NK Cells & Killer Immunoglobulin-Like Receptors: Function and Clinical Relevance

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Functions of NK cells

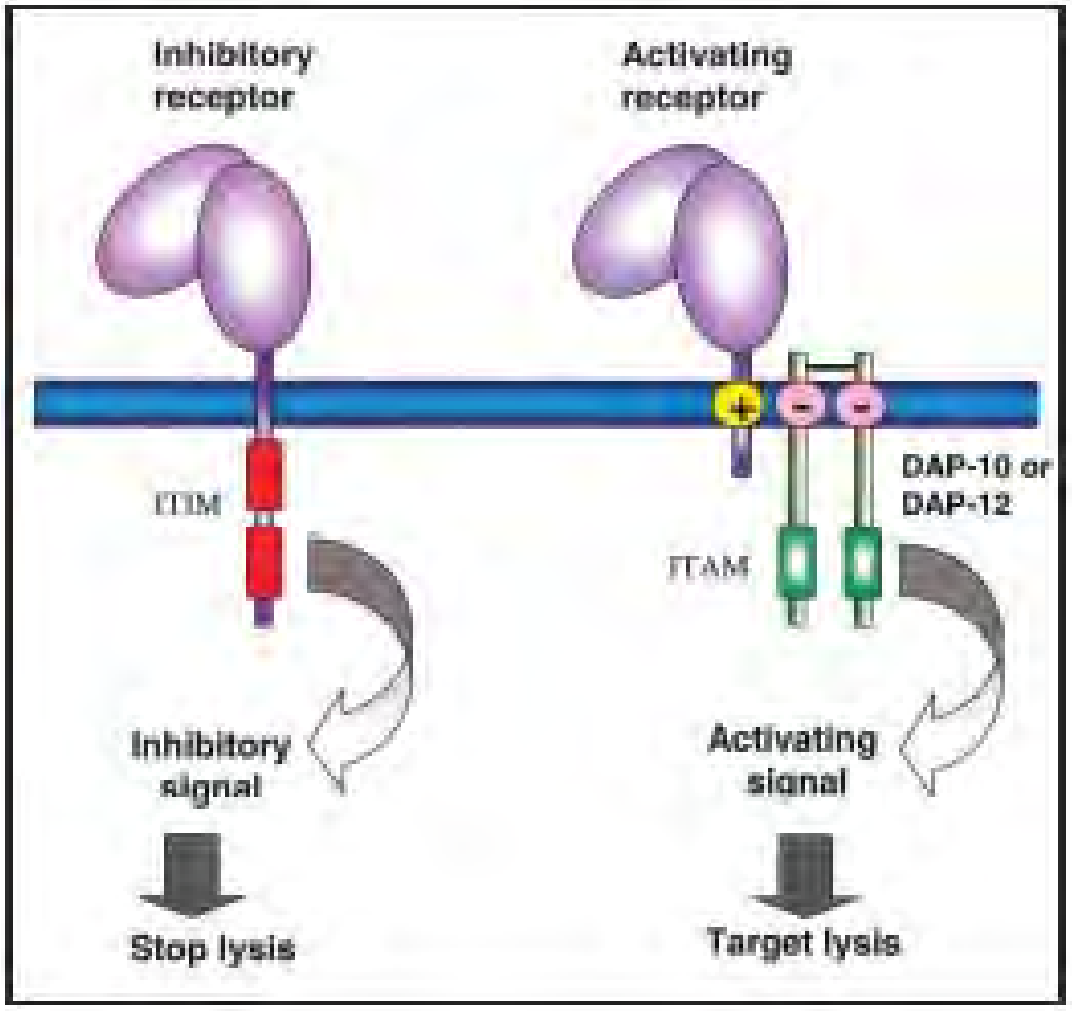
- Cytotoxicity against abnormal cells
  - tumour cells.
  - virus infected cells.

- Cytokine secretion – IFNg, TNF
  - Activation of macrophages, DC.
  - Influence T1/T2 balance by interaction with dendritic cells.
  - IFNg essential for Treg generation following NK:DC interaction.

- Editing/termination of immune responses
  - Kill activated DC, macrophages

  => Interface between innate and adaptive immunity.

NK Cell activation is controlled by the balance of activating and inhibitory signals

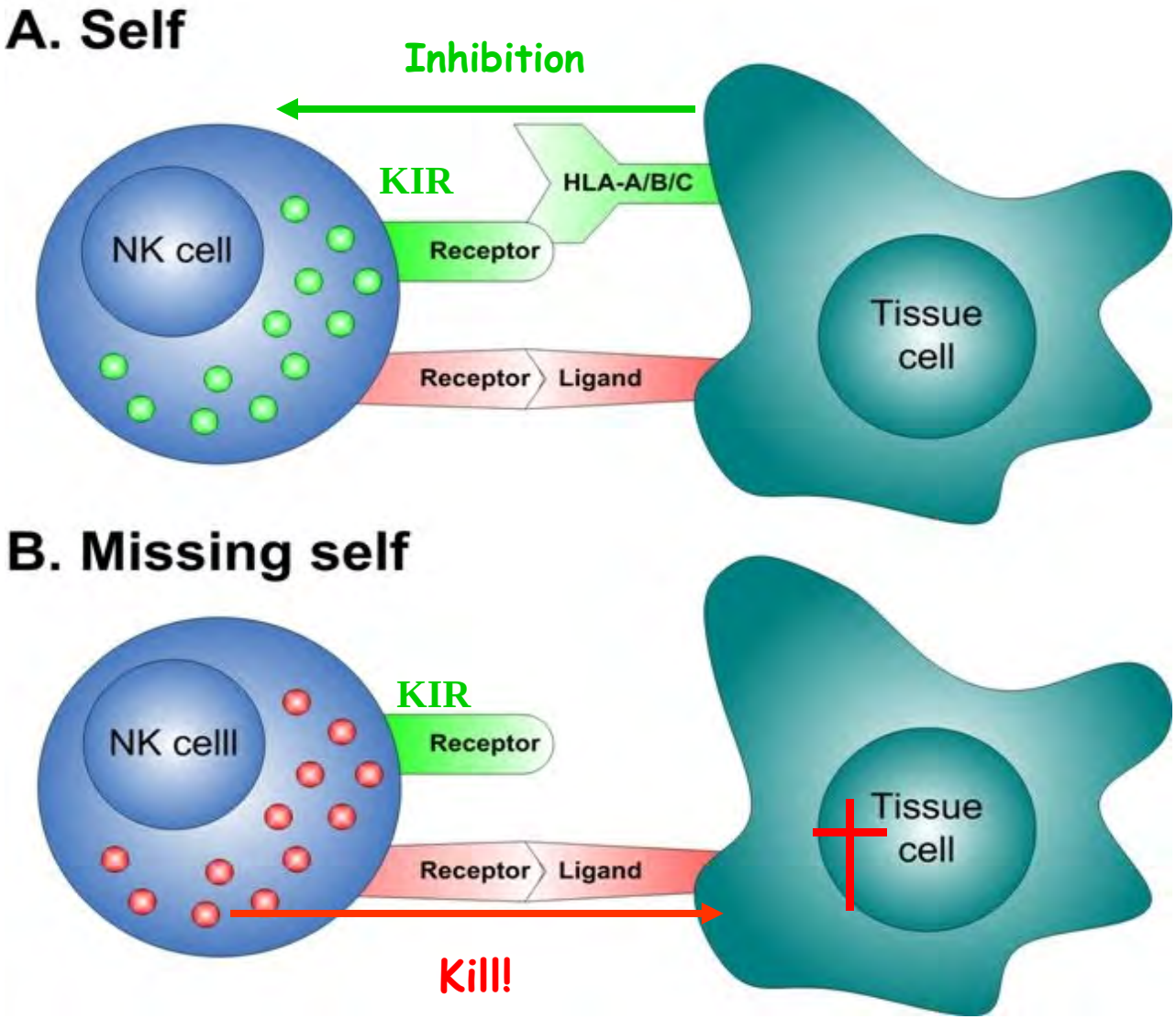


Inhibitory signals tend to be dominant

From Rajalingam, 2003

”Missing Self” or ”Two receptor” model of NK cytotoxicity

NK cells are inhibited by self HLA class I



The KIR – HLA Lottery

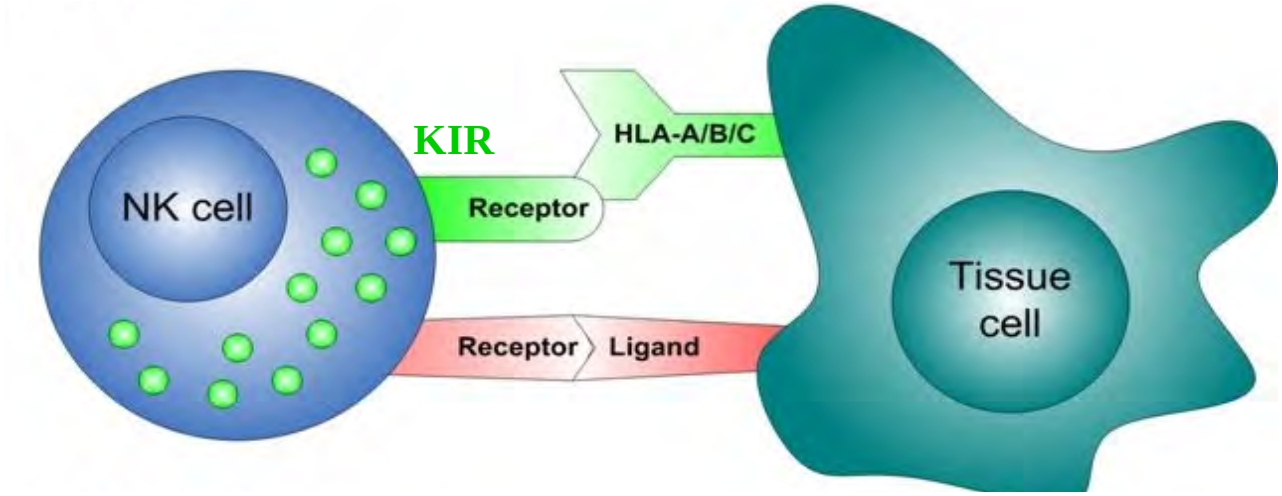
KIR genes
inherited on chromosome 19

HLA alleles
inherited on chromosome 6

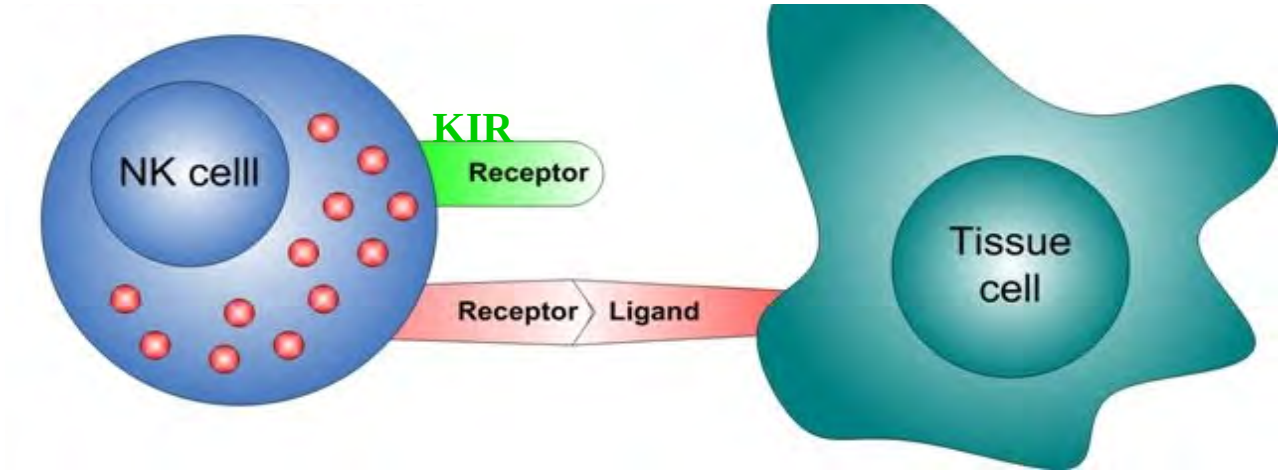
The KIR genes do NOT always match the HLA alleles!
We can have inhibitory KIR receptors but not the HLA ligand!

