PACT: Translational Development & Scale Up
NK cells

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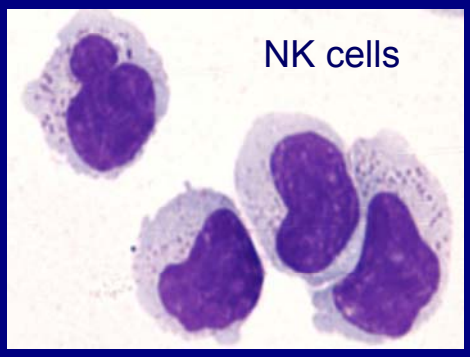
How do you know when your pre-clinical work in cell therapy is ready for the clinic?

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, 14448
- **Survivor, one random FDA audit**

Make Sure what you are studying is important and has relevance to health and disease

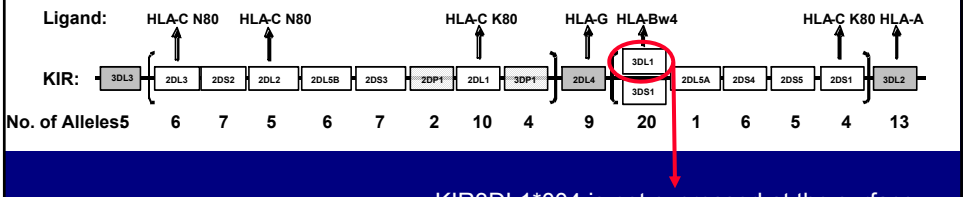


- Cancer treatment and tumor surveillance
- Infection disease control
- Autoimmunity
- Pregnancy (placental angiogenesis)

NK cell functions

- Killing targets
- Produce cytokines
  - Interferon-γ
  - Tumor necrosis factor
  - Many others

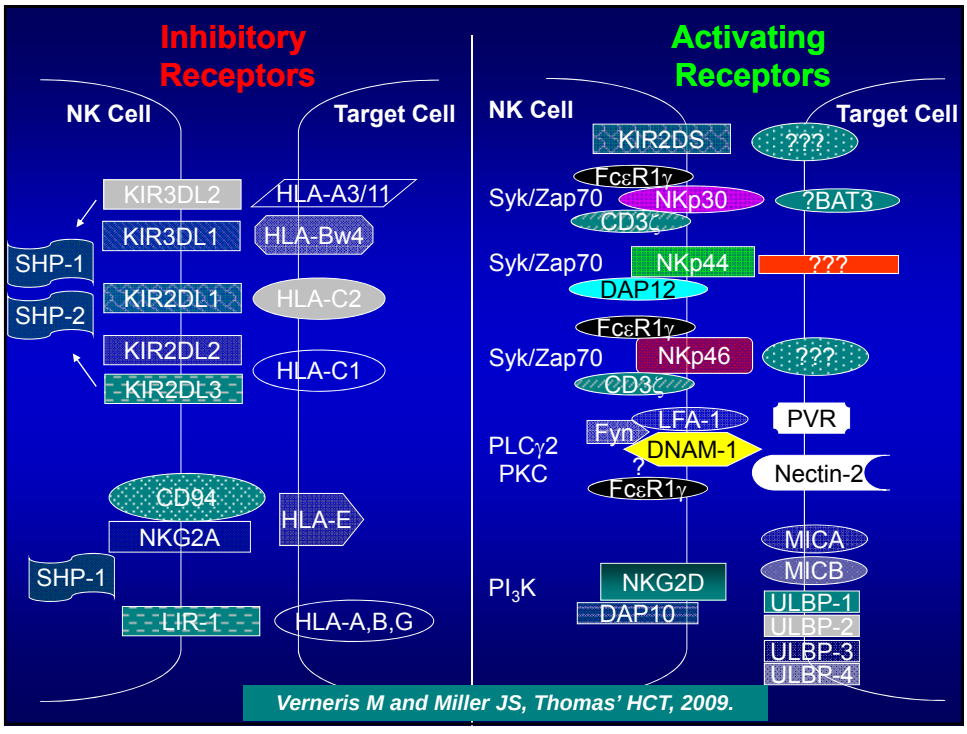
Biology needs to be relatively established:
Chr. 19 determines the personality of NK cells - Killer-immunoglobulin receptor (KIR) gene locus



