Friending KIR

Council Meeting 2016

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Disclosures

The following faculty and planning committee staff have the following financial disclosures:

Name	Institution	Disclosure
Carolyn Hurley	Georgetown University Medical Center	Holds a patent related to HLA testing with ThermoFisher

Learning objectives

At the conclusion of this session, attendees will be able to:

- Explain how NK cells function when infused into recipients.
- Describe how KIR biology relates to the outcome of hematopoietic stem cell transplantation.
- Use web-based tools to incorporate KIR into donor selection.

Friending KIR

My Focus

- Biology of KIR and how it relates to hsc transplantation

Topics Not Covered Today

- Comprehensive overview of KIR literature related to transplant outcome
- Guidelines to select donors based on KIR

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Topics for Today

- What do natural killer (NK) cells do?
- What role does KIR play in NK response?
- Who is in the KIR family?
- What are KIR ligands?
- KIR in transplantation

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Natural Killer (NK) Cells Target Tumor or Virally Infected Cells

www.nhs.uk
NK cells attacking a tumor cell (red)

- Type of lymphocyte
  - Similar to cytotoxic T cells
- Circulate in blood / tissues
- Kill unhealthy cells
- Release cytokines to activate other immune cells to target unhealthy cells

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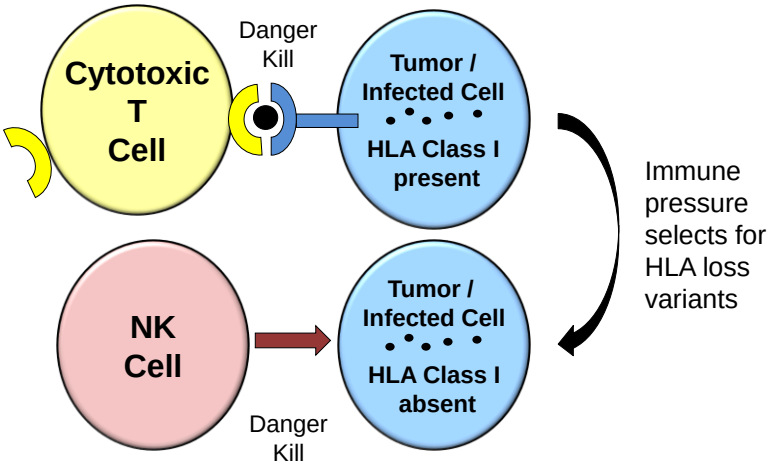
Natural Killer (NK) Cells Provide Backup to Prevent "Escape" of Unhealthy Cells



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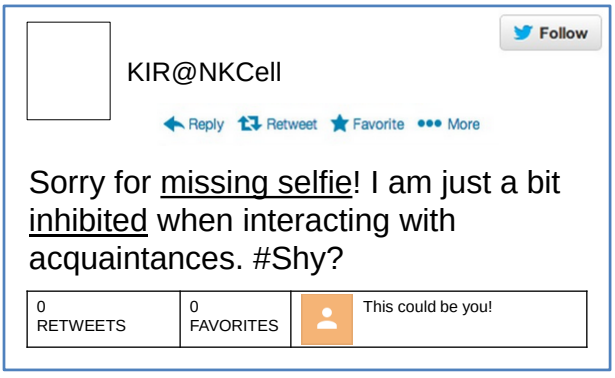
What Role Does KIR Play in NK Response?



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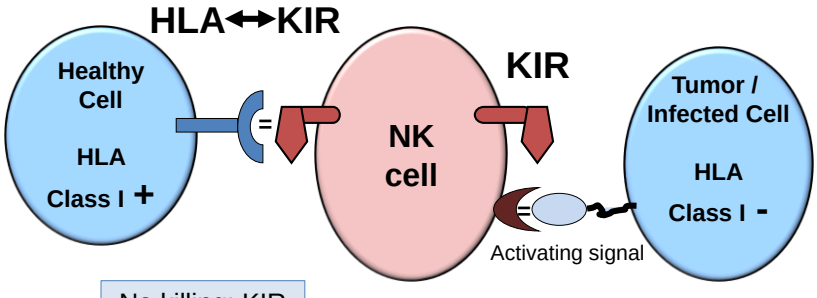
Killer Cell Immunoglobulin-Like Receptors on NK Cells Detect "Missing Self"