Armed NK Cells with a single inhibitory receptor for a non-self ligand are NOT ALLOWED

Inhibition encountered during development → armed NK cell



Inhibition not encountered during development → disarm

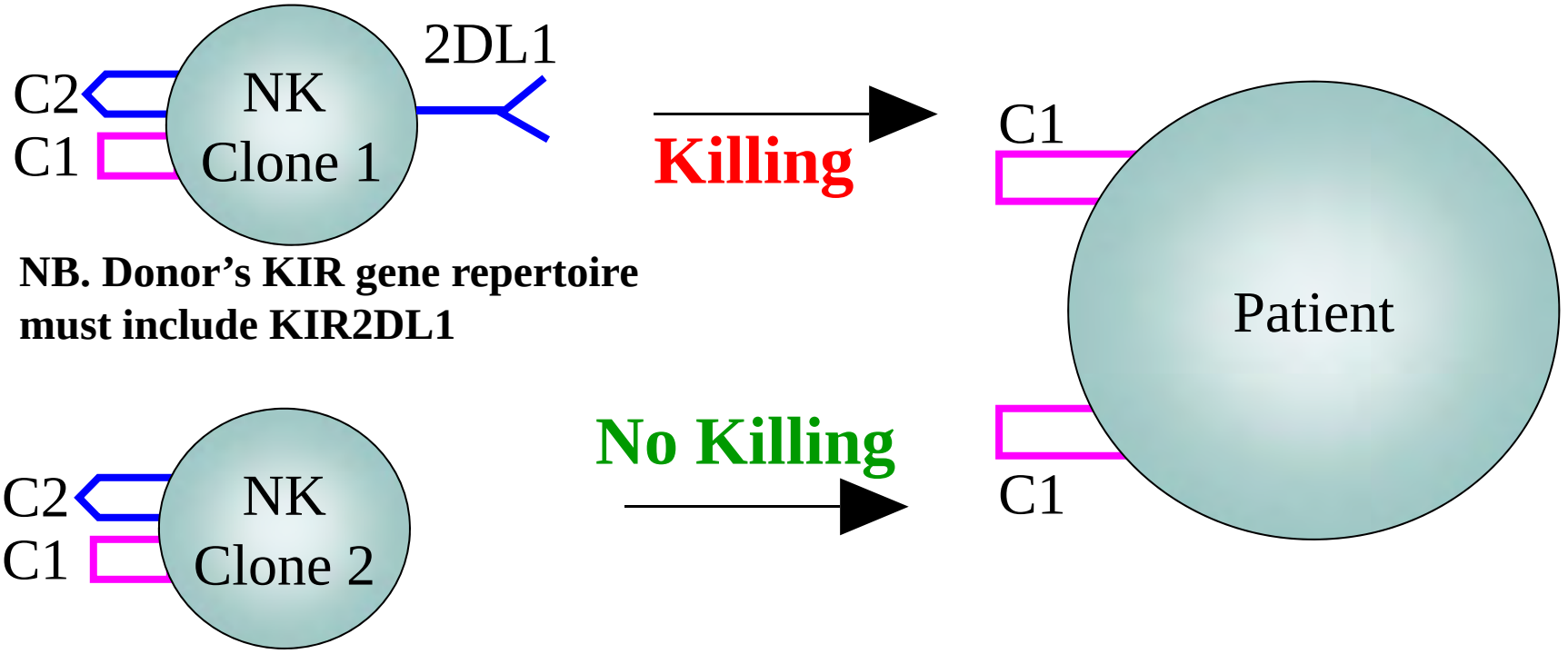


NK ALLOREACTIVITY

What is NK Alloreactivity?

Donor NK cells kill patient cells if patient cells lack the HLA alleles that are ligands for the donor's inhibitory KIR receptors

NK Alloreactivity: Donor must also have the Relevant Inhibitory KIR to recognise an HLA mismatch (missing self)



NB. Donor's KIR gene repertoire must include KIR2DL1

Donor's KIR gene repertoire does not include 2DL1.
Unable to recognise lack of C2
=> No killing

NK ALLOREACTIVITY
in
HAEMATOPOIETIC STEM CELL TRANSPLANTATION

Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants

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Katia Perruccio,[1] Warren D. Shlomchik,[2] Antonella Tosti,[1]
Sabrina Posati,[1] Daniela Rogaia,[1] Francesco Frassoni,[3]
Franco Aversa,[1] Massimo F. Martelli,[1] Andrea Velardi[1]*

Science (2002), 295: 2097-2100

Haploidentical BMTx with Potential NK Alloreactivity in the GvHD Direction Results in Less GvHD, Less Rejection and Less Relapse (AML)

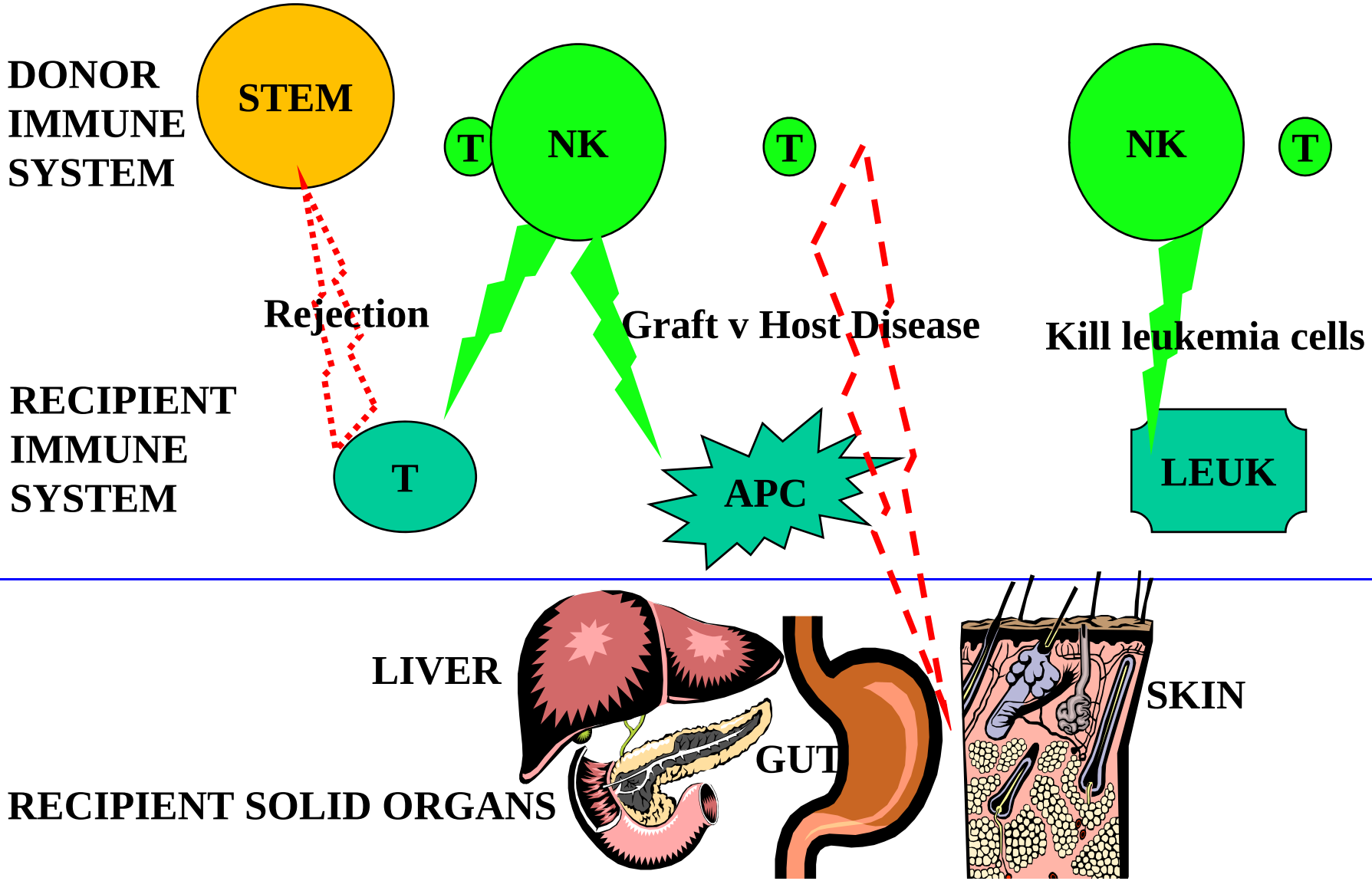
	C1, C2, Bw4 MATCHED	MISMATCHED
KIR ligand incompatibility in GVH direction	No	Yes
Number of transplants	58	34
Donors displaying antirecipient NK clones	1/58	34/34*
Disease		
ALL	21	14
AML	37	20
Transplantation outcomes		
Rejection	15.5%	0%*
Acute GVHD, ≥ grade II	13.7%	0%*
Probability of relapse at 5 years		
ALL	90%	85%
AML	75%	0%**

$P \leq 0.01$; $^{**}P < 0.0008$ (22).

