for indolent leukemias (CLL), follicular lymphoma
Genetic Polymorphism Results in the Display of Unique Peptides by Cell Surface MHC Molecules

- Donor T Cells
  - Tolerance
  - Donor Cells
  - Activation

- Nonsynonymous SNPs
- 5’ “untranslated regions”
- Differential gene expression
- Peptide splicing/reassortment
Minor H Antigens - Characteristics of Effective Targets for Cancer Immunotherapy

- Antigens are “foreign” to donor T cells
- T cell responses are of high avidity
- T cell responses may be multivalent
- Responses typically involve both CD8 and CD4 T cells

Can the GVL effect for acute leukemias be augmented and separated from GVHD?
Tissue Expression of Minor H Antigens May Provide A Basis for Segregating GVHD and GVL Responses
Isolation of T Cells Specific For Recipient Minor H Antigens After In Vivo Priming

PBMC from recipient post transplant

γ-irradiated APC (PBMC or leukemia) from the recipient pre transplant

Assay for cytolytic activity

Clone reactive T cell lines and characterize T cell clones for phenotype, HLA restricting allele, tissue expression
Part 1. Lost in Translation

Leukemic Stem Cell

Self Renewal

CD34+CD38-

Clonogenic Leukemic Progenitors

"Hematopoietic Lineage"-Restricted Minor H Antigen

AML +/- CTL

375 cGY

% Engraftment

Control MHAg CTL

• Bonnet D. et al. Proc Natl Acad Sci 1999
Adoptive Transfer of Minor H Antigen-Specific T Cell Clones To Treat Post Transplant Leukemic Relapse

Outcome - Post transplant Relapse

- Enroll patients with advanced leukemia (>2nd CR, refractory disease)
- Isolate CD8+ T cell clones from recipients early post transplant
- Select clones that lyse recipient hematopoietic cells (including leukemia) BUT NOT skin fibroblasts
- QC testing & cryopreservation
- Treat patients at relapse with escalating doses of T cells
- Evaluate toxicity, T cell migration and persistence, and antitumor activity
- Early TRM or relapse -- 32% of enrolled patients
- 7 patients relapsed and received T cell infusions
- 3 patients relapsed and had T cells available - not treated
- 6 patients have T cells available and remain at risk
UPN-15652
- primary refractory Ph⁺ ALL
- MRD SCT with Cy/TBI
- Isolated minor H antigen-specific CTL clones
- Relapse 7 months post SCT (>90% blasts)
- Cytoreduction with Mitoxantrone/VP16

CTL Clone 11C6

% Specific Lysis

- Recipient LCL
- Recipient Fibroblasts
- Leukemic Blasts
- Donor LCL
- Donor Fibroblasts
**Toxicity:**

* Skin GVHD (PCR -ve for CTL)  
* Lung Toxicity

**T Cells**

![Graph showing T Cells over days with markers for toxicity]

**WBC**

![Graph showing WBC and Blasts over days]

**Blasts in BM**

<table>
<thead>
<tr>
<th>Day</th>
<th>WBC (x10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
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<td>10</td>
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<td>20</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
</tr>
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</table>

**Blasts in BM**

- 90% at Day 0  
- >15% at Day 57
Transferred CTL Localize At Site of Tissue Injury and Modulate TcR Expression

Modulation of Vβ 13 TcR Expression in bronchoalveolar lavage fluid

Pre-Inf

4 hr Post T Cells

MP

2 days Post T Cells

Detection of CTL by clone-specific PCR

PBMC
+ve pre BAL +3 +12 +21 +66
**Toxicity:**

- Skin GVHD (PCR -ve for CTL) *
- Lung Toxicity *

**T Cells**

- Methylprednisolone

**Graph:**

- WBC
- Blasts

**Legend:**

- Blasts in BM: 90% >15% <5%
Post chemotherapy - Relapse

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

UPN15652

Post T cell infusions - Remission

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
### Patient 3 ALL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre</th>
<th>Post CTX</th>
<th>Post T Cells</th>
<th>Outcome</th>
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</thead>
<tbody>
<tr>
<td>#3 ALL</td>
<td>90%</td>
<td>80%</td>
<td>&lt;5% CR</td>
<td>No GVHD, toxicity</td>
</tr>
</tbody>
</table>