No killing; KIR inhibits NK cell

No KIR ligand (HLA) removes inhibition; activating ligands on target trigger killing

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Summary NK & KIR Function

- NK cells kill malignant cells or cells infected by viruses
- NK cells are prevented from killing healthy cells
  - KIR binds to HLA class I proteins (ligand) and inhibits killing
- Loss of HLA class I removes inhibition allowing NK cell to target unhealthy cell for killing
- NK cell must also receive activation signals from target to be stimulated to kill or release cytokines

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Transplant Implications

- Both T and NK cells can kill malignant cells (GvL)
- NK cells are first immune cell to reach normal levels after transplant but take ~6 months to become fully functional
- T cell content in graft influences NK development
  - T cell activation may mask or reduce NK effect

GvL, graft vs leukemia

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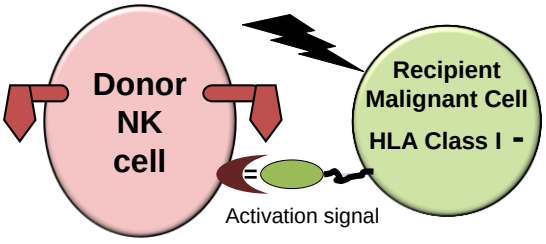
Transplant Implications

Donor NK Cells Can Target Recipient Malignant Cells



- No KIR ligand (HLA) removes inhibition
- Target cell must activate NK cell
  - Explains differential sensitivity of different cancers (AML, not ALL)

GvL, graft vs leukemia

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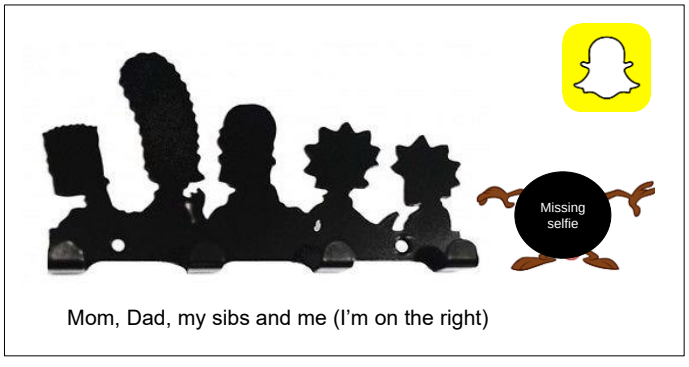
Meeting the KIR Family



Mom, Dad, my sibs and me (I'm on the right)

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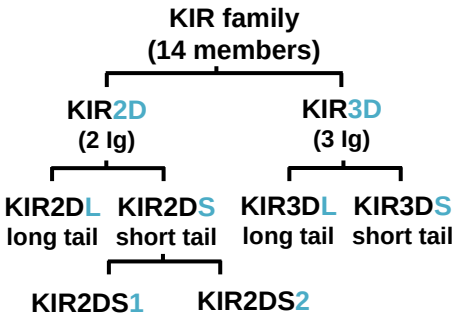
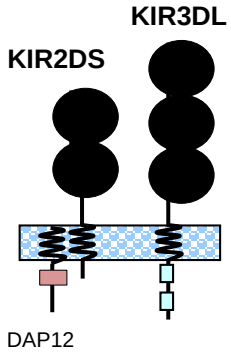
KIR Is a Family of 14 Proteins





- KIR2DL1-2DL5, KIR2DS1-2DS5, KIR3DL1-3, KIR3DS1
- Long tail, inhibitory; short, activating

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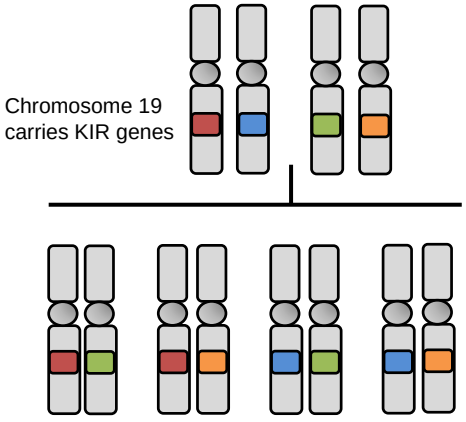
Inheritance of KIR Genes