EFS-AML	5%	60%

Ruggeri et al, Science, 295, 2097, 2002

Benefits of Donor NK Alloreactivity



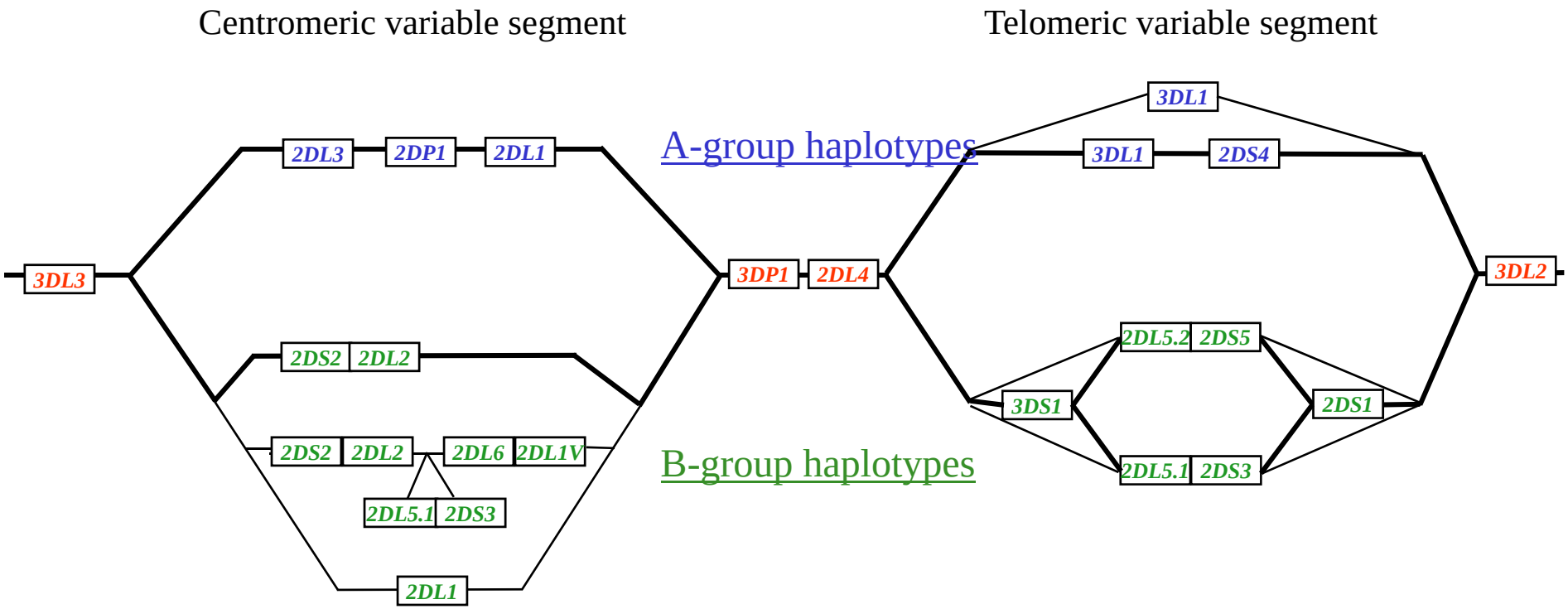
Does KIR ligand mismatching improve outcome in conventional MUD transplants?

Reference	Subgroup	M:L:O[a]	T-dep (%)[b]	ATG (%)[c]	Rej[d]	GVHD[e]	Relapse[f]	Surv[g]
Davies [15]		71:35:59	34[h]	?	–	X	–	XXX
Yabe [8••]	NO ATG	1173:617:0	0	6		XXX	–	XXX
Morishima [5••]		577 AML	0	0	XX	XXX	–	XXX
Morishima [5••]		596 CML	0	0	XX	XXX	–	XXX
Morishima [5••]		617 ALL	0	0	XX	–	XX	X
Farag [6•]		1397:0:0	22[h]	14		XXX	XX	XXX
De Santis [16]		42:17:45	9[h]	?	–	XX	XX	XX
Bornhauser [17]		118:0:0	19	100	–	–	XX	–
Schaffer [18]		132:54:0	7	100	–	–	–	XXX
Kroger [19]		90:52:0	0	100	–	–	XX	XX
Lowe [20]		69:33:3	100[h]	100	–	–	–	X
Miller [21]	EM[i]	534:0:0	+/–	+/–		–		
Miller [21]	EC1[j]	479:0:0	+/–	+/–		–		
Miller [21]	IM[k]	702:0:0	+/–	+/–		–		
Hsu [7•]		?	0	?			–	–
Sun [23]		65:0:0	0	0		–	–	–
Elmaagacli [24]		236:0:0	0	?	–	–	✓✓	–
Giebel [25]		87:38:5	0	100	–	✓	✓	✓✓✓
Beelen [27]		137:0:0	0	0	XX	–	✓✓	–
Kroger [28]		0:0:73	0	100	–		✓✓✓	–
Yabe [8••]	ATG	?	0	100		✓✓	–	–

Studies are arranged to group together those finding similar associations with outcome.
+/– = heterogeneity with respect to this variable, '?': data not provided, X: deleterious effect found, '✓': beneficial effect found, –: no effect found at $p < 0.10$, one tick or cross = $p < 0.1$, two ticks or crosses = $p < 0.05$, three ticks or crosses = $p < 0.01$; blank entries under columns headed "Rej", "GVHD", "Relapse" and "Surv" indicate that these outcomes were not reported on in that publication.

Two Major KIR Haplotype Groups -differing in number of activating KIR

Alternate configurations of the KIR gene complex



From Martin et al, Gene, 2004

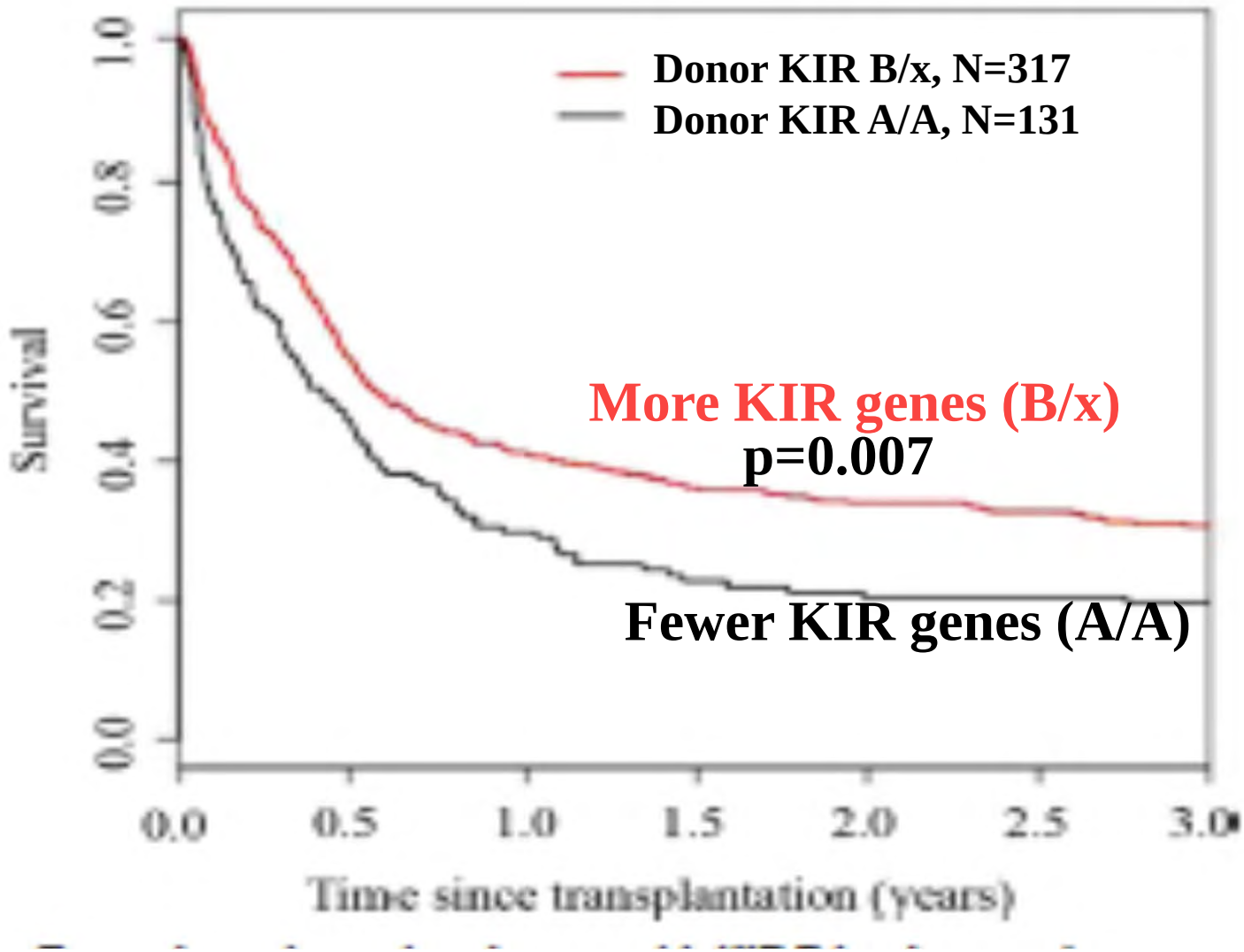
Retrospective studies of number of KIR genes (B-haplotype) present in donor and outcome in MUD transplants.

Studies investigating the effects of a greater number of donor KIR genes on outcomes in matched unrelated donor or HLA identical sibling transplants.

Reference	Donor	M:L:O[a]	T-Dep (%)[b]	ATG[c]	Rej[d]	GVHD[e]	Relapse[f]	Surv[g]
De Santis [16]	MUD	42:17:45	9	?	–	✓✓✓	–	✓✓✓
Cook [29]	SIB	112:0:0	?	?				✓✓✓
Cooley [9•]	MUD	448:0:0	0	?			–	✓✓✓
Savani [32]	SIBS	39:15:0	100	?		✓✓	✓✓✓	✓✓✓
Verheyden [33]	SIBS	49:16:0	52	0		–	✓✓	–
Kim [34]	MUD	30:7:7	?	?		✓		
Kroger [19]	MUD	90:52:0	0	100			XXX	XXX
Mcqueen [35]	SIBS	113:61:0	?	?		XX		XX
Triplett [36]	MUD	59:0:0	13 + 27	100				XX
Giebel [11••]	MUD/SIB	78:22:0	0	68		–	XX	XX
Clausen [37]	SIB	?	0	0				XX
Yabe [8••]	MUD	$n = 187$	0	6		XX	–	–
Sun [23]	MUD	65:0:0	0	0		–	–	–
Hsu [10]	MUD/SIB	?	0	?			–	–