**Bone Marrow Blasts**

- **Infusion #**
  - 3: + PBL M
  - 4: PBL M
  - 6: PBL M

**Clone-specific TcR V beta PCR**
Results

• Toxicity

  • 3/7 patients (2 lung, 1 GVHD)

• Transfer efficiency and migration

  • high levels of transferred T cells in the blood and bone marrow (>8% of PBMC), t1/2 of transferred cells in the blood ~ 7 days

• *Efficacy

  • 4/6 evaluable patients achieved CR, including 2 with persistent disease after chemotherapy.

  • 6/7 patients subsequently progressed 4 -15 months after therapy, 1 patient remains alive >39 months
• Isolation of minor H antigen specific T cells from patients transplanted for advanced leukemia is feasible but problematic

• Separation of GVL effect from toxicity cannot be achieved by selecting clones based on in vitro cytotoxicity against non-hematopoietic targets

3. Adoptively transferred CD8+ T cell clones exhibit limited persistence in vivo

   Mechanisms responsible may include:
   • high antigen load - activation induced cell death
   • inadequate T helper responses/prosurvival cytokines
   • intrinsic defect due to culture and/or differentiation
   • other
Part 2. Back to Basics
Identification of the Genes that Encode Minor H Antigens

Recipient EBV-LCL → cDNA → cDNA Library → Plasmid Vector Encoding HLA-Alele

mRNA For mHAg → pEAK10 Plasmid Vector

IFN-γ (ELISA) → COS Cells

CTL Clone
UDP Glycosyltransferase 2 Family, Polypeptide B17 (UGT2B17) Encodes a Minor H Antigen Presented by HLA A29

CTL Clone PL-8

Construct I (52-513)
Construct II (493-993)
Construct III (493-564)

UGT2B17 cDNA

ATG

TAA

ATG

TAA

ATG

TAA

(−)

(+)

(−)

Recognition of Donor LCL Precultured with Synthetic Peptide

Specific lysis (%)

0 10 20 30 40 50 60

Peptide concentration (nM)

0 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4}

HLA-A29 Anchors

1 2 3 4 5 6 7 8 9

E Y
Immunogenicity of UGT2B17 Results from Differential Transcription in Recipient versus Donor Cells

Northern Blot Analysis of Total RNA

<table>
<thead>
<tr>
<th></th>
<th>Recipient LCL</th>
<th>Donor LCL</th>
<th>Unrelated HLA-A29+ LCL</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ag+</td>
</tr>
<tr>
<td>UGT2B17</td>
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<td></td>
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</tr>
<tr>
<td>GAPDH</td>
<td></td>
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</tbody>
</table>
SSP-PCR Primers exon 1 (a)

SSP-PCR Primers exon 1 (b)

PCR Primers for 5' upstream

SSP-PCR Primers for exon 6

11% of Caucasian donors are deficient in UGT2B17
**Tissue Expression Matters**

SSP-PCR for UGT2B17 cDNA from Human Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>UGT2B17</th>
<th>GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
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<tr>
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<tr>
<td>Pancreas</td>
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<tr>
<td>Spleen</td>
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<td>Thymus</td>
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<td>Prostate</td>
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<tr>
<td>Testis</td>
<td></td>
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<tr>
<td>Ovary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
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<td></td>
</tr>
</tbody>
</table>

- the recipient had acute GI/liver GVHD and cGVHD involving the GI tract
DRN-7 CTL Recognize A Minor H Antigen Presented By HLA -A3 And Encoded by the SP110 gene

CTL Clone DRN-7

Donor SP110 - G

Genotype

PCR-RFLP

% Specific Lysis

DRN  CAN  SEB  ABB  WGB  RLB  MDK  TM  WAS  JDS  JR

+ + +
What is the SP110 epitope?

