Case Study: How do you know when your pre-clinical work in cell therapy is ready for the clinic?

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The simple answer:

- You are never as ready as you want
- Always multiple your planned time to REALLY opening by a factor of 2-3 to get a realistic answer
- ...But there are some guiding principles

- My credentials: Sponsor BB-IND 5708, 6544, 6545, 8847, 10430, 10530, 13659
- **Survivor, one random FDA audit**
Make Sure what you are studying is important and has relevance to health and disease

- Cancer treatment and tumor surveillance
- Infection disease control
- Autoimmunity
- Pregnancy (placental angiogenesis)

**NK cell functions**

- Killing targets
- Produce cytokines
  - Interferon-γ
  - Tumor necrosis factor
  - Many others
Biology needs to be relatively established:
Chr. 19 determines the personality of NK cells - Killer-immunoglobulin receptor (KIR) gene locus

From Peter Parham

KIR3DL1*004 is not expressed at the surface
Mice do not have KIR
NKG2 family recognizes HLA-E
Inhibitory Receptors

NK Cell
- KIR3DL2
- KIR3DL1
- KIR2DL1
- KIR2DL2
- KIR2DL3
- CD94
- NKG2A
- LIR-1
- SHP-1
- SHP-2

Target Cell
- HLA-A3/11
- HLA-Bw4
- HLA-C2
- HLA-C1
- HLA-E
- HLA-A,B,G

Activating Receptors

NK Cell
- KIR2DS
- FceR1γ
- Syk/Zap70
- NKP30
- NKP44
- NKP46
- CD3ε
- DAP12
- PLCγ2
- PKC
- PI3K
- LFA-1
- Syk/Zap70
- DAP10
- DNAM-1
- Fyn
- PLCγ2
- Nectin-2

Target Cell
- ???

NK cell receptors define the NK cell repertoire

KIR⁻/NKG2A⁻ subset: 19.4 ± 2.8% of CD56⁺dim NK cells healthy donors (n=26)
Know the literature!
Any human experience?
## Transplant Trials Exploring NK Cell Alloreactivity

<table>
<thead>
<tr>
<th></th>
<th>Transplant</th>
<th>Graft</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>Ruggeri et al</td>
<td>Haploidentical KIR-L Mismatch</td>
<td>TCD</td>
<td>Benefit in AML</td>
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<td>Science 3/2002</td>
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<td>Davies et al</td>
<td>URD KIR-L Mismatch</td>
<td>UBM</td>
<td>No Benefit</td>
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<tr>
<td>Giebel et al</td>
<td>URD KIR-L Mismatch</td>
<td>In Vivo TCD</td>
<td>Benefit</td>
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<td>Blood 8/2003</td>
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NK cells after transplant are increased

Cooley et al
Blood 106:4370, 2005
Pick your questions carefully and stick with it for the long-term!
How can we best exploit NK cells in cancer?

Adoptive Transfer vs. Transplant

Pros and cons:
- Safer
- Transient
- Can expand in vivo (IL-2)

- More TRM
- Permanent
- Too risky 2° GVHD risk

Pick your questions carefully and stick with it for the long-term!
Build on your own experience in small, manageable steps!
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

NK cells more activated using this approach
React to the data appropriately and remember that the only thing that matters is clinical outcomes!
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)
Alter you plan based on new biology to explain failures!
Hypothesis:
Autologous NK Cell Therapy Failed Due to Inhibitory Receptors that Recognize MHC

KIR - MHC match -> No Killing
KIR - MHC mismatch -> Lysis occurs

To Kill or not to kill

apoptosis
2302 IND #’s later: Adoptive Transfer of Human Haploidentical NK Cells

Eligible patients: Poor prognosis with refractory AML

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

PB NK-enriched

CD3-depletion

IL-2

BB-IND 8847

Miller et al, Blood 105:3051-3057, 2005
Make sure you have readouts other than clinical outcomes!
In vivo expansion of haploidentical NK cells in AML

After cell infusions

<table>
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<tr>
<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>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>100</td>
<td>10%</td>
<td>1%</td>
<td>0.1%</td>
<td>0.01%</td>
<td>0.001%</td>
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- HLA-B7
- B-act
- Donor Specific HLA-A31
- β-actin
Clinical Update and Long-term Follow-up

- 10 of 32 (31%) remissions
  - 3 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 received no further therapy but relapsed within 4-11 months (probably not curative)

- Data suggests that in vivo expansion important for efficacy

Pick surrogate markers wisely to move forward!
Endpoint Definitions:

- **In Vivo NK Cell Expansion**
  - $\geq 100$ donor-derived NK cells per $\mu$L blood 12-14 days after NK cell infusion
  - $\text{ANK cells/}\mu\text{l} = (\text{ALC}) \times (\% \ CD56^+/{CD3^-} \ lymphocytes) \times (\% \ donor \ by \ VNTR)$