4 potential combinations of parental chromosomes

- 2 copies chromosome 19
- In families, only 25% of HLA-identical siblings are also KIR identical
- Unrelated donors and recipients who are HLA identical are not necessarily identical for KIR genes

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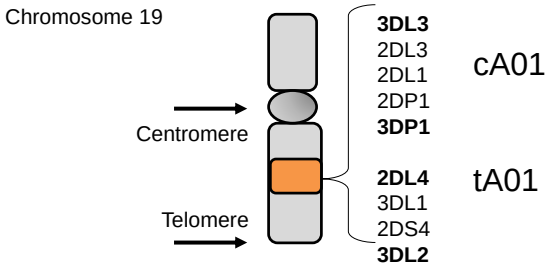
KIR Genes Cluster to Form Haplotypes



- Not all KIR genes are found in a haplotype (one version Chr 19)
- **Framework** genes—in all haplotypes
- Pseudogenes: 2DP1, 3DP1
- 2 gene clusters, telomere (t) & centromere (c)

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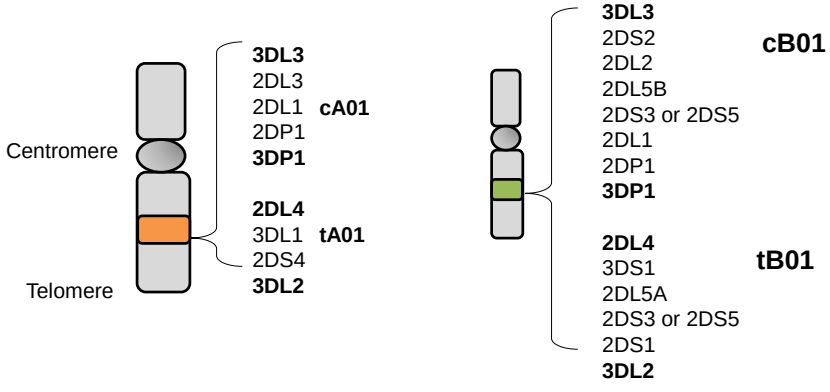
Haplotypes Vary in KIR Gene Content



- "**A**" (more conserved in gene content, more inhibitory (L) genes) vs "**B**" (more variable in gene content, more activating (S) genes)
- Two copies of 2DL5 gene

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Definition of A vs B found at https://www.ebi.ac.uk/ipd/kir/introduction.html

Common KIR Haplotypes

cA01
3DP1
2DP1
2DL1
2DL3
3DL3

cB01
3DP1
2DP1
2DL1
2DS3 or 2DS5
2DL5B
2DL2
2DS2
3DL3

cB02
3DP1
2DL2
2DS2
3DL3

tA01
3DL2
2DS4
3DL1
2DL4

tB01
3DL2
2DS1
2DS3 or 2DS5
2DL5A
3DS1
2DL4

Common gene combinations

cA01+tA01
cB01+tA01
cB02+tA01
cA01+tB01
cB01+tB01
cB02+tB01

Also less common haplotypes with insertions or deletions

Remember 2 copies of chromosome 19 !

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https://www.ebi.ac.uk/ipd/kir/sequenced_haplotypes.html

Summary KIR Family

- 14 KIR, some inhibitory, some activating
- Named by number Ig domains (2D/3D) on outside of NK cell and length of tail inside cell (L/S)
- Different subsets of KIR genes carried on chromosome 19 forming haplotypes
- Genes found in 2 clusters, c and t
- Random association between c and t clusters
- B haplotypes vary more in gene content, more activating genes

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Transplant Implications

- Certain KIR proteins appear to be important in mediating GvL
- Selecting a donor with these KIR genes may improve outcome
- Being HLA matched does not mean KIR matched

