NK Cell Therapeutics for Cancer

Jeffrey S. Miller, M.D.

University of Minnesota Cancer Center
Associate Director of Experimental Therapeutics
Sponsor BB-IND 5708, 6544, 6545, 8847, 10430, 10530
Survivor, one random FDA audit
Division of Heme/Onc/Transplant
Minneapolis, MN
Chr. 19 determines the personality of NK cells: Killer-immunoglobulin receptor (KIR) gene locus

KIR3DL1*004 is not expressed at the surface

From Peter Parham
The interest in therapeutic uses of NK cells has been growing since in 2002

<table>
<thead>
<tr>
<th>Transplant</th>
<th>Graft</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davies et al, Blood 11/2002</td>
<td>URD KIR-L Mismatch</td>
<td>UBM</td>
</tr>
<tr>
<td>Giebel et al, Blood 8/2003</td>
<td>URD KIR-L Mismatch</td>
<td><em>In Vivo</em> TCD</td>
</tr>
</tbody>
</table>
How can we best exploit NK cells?

Adoptive Transfer ? Transplant

Pros and cons

Safer
Transient
Can expand in vivo (IL-2)

More TRM
Permanent
Too risky 2° GVHD risk
Outpatient Subcutaneous IL-2 Promotes In Vivo NK Cell Expansion

…but NK cells are not maximally activated

Miller et al, Biol Blood Marrow Transplant 3:34, 1997
837 IND #’s later: Autologous NK Administration in Cancer Patients

Recovery from autologous HCT

IL-2

PB

IL-2

NK

NK cells more activated using this approach

BB-IND 6545
NK Cell-based Autologous Immunotherapy to Prevent Relapse (HD, NHL, BC)

*Burns et al.*, Bone Marrow Transplant, 32:177-186, 2003

**Conclusions**

Enhanced activation of NK cells

A matched paired analysis with our data and data from the IBMTR showed no apparent efficacy (survival or time to disease progression)
Hypothesis:
Autologous NK Cell Therapy Failed Due to Inhibitory Receptors that Recognize MHC

KIR - MHC class I:
No Killing

KIR - MHC mismatch:
Lysis occurs

To Kill or not to kill

Hypothesis:
Autologous NK Cell Therapy Failed Due to Inhibitory Receptors that Recognize MHC

KIR - MHC class I:
No Killing

KIR - MHC mismatch:
Lysis occurs

To Kill or not to kill

apoptosis
2302 IND #'s later: Related Donor Haploidentical NK Infusions After High Dose Chemotherapy

PB → NK → TCD IL-2 → IL-2 → HD Rx

HD Rx:
- Cy 60 mg/kg x 2
- Flu 25 mg/m² x 5
- 2-8 x 10⁷ MNC/kg
- 10 MU QOD x 6

BB-IND 8847
Patients and Eligibility

- Poor prognosis AML
  - Primary refractory disease
  - Relapsed disease not in CR after 1 or more cycles of standard re-induction therapy
  - Secondary AML from MDS
  - Relapsed AML ≥ 3 months after HCT.
- No active infections
Higher Numbers of Functional NK Cells in Patients with CR After Adoptive Transfer

% NK cells at Day 14-28

NK cells did not expand with lower dose preparative regimens

Correlates with an increase in IL-15 and IL-7

Miller et al, Blood 105:3051, 2005
In vivo expansion of haploidentical NK cells in AML

<table>
<thead>
<tr>
<th></th>
<th>100</th>
<th>10</th>
<th>1</th>
<th>0.1</th>
<th>0.01</th>
<th>0.001</th>
<th>No Donor</th>
<th>D1</th>
<th>D2</th>
<th>D7</th>
<th>D14</th>
<th>D28</th>
<th>H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-act</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor Specific HLA-A31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Long-term Follow-up

- 10 of 32 (31%) remissions
- No correlation with KIR-L mismatch
- 3 of 10 total CRs went on to receive allo transplant
  (1 sib, 2 UCB) with DFS > 2.5 years
- 3 died of toxicity without relapse (1 meningitis, 1 CNS, 1 PTLD)
- 4 of 10 CRs lasted 4-11 months (probably not curative)
The best strategy may be to combine adoptive transfer and in vivo expansion followed by HCT

Adoptive Transfer + Transplant

The best of both worlds?
Haplo related donor RIC strategy to combine NK cells and HCT for patients with refractory AML