- **Leukemia Clearance**
  - $<1\%$ blasts on BMBx Day +12 after NK infusion

- **Remission**
  - No evidence of leukemia after donor neutrophil engraftment
4812 IND #’s later: Combination of Haploidentical Related Donor NK Cells with HCT for Patients with Refractory AML

Haplo Donor Apheresis
CD3⁻/CD19⁻ NK product
(2-8 x 10⁷ MNC/kg)
IL-2 Overnight (1000 U/ml)

Measure NK cell Expansion before CD34+ graft

Preparative Regimen
Haplo NK
In vivo NK Expansion

IL-2 10 MU SQ EOD x 6 doses

Haplo Donor Apheresis

Preparative Regimen

Haplo NK

In vivo NK Expansion

FLU

IL-2 10 MU SQ EOD x 6 doses

Measure NK cell Expansion before CD34+ graft

Preparative Regimen
Haplo NK
In vivo NK Expansion
Be flexible!

We identified definitive clinical toxicity to B-cell contaminants of our NK cell therapy

- PTLD
- Passenger lymphocyte syndrome
  - ACTION→CD19 depletion of all NK cell products
Anticipate failure and your next move!
Problems and future directions

- Immune deficiency after CD34+ HCT leads to high TRM because of profound immunodeficiency

- Failure to achieve CR in some
  - Target sensitizing agents (e.g. bortezomib)
  - IL-15 may be better for expansion?
  - Receptors other than KIR
    - Insertion of receptors by gene therapy
  - Suppressive mechanisms
  - In vivo vs. ex vivo expansion
  - Donor factors
Emerging questions: NK cell expansion In Vivo versus Ex Vivo

- Do these approaches differentially affect:
  - Specific Function
  - Receptor repertoires (education)
  - Survival in vivo
  - Homing to tumor
  - Efficacy!!!!!!
Can we define an NK cell Super-donor?
• **Hypothesis:** Evaluation of *KIR B* haplotypes for specific gene motifs will inform selection of “good” *KIR* donors to improve the effectiveness of NK cell therapy
Killer-Immunoglobulin Receptor (KIR) Gene Locus

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

Group-B Haplotype:
Presence of at least one of above
Demographics (n=448)

<table>
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<tr>
<th>Year of Transplant</th>
<th>1998-2003</th>
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<tr>
<td>HLA Matching</td>
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<tr>
<td>10/10</td>
<td>209 (47%)</td>
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<tr>
<td>9/10</td>
<td>95 (22%)</td>
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<tr>
<td>8/10</td>
<td>90 (20%)</td>
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<tr>
<td>≤7/10</td>
<td>44 (12%)</td>
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<tr>
<td>HLA Mismatched Group</td>
<td></td>
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<tr>
<td>GvH KIR-Ligand MM</td>
<td>70 (29%)</td>
</tr>
<tr>
<td>KIR-Ligand Match</td>
<td>169 (71%)</td>
</tr>
<tr>
<td>Mean Age (range)</td>
<td>34 (1-61)</td>
</tr>
<tr>
<td>Disease Status at Transplant</td>
<td></td>
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<tr>
<td>1\textsuperscript{st} CR (Early)</td>
<td>86 (18%)</td>
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<tr>
<td>2\textsuperscript{nd} or &gt; CR (Intermediate)</td>
<td>160 (36%)</td>
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<tr>
<td>1\textsuperscript{st} or &gt; Relapse/PIF (Advanced)</td>
<td>202 (46%)</td>
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KIR B/x Genotype Donors Confer Improved Relapse Free Survival in AML

Cooley et al., Blood 2009
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”

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

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<tr>
<th>MCW</th>
<th>Indiana</th>
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<tr>
<td>William Drobyski, MD</td>
<td>Sharif Farag, MD</td>
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<tr>
<td>David Margolis, MD</td>
<td>Washington U</td>
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<tr>
<td><strong>Moffitt</strong></td>
<td>John Dipersio, MD</td>
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<tr>
<td>Claudio Anasetti, MD</td>
<td>U of Penn</td>
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<tr>
<td><strong>OSU</strong></td>
<td>David Porter, MD</td>
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<td>Steven Devine, MD</td>
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<td><strong>Emory</strong></td>
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<td>Ned Waller, MD</td>
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  - Sue Fautsch
  - Julie Curtsinger
  - Rosanna Warden
  - Liz Narten
  - Wade Johnson
  - Dave Ankarlo

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- CTO/Research Nurses (Lewis/Nicklow)

- U of MN Faculty
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  - Sarah Cooley
  - Phil McGlave
  - Arne Slungaard
  - Linda Burns
  - Claudio Brunstein
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