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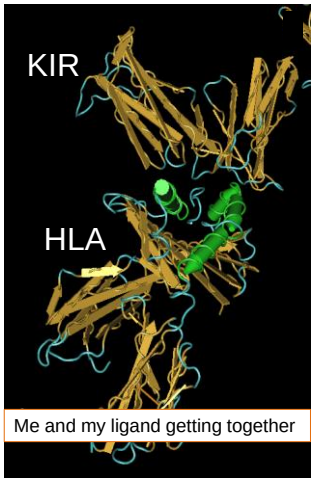
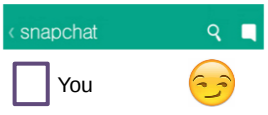
KIR and Their "Significant Others"





1EFX; Boyington et al Nature 405:537, 2000

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To KIR2DL Proteins, There Are Only 2 HLA-C Types

2DL2 & 2DL3

Cg1 - 80 N (asparagine)

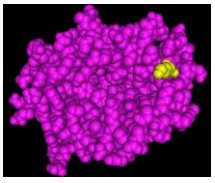
- C*01:02:01
- C*03:02:01
- C*07:01:01:01
- C*08:01:01
- C*12:02:01
- C*14:02:01:01
- C*16:01:01:01
- Etc

2DL1

Cg2 - 80 K (lysine)

- C*02:02:01
- C*04:01:01:01
- C*05:05
- C*06:02:01:01
- C*15:02:01:01
- C*16:02:01
- C*17:01:01:01
- Etc

2DL2 weakly binds Cg2



Top view HLA

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To KIR3DL1, There Are Only Two HLA-B Types

3DL1

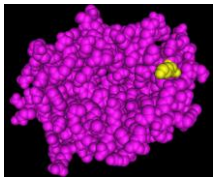
Bw4+

- B13, B27, B37, B38, B44, B47, B49, B51, B52, B53, B57, B58, B59, B63, B77
- A23, A24, A25, A32

Residue 77	Residue 80
Asparagine (N)	Isoleucine (I)
Asparagine (N)	Threonine (T)
Aspartic acid (D)	Threonine (T)
Serine (S)	Threonine (T)

Bw4-

HLA-Bw6 and most HLA-A do not interact with KIR



Top view HLA

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Some KIR Bind HLA Subsets

KIR	HLA	Type
2DL1	HLA-C (group 2)	Inhibitory
2DL2	HLA-C (group 1, low g2)	Inhibitory
2DL3	HLA-C (group 1)	Inhibitory
3DL1	HLA-Bw4	Inhibitory

- Function of other iKIR proteins (2DL4, 2DL5, 3DL2, 3DL3) is less clear
- Some stimulatory KIR bind HLA but ligands not well understood

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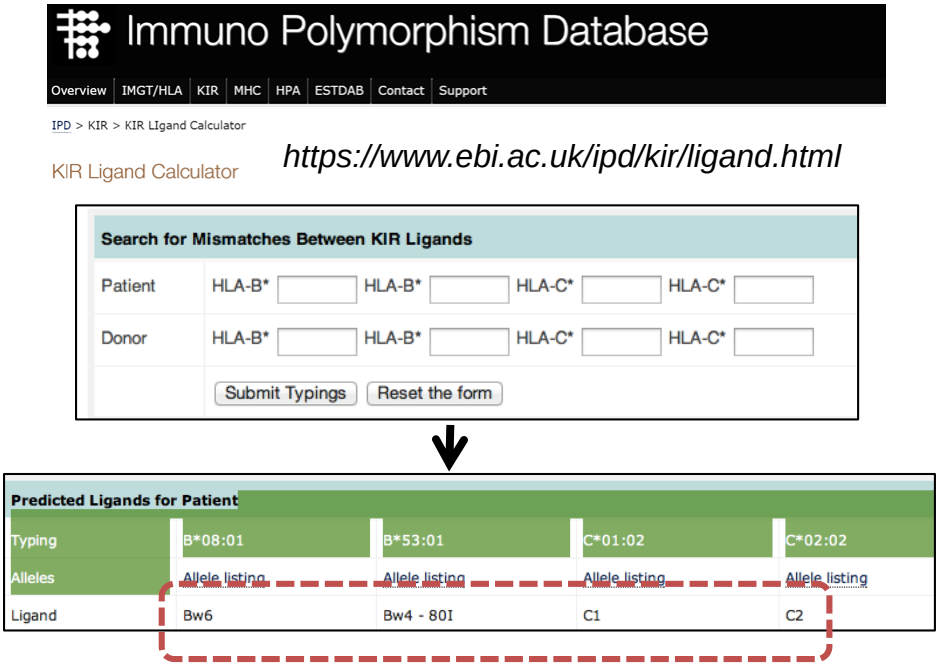

