

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

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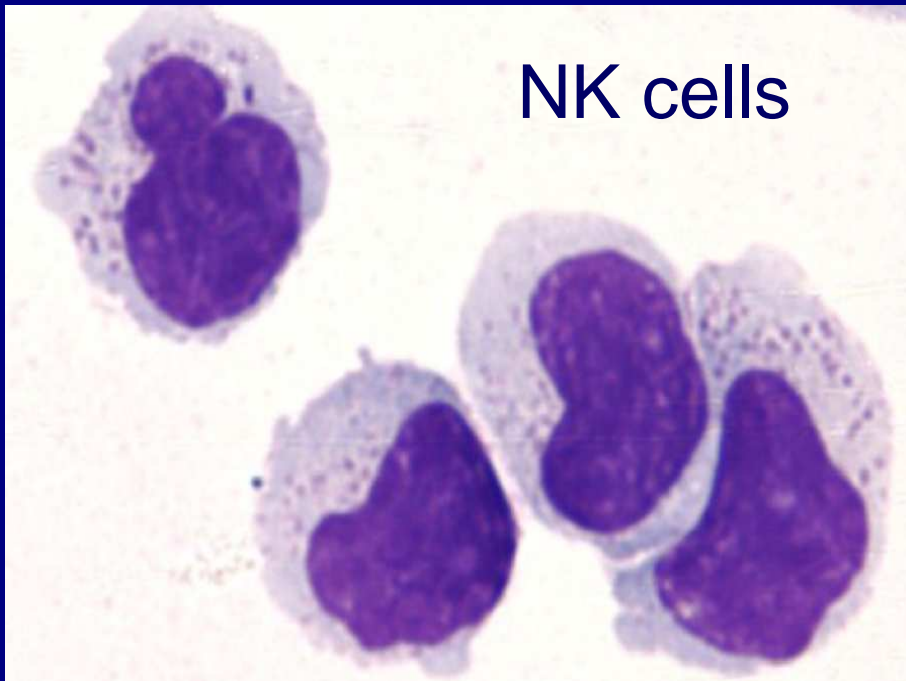
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The simple answer:

- 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

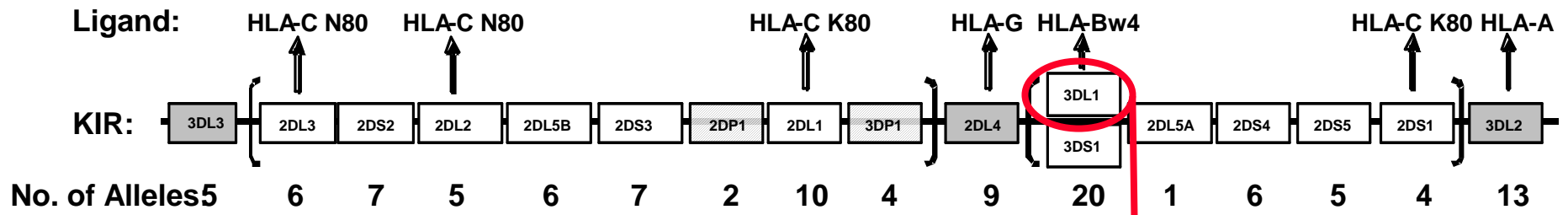


- Cancer treatment and tumor surveillance
- Infection disease control
- Autoimmunity
- Pregnancy (placental angiogenesis)