- 20 mer peptide containing G-->R substitution sensitized donor LCL for recognition by CTL but only at high concentrations (>10 μg/ml)

- No synthetic 8 - 12 mer peptide comprised within the 20 mer sequence could be identified that sensitized target cells for recognition by the CTL clone

- Consistent with a requirement for a posttranslational modification for epitope generation
Immune recognition of a human renal cancer antigen through post-translational protein splicing

Ken-ichi Hanada¹, Jonathan W. Yewdell² & James C. Yang¹

¹Surgery Branch, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, Building 10, Room 2B42, Bethesda, Maryland 20892, USA
²Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA

An Antigenic Peptide Produced by Peptide Splicing in the Proteasome

Nathalie Vigneron,¹* Vincent Stroobant,¹* Jacques Chapiro,¹
Annie Ooms,² Gérard Degiovanni,² Sandra Morel,¹†
Pierre van der Bruggen,¹ Thierry Boon,¹
Benoît J. Van den Eynde¹‡

www.sciencemag.org  SCIENCE  VOL 304  23 APRIL 2004
Does the Proteasome Generate The SP110 Epitope?
Is the SP110 epitope a product of peptide splicing?

STPKRRHKKKS

STPKSLPRGTASSR

STPK + SLPRGTASSR

STPK + SLPRGTASSR

SLPRGTASSR

STPK

Interferon gamma (ng/ml)

But ---- STPKSLPRGT did not sensititize target cells for recognition
HLA A3 binding motif

STPKRRHKKKSLPRGTA

ASSR

HLA A3 binding motif

L _ _ _ _ _ K

STPK  SLPRGT  →  SLPRGTSTPK
Peptide Splicing With A Twist -- Rearrangement of SP110 Encoded Peptides Creates The DRN-7 Epitope
The Putative DRN-7 Epitope SLPRGTSTPK Co-elutes With The Naturally Processed Epitope
PANE-1 transcript k -- a B-lineage minor H antigen

Clone KSN-7A7

% Lysis

Target Cell

1.E-03 1.E-02 1.E-01 1.E+00 1.E+01 1.E+02 1.E+03

[Peptide], nM

% Specific Lysis

RVWDLPGVLK on GAO LCL
RVWDLPGVLK on T2-A3
SLPRGTSTPK on GAO LCL
SLPRGTSTPK on T2-A3
PANE-1 is selectively expressed in B-lineage cells
Expression of PANE-1 by q-PCR

<table>
<thead>
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<th>Tissue Source</th>
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<tr>
<td>Colon</td>
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<tr>
<td>Small Intestine</td>
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<td>Lung</td>
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<tr>
<td>Heart</td>
<td></td>
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<tr>
<td>Brain</td>
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Relative Level of Expression
<table>
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<tr>
<th>Minor Histocompatibility Antigen</th>
<th>HLA restriction</th>
<th>Gene/chromosome</th>
<th>Peptide sequence</th>
<th>Tissue distribution</th>
<th>Identification technique</th>
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<td>HA-1a</td>
<td>HLA A201</td>
<td>KIAA0223/19 p13</td>
<td>VLHDDLLEA</td>
<td>Hematopoietic</td>
<td>HPLC with mass spectometry</td>
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<tr>
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<td>HLA A201</td>
<td>MYOG1/7</td>
<td>YIGEVLSV</td>
<td>Hematopoietic</td>
<td>HPLC with mass spectometry</td>
</tr>
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<td>HA-2a,b</td>
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<td>HPLC with mass spectometry</td>
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<tr>
<td>HA-8a</td>
<td>HLA A201</td>
<td>KIAA0020/9</td>
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<td>HPLC with mass spectometry</td>
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<td>HB-1a,b</td>
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<td>HLA B4403</td>
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<td>DYLQYVKQI</td>
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<td>Genetic linkage analysis</td>
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<td>UTY</td>
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<td>HLA DRB3</td>
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<td>C2orf18</td>
<td>RDWDLPVGLVK</td>
<td>B-cell (CLL)</td>
<td>HPLC/mass spectometry</td>
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<td>SP110</td>
<td>HLA A3</td>
<td>SP110</td>
<td>SLPRGTVSTP^</td>
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<td>SNP (L-&gt;H)</td>
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</tr>
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</table>
• GVHD is common after unmodified allogeneic HCT requiring the administration of immunosuppression, poor platform for immunotherapy to augment GVL effect