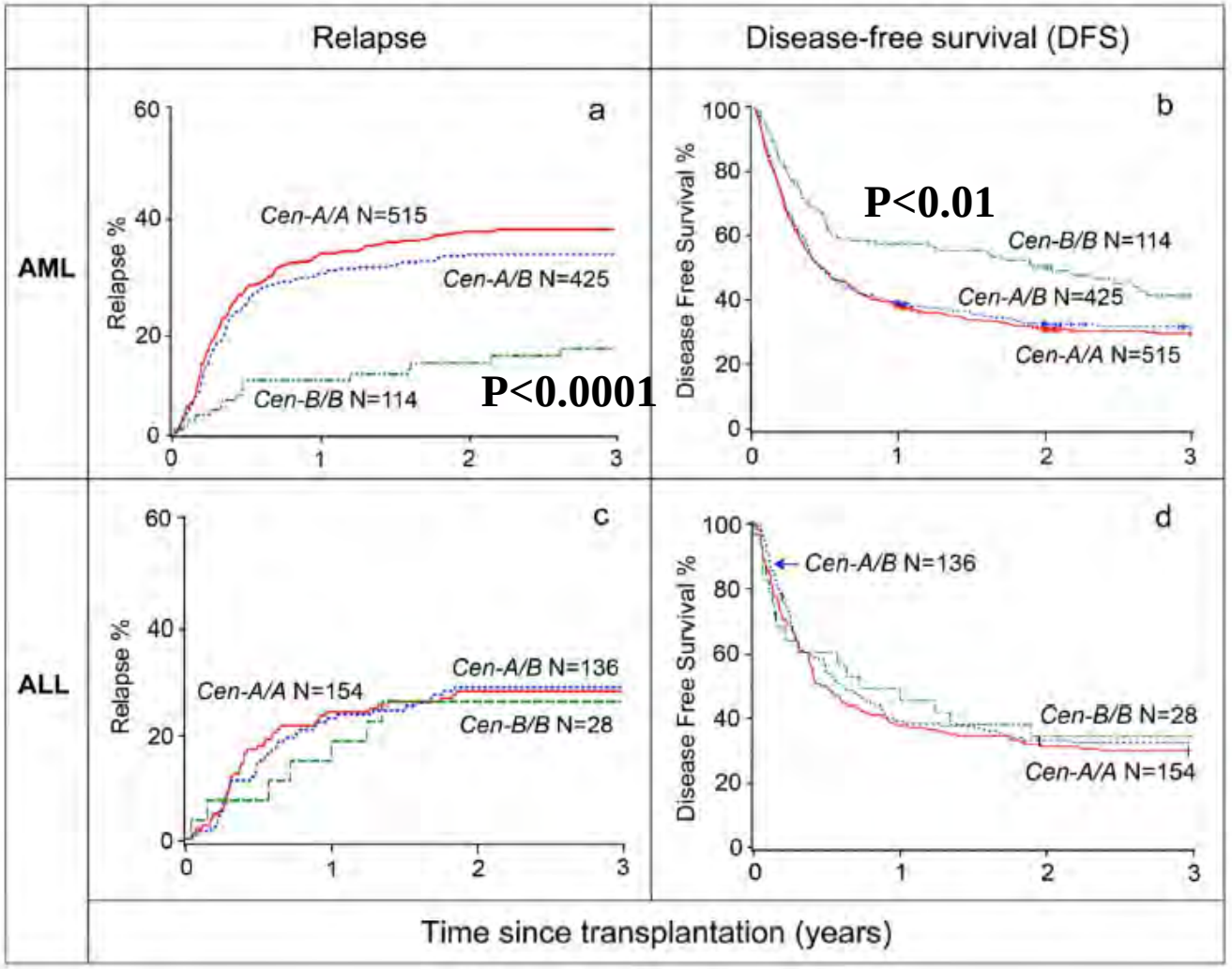
Studies are arranged to group together those finding similar associations with outcome.
'?': data not provided, X: deleterious effect found, '✓': beneficial effect found, –: no effect found at $p < 0.10$, one tick or cross = $p < 0.1$, two ticks or crosses = $p < 0.05$, three ticks or crosses = $p < 0.01$; blank cells for rejection, GVHD, relapse and survival indicate that these outcomes were not reported on.

KIR B-haplotype donors result in improved survival in multicentre study of AML



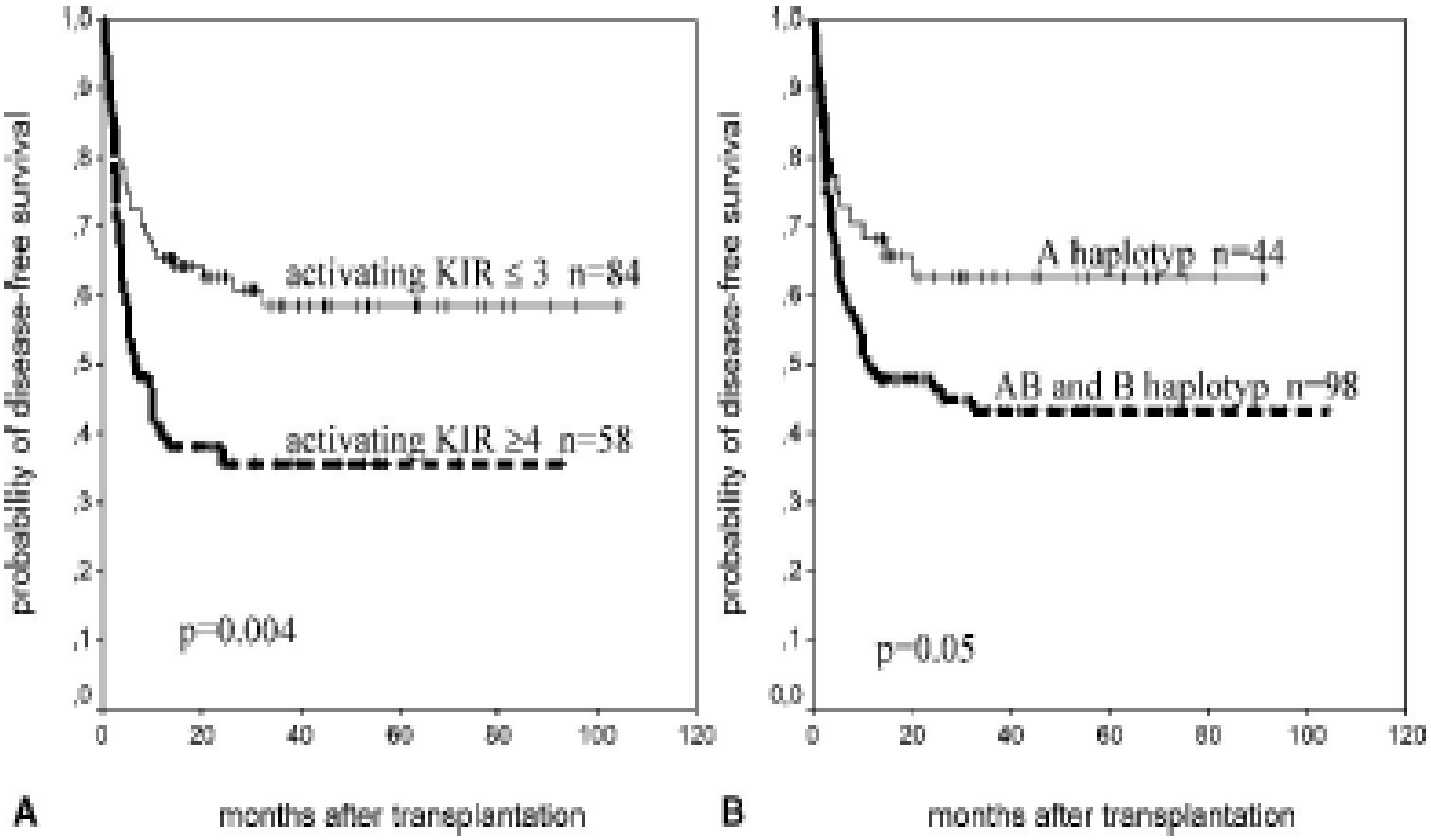
Cooley et al Blood, 2009

Donors with more KIR genes have beneficial effect on relapse and survival in AML



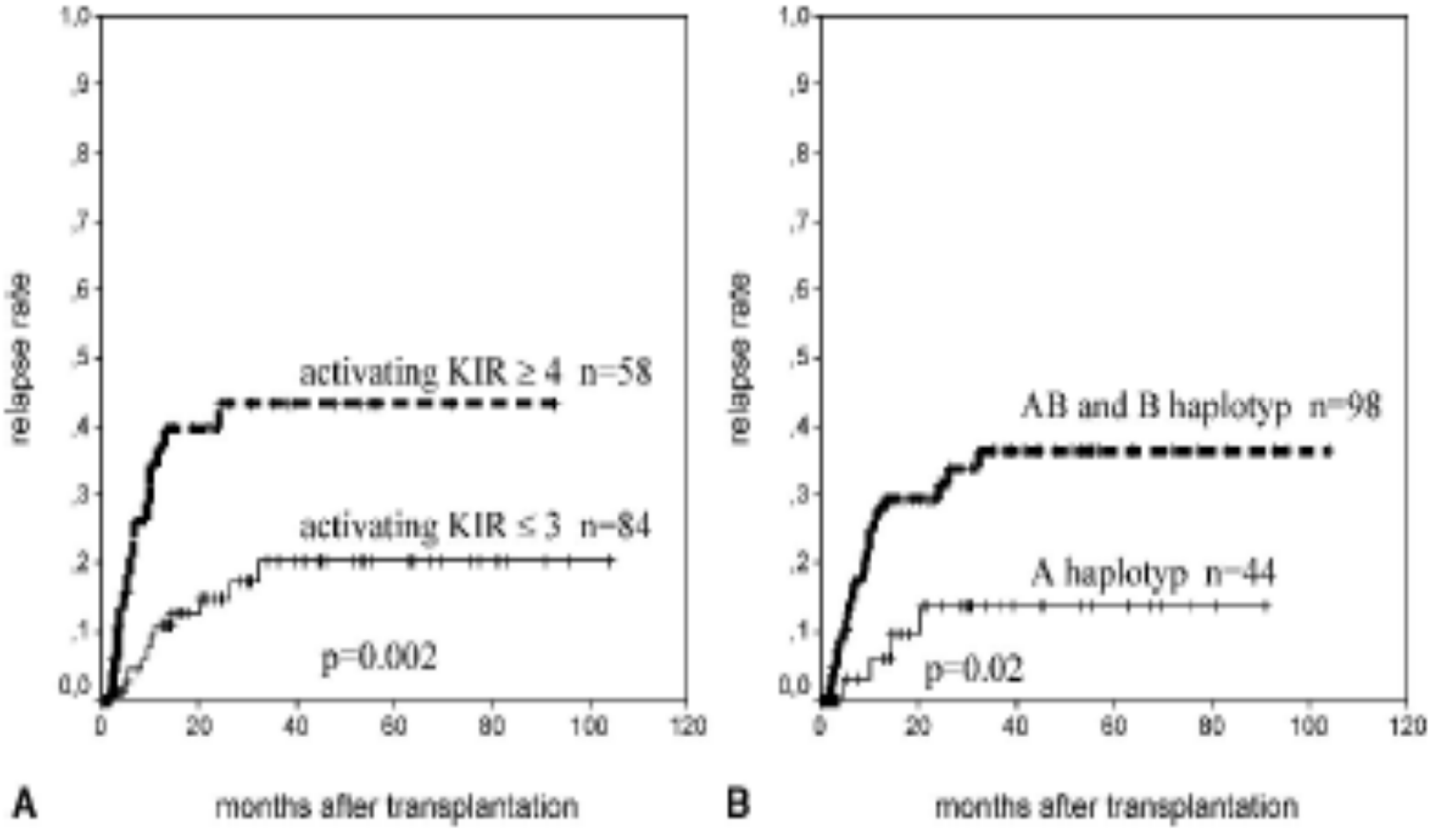
Cooley et al, Blood, 2010

Donors with fewer KIR genes result in improved survival



Kroger et al, Transplantation, 2006

Donors with fewer KIR genes protect against relapse



Kroger et al, Transplantation, 2006

How can we explain such contrasting results in different transplant centres?