KIR3DL1*004 is not expressed at the surface

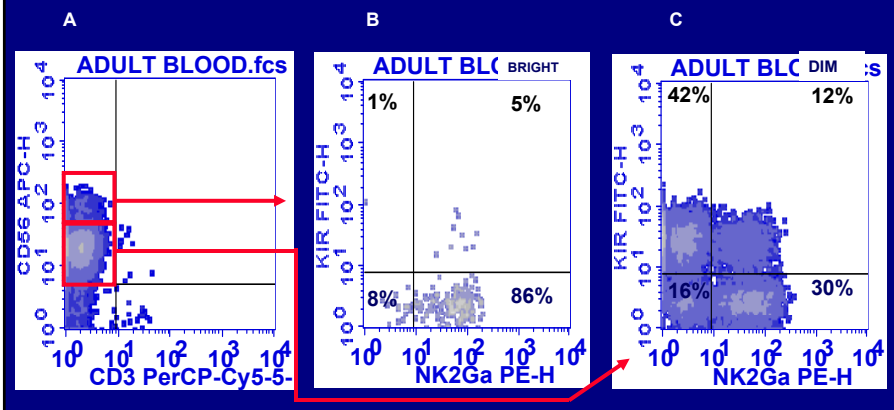
Mice do not have KIR
NKG2 family recognizes HLA-E

From Peter Parham



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

NK cell receptors define the NK cell repertoire



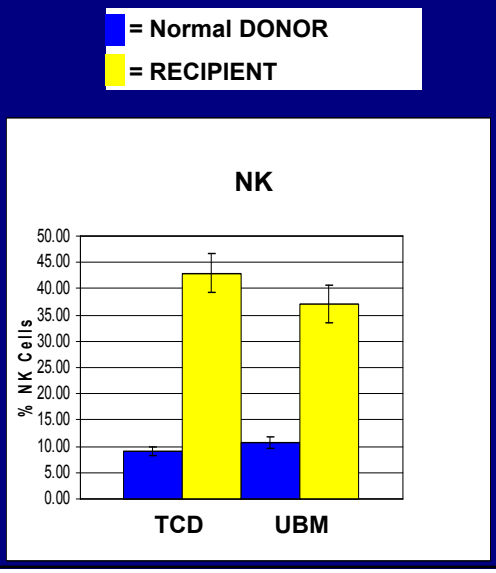
KIR^{-}/NKG2A^{-} subset: 19.4 ± 2.8% of CD56^{+dim} NK cells healthy donors (n=26)

Know the literature!
Any human experience?