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

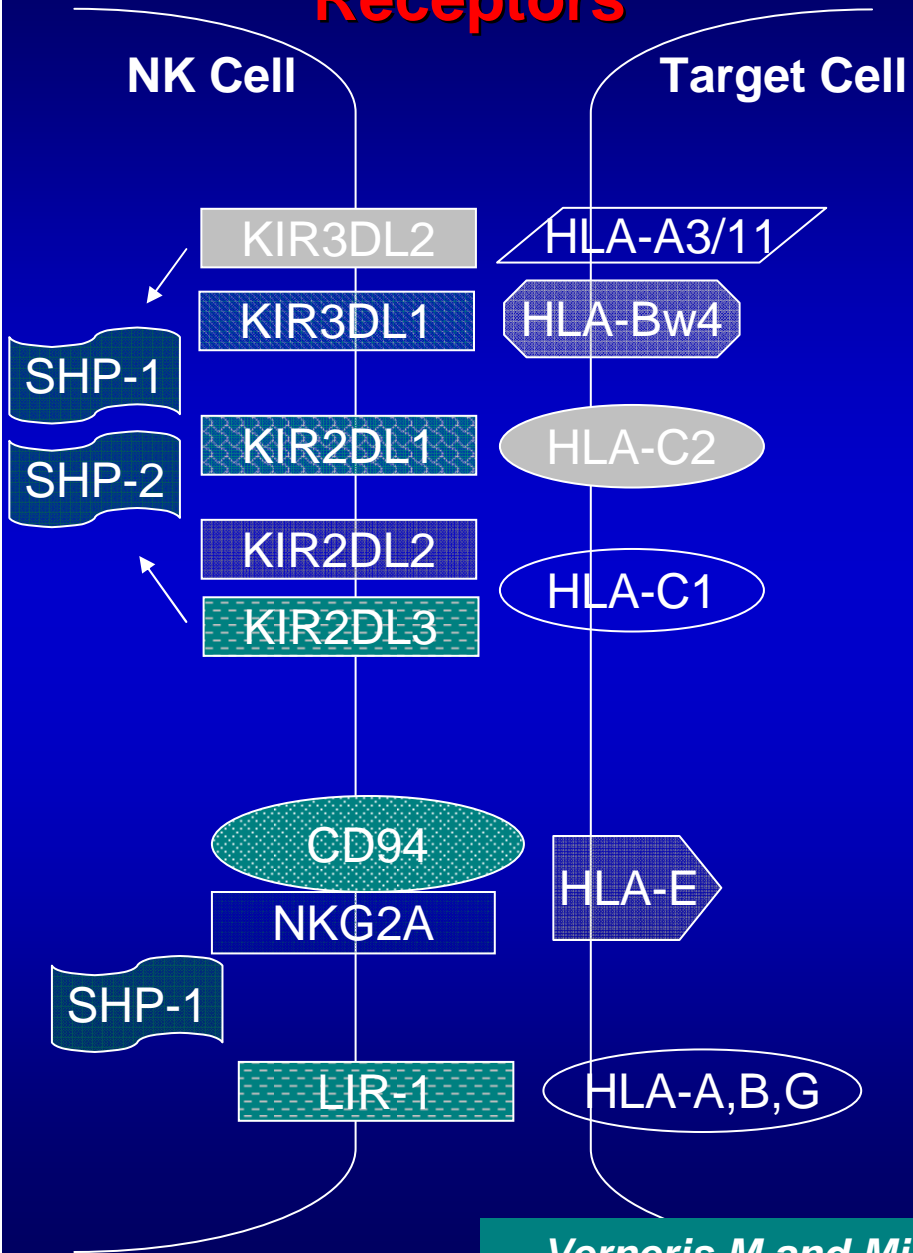


KIR3DL1*004 is not expressed at the surface

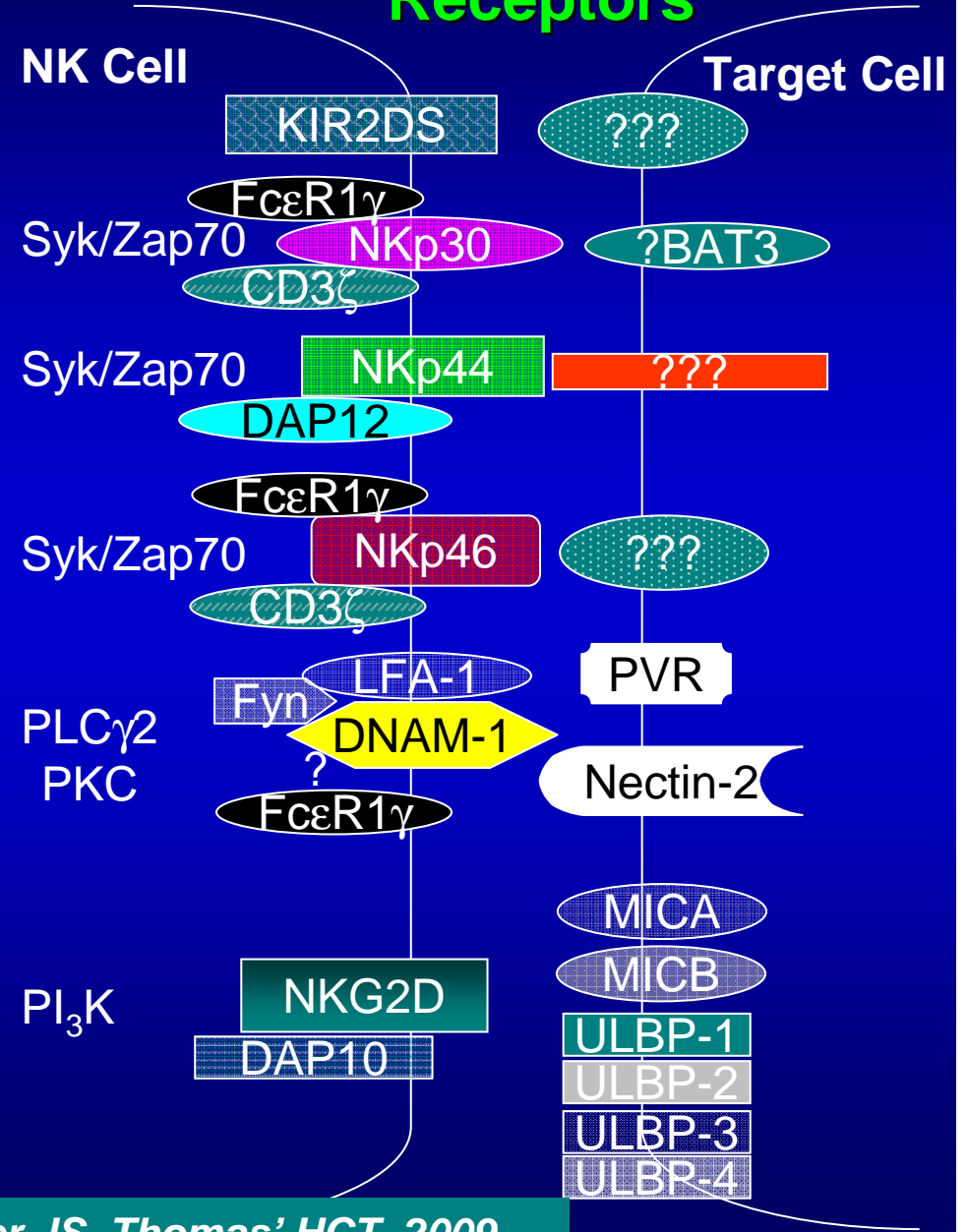
Mice do not have KIR
NKG2 family recognizes HLA-E