The size effect of donor KIR genotype appears at least as big as mismatching one HLA locus. Therefore we would like to use it for donor selection but which effect will we get?

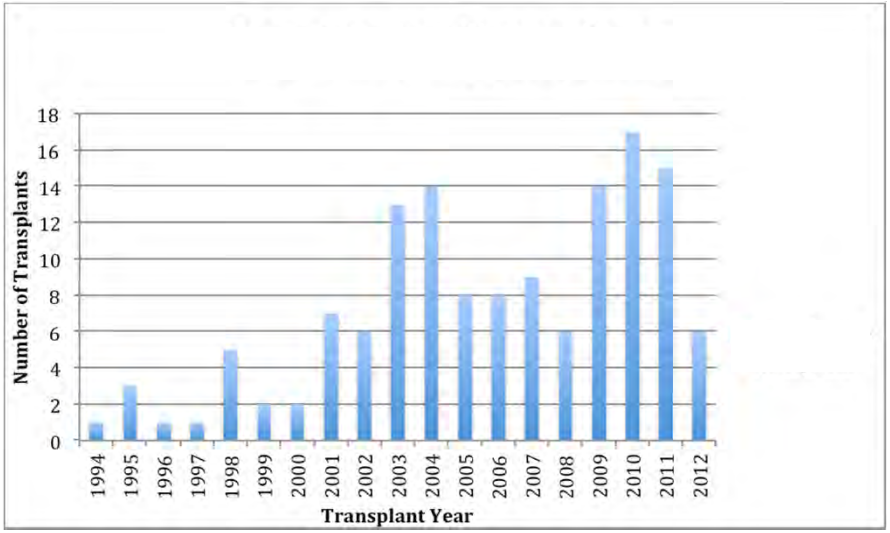
Could there be something in the transplant protocol that interacts with KIR genotype?

Why the Different Effects?

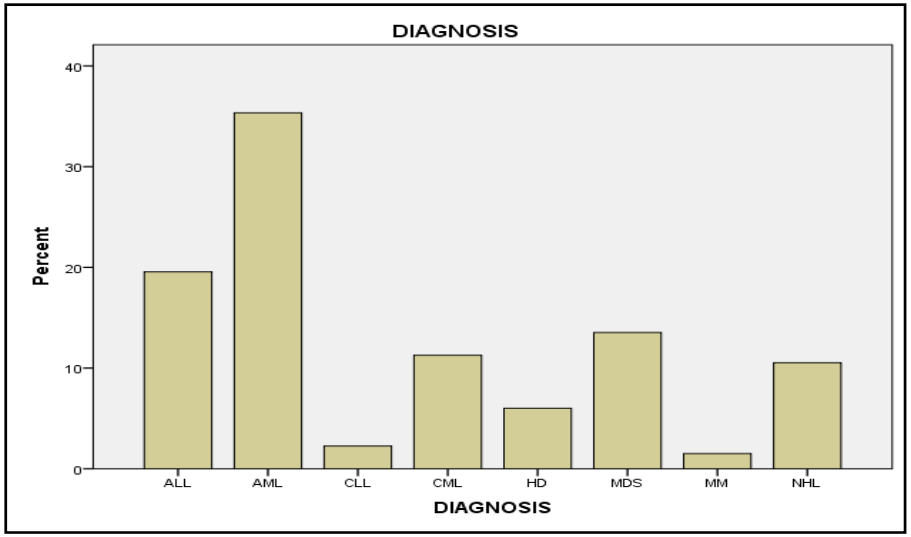
- **Heterogeneous disease groups**
  - Myeloid vs lymphocytic leukemias, other diseases
- **Stem Cell Source**
  - T replete vs T depleted
  - Use of ATG
  - BM vs Peripheral Stem cells
- **Conditioning, GVHD prophylaxis**
- **HLA Matching**
- **Limited Power**
  - Univariate vs multivariate analysis

Could differences in transplant protocol be responsible for the different effects??

Year of 133 MUD Transplants Performed at Royal Perth Hospital



Diagnoses of 133 MUD Transplants (RPH)

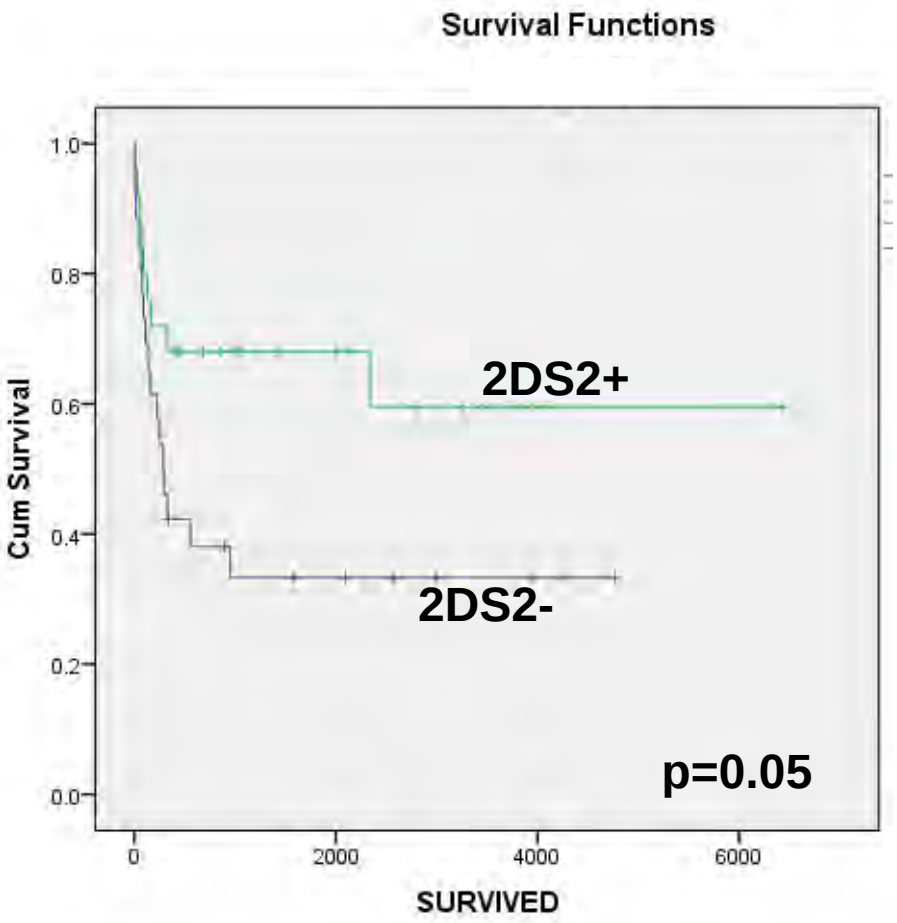


Transplant Variables Relevant to NK Cells

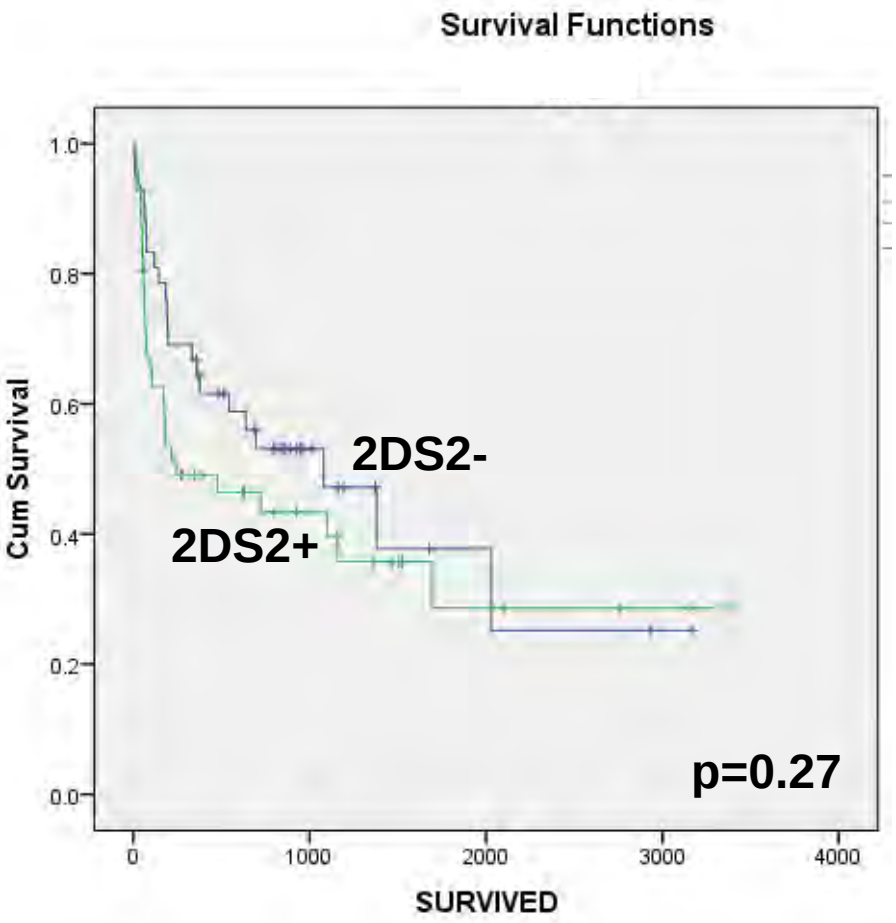
- **Cytomegalovirus (CMV) status & CMV Prophylaxis**
  - NK cells (KIR) are important for control of CMV reactivation.
  - CMV+ patients have worse outcomes.
- **Peripheral blood or Bone Marrow**
  - PBSC grafts have more lymphocytes (NK cells)
- **Conditioning Drugs and Total Body Irradiation (TBI)**
  - TBI and cytotoxic drugs up-regulate stress ligands on leukaemia cells for NK cell receptors.
  - Increased cytotoxicity towards tumor cells.

TBI may have a weak interaction with KIR2DS2 -of the kind we are looking for.