Transplant Trials Exploring NK Cell Alloreactivity

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

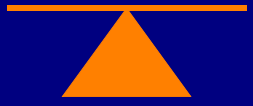
NK cells after transplant are increased



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 ? Transplant

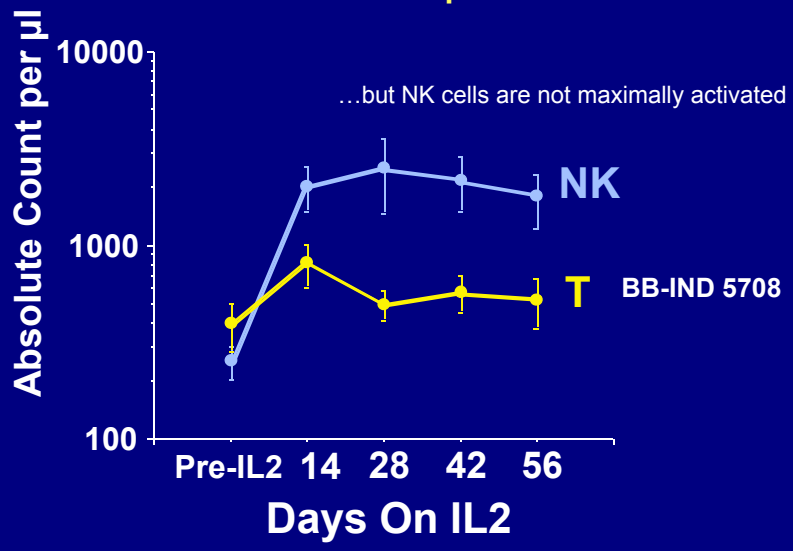


Pros and cons

Safer	More TRM
Transient	Permanent
Can expand in vivo (IL-2)	Too risky 2^{o} GVHD risk

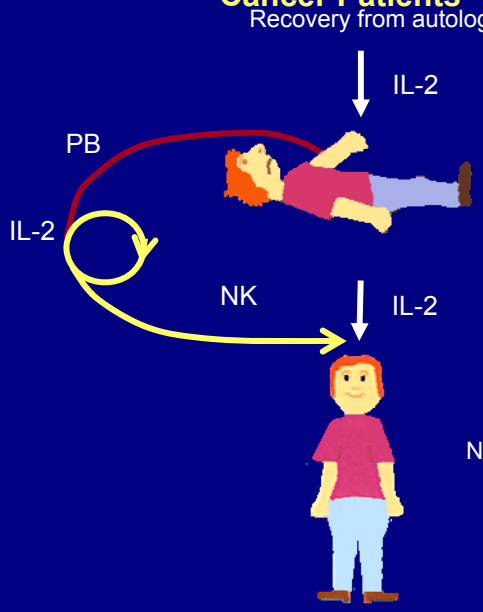
Build on your own experience in small, manageable steps!

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



BB-IND 6545

NK cells more activated using this approach

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

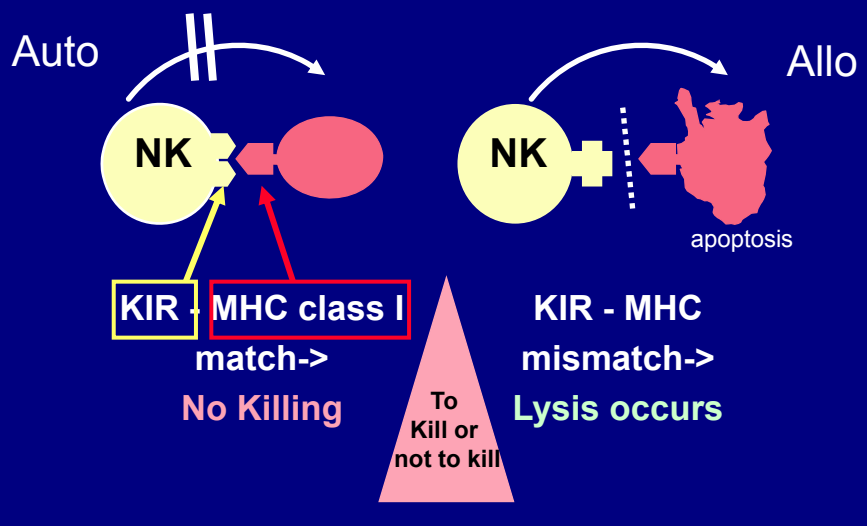
Conclusions

Enhanced activation of NK cells

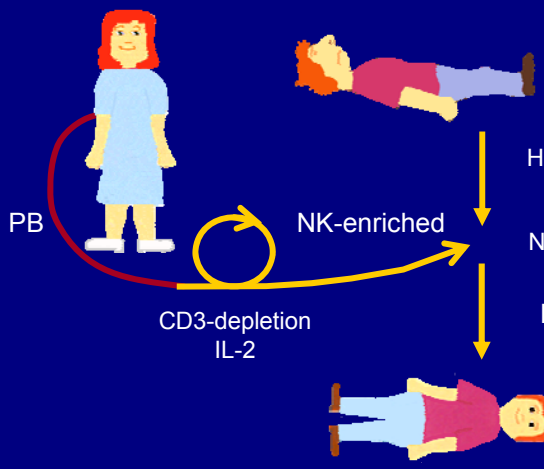
A matched paired analysis with our data and data from the IBMTR showed no apparent efficacy (survival or time to disease progression)