From Peter Parham

Inhibitory Receptors

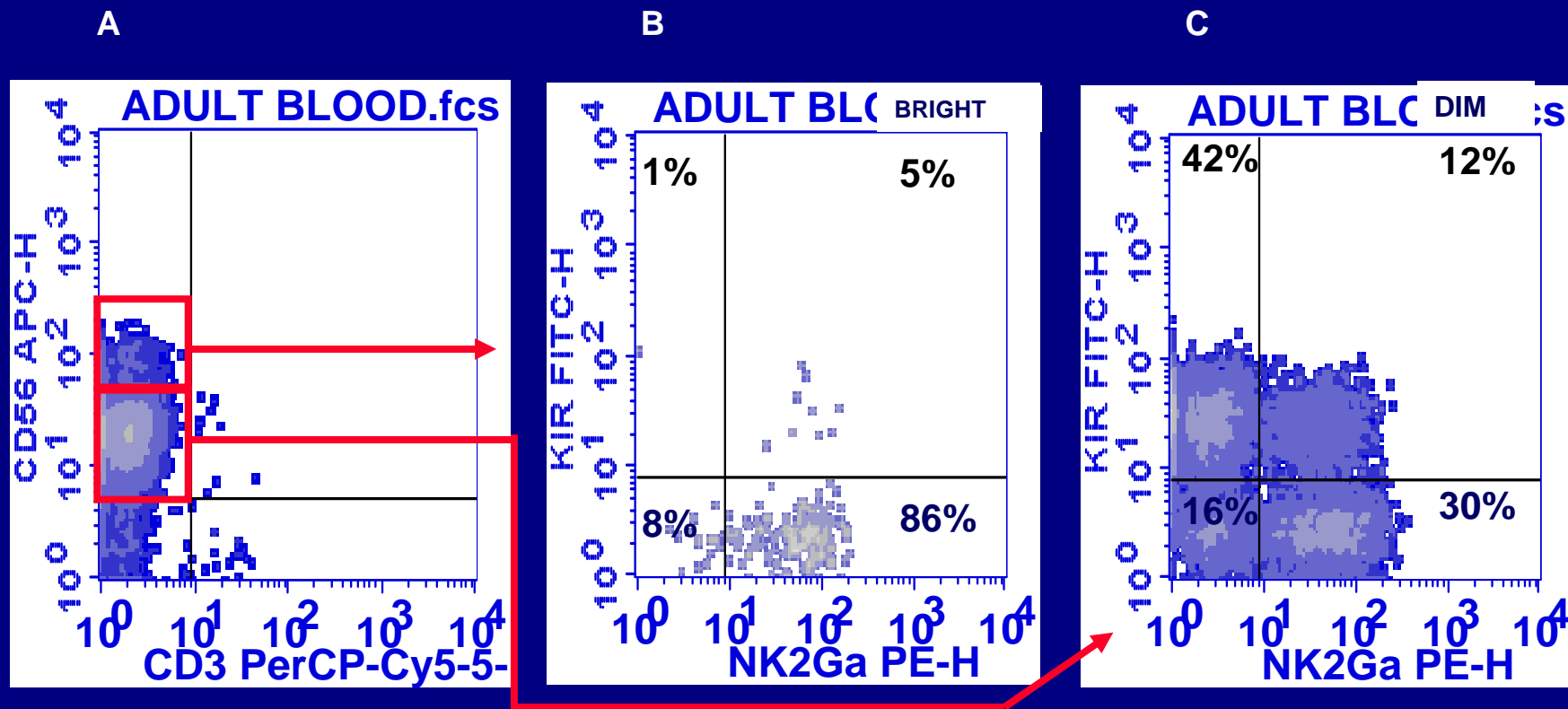


Activating Receptors



Verneris M and Miller JS, Thomas' HCT, 2009.

NK cell receptors define the NK cell repertoire



KIR⁻/NKG2A⁻ subset: $19.4 \pm 2.8\%$ of CD56^{dim} NK cells healthy donors (n=26)

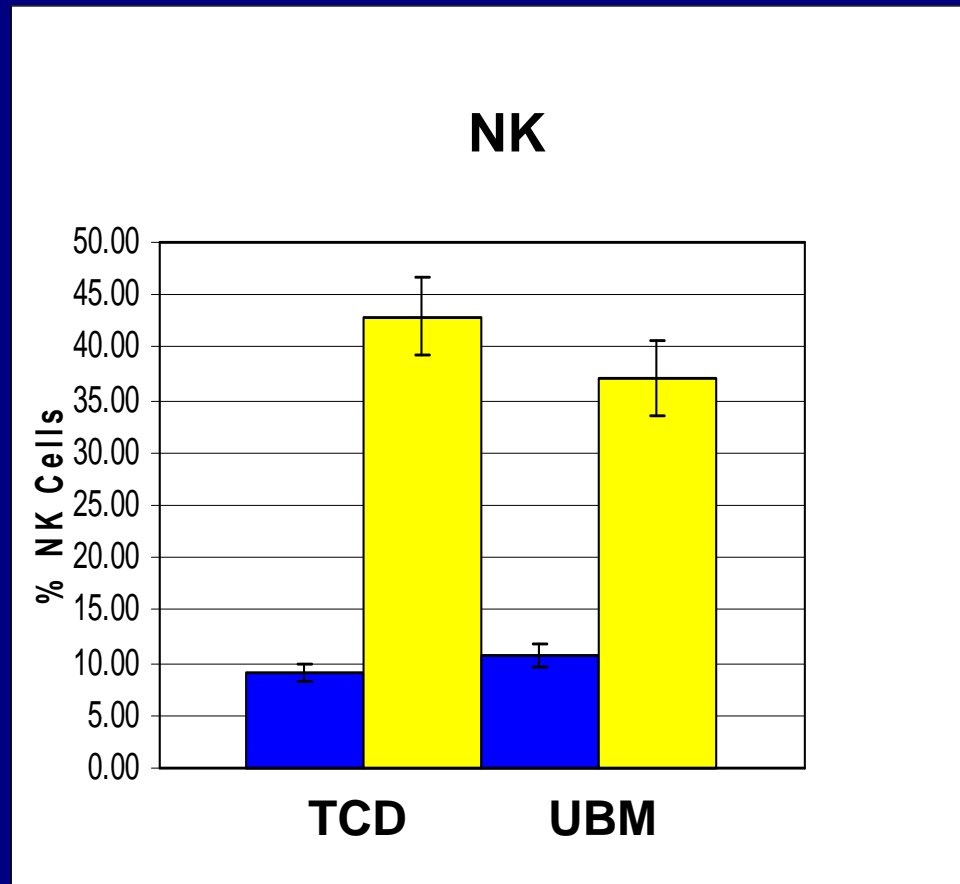
Know the literature!
Any human experience?

Transplant Trials Exploring NK Cell Alloreactivity

	Transplant	Graft	Outcome
Ruggeri <i>et al</i> Science 3/2002	Haploidentical KIR-L Mismatch	TCD	Benefit in AML
Davies <i>et al</i> Blood 11/2002	URD KIR-L Mismatch	UBM	No Benefit
Giebel <i>et al</i> Blood 8/2003	URD KIR-L Mismatch	<i>In Vivo</i> TCD	Benefit

NK cells after transplant are increased

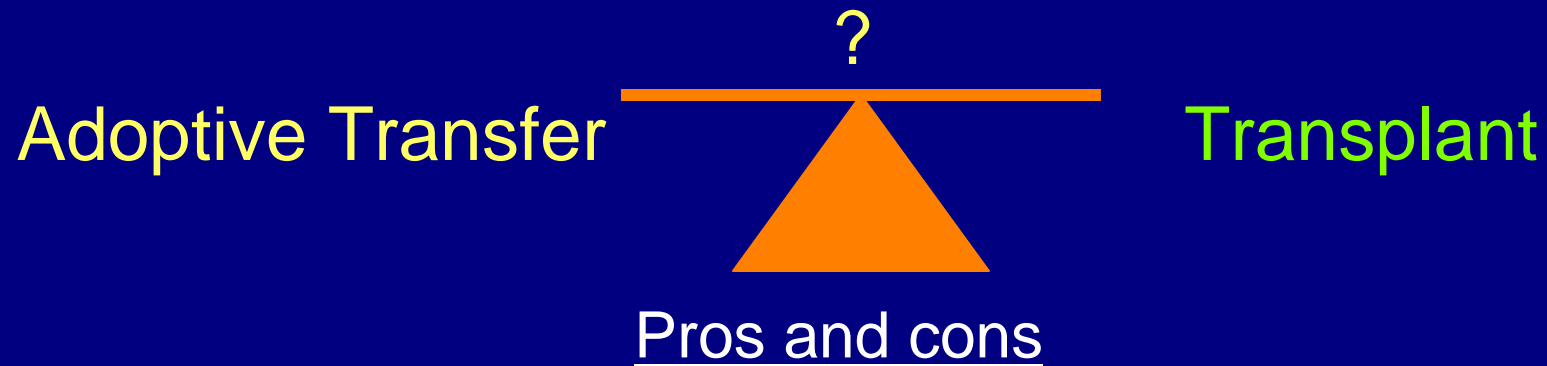
■ = Normal DONOR
■ = RECIPIENT



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?