**Patient Eligibility**
- >45 years
- Refractory AML

**Haplo donor**
- CD3 deplete
- IL-2 Overnight (1000 U/ml)

**Same haplo donor**
- Potent TCD
- CD34 selection

**KIR-L MM if possible**

**TIME**

No post-transplant immunosuppression
Haplo related donor RIC strategy to combine NK cells and HCT for patients with refractory AML

**Patient Eligibility**
- >45 years
- Refractory AML

**Haplo donor**
- Same haplo donor
- Potent TCD
- CD34 selection

**KIR-L MM if possible**

**IL-2 x 6 doses for In vivo NK expansion**

**TIME**
- -25
- -19
- -18
- -17
- -16
- -15
- -14
- -13
- -12
- -1
- 0
- 14
- 28

**BMBx**
- 25
- 19
- 18
- 17
- 16
- 15
- 14
- 13
- 12
- 1
- 0

**FLU**
- 25
- 19
- 18
- 17
- 16
- 15
- 14
- 13
- 12

**CY, TBI 200**

**No post-transplant immunosuppression**
Haplo related donor RIC strategy to combine NK cells and HCT for patients with refractory AML

**Patient Eligibility**
- >45 years
- Refractory AML

**Haplo donor**
- CD3 depletion
- IL-2 Overnight (1000 U/ml)

**Same haplo donor**
- Potent TCD
- CD34 selection

**KIR-L MM if possible**

**TIME**

**BMBx**
- -25
- -19
- -18
- -17
- -16
- -15
- -14
- -13
- -12

**FLU**
- -19
- -18
- -17
- -16
- -15
- -14
- -13
- -12

**CY**
- -17
- -16

**TBI 400**
- -16

**IL-2 x 6 doses for In vivo NK expansion**

**No post-transplant immunosuppression**
Haplo related donor RIC strategy to combine NK cells and HCT for patients with refractory AML

Patient Eligibility
- >45 years
- Refractory AML

KIR-L MM if possible

Haplo donor
- CD3/CD19 deplete
- IL-2 Overnight (1000 U/ml)

Same haplo donor
- Potent TCD
- CD34 selection

Patient Eligibility
- >45 years
- Refractory AML

IL-2 x 6 doses for In vivo NK expansion

No post-transplant immunosuppression
Where do we go from here?

• Improve Donor choice
• Improve NK cell activation
  – Interrupt inhibitory receptor mechanisms
• Increase target sensitivity
  – Bortezomib
Killer-Immunoglobulin Receptor (KIR) Gene Locus

Group-A Haplotype:
Absence of 2DL5, 2DS2, 2DS1, 2DS3, 2DS5, 3DS1

Group-B Haplotypes: Presence of at least one of above
Where do we go from here?

• Improve Donor choice
• Improve NK cell activation
  – Interrupt inhibitory receptor mechanisms
• Increase target sensitivity
  – Bortezomib
NK Cell Target Cell

Inhibitory Receptors

KIR3DL2
KIR3DL1
SHP-1
SHP-2
KIR2DL1
KIR2DL2
KIR2DL3

CD94
NKG2A
LIR-1

HLA-A3/11
HLA-Bw4
HLA-C2
HLA-C1
HLA-E
HLA-A

Verneris and Miller
Where do we go from here?

- Improve Donor choice
- Improve NK cell activation
  - Interrupt inhibitory receptor mechanisms
- Increase target sensitivity
  - Bortezomib
SENSITIZATION OF TUMOR CELLS TO NK CELL-MEDIATED KILLING BY PROTEASOME INHIBITION

RUNNING TITLE: BORTEZOMIB INCREASES NK CELL KILLING

William H.D. Hallett*, Erik Ames*, Milad Motarjemi*, Isabel Barao*, Anil Shanker†, David L. Tamang*, Thomas J. Sayers†, Dorothy Hudig* and William J. Murphy*
Haplo related donor RIC strategy to combine NK cells and HCT for patients with refractory AML

Same haplo donor
Potent TCD
CD34 selection

Patient Eligibility
• >45 years
• Refractory AML

KIR-L MM if possible

Haplo donor

CD3/CD19 deplete
IL-2 Overnight
(1000 U/ml)

BMBx

IL-2 x 6 doses for
In vivo NK expansion

BMBx

BMBx

BMBx

FLU

FLU

FLU

FLU

FLU

FLU

FLU

FLU

FLU

CY

CY

TBI 400

Bortezomib

>45 years
Refractory AML

Patient Eligibility

No post-transplant immunosuppression

TIME


-1 0 14 28
Lessons and Issues

• Important strategic decisions
  – Do the right thing, do not forget the patient
  – Well-intended improvements may lead to failures (pure NK cells not clinically active)
  – Put as few people at risk as possible
  – Minimize patients exposed to therapies that will not work
  – BE FLEXIBLE
  – Do not do it alone