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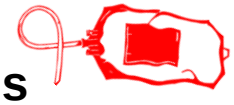
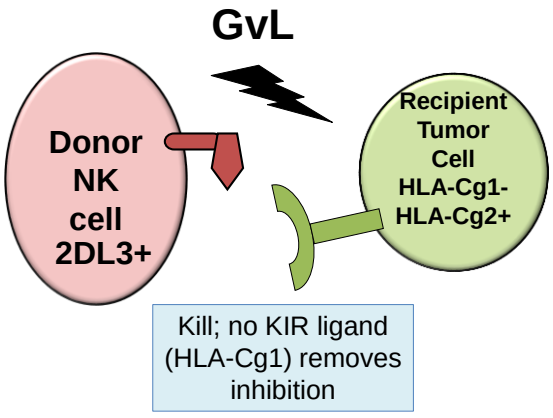
Transplant Implications

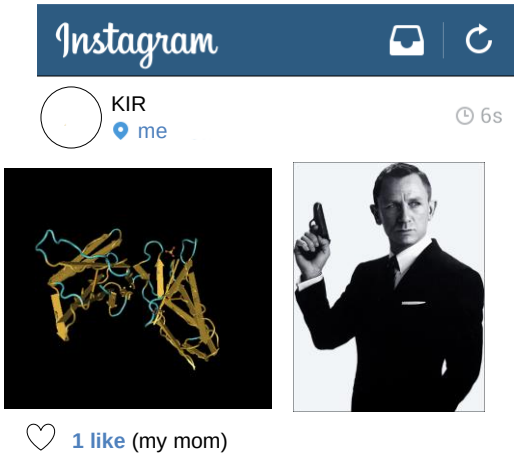


Absence of <u>Specific</u> HLA Types in Recipient May Lead to NK Activation



2DL3 binds Cg1

Licensing of NK Cells



NK Cells Must be Licensed to Kill

- Inhibitory KIR+ NK must interact with their ligand during maturation in order to be licensed to kill unhealthy cells in the future
  - 3DL1+ NK cells in Bw4+ donor are licensed to kill
  - 3DL1+ NK cells in Bw4- donor are not licensed and respond only weakly to HLA loss
  - Same for 2DL1, 2DL2, 2DL3
- Protects healthy cells from killing in iKIR+ but ligand negative host

Licensing = education = tolerance

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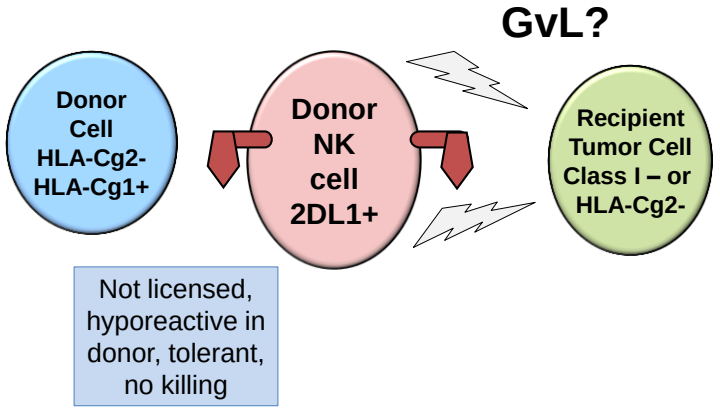
Transplant Implications

Licensing in Donor May Impact NK Response in Recipient



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2DL1 ligand is Cg2

What Happens to Donor NK Cells in Recipient?