Safer

Transient

Can expand in vivo (IL-2)

More TRM

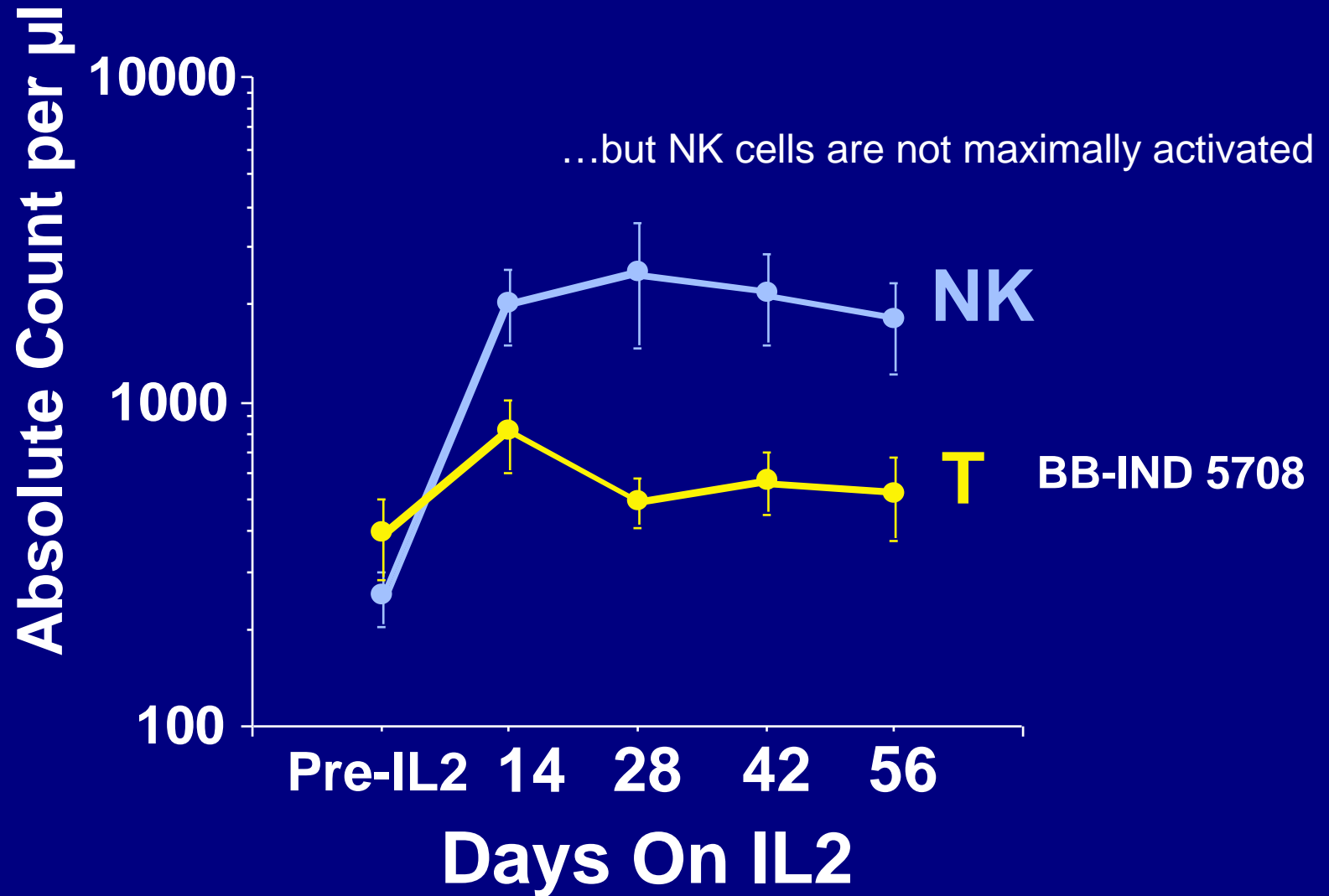
Permanent

Too risky 2°

GVHD risk

**Build on your own
experience in small,
manageable steps!**

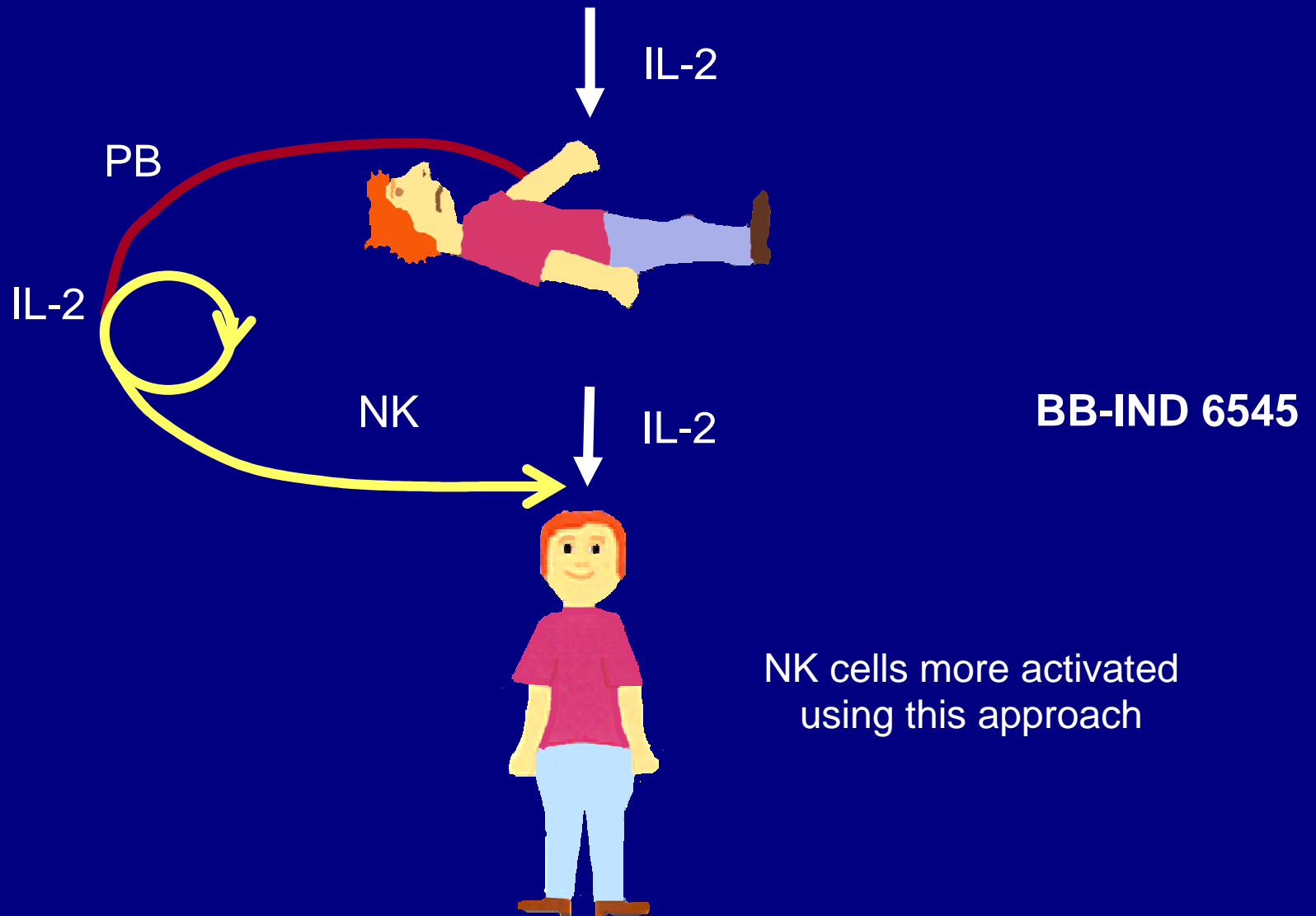
Outpatient Subcutaneous IL-2 Promotes In Vivo NK Cell Expansion



