Therapy for Leukemia with Antigen-Specific T Cells

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Fred Hutchinson Cancer Research Center
ISCT 2010 Annual Meeting
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T cells recognize short peptides presented on the cell surface by MHC molecules.

MHC-association “peptidome” comprises ~10,000 distinct peptides derived from:
- nuclear proteins
- cytoplasmic proteins
- membrane proteins
- cryptic proteins...

MHC Class I with peptide
MHC Class II with peptide

αβ TCR
Peptide – MHC Class I
Targets for T cell therapy on leukemic cells

- **Tumor-associated antigens:**
  - PR1
  - WT1

- **Tumor-specific antigens:**
  - Cancer-testis genes such as NY-ESO-1
  - PRAME
  - BCR/ABL junctional peptides

```
...VLQELNVTV...
Elastase_{168-176}
Proteinase 3_{168-176}
→ HLA-A*0201

...RMFPNAPYL...
WT1_{126-134}
→ HLA-A*0201
```
Cured by cancer immunotherapy

Patient Reunion 2005
Minor histocompatibility antigens as targets for T cell therapy after allogeneic HCT

Peptide/MHC complexes

Donor T Cell

Recipient Cell

Tissue expression:
- Broad: GVHD & GVL?
- Narrow: GVL?
Variation in both genome sequence and structure create minor histocompatibility antigens

**Sequence variation: SNPs**

**KIAA0020** (HLA-A*0201)

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<th>GAC</th>
<th>AAA</th>
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**HB-1** (HLA-B*4402,4403)

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<td>L</td>
<td>Y</td>
<td>V</td>
<td>W</td>
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**Structural variation: gene deletions**

e.g. **UGT2B17**

![Diagram showing gene deletions and variation](image)
Reordering and splicing of noncontiguous peptides from SP110 create a minor H antigen

**SP110** – Chromosome 2q

<table>
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<th>Donor</th>
<th>TCAACTCCAAAAAGGAGACATAAGAAAAAAAGCCTCCAGAGGGACA...</th>
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<tr>
<td>Recip</td>
<td>TCAACTCCAAAAAGGAGACATAAGAAAAAAAGCCTCCAGAGGGACA...</td>
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<tr>
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</tr>
<tr>
<td><strong>S T P K R R H K K K S L P R G T</strong></td>
<td>(+)</td>
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SLPRGTSTPK

For the immunologist, the practical conclusion is that the complete spectrum of peptides presented by a given cell type cannot be deduced from the predicted sequence of the major products of the active genes.

How many minor histocompatibility antigens are there?

- > 150,000 nonsynonymous coding SNPS in HapMap
- Several dozen genes for which one or more exons are ablated by common (MAF > 5%) deletion polymorphisms
Adoptive therapy with CD8\(^+\) minor H antigen-specific T cells to augment GVL

**Study population**: Hematologic or molecular relapse of AML/ALL/MDS after HLA-identical allogeneic HCT

**Objectives**: 1 : Evaluate safety of T cell therapy  
2 : Evaluate *in vivo* persistence, migration, and antileukemic efficacy of adoptively transferred CTL

**FHCRC Protocol 1334**

**Withdrawal of immune suppression; Chemotherapy**

<table>
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<th>CTL Infusions</th>
<th>CTL Infusions</th>
<th>IL-2</th>
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<tr>
<td>-2</td>
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<tr>
<td>4</td>
<td>11</td>
<td>21</td>
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<tr>
<td>28</td>
<td>≥35</td>
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*patients with hematologic relapse

Generation of donor-derived T cell clones specific for recipient minor H antigens

HCT Recipient

Posttransplant PBMC

HCT Donor

Ex vivo Expansion to ~ 3 – 6 x 10^9 cells

17 – 22 Weeks: 120 – 150 days

HCT Donor

Minor Antigen-Specific T Cell Clones

Polyclonal Minor Antigen-Specific T Cells

HCT Recipient
Selection criteria for CTL clones to be used in adoptive GVL therapy
Protocol 1334 – Summary of enrolled patients

- 71 patient/donors enrolled, samples acquired; 70 underwent HCT
- CD8⁺ minor H antigen-specific CTL clones suitable for use in therapy generated for 25 recipients (35%)

Grade 2-4 acute GVHD

Clinical extensive chronic GVHD
Protocol 1334 – Summary of patients treated with CD8+ T cells