Alter your 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



Eligible patients: Poor prognosis with refractory AML

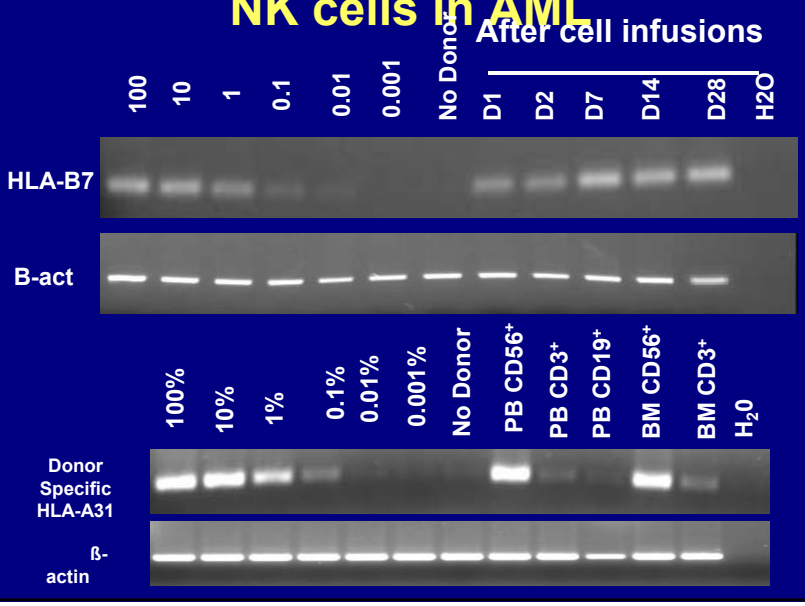
HD Rx	Cy 60 mg/kg x 2 Flu 25 mg/m^2 x 5
NK cells	2-8 x 10^7 MNC/kg
IL-2	10 MU SQ QOD x 6

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



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

Cooley et al, Blood 112:307, 2008

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
  - ANK cells/µl = (ALC) x (% $CD56^+/CD3^-$ lymphocytes) x (% donor by VNTR)
- **Leukemia Clearance**
  - <1% blasts on BMBx Day +12 after NK infusion
- **Remission**
  - No evidence of leukemia after donor neutrophil engraftment

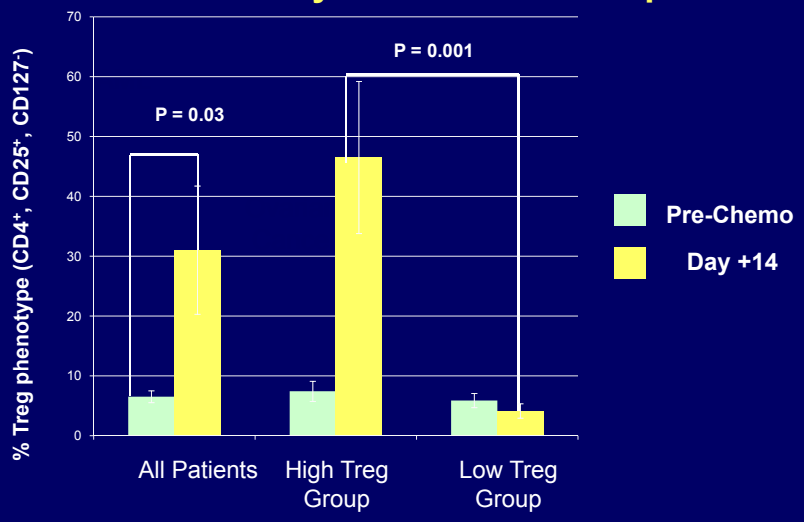
Be flexible!