- Do unlicensed NK cells become licensed?
  - Environment with cell damage, lots of cytokines, reactivation CMV could result in licensing
- Does HLA of recipient influence licensing?
  - 2DL2+ NK cells may need HLA-Cg1 interactions to become licensed in recipient
  - Cooley et al. J Immunol. 2014,192(10):4592-600

CMV, cytomegalovirus
2DL2 ligand is Cg1

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Summary iKIR Ligands & Licensing

- Inhibitory KIR bind HLA molecules (their ligands)
- Specific KIR bind specific HLA
  - 3DL1 – Bw4+
  - 2DL1 – Cg2
  - 2DL2 and 2DL3 – Cg1
  - 2DS1 – Cg2
- Interaction with their ligand during NK maturation give inhibitory KIR a license to kill unhealthy cells
- Licensing may also take place in recipient

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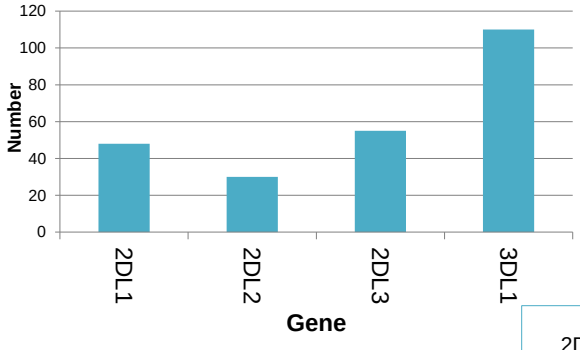
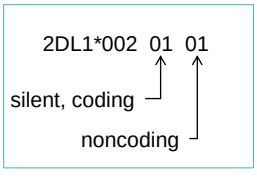
Alleles of KIR

KIR Genes Are Polymorphic



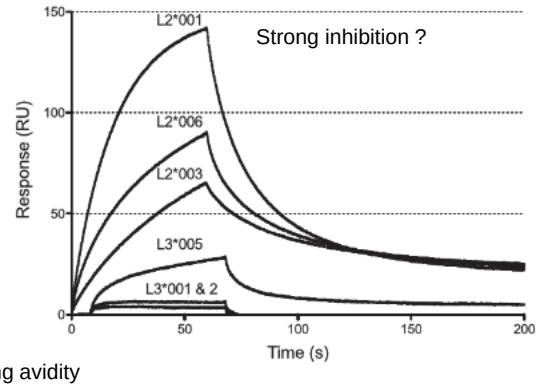
Nomenclature

- 2DL1*001
- 2DL1*0020101
- 2DL1*0020102
- 2DL1*00301
- 2DL1*0030201
- 2DL1*004
- 2DL1*005
- etc



IPD-KIR database; www.ebi.ac.uk/ipd/kir/

2DL2 & 2DL3 Allelic Products Bind to C*03:04 (g1) With Different “Strengths”



Biacore binding avidity

Frazier et al. J. Immunol. 190:6198, 2013

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Summary KIR Alleles

- Allelic variants differ in amount on cell surface and/or in strength of interaction with their HLA ligand
- May alter strength of inhibitory signal

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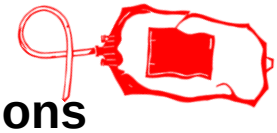

Transplant Implications

- Donor selection may include selection for presence of specific KIR alleles based on strength of their inhibitory signal
  - Which hypothesis is correct?
    - Weak signal more easily overcome to activate NK cell?
    - Strong signal might make the NK cell more effective at killing by giving it greater license to kill?