Part 3 - A Naïve Answer (?)
Memory CD4+ T cells do not induce graft-versus-host disease

Britt E. Anderson,1 Jennifer McNiff,2 Jun Yan,3 Hester Doyle,3 Mark Mamula,3 Mark J. Shlomchik,1,4 and Warren D. Shlomchik1,5

1Section of Immunobiology,
2Department of Dermatology,
3Section of Rheumatology,
4Department of Laboratory Medicine, and
5Section of Medical Oncology, Yale University School of Medicine, New Haven, Connecticut, USA

Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality in allogeneic stem cell transplantation (alloSCT). Donor T cells that accompany stem cell grafts cause GVHD by attacking recipient tissues; therefore, all patients receive GVHD prophylaxis by depletion of T cells from the allograft or through immunosuppressant drugs. In addition to providing a graft-versus-leukemia effect, donor T cells are critical for reconstituting T cell–mediated immunity. Ideally, immunity to infectious agents would be transferred from donor to host without GVHD. Most donors have been exposed to common pathogens and have an increased precursor frequency of memory T cells against pathogenic antigens. We therefore asked whether memory CD62L−CD44+ CD4+ T cells would induce less GVHD than unfractionated or naive CD4+ T cells. Strikingly, we found that memory CD4 cells induced neither clinical nor histologic GVHD. This effect was not due to the increased number of CD4−CD25+ regulatory T cells found in the CD62L−CD44+ fraction because memory T cells depletion of these cells did not cause GVHD. Memory CD4 cells engrafted and responded to antigen both in vivo and in vitro. If these murine results are applicable to human alloSCT, selective administration of memory T cells could greatly improve post-transplant immune reconstitution.


Chen B et al. Blood 2004
Xystrakis E. et al Eur J Immunol 2004
Zhang Y et al J Immunol 2005
In the absence of priming of the donor to alloantigens, the overwhelming majority of alloreactivity should reside in the naïve T cell pool.
Direct Analysis of Minor H Antigen Alloreactivity In Naïve and Memory Subsets of CD8\(^+\) T Cells

- 5 HLA identical sibling pairs

- Purified naïve (Tn -- CD45RA, CD62L+) and memory (Tm -- CD45RO) T cells

- Limiting dilution assay using purified Tn and Tm cells as responder cells, recipient dendritic cells as the APC, and IL12/IL15

- Recipient and donor DC or CD40L B cells as target cells, validation of positive wells
Naïve T Cell Purification
- T cell clones specific for broadly expressed and lineage restricted minor H antigens
Stem Cells + Memory T cells

Conditioning Decreased posttransplant immunosuppression

Predicted Outcome:
• rapid immunologic recovery
• few infections
• little or no GVHD

How to restore the GVL effect?
- improved platform for immunotherapy
- vaccinate donors to elicit lineage restricted minor H antigen specific memory T cells
  OR
- T cells specific for leukemia associated or minor H antigens isolated from naïve donor T cells could be adoptively transferred
Pilot trial of naïve T cell depletion to reduce GvHD

- HLA id siblings (acute leukemia, donor parity/transfusion hx)
- high stem cell dose
- CD34 selection, CD62L depletion (removes all naive and central memory cells) -- administer defined dose of memory T cells
- FK506/MTX initially, if reduction in GvHD, test FK506 alone (MTX may inhibit homeostatic proliferation of memory T cells and delay immune reconstitution to pathogens)

End points
- engraftment
- relapse
- infections
- GVHD
- immune reconstitution (CMV, EBV, HSV, aspergillus)
• Genotyping for relevant minor H antigens based on HLA type
• Vaccination of the donor to one or more antigens
• Preparation of stem cell graft (naïve T cell depletion)
• Vaccination of recipient to boost leukemia associated minor H antigen-specific T cell responses
Edus H. Warren
Marc Gavin
Jeff Mito
Michele Brown

Marie Bleakley
Audrey Mollerup
Thomas Manley
Larry Anderson
Tory Yamamoto

Nathalie Vigneron
Benoit van den Eynde
(Ludwig Institute, Brussels)

Tony Brickner
Vic Engelhard
(University of Virginia)

Warren Shlomchik
(Yale University)

Tetsuya Nishida