• Regulatory authorities
  – Work with the FDA and they will work with you
  – Be concrete, realistic and logical about your goals
  – Do not do it alone

• Funding of the project:
  – Huge issue but if science is solid NIH/NCI still good investors
  – If tied to therapeutics, clinical partners must also be willing to invest

• Lessons learned
  – The field is narrowing…decide your contribution and make sure it is realistic
  – Specialized ETU's needed for clinical implementation
  – Make sure you have lab endpoints to teach you something when your trial fails and most of them will
  – COMBINATIONS ARE THE KEY TO SUCCESS…this is a challenge!
# P01 (PI: Jeffrey S. Miller)

“NK Cells and their receptors in unrelated donor transplantation”

<table>
<thead>
<tr>
<th>University of Minnesota</th>
<th>NMDP/CIBMTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeffrey S. Miller, MD</td>
<td>Stephen Spellman</td>
</tr>
<tr>
<td>Daniel J. Weisdorf, MD</td>
<td>Michael Haagenson</td>
</tr>
<tr>
<td>Sarah Cooley, MD</td>
<td>John Klein, PhD</td>
</tr>
<tr>
<td>Michael Verneris, MD</td>
<td></td>
</tr>
<tr>
<td>Chap T. Le, PhD</td>
<td></td>
</tr>
<tr>
<td>Tracy Bergemann, PhD</td>
<td></td>
</tr>
<tr>
<td><strong>Stanford University</strong></td>
<td><strong>Dennis Confer, MD</strong></td>
</tr>
<tr>
<td>Peter Parham, PhD</td>
<td><strong>Martin Meiers</strong></td>
</tr>
<tr>
<td><strong>Children’s Hospital and Research Institute, Oakland</strong></td>
<td><strong>Tao Wang, PhD</strong></td>
</tr>
<tr>
<td>Elizabeth Trachtenberg, PhD</td>
<td></td>
</tr>
<tr>
<td><strong>Anthony Nolan Research Inst.</strong></td>
<td></td>
</tr>
<tr>
<td>Steven G.E. Marsh, PhD</td>
<td></td>
</tr>
<tr>
<td><strong>Fred Hutchinson CRC</strong></td>
<td></td>
</tr>
<tr>
<td>Daniel Geraghty, PhD</td>
<td></td>
</tr>
</tbody>
</table>

**Affiliated Clinical Sites**

<table>
<thead>
<tr>
<th>MCW</th>
<th>Indiana</th>
</tr>
</thead>
<tbody>
<tr>
<td>William Drobyski, MD</td>
<td>Sharif Farag, MD</td>
</tr>
<tr>
<td>David Margolis, MD</td>
<td></td>
</tr>
<tr>
<td><strong>Moffitt</strong></td>
<td><strong>Washington U</strong></td>
</tr>
<tr>
<td>Claudio Anasetti, MD</td>
<td>John Dipersio, MD</td>
</tr>
<tr>
<td><strong>OSU</strong></td>
<td><strong>U of Penn</strong></td>
</tr>
<tr>
<td>Steven Devine, MD</td>
<td>David Porter, MD</td>
</tr>
<tr>
<td><strong>Emory</strong></td>
<td><strong>City of Hope</strong></td>
</tr>
<tr>
<td>Ned Waller, MD</td>
<td>Steve Forman, MD</td>
</tr>
<tr>
<td><strong>City of Hope</strong></td>
<td></td>
</tr>
</tbody>
</table>
Acknowledgements

• Miller Lab
  – Valarie McCullar (Research)
  – Todd Lenvik
  – Robert Godal
  – Frank Cichocki
  – Purvi Gada
  – Gong Yun
  – Karen Peterson
  – Michelle Pitt
  – Becky Haack
  – Sue Fautosch (Translational)
  – Julie Curtsinger
  – Rosanna Warden
  – Liz Narten
  – Michelle Gleason

• HLA typing lab - Harriet Noreen

• CTO/Research Nurses (Dixie Lewis/Roby Nicklow)

• U of MN Faculty
  – Dan Weisdorf
  – Sarah Cooley
  – Phil McGlave
  – Arne Slungaard
  – Linda Burns
  – Claudio Brunstein
  – Veronika Bachenova
  – John Wagner
  – Bruce Blazar
  – Michael Verneris
  – Dave McKenna (GMP Facility)
  – Chap Le/Tracy Bergemann (Biostat)