TBI + Transplants

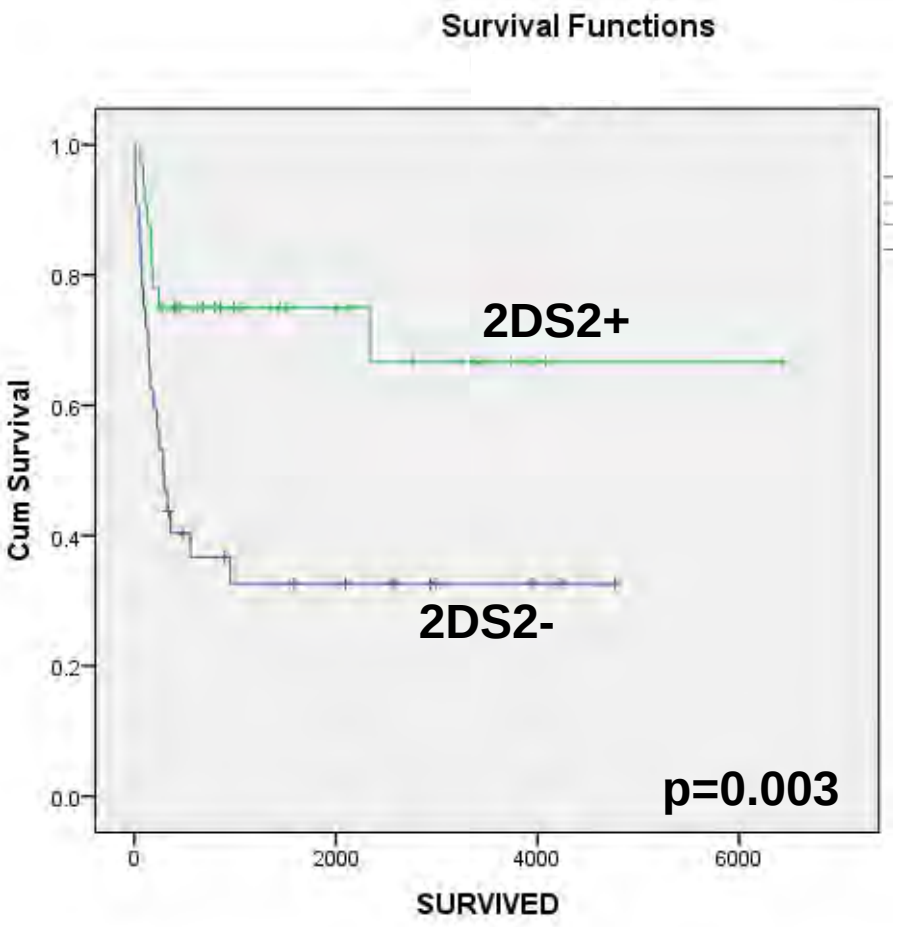


TBI- Transplants

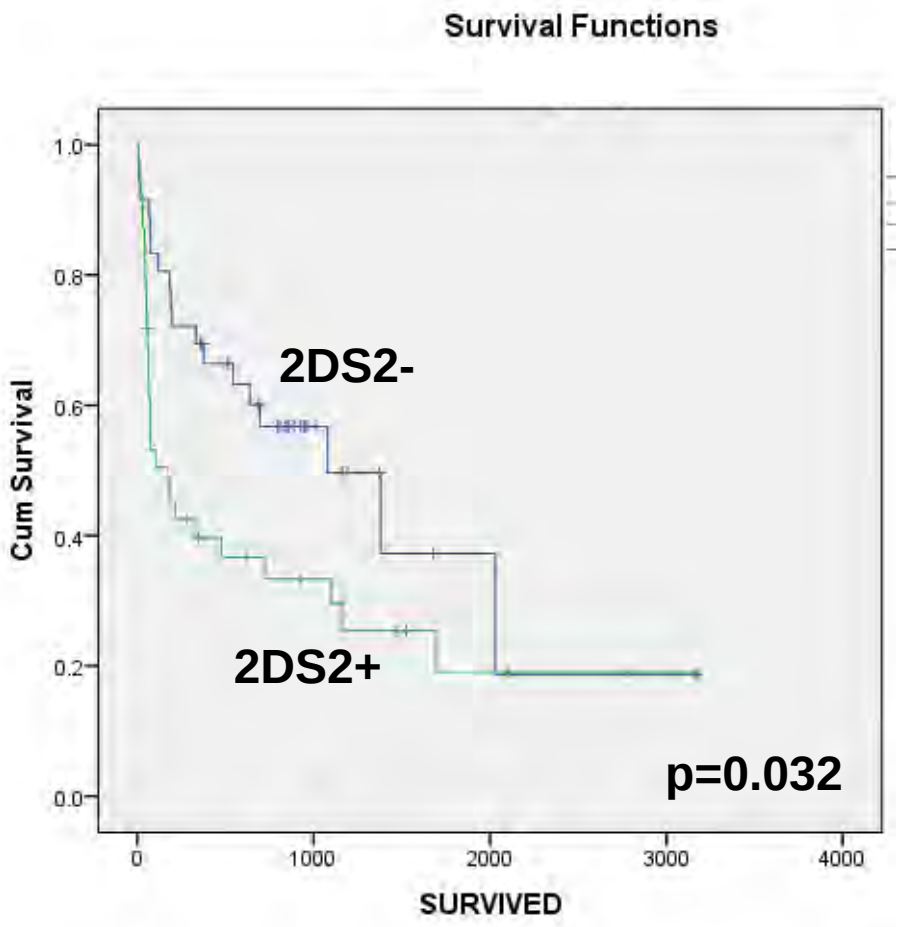


Cyclophosphamide has a strong interaction with KIR2DS2 -of the kind we are looking for.