Miller et al, Biol Blood Marrow Transplant 3:34, 1997

837 IND #'s later: Autologous NK Administration in Cancer Patients

Recovery from autologous HCT



**React to the data
appropriately and
remember that the only
thing that matters is
clinical outcomes!**

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

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

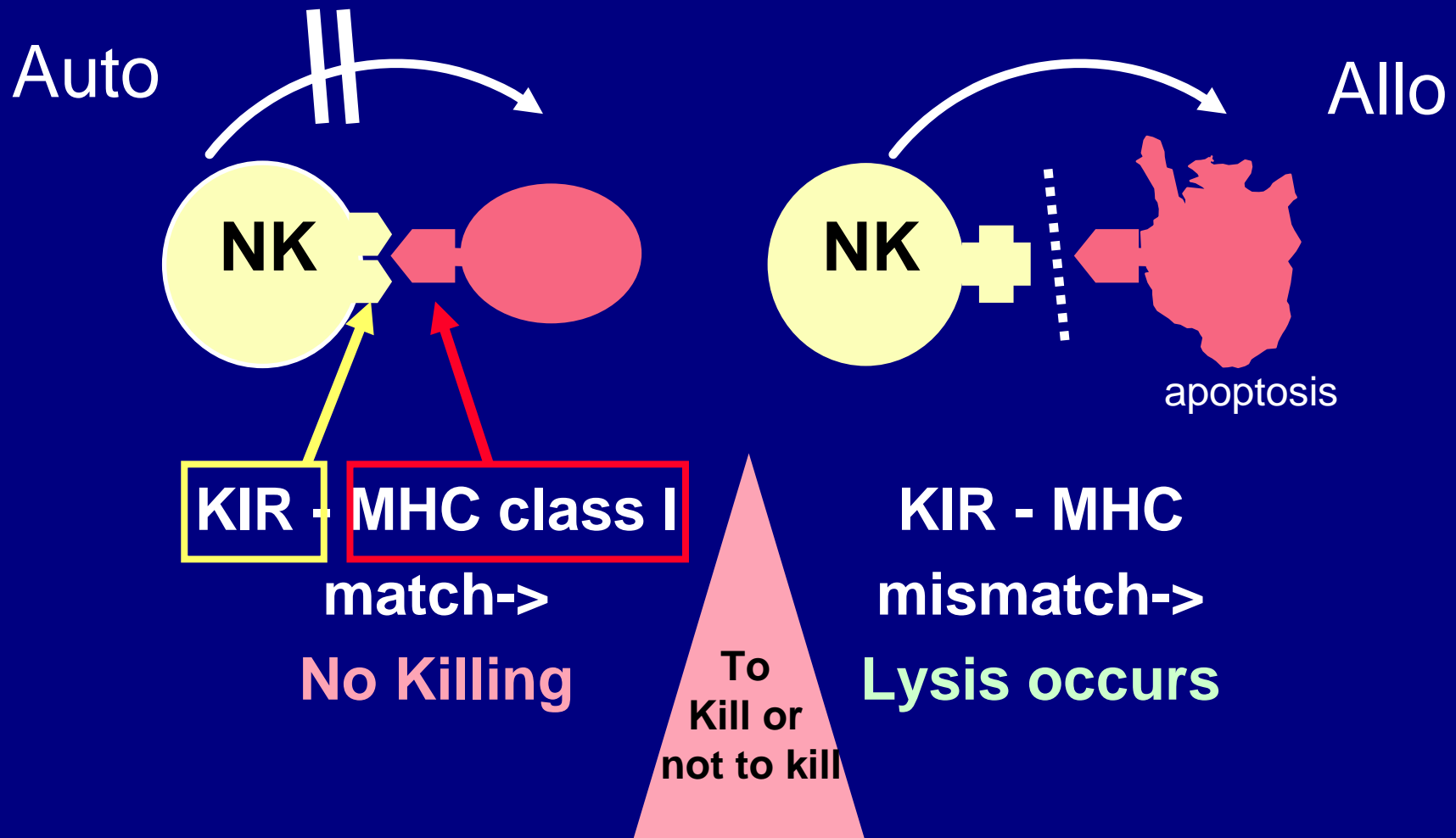