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**

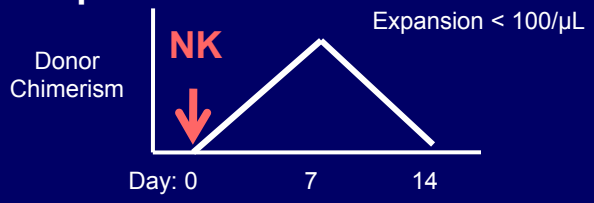
Anticipate failure and your next move!

Treg expansion after IL-2 and NK cell therapy in ovarian cancer may inhibit NK cell expansion



Problems and future directions

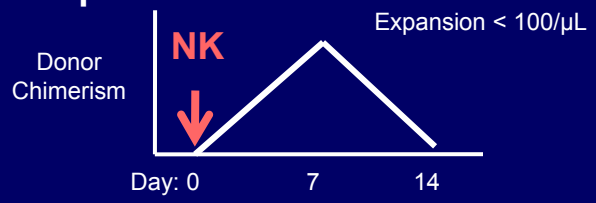
- **In adoptive transfer for solid tumors:**



- **Overcoming Suppressive mechanisms**
  - **IL-2/DT (Ontak) binds to CD25^{hi} cells and may provide transient but potent Treg depletion**
  - **Cyclosporine (CSA) may provide benefit based on the premise that Teff and Treg, which may "reject" NK cells, are more sensitive to CSA than NK cells**

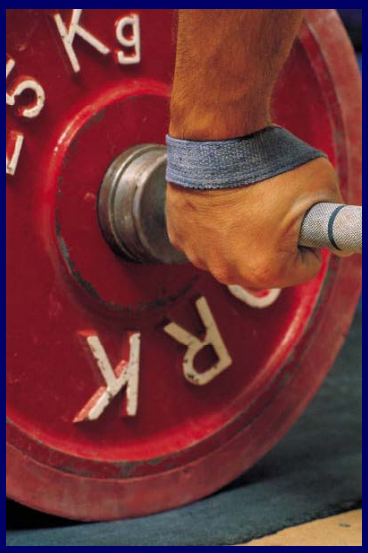
Problems and future directions

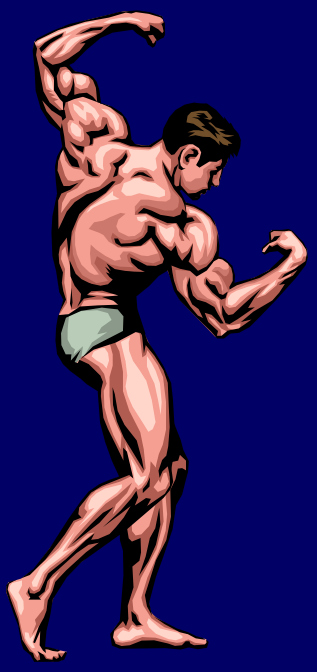
- **In adoptive transfer for solid tumors:**



- **Other strategies to improve efficacy:**
  - **Target sensitizing agents (e.g. bortezomib)**
  - **IL-15 (FDA approval [IND 14448] granted for NCI GMP product)**
  - **Inhibitory receptor blockade (anti-KIR, anti-NKG2A)**
  - **The DONOR**

Can we define an NK cell Super-donor?