Cy+ Transplants

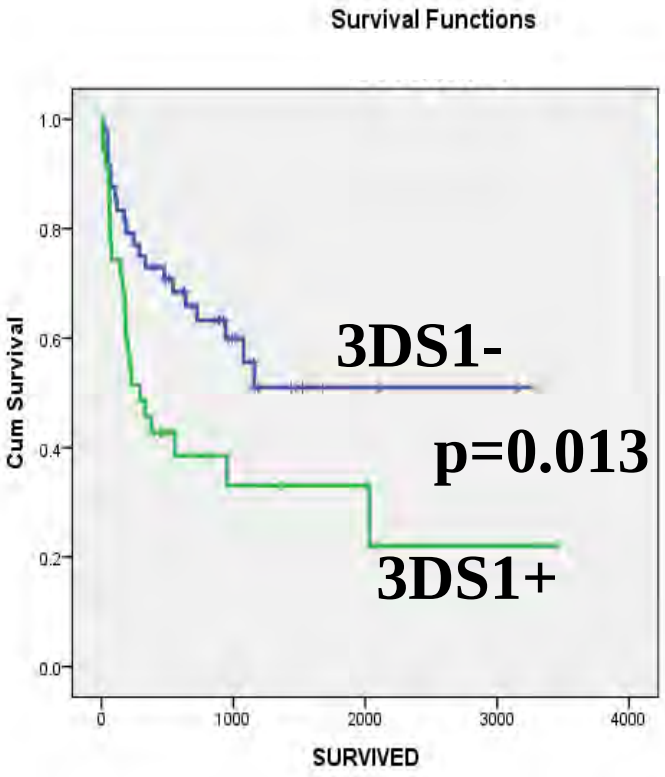


Cy- Transplants

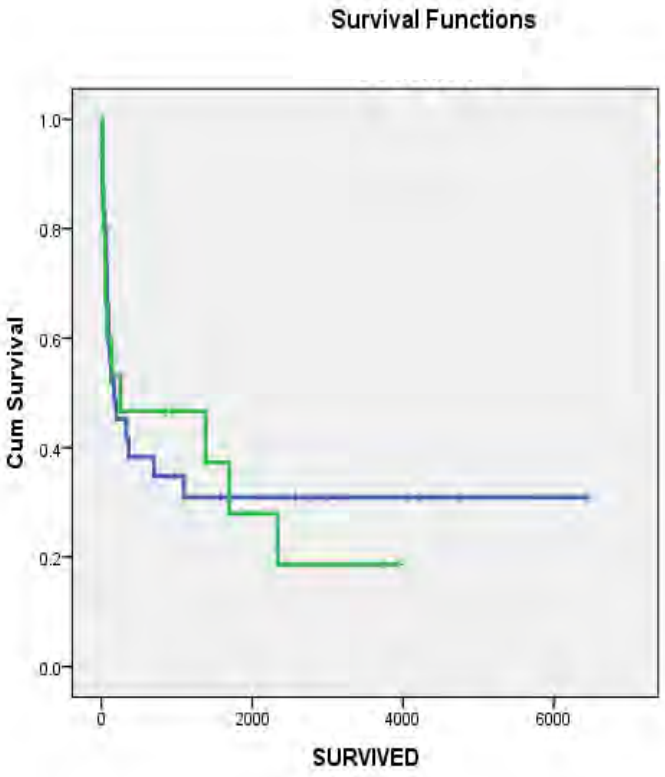


Absence of KIR3DS1 is beneficial in PBSC transplants

PBSC

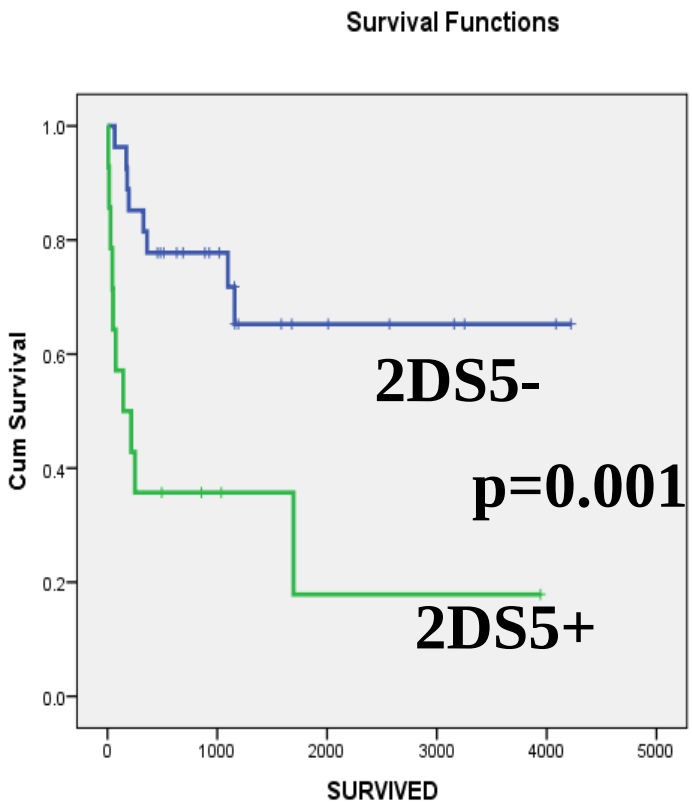


Marrow

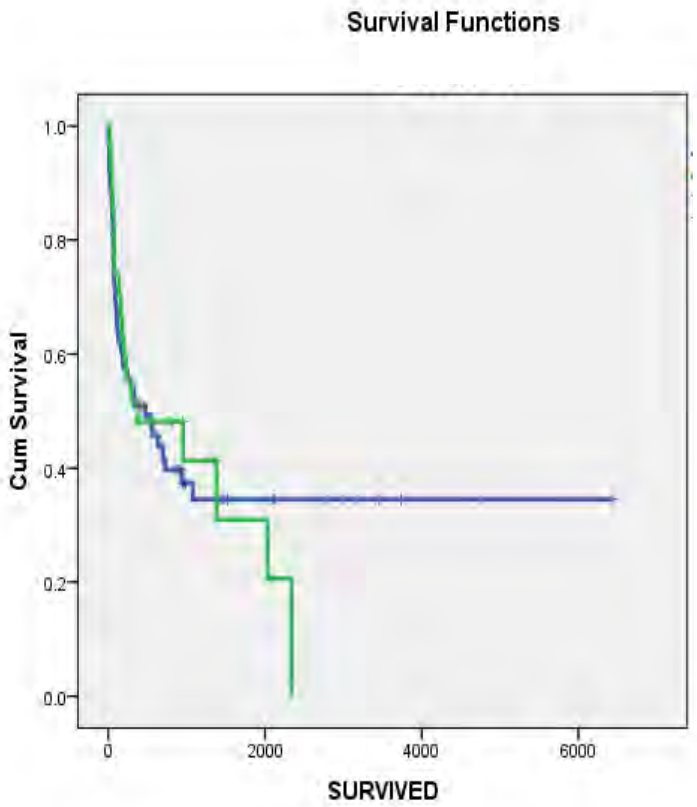


Absence of KIR2DS5 is beneficial in CMV Neg transplants

CMV- Transplants



CMV+ Transplants



Telomeric KIR Genes Interact with Source and CMV Status

Centromeric KIR Interact with Conditioning Agents

KIR Genes	Peri. Blood	Bone Marrow	Tx CMV-[1]	Tx CMV+[2]	Cy – 3	Cy + 4	Bu – 5	Bu + 6	Flu – 7	Flu + 8	Mel – 9	Mel + 10	TBI - 11	TBI + 12
2DL2 CenB	0.944	0.751	0.837	0.777	0.032	0.002	0.287	0.577	0.196	0.036	0.020	0.064	0.151	0.028
2DL5	0.032	0.386	0.025	0.621	0.123	0.605	0.489	0.659	0.859	0.238	0.847	0.179	0.315	0.943
2DS1	0.028	0.863	0.005	0.592	0.088	0.968	0.450	0.294	0.373	0.467	0.880	0.102	0.174	0.857
2DS2 CenB	0.944	0.690	0.837	0.732	0.032	0.002	0.309	0.577	0.198	0.038	0.024	0.064	0.151	0.034
2DS3	0.279	0.238	0.454	0.602	0.170	0.069	0.945	0.945	0.417	0.129	0.184	0.281	0.204	0.122
2DS5	0.039	0.674	0.001	0.937	0.328	0.120	0.100	0.485	0.063	0.878	0.147	0.318	0.481	0.073
3DS1	0.008	0.946	0.008	0.952	0.049	0.718	0.372	0.139	0.284	0.257	0.454	0.083	0.096	0.593
KIR A/A vs B/X	0.302	0.739	0.065	0.430	0.118	0.354	0.684	0.598	0.998	0.215	0.624	0.181	0.289	0.798
High KIR	0.023	0.605	0.009	0.633	0.173	0.815	0.496	0.442	0.591	0.298	0.984	0.211	0.212	0.860
aKIR1 (>1)	0.376	0.664	0.065	0.435	0.146	0.352	0.707	0.634	0.940	0.215	0.628	0.238	0.320	0.770
aKIR2 (>2)	0.046	0.460	0.025	0.555	0.238	0.741	0.514	0.721	0.866	0.298	0.908	0.295	0.358	0.893

Table 15. P-values of all the conditioning variables with individual KIR genes on survival rate.

[1]Transplants with both patient and donor CMV negative, [2]Transplants with at 1 patient or donor CMV positive, [3]Transplant regimens with no cytophosphamide, [4]Transplants with cytophosphamide, [5]Transplant regimens with no busulphan, [6]Transplant regimens with busulphan, [7]Transplant regimens with no fludarabine, [8]Transplant regimens with fludarabine, [9]Transplant regimens with no melphalan, [10]Transplant regimens with melphalan, [11]Transplant regimens with no total body irradiation, [12]Transplant regimens with total body irradiation.

CONCLUSIONS

- Interactions between KIR genes and other transplant variables may explain the conflicting reports of the effect of donor KIR genotype.

- Cyclophosphamide is used less now than previously and may be the main factor that interacts with the centromeric B haplotype genes (2DS2/2DL2)

- Given the history of this field, these findings need confirmation!!

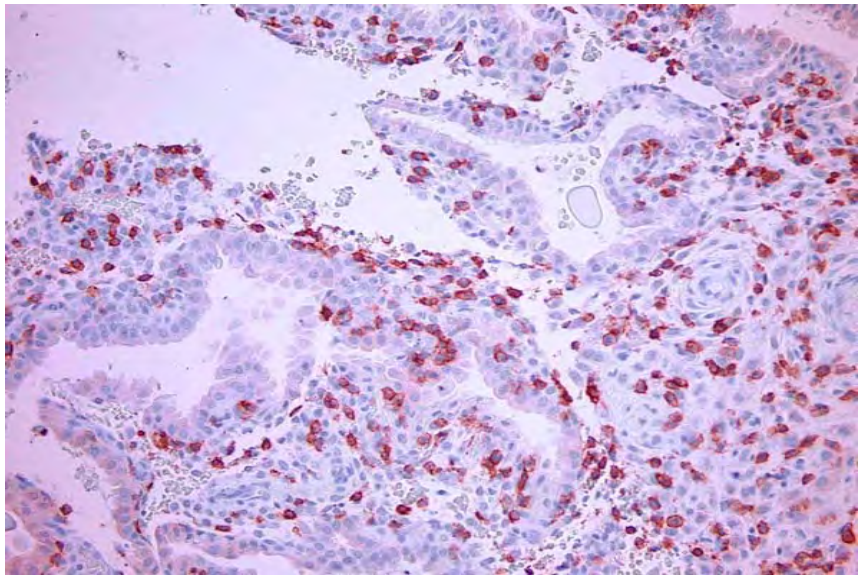
KIR IN DISEASE

PRE-ECLAMPSIA

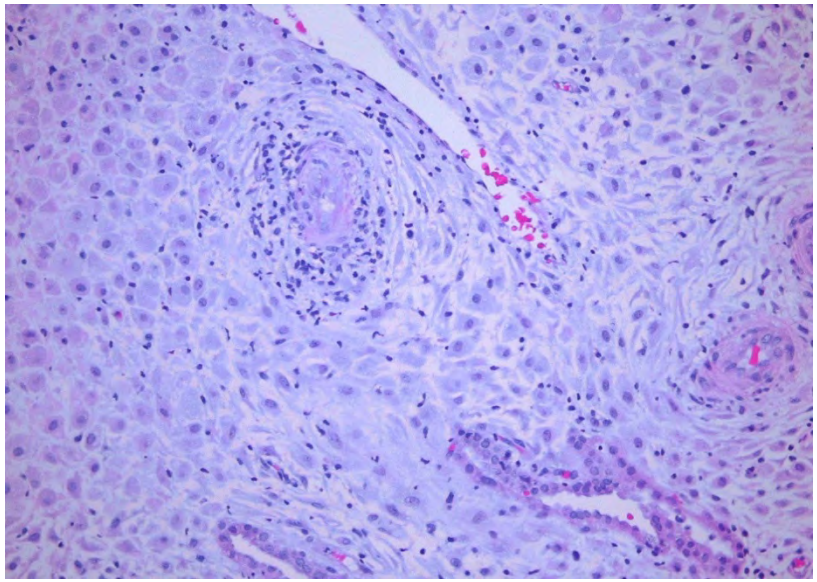
&

RECURRENT SPONTANEOUS ABORTION

Decidual NK Cells in pregnancy



Decidual NK Cells



Especially around spiral arteries

- NK cells constitute 50-90% of lymphocytes in decidua
- Express highest amount of CD56 and are skewed towards cytokine production
- On embryo implantation & placentation, uterine NK cells cooperate with extravillous trophoblasts to remodel the spiral arteries
- extravillous trophoblasts express the MHC class I molecules, HLA-C, E and G, all good ligands for NK cell receptors
- Paternal HLA-C is expressed on trophoblast cell surface

Combinations of Maternal KIR and Fetal HLA-C Genes Influence the Risk of Preeclampsia and Reproductive Success

Susan E. Hiby,[1] James J. Walker,[2] Kevin M. O'Shaughnessy,[3] Christopher W.G. Redman,[4] Mary Carrington,[5] John Trowsdale,[1] and Ashley Moffett[1]

J Exp Med, 2004

Human Reproduction Vol.23, No.4 pp. 972–976, 2008
Advance Access publication on February 8, 2008
doi:10.1093/humrep/den011

Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage

S.E. Hiby[1], L. Regan[2], W. Lo[2], L. Farrell[1], M. Carrington[3] and A. Moffett[1,4]