Conclusions

Enhanced activation of NK cells

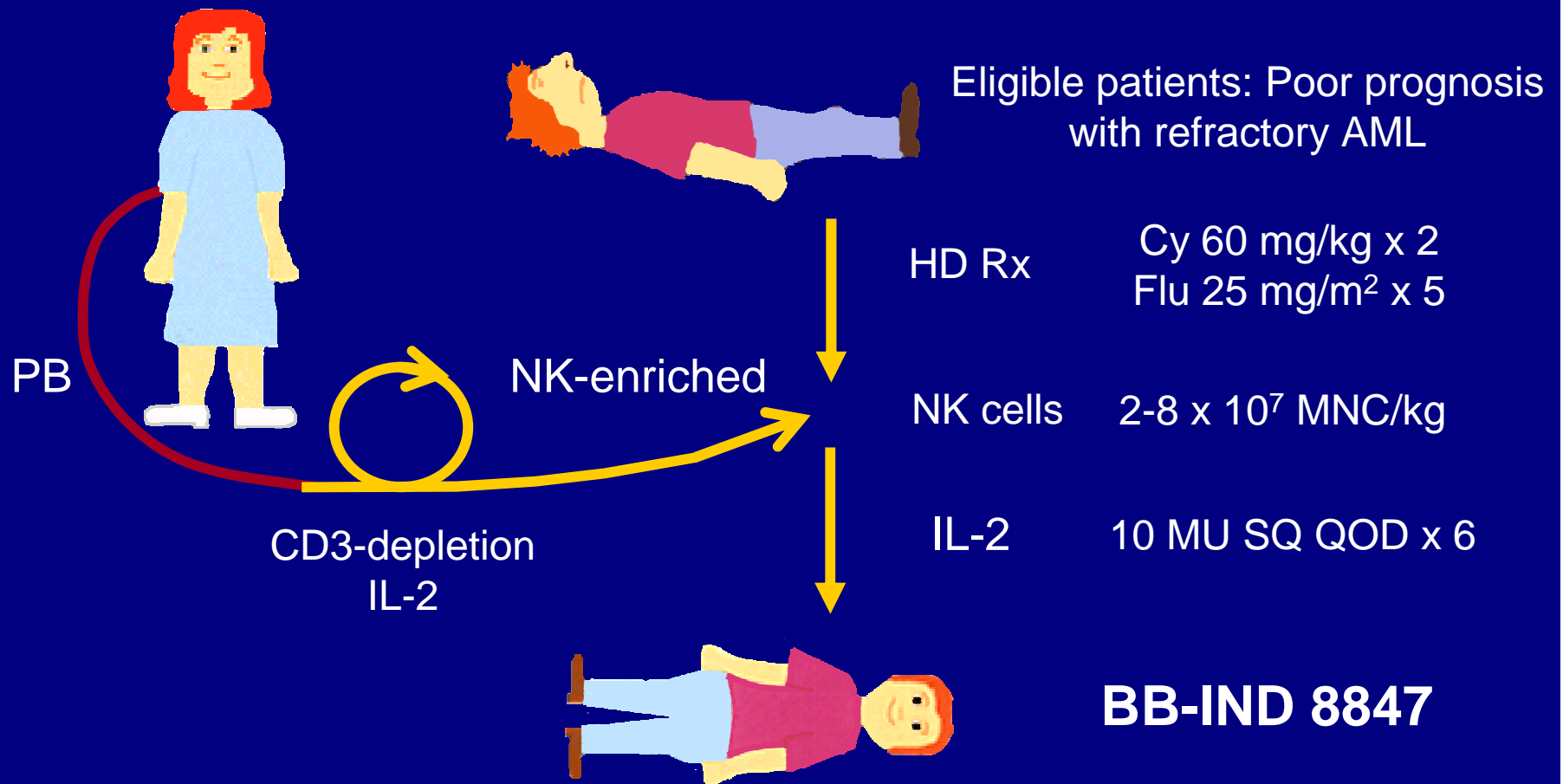
A matched paired analysis with our data and data from the IBMTR showed no apparent efficacy (survival or time to disease progression)

**Alter you plan based on
new biology to explain
failures!**

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

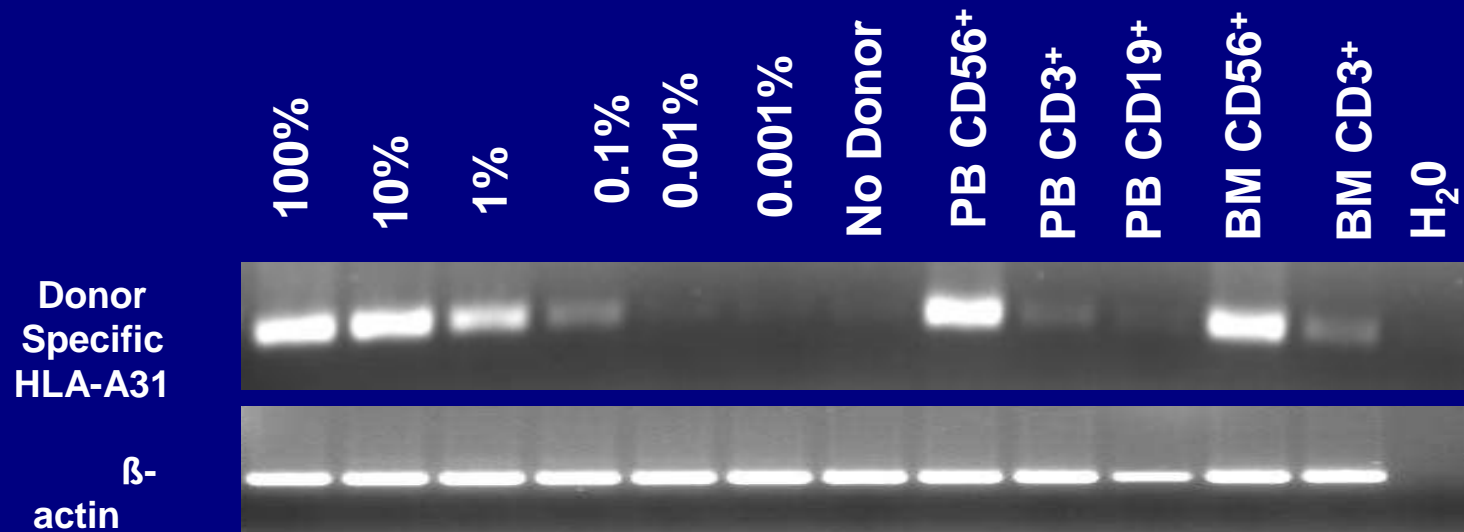
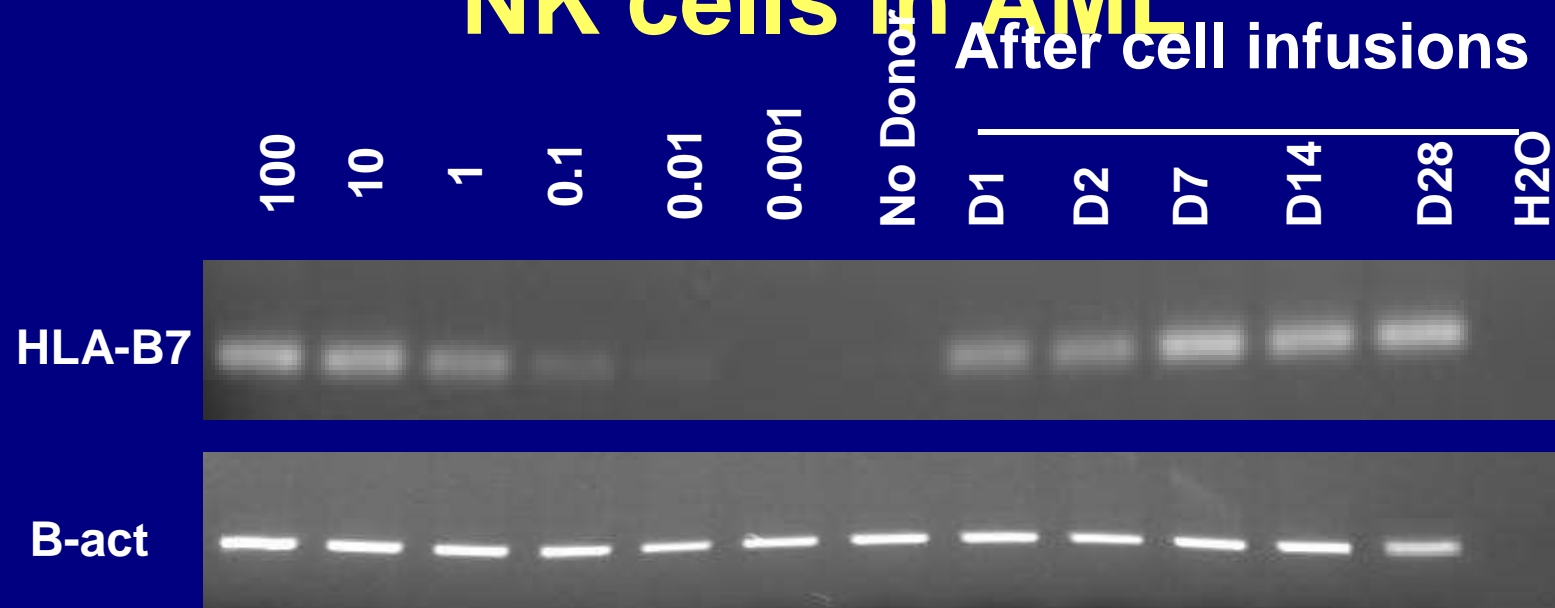