- 46 infusions of 8 different CD8+ minor H antigen-specific CTL clones administered to 7 patients
- Highest single dose administered (range): $2.25 \times 10^9$ – $6.6 \times 10^9$ cells
- Transferred CTL detected in blood and bone marrow in 5 of 7 patients, and persisted \textit{in vivo} up to 21 days after infusion
- Toxicity:
  - fever/chills universal
  - pulmonary toxicity in 3 patients – clearly attributable to T cell therapy in at least 2 patients
  - GVHD requiring immunosuppressive Rx developed in 3 patients, all of whom had been on GVHD Rx at the time of relapse
In vivo persistence of adoptively transferred CTL and migration to bone marrow

T cell clone-specific Q-PCR
Clone 11C6-109
Gene: P2RX7

Clone 68H7-819
Gene: DDX3Y

Clone 50F5-448
Gene: DPH1

UPN 15652 clinical course – Ph⁺ ALL

Legend
- Morphology
  - Flow cytometry
  - Cytogenetics
- Relapse
- Remission
- Unavailable/nondiagnostic

Chemotherapy
- CTL, Dose < $10^8$
- CTL, Dose $10^8-10^9$
- CTL, Dose > $10^9$
- Interleukin-2

Histologic oral GVHD
- Histologic skin GVHD
- Histologic gut GVHD
- Death
Acute pulmonary toxicity – UPN 15652

- Infusion #6 – cell dose $2.25 \times 10^9$
- Noncardiogenic pulmonary edema
- Treated with intubation, mechanical ventilation, methylprednisolone 2 mg/kg day
- Prompt resolution
In vivo tracking of CTL 11C6 TCRβ CDR3 detects high levels in bronchoalveolar lavage fluid

**CTL 11C6 TCRβ CDR3**

**β₂-microglobulin**
CD8\textsuperscript{+} T cells from BAL show TCR downregulation, suggesting recent antigen encounter.

- **CD8\textsuperscript{+} CTL prior to infusion**
- **Mononuclear cells recovered from BAL fluid**
P2RX7, the target of 11C6-109, is specifically expressed in the pulmonary alveolar epithelium.

Keith Loeb, FHCRC
Evidence for antigen loss and immune escape in UPN 15652

6 infusions of mHAg-specific T cells
No change in surface phenotype of leukemic cells between 1\textsuperscript{st} and 2\textsuperscript{nd} relapse

- 1\textsuperscript{st} relapse
- 2\textsuperscript{nd} relapse

- CD80
- CD54 (ICAM-1)
- CD86
- CD58 (LFA3)
- PDL1
- CD44
- HLA-pan class I
- CXCR4
- HLA-A29
UPN 19492 clinical course – B-ALL

**Legend**

- Morphology
- Flow cytometry
- Cytogenetics
- □ Relapse
- □ Remission
- □ Unavailable/nondiagnostic

<table>
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<th>6 months</th>
<th>9.1 months</th>
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**HCT**

**Relapse**

- 0 months
- 1 month
- 2 months
- 3 months
- 4 months
- 5 months
- 6 months

**Months Post Relapse**

- Chemotherapy
  - CTL, Dose < $10^8$
  - CTL, Dose $10^8-10^9$
  - CTL, Dose > $10^9$
- Interleukin-2
- Histologic oral GVHD
- Histologic skin GVHD
- Histologic gut GVHD
- Death

**Interpreted Diagram:**

- The timeline represents the clinical course of UPN 19492 with key events marked at specific time points.
- The timeline includes phases such as HCT (stem cell transplant) and relapse.
- Treatment interventions like chemotherapy and immunotherapy are indicated.
- The legend provides a key to interpret the various symbols used in the diagram.
CTL clone 68H7-819 recognizes an H-Y antigen encoded by the Y chromosome gene \textit{DDX3Y}

Rosinski \textit{et al.}, \textit{Blood} 2008; 11:4817-4826
DDX3Y is expressed in most primary male hematologic malignancies

Representative data from 187 primary male hematologic malignancies shown.

* KG1, male AML cell line
DDX3Y is expressed at high levels in blood stem cells
The *DDX3Y*-encoded H-Y antigen is expressed in CML and AML stem cells.