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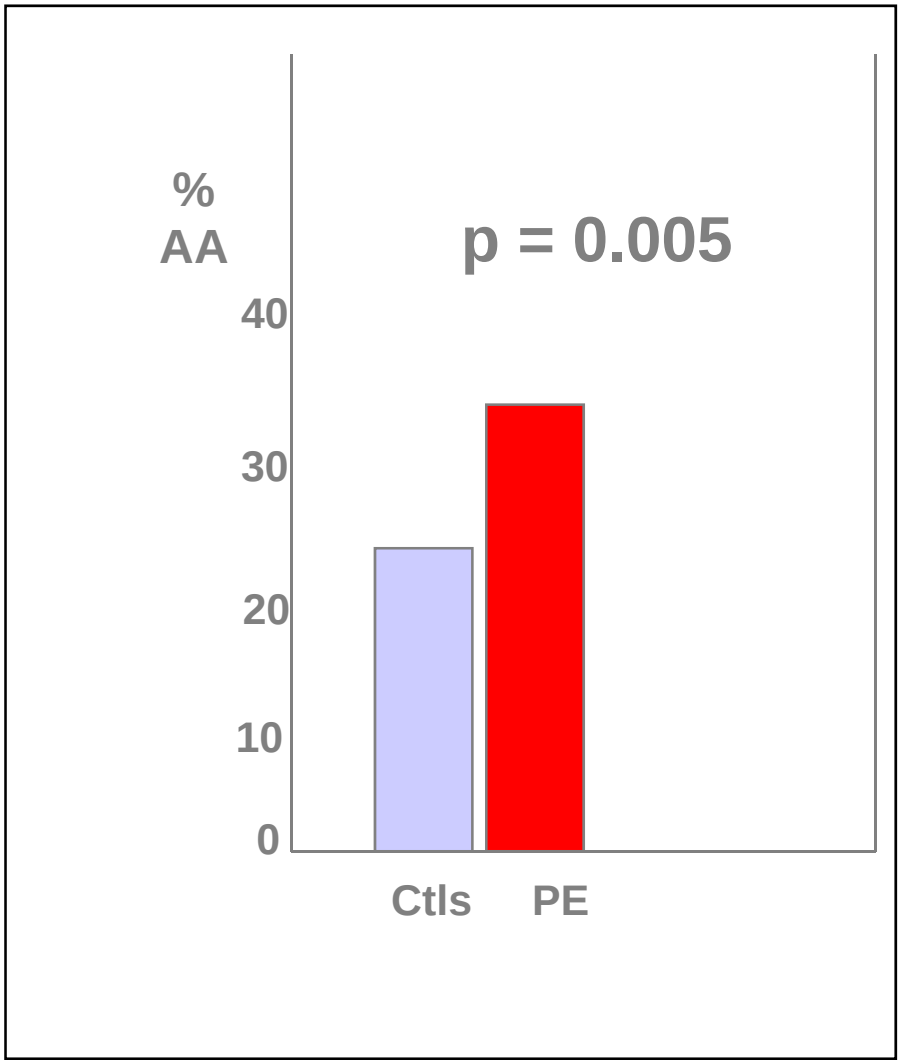
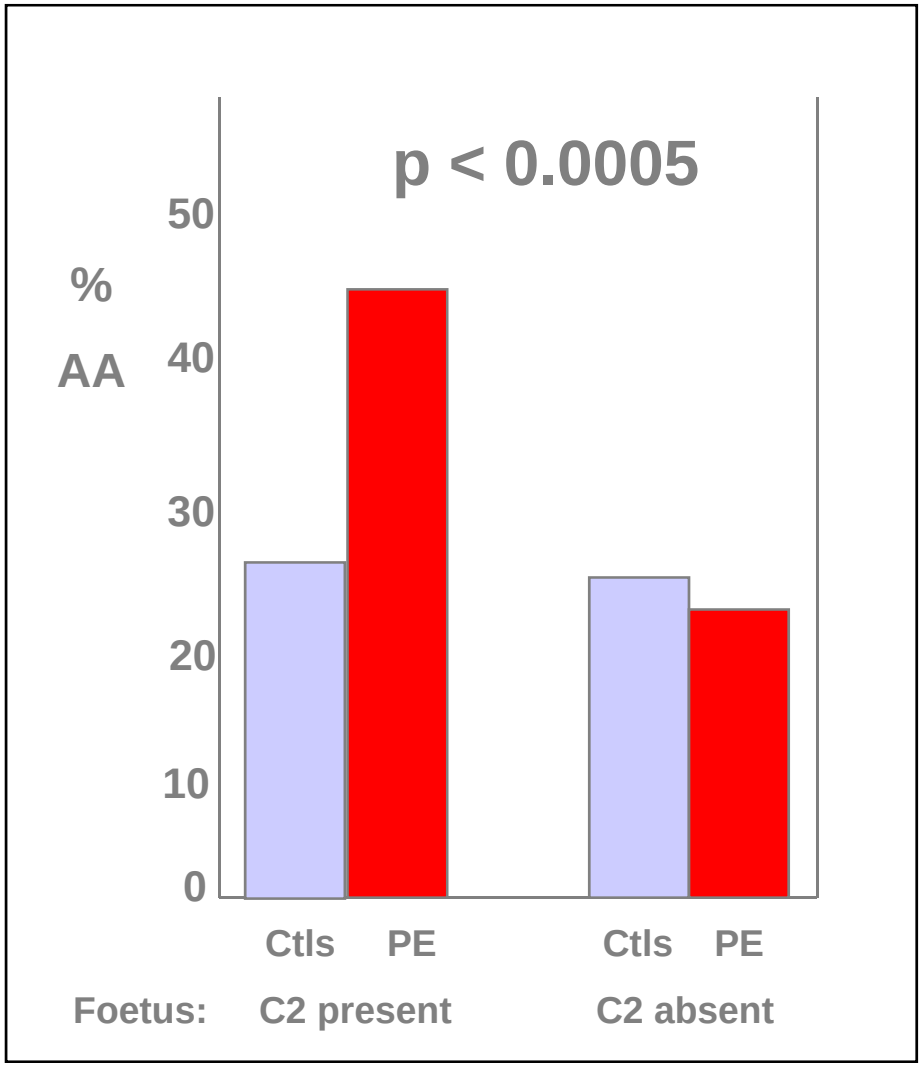
Pre-eclampsia and recurrent miscarriage

Pre-eclampsia and recurrent miscarriage are attributed to inadequate trophoblast invasion

$\longrightarrow$ could the interaction between uterine NK cells and extravillous trophoblast be important in such diseases

Homozygosity for the KIR "A Haplotype" in the mother predisposes to PE, risk is further increased when foetus expresses C2 epitope





Hiby et al, PNAS 2004

The risk of pre-eclampsia was greater when the fetus had more copies of HLA-C2 than mother and disease was more prevalent when the fetus expressed paternal HLA-C2

Maternal *KIR AA* frequency is increased in affected compared with control pregnancies when the fetus has more *C2* genes than the mother or when fetal *C2* is inherited paternally

Parameter	OR[A]	*P*	*n* (affected/controls)
Effect of relative dose of maternal and fetal *C2* genes[B]			
Fetus had fewer *C2* genes than the mother	0.97	1.00	177/85
Fetus had the same number of *C2* genes	1.43	0.06	364/233
Fetus had more *C2* genes than the mother	2.09 (1.24–3.51)	0.007	188/105
Effect of origin of fetal *C2* genes[C]			
Paternal origin	2.02 (1.14–3.58)	0.022	135/90
Maternal origin	1.11	0.90	91/61

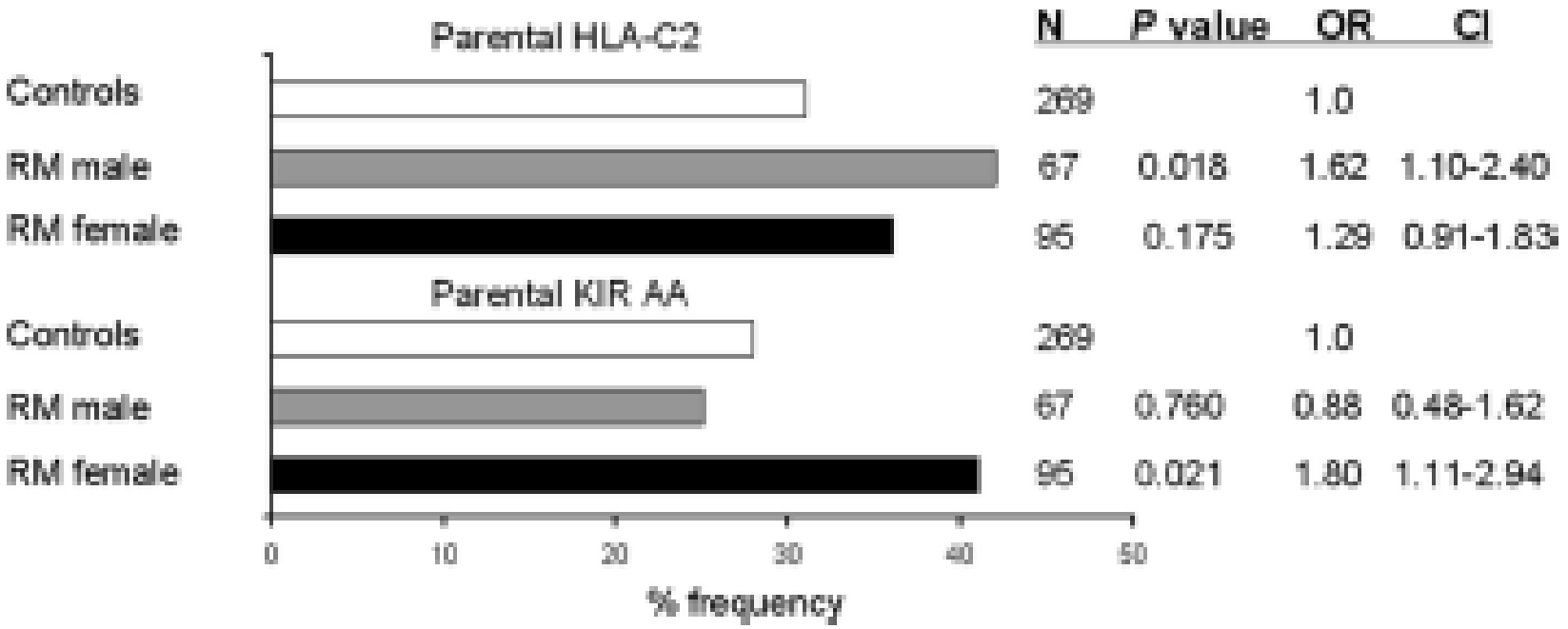
[A]Where shown, values in parentheses denote 95% CI. [B]See Figure 5A for groupings. ORs and *P* values were calculated for the relative frequency of the maternal *KIR AA* genotype in control and affected pregnancies. [C]See Figure 5B for groupings.

The telomeric-B of the KIR B haplotype protect against disorders of pregnancy, particularly when the fetus has an HLA-C2 gene

Maternal *KIR B* regions present[A]	*KIR* genotype frequencies (%) in all controls and affected cases		Maternal *KIR* frequencies (%) in pregnancies with fetal *C2*		Maternal *KIR* frequencies (%) only in pregnancies with fetal *C1*	
	Controls	Affected	Controls	Affected	Controls	Affected
	(*n* = 592)	(*n* = 975)	(*n* = 235)	(*n* = 513)	(*n* = 188)	(*n* = 338)
None (*KIR AA*)	27.5	36.9[C]	17.0	23.4[G]	11.8	13.0
Cen-B alone	27.4	30.1	14.2	17.7	12.5	12.1
Tel-B alone	19.3	14.6[D]	11.1	9.4	8.7	6.4
Cen-B plus *Tel-B*	25.8	18.5[E]	13.2	9.8	11.3	9.2
All with *Tel-B*[B]	45.1	33.0[F]	24.3	19.2[H]	20.0	15.6
Trend test	$P < 0.001$		$P = 0.002$		NS	

[A]All affected women (preeclampsia, FGR, and RM) were grouped according to whether they had any *KIR B* haplotype genes in the centromeric (*Cen-B*) and/or telomeric (*Tel-B*) region. The frequency of these *KIR* genotypes was compared in affected and control pregnancies. [B]Includes both *Tel-B* alone and *Cen-B* plus *Tel-B* groups. (Separate results are shown for each pregnancy disorder in Supplemental Figure 6). The trend from no *KIR B* genes (*AA* genotype) to possession of *Tel-B* genes was highly significant ($P < 0.001$). Preeclampsia and FGR pregnancies were divided into those with a *C2* carrier fetus and those with a *C1/C1* fetus. Reduced group sizes were due to omission of the women with RM plus some patients from the affected cohorts in which the baby was not available. The trend from *AA* genotype to presence of *Tel-B* region *KIR* was significant ($P = 0.002$) only when there was a *C2* allele present in the fetus. [C]$P = 1.3 \times 10^{-4}$, OR 1.54 (1.23–1.92). [D]$P = 0.019$, OR 0.71 (0.55–0.94). [E]$P = 7.4 \times 10^{-4}$, OR 0.65 (0.51–0.83). [F]$P = 1.7 \times 10^{-6}$, OR 0.60 (0.49–0.74). [G]$P = 0.01$, OR 1.49 (1.10–2.01). [H]$P = 0.039$, OR 0.74 (0.56–0.97).

Recurrent miscarriage was found to be associated with the KIR "AA" Haplotype in the mother and an increase frequency of HLA-C2 in RM couples



- The increased frequency of HLA-C2 in RM couples predicts an increase of HLA-C2 in the foetus

THANK YOU