2302 IND #'s later: Adoptive Transfer of Human Haploidentical NK Cells



**Make sure you have
readouts other than
clinical outcomes!**

In vivo expansion of haploidentical NK cells in AML



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!**

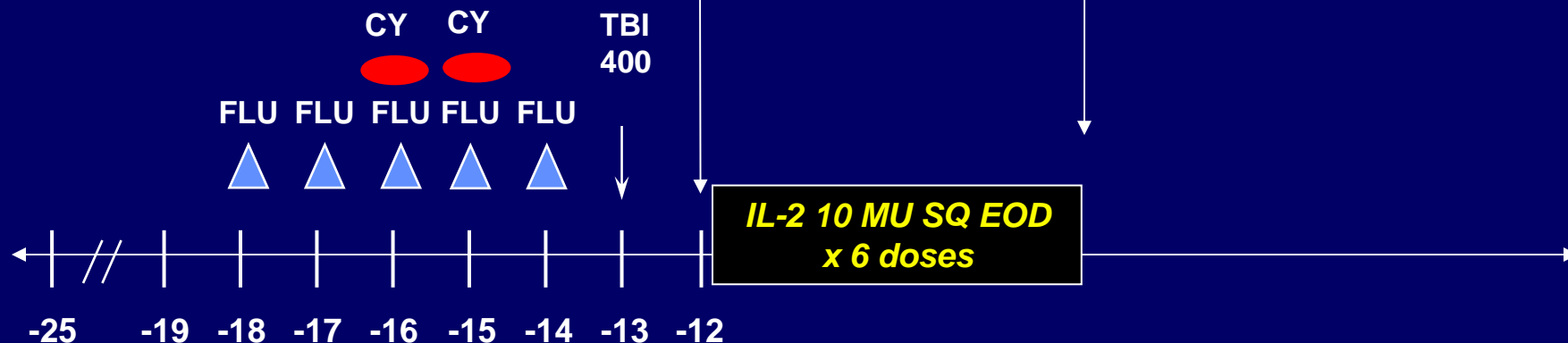
Endpoint Definitions:

- **In Vivo NK Cell Expansion**
 - ≥ 100 donor-derived NK cells per μL blood 12-14 days after NK cell infusion
 - $\text{ANK cells}/\mu\text{l} = (\text{ALC}) \times (\% \text{ CD56}^+/\text{CD3}^- \text{ lymphocytes}) \times (\% \text{ donor by VNTR})$
- **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
($2-8 \times 10^7$ MNC/kg)
IL-2 Overnight (1000 U/ml)**