DDX3Y-specific T cells

Primary leukemia cells

Culture overnight

350 cGy

Evaluate survival, leukemic engraftment

Days post injection

Proportion surviving

Days post injection: 0, 7, 14, 21, 28, 35

Proportion surviving: 0, 0.2, 0.4, 0.6, 0.8, 1

PCR for human Y-chromosome DNA

Rosinski *et al.*, *Blood* 2008; 11:4817-4826
UPN 15408 clinical course - AML

Legend
- Morphology
- Flow cytometry
- Cytogenetics
- ■ Relapse
- □ Remission
- □ Unavailable/nondiagnostic
- ★ Histologic oral GVHD
- ★ Histologic skin GVHD
- ★ Histologic gut GVHD
- ♦ Death

Timeline:
- Months Post Relapse:
  - 0 months: HCT
  - 6 months: Relapse
  - 7 months: Chemotherapy
  - 8 months: CTL, Dose < 10^8
  - 9 months: CTL, Dose 10^8-10^9
  - 10 months: CTL, Dose > 10^9
  - 11 months: Interleukin-2
  - 12.5 months: Death

Timeline:
- 43 months: 15408
Defective *DPH1*-encoded antigen expression in UPN 15408 leukemic cells: immune evasion?

Surface phenotype of UPN 15408 leukemic cells

![Graphs showing expressions of various markers: HLA class I, PDL1, CD80, CD86, CD54, CD58, CD44, CXCR4.](image)
Expression of minor H antigen-encoding genes in lung tissue correlates with pulmonary toxicity
FHCRC Protocol 1334 – lessons learned

• High prevalence of acute and, more importantly, chronic GVHD → New transplant platforms? Steroid-resistant CTL?

• Inordinately long time required for generation of mHAg-specific T cells from recipient → generate cells directly from donor

• Short *in vivo* persistence of adoptively transferred cells → transfer cells that will reconstitute both effectors and long-term immunologic memory

• Lack of reactivity with recipient fibroblasts is not always a sufficiently stringent criterion for selecting CTL that can be transferred safely

• Inadequate antileukemic activity of transferred cells → identify better target antigens
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Jerome Ritz
David Ginsburg

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National Cancer Institute
Burroughs Wellcome Fund

UW Royalty Research Foundation
J. Orin Edson Fund for Immunotherapy Research
DDX3Y shows exquisitely cell-type specific expression in the testis.

**ARTICLES**

Generation of pluripotent stem cells from adult human testis

Sabine Conrad¹, Markus Renninger², Jörg Hennenlotter³, Tina Wiesner², Lothar Just³, Michael Bonin⁴, Wilhelm Aicher²,³, Hans-Jörg Bühring², Ulrich Mattheus³, Andreas Mack³, Hans-Joachim Wagner², Stephen Minger³, Matthias Matzkies³, Michael Reppel⁵, Jürgen Hescheler⁶, Karl-Dietrich Sievert³, Arnulf Stenzi³ & Thomas Skutella¹,⁶

Several cancer-testis genes are exclusively expressed in spermatogonia

MAGEA4

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NY-ESO-1 is likewise expressed selectively in spermatogonia

Jungbluth et al., Int. J. Cancer 92:856-860, 2001
### Functions of H-Y genes

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**Zfx Controls the Self-Renewal of Embryonic and Hematopoietic Stem Cells**

Jose M. Galan-Caridad,1,2 Sivan Harel,1,2 Teresa L. Arenzana,1,2 Z. Esther Hou,1 Fiona K. Doetsch,2 Leonid A. Mirny,2 and Boris Reizis1,2

**Cell** 129:345-357 (2007)

**UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development**

Karl Agger1,2, Paul A. C. Cloos1,2, Jesper Christensen1,2, Diego Pasini1,2, Simon Rose1, Juri Rappaport1,2, Irina Isaeva1, Ely Canaan1, Anna Elisabetta Salcini2 & Kristian Helin1,2

**Nature** 449:731-735 (2007)

**The X-Linked Mental Retardation Gene SMCX/JARID1C Defines a Family of Histone H3 Lysine 4 Demethylases**

Shigeki Iwase,1,2 Fei Lan,1,2 Peter Baylis,1,2 Luis de la Torre-Ubieta,1,2 Maite Huarte,1,2 Hank H. Qi,2 Johnathan R. Whetstine,1 Azad Bonni1, Thomas M. Roberts,1 and Yang Shi1,2

**Cell** 128:1077-1088 (2007)