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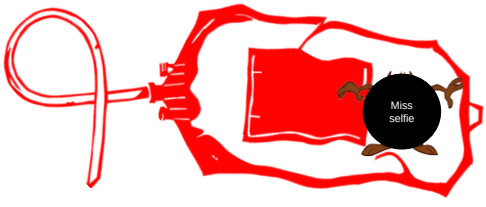

KIR & Hematopoietic Progenitor Cell Transplantation

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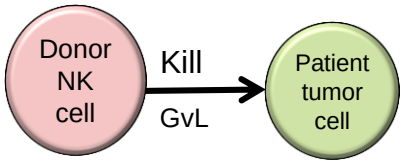
1st Clinical Study –KIR Ligand Incompatibility Reduces Relapse



KIR ligand incompatibility in GVH direction	No	Yes
Number of transplants	58	34
Probability of relapse at 5 years		
ALL	90%	85%
AML	**75%**	**0%****

P . 0.01; **P . 0.0008 (22)

HLA-Bw4 +
B*44:02, *07:02

HLA-Bw4 –
B*08:01, *07:02
Missing self

- Haploidentical transplants, extensive T cell depletion
- Focused on absence in recipient of donor class I allele group recognized by KIR (KIR ligand incompatibility)
- Graft vs host direction
- Did not type donor KIR

Ruggeri et al. Science 295:2097, 2002

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Tool Predicts KIR Ligand Incompatibility

https://www.ebi.ac.uk/ipd/kir/ligand.html

Predicted Ligands for Patient				
Typing	B*07:02	B*08:01	C*07:01	C*07:02
Alleles	Allele listing	Allele listing	Allele listing	Allele listing
Ligand	Bw6	Bw6	C1	C1
Exceptions				
Predicted Ligands for Donor				
Typing	B*07:02	B*08:01	C*07:01	C*02:02
Alleles	Allele listing	Allele listing	Allele listing	Allele listing
Ligand	Bw6	Bw6	C1	C2
Exceptions				
Mismatching in the GvH Direction				
HLA-B	KIR ligands are matched			
HLA-C	KIR ligands are (mis)matched in the GvH Direction (C2)			
Mismatching in the HvG Direction				
HLA-B	KIR ligands are matched			
HLA-C	KIR ligands are matched			

In summary, these ligands will be (mis)matched in the GvH direction and matched in the HvG direction.

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Deciphering KIR Laboratory Reports - Selecting Based on KIR Ligand Incompatibility
Which Donor Would You Choose?

	HLA-A	HLA-B	HLA-C	HLA-DRB1
Recipient	02:01, 11:01	27:05, 44:03	01:02, 16:01	14:01, 13:02
Donor 1	02:01, 11:01	27:05, 44:03	01:02, 16:01	14:01, 13:02
Donor 2	02:01, 11:01	27:05, 44:03	02:02, 16:01	14:01, 11:01
Donor 3	02:01, 11:01	18:01, 44:03	01:02, 16:01	14:01, 13:02
Donor 4	02:01, 11:01	27:05, 44:03	02:02, 16:01	14:01, 13:02

calculator: *https://www.ebi.ac.uk/ipd/kir/ligand.html*

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Deciphering KIR Laboratory Reports - Selecting Based on KIR Ligand Incompatibility

	HLA-A	HLA-B	HLA-C	HLA-DRB1
Recipient	02:01, 11:01	27:05, 44:03	01:02, 16:01	14:01, 13:02
Donor 1	02:01, 11:01	27:05, 44:03	01:02, 16:01	14:01, 13:02
Donor 2	02:01, 11:01	27:05, 44:03	02:02, 16:01	14:01, 11:01
Donor 3	02:01, 11:01	18:01, 44:03	01:02, 16:01	14:01, 13:02
Donor 4	02:01, 11:01	27:05, 44:03	02:02, 16:01	14:01, 13:02

HLA-B residues 77--80
27:05 D—T Bw4
44:03 N—T Bw4
18:01 S—N Bw6

HLA-C residue 80
01:02 N Cg1
16:01 N Cg1
02:02 K Cg2

- Donor must be mismatched
- Focus on B and C
- Determine Bw4 and Cg1/2 status
- GvH direction (D -> R)
- Expect KIR2DL1 to lose inhibition (Cg2)

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Questions Arising

- Can NK cells function in T replete transplant?
- Is it critical to have HLA mismatch to get KIR effect?
- Will donor selection improve if presence/absence of specific KIR is known?
  - What KIR genes are most important?