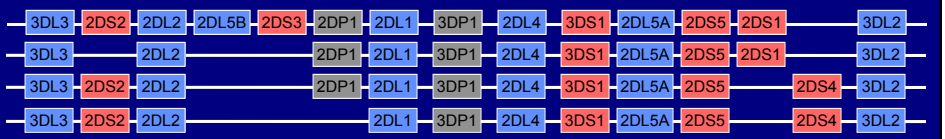




Killer-Immunoglobulin Receptor (KIR) Gene Locus

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

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



- **Hypothesis:** Evaluation of *KIR B* haplotypes at higher resolution will inform selection of "good" *KIR* donors to improve the effectiveness of unrelated donor HCT and NK cell adoptive transfer
- **Study Cohort:** 1409 Donor/Recipient pairs from URD HCT for acute leukemia
  - DNA from NMDP Sample Repository
  - Mature outcome data from CIBMTR

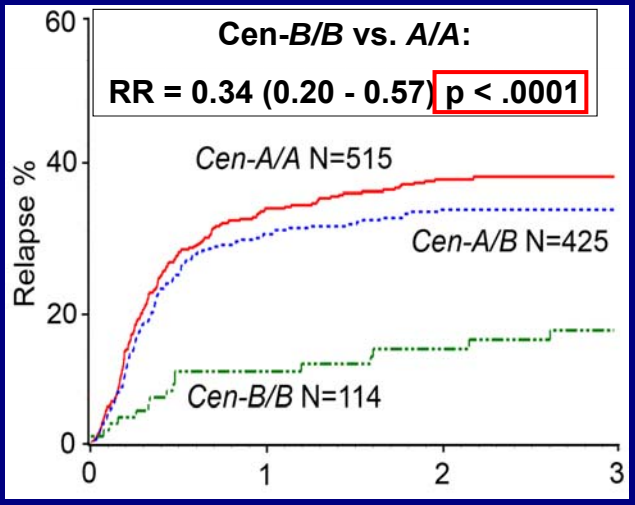
KIR Genotype Assignment

- Donor DNA samples were typed for 15 individual KIR genes
  - Validated single nucleotype polymorphism (SNP)-based MALDI-TOF assay (CHORI: Elizabeth Trachtenberg, PhD
  - Trachtenberg et al. *Immunogenetics.* 2007 Jul;59(7):525-37
- KIR Genotype Assignment (gene content) — Libby Guethlein & Peter Parham
  - Haplotypes: *A/A* or *B/x*
    - *B/x* Haplotype: Presence of *2DS2, 2DL5, 2DS3/S5, 3DS1, 2DS1*
  - Centromeric/Telomeric Segments: *Cen/Tel A/A, A/B, B/B*
  - *B*-content Score *(0-4)*

B content score	Cen	Tel
0	A/A	A/A
1	A/A	A/B
	A/B	A/A
2	A/A	B/B
	A/B	A/B
	B/B	A/A
3	A/B	B/B
	B/B	A/B
4	B/B	B/B

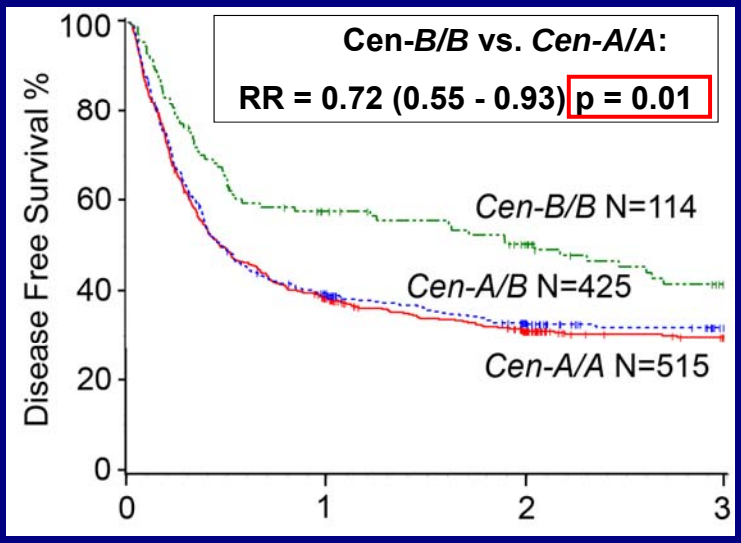
Demographics	AML n = 1086	ALL n = 323	Total n = 1409
Age (median)	18.5 (0.8-55)	38.9 (1-70)	
Disease Status			
Early	27%	25%	370
Intermediate	32%	49%	508
Advanced	41%	26%	531
HLA matching			
10/10	41%	46%	595
<10/10	59%	54%	814
Year of Transplant = 1988-2006	All Myeloablative, T-cell Replete Other Variables: Graft Type, Time from Diagnosis to Transplant, Race, D/R Sex Match, CMV Serostatus, KPS		