**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!**

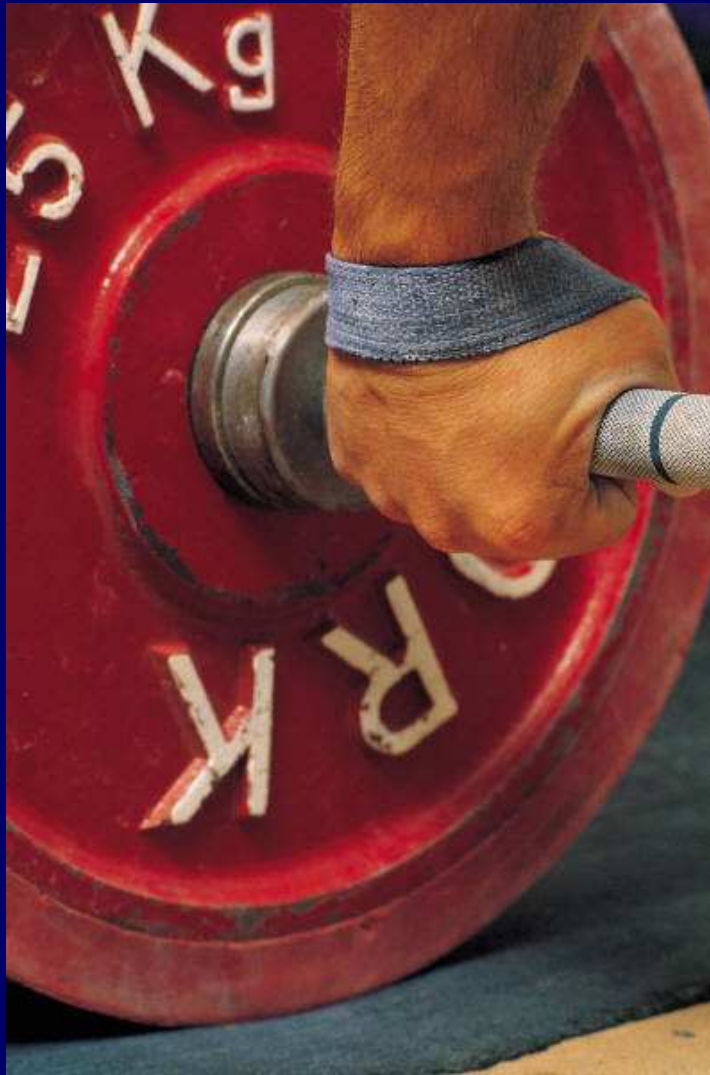
Problems and future directions

- 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

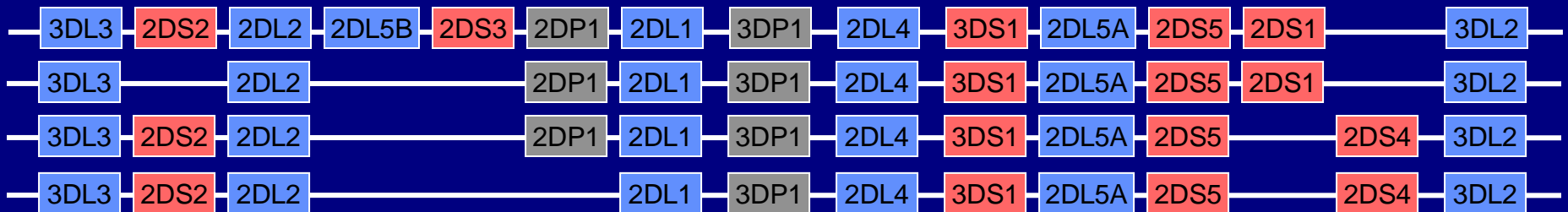
Killer-Immunoglobulin Receptor (KIR) Gene Locus

Group-A Haplotype:

Absence of 2DL5, 2DS2, 2DS1, 2DS3, 2DS5, 3DS1



Group-B Haplotypes: Presence of at least one of above



Demographics (n=448)

Year of Transplant	1998-2003
HLA Matching	
10/10	209 (47%)
9/10	95 (22%)
8/10	90 (20%)
≤7/10	44 (12%)
HLA Mismatched Group	
GvH KIR-Ligand MM	70 (29%)
KIR-Ligand Match	169 (71%)
Mean Age (range)	34 (1-61)
Disease Status at Transplant	
1 st CR (Early)	86 (18%)
2 nd or > CR (Intermediate)	160 (36%)
1 st or > Relapse/PIF (Advanced)	202 (46%)

KIR B/x Genotype Donors Confer Improved Relapse Free Survival in AML

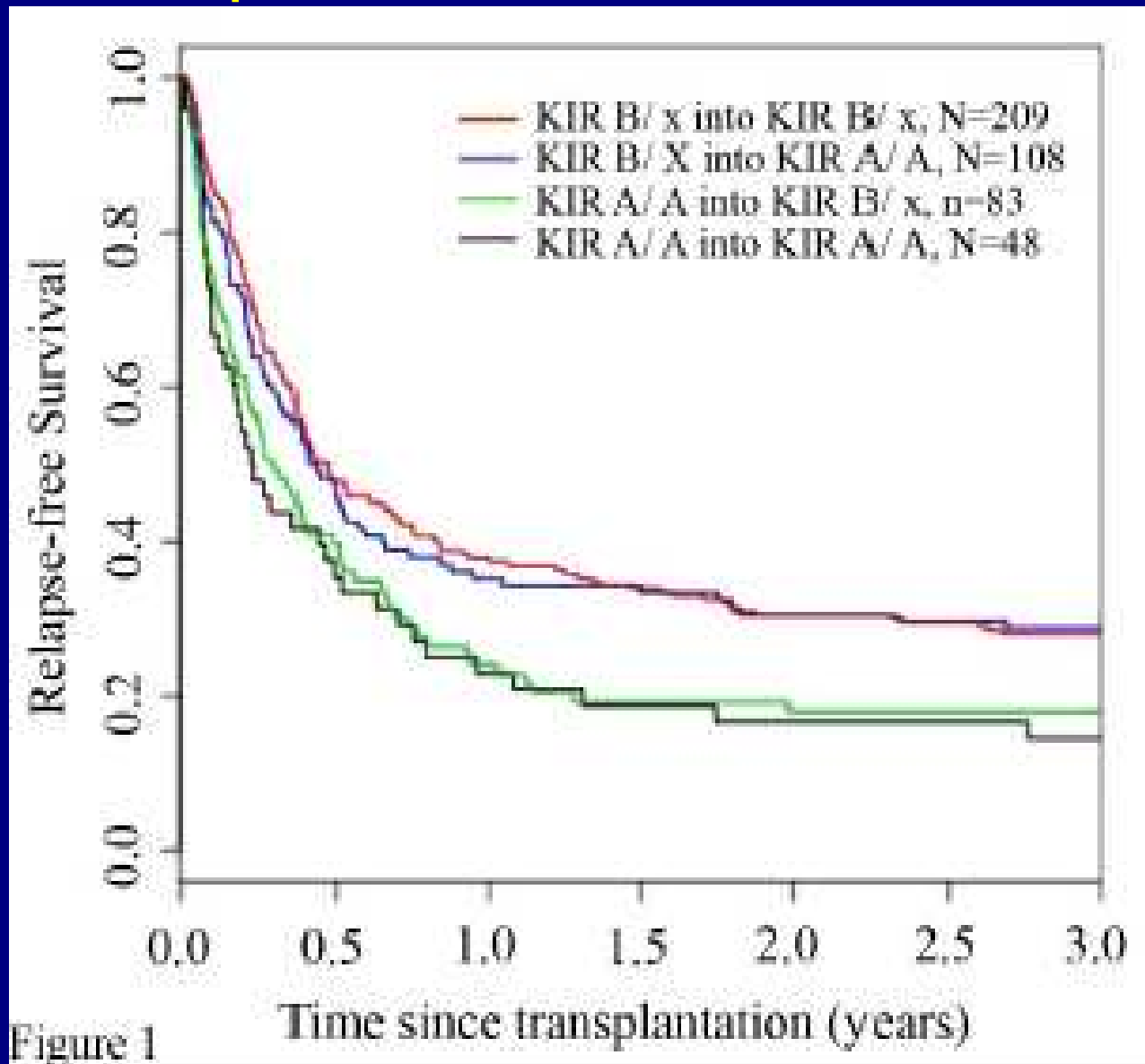


Figure 1

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 will 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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