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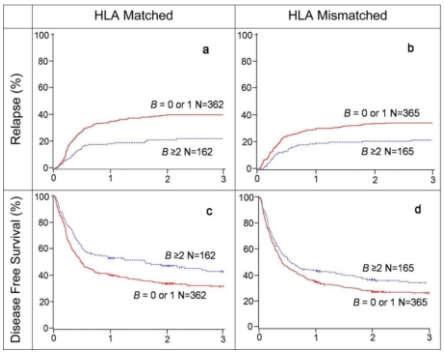
Improved Survival in AML - KIR CenB Haplotypes Had Strongest Effect



- AML
- Unrelated donor
- Myeloablative conditioning
- T replete transplants
- Impact observed in both HLA matched and mismatched transplants

Cen B
3DL3
2DS2
2DL2
2DL5B
2DS3 or 2DS5
2DL1
3DP1

Tel B
2DL4
3DS1
2DL5A
2DS3 or 2DS5
2DS1
3DL2

Cooley et al. Blood 116 (14):2411-2419, 2010
Cooley et al. J Immunol. 2014 May 15;192(10):4592-600

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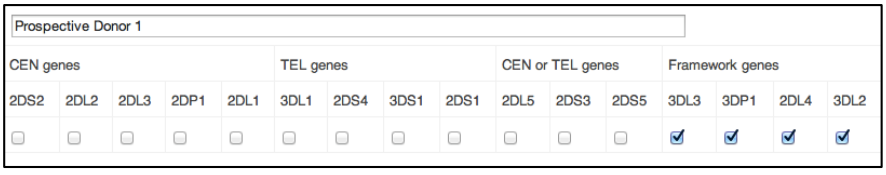
Deciphering KIR Laboratory Reports - CenB Haplotype Donor

	Donor 1	Donor 2	Donor 3	Donor 4
2DL1	+	+	+	+
2DL2			+	+
2DL3	+	+		+
2DL4	+	+	+	+
2DL5		+	+	+
2DS1		+	+	+
2DS2			+	+
2DS3		+	+	
2DS4	+		+	+
2DS5			+	+
3DL1	+		+	+
3DL2	+	+	+	+
3DL3	+	+	+	+
3DS1		+	+	+
2DP1	+		+	+
3DP1	+	+	+	+

KIR B Haplotype Predictor

Given a list of KIR genes present, how can we determine if A or B haplotypes ?

https://www.ebi.ac.uk/ipd/kir/donor_b_content.html?



Deciphering KIR Laboratory Reports - CenB Haplotype Donor

Donor 1	Donor 2	Donor 3	Donor 4
cA01/cA01	cA01/cA01	cB01/cB02	cA01/cB01
tA01/tA01	tB01/tB01	tA01/tB01	tA01/tB01
Neutral	Better	Best	Better
	Note "rare" because 2DP1 not present*		

*Not typed or incorrectly typed as negative or unusual haplotype

Summary KIR B Haplotypes in Transplant

- Selecting donor with KIR B haplotypes, especially B centromeric cluster improves survival
  - Still not clear which KIR genes are important
- Impact observed in T replete transplants
- Impact observed in HLA matched transplants, not necessary to mismatch HLA
- Impact in HLA mm transplant may require T depletion
- Recipient HLA providing licensing may be important

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Role of KIR In Transplant Is Complicated

Donor

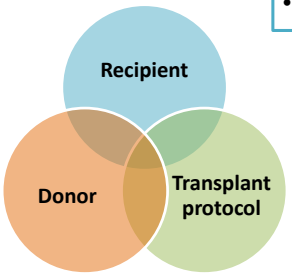
- KIR genes / haplotypes/ alleles
- HLA licensing

Recipient

- Malignant disease
- HLA ligands
- HLA licensing
- CMV serostatus
- GvHD

Transplant

- Graft source
  - T cell content
- Conditioning
- HLA matching
- Immune suppressive drugs



Foley et al. The biology of NK cells and their receptors affects clinical outcomes after hematopoietic cell transplantation (HCT). Immunol. Review 258:45, 2014

Ullah MA et al. Functional Reconstitution of Natural Killer Cells in Allogeneic Hematopoietic Stem Cell Transplantation. Front. Immunol. 7:144, 2016

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Friending KIR

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