High-affinity allo-restricted TCR for adoptive T cell therapy: selection and characterization

Dolores J. Schendel
SITC, November 2011
The following relationships exist related to this presentation:

*My center holds IP on methods to generate high avidity T cells and selected TCRs derived using these methods*
DC priming of high avidity CD8$^+$ T cells

**HLA-A2$^{\text{pos}}$ donor**

HLA-A2 is an *endogenous* gene. High affinity TCRs are deleted to protect against autoimmunity.

**HLA-A2$^{\text{neg}}$ donor**

HLA-A2 is a *transgene*. Donor still has high affinity TCRs.
Advantages of DC priming strategy

HLA-A2\textsuperscript{neg} donor

- optimal priming capacity of DC
- use any donor negative for selected MHC allele
- use any allogeneic class I or class II allele
- use any antigen available as cDNA
HLA-A2+ allo-restricted tyrosinase-specific CTL
Higher intensity multimer binding by allo-primed CTL

**HLA-A2\textsuperscript{pos} donor**

**HLA-A2\textsuperscript{neg} donor**

low intensity multimer binding

higher intensity multimer binding

0.39% MFI 3,963

0.43% MFI 17,110

auto-DC

endo A2

tyrosinase-RNA

HLA-A2 RNA

tyrosinase-RNA

tg A2

CD8
Distribution of self- and allo-restricted CTL clones

<table>
<thead>
<tr>
<th>reactivity</th>
<th>self-restricted (HLA-A2⁺ donors)</th>
<th>allo-restricted (HLA-A2⁻ donors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no reactivity</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>allo-A2</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>A2-tyrosinase</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>total number</td>
<td>38</td>
<td>51</td>
</tr>
</tbody>
</table>

55% | 16% | 0%  | 53% | 45% | 31% | 100% | 100%
Specific recognition of melanoma lines

![Graph showing IFN-γ levels in tumor cell lines with HLA-A2 self-restricted and HLA-A2 allo-restricted categories.](graph.png)

**tumor cell lines**
Allo-restricted clones show greater peptide sensitivity

- C115
- C58

**Graphs:**
- Self-restricted
- Allo-restricted

**Horizontal axis:** Tyr-peptide loaded T2 cells

**Vertical axis:** % relative lysis

**Y-axis:**
- 0
- 20
- 40
- 60
- 80
- 100

**X-axis:** [M]
- $10^{-11}$
- $10^{-10}$
- $10^{-9}$
- $10^{-8}$
- $10^{-7}$
- $10^{-6}$
- $10^{-5}$

**Legend:**
- C115
- C58

**Notes:**
- Relative half-maximal lysis [M]
- Self-restricted
- Allo-restricted

**Statistical Significance:** *
Self- and allo-restricted CTL have identical specificities but different functional capacities.
Transgenic TCR expression in PBL

T cell clone → T cell culture in vitro → Expanded clonal T cells

TCR mRNA → TCR cDNA into retrovirus → Tg-TCR recipient cells
Transgenic TCR expression in recipient cells

Jurkat 76 unmod.  
Jurkat 76 tgC115  
Jurkat 76 tgC58  
PBL unmod.  
PBL TCR-C115  
PBL TCR-C58  

self-restricted  
allo-restricted
Tumor-specific recognition by TCR-transgenic PBL

HelmholtzZentrum münchen
German Research Center for Environmental Health
Allo-TCR shows superior peptide sensitivity

**Functional avidity**

**tyrosinase\textsubscript{369-377} pulsed T2 cells**
Lessons learned from tyrosinase-specific T cells

- Allo-restricted CTL have superior peptide sensitivity
- Multimer binding does not necessarily correlate with peptide sensitivity and superior cell function
- PBL transduced with the allo-restricted TCR show superior functions
- DC priming can provide high affinity TCR for adoptive T cell therapy
Repertoire of allo-restricted TCR in development

Tyrosinase
Melan A
Survivin
HMMR
WT-1
NY-ESO-1 (non-HLA-A2 restricted)

Cancer-germline: 10 TAAs in progress
HLA-A2\(^+\) allo-restricted survivin-specific T cells
Ranking of tumor-associated antigens for vaccine development

- therapeutic function
- immunogenicity
- specificity
- oncogenicity
- expression level & positive cells
- stem cell expression
- nr. of patients with Ag positive cells
- nr. of epitopes
- cellular location of expression

Cheever MA et al., CCR 2009
HLA-A2 self-restricted and allo-restricted survivin-specific T cell lines
Screening of survivin-specific self-restricted and allo-restricted T cell clones

![Graph showing specific lysis]
No detection of self-restricted survivin-specific CTL

<table>
<thead>
<tr>
<th></th>
<th>A2⁺ (self-restricted)</th>
<th>A2⁻ (allo-restricted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no reactivity</td>
<td>46 100%</td>
<td>9 12%</td>
</tr>
<tr>
<td>alloreactive</td>
<td>0 0%</td>
<td>44 60%</td>
</tr>
<tr>
<td>survivin-reactive</td>
<td>0 0%</td>
<td>22 28%</td>
</tr>
<tr>
<td>total number</td>
<td>46 100%</td>
<td>74 100%</td>
</tr>
</tbody>
</table>
CTL display broad range of peptide sensitivities

peptide sensitivity of survivin$_{96-104}$-pulsed T2 cells
Transgenic TCR are well expressed on PBL

![Histograms showing TCR expression]

- PBL: 0%
- + TCR-A71: 72%
- + TCR-A66: 76%
- + TCR-A72: 78%
TCR-transgenic PBL kill survivin-positive tumor cells
Tg-TCR recognition is survivin-peptide dependent
HLA-A2\(^+\) PBL with tg-TCR undergo high apoptosis
Survivin-specific tg-TCR kill HLA-A2+ PBL
Activated PBL express high levels of survivin transcripts

![Image]

<table>
<thead>
<tr>
<th>target PBL</th>
<th>A2(^{-}) (PHA)</th>
<th>A2(^{-}) (CD3/CD28)</th>
<th>A2(^{+}) (unstimulated)</th>
<th>A2(^{+}) (PHA)</th>
<th>A2(^{+}) (CD3/CD28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>survivin</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>(\beta_2m)</td>
<td>_</td>
<td>_</td>
<td>_</td>
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</tr>
</tbody>
</table>
HLA-A2+ CTL are killed by TCR-transgenic PBL

![Graph showing specific lysis (%) of CTL against different target cells: JB4 (A2-), A42 (A2+), Tyr-F8 (A2+), FaLe (A2+) with various conditions: PBL, + TCR-A71, + TCR-A66, + TCR-A72, + GFP.]

- **target CTL**
  - JB4
  - A42
  - Tyr-F8
  - FaLe

- **Western Blots**
  - Survivin
  - β2m
Lessons learned from survivin-specific TCR

- Self-restricted survivin-specific T cells were not found
- Allo-restricted survivin-specific T cells were frequent and some had very high peptide sensitivities
- PBL transduced with the allo-restricted TCR showed excellent and specific effector cell functions
- MHC-restricted fratricide eliminates survivin as a tg-TCR specificity and raises questions regarding its use as a vaccine antigen
Wider implications for MHC-restricted fratricide?

CD8-sorted

1. EGFR
2. MART-1/melan A
3. NY-ESO-1
4. Tyrosinase
5. WT-1
6. EphA2
7. PSA
8. FLT3
9. CASP-1
10. CYP1B1
11. PRAME
12. EPCAM
13. CA9
14. hTERT
15. RGS5
16. MUC-1
17. VEGF
18. p53
19. c-myc
20. HMMR
21. NPM1
22. Survivin
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