KIR Cen-B/B Donors Associated with the Lowest Relapse Risk



Cooley et al, Blood 2010

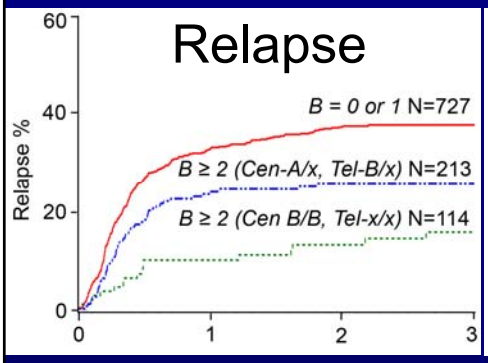
KIR Cen-B/B Donors Increase Relapse-free Survival

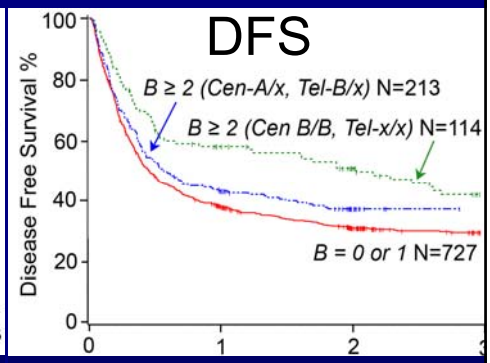


Donors with more *KIR B*-gene content decrease relapse and improve survival

***B* ≥2 (*Cen-A/x, Tel-B/x*)**
RR = 0.64 (0.48 – 0.86) p = 0.003
***B* ≥2 (*Cen-B/B, Tel-x/x*)**
RR = 0.33 (0.20 – 0.55) p < 0.0001

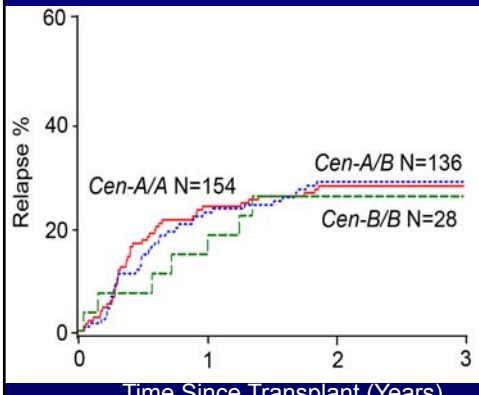
***B* ≥2 (*Cen-A/x, Tel-B/x*)**
RR = 0.84 (0.70 – 1.01) p = 0.07
***B* ≥2 (*Cen-B/B, Tel-x/x*)**
RR = 0.70 (0.55 – 0.90) p = 0.007



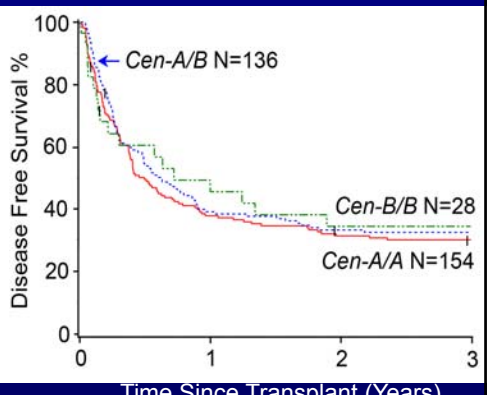


Donor *KIR* genotype does not affect outcomes for ALL

Relapse



Survival



Suggests a unique interaction between NK cells and AML targets

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!!!!!!!**

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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