Friending KIR
Council Meeting 2016

Presenter(s) – Carolyn Hurley, PhD, D(ABHI), Professor, Department of Oncology, Georgetown University Medical Center

Disclosures

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Disclosure</th>
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</thead>
<tbody>
<tr>
<td>Carolyn Hurley</td>
<td>Georgetown University Medical Center</td>
<td>Holds a patent related to HLA testing with ThermoFisher</td>
</tr>
</tbody>
</table>
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

You have 0 friends. Do you really need a Facebook account?
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

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

- 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

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

Cytotoxic T Cell

NK Cell

Tumor / Infected Cell

Danger
Kill

HLA Class I present

Immune pressure selects for HLA loss variants

Tumor / Infected Cell

Danger
Kill

HLA Class I absent
What Role Does KIR Play in NK Response?

KIR@NKCell

Sorry for missing selfie! I am just a bit inhibited when interacting with acquaintances. #Shy?

This could be you!

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

Healthy Cell

HLA

Class I +

No killing; KIR inhibits NK cell

KIR

HLA

Class I -

Tumor / Infected Cell

Activating signal

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

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
Transplant Implications
Donor NK Cells Can Target Recipient Malignant Cells

GvL

- 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

Meeting the KIR Family

Mom, Dad, my sibs and me (I’m on the right)
KIR Is a Family of 14 Proteins

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

Inheritance of KIR Genes

- 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
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)

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

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

<table>
<thead>
<tr>
<th>CA01</th>
<th>3DP1</th>
<th>2DP1</th>
<th>2DL1</th>
<th>2DL3</th>
<th>3DL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB01</td>
<td>3DP1</td>
<td>2DP1</td>
<td>2DL1</td>
<td>2DS3 or 2DS5</td>
<td>2DL5B</td>
</tr>
<tr>
<td>CB02</td>
<td>3DP1</td>
<td>2DL2</td>
<td>2DS2</td>
<td>3DL3</td>
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<table>
<thead>
<tr>
<th>TA01</th>
<th>3DL2</th>
<th>2DS4</th>
<th>3DL1</th>
<th>2DL4</th>
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<tbody>
<tr>
<td>TB01</td>
<td>3DL2</td>
<td>2DS1</td>
<td>2DS3 or 2DS5</td>
<td>2DL5A</td>
</tr>
</tbody>
</table>

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!

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
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

KIR and Their “Significant Others”

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

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

**Bw4-**
- HLA-Bw6 and most HLA-A do not interact with KIR

<table>
<thead>
<tr>
<th>Residue 77</th>
<th>Residue 80</th>
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<tbody>
<tr>
<td>Asparagine (N)</td>
<td>Isoleucine (I)</td>
</tr>
<tr>
<td>Asparagine (N)</td>
<td>Threonine (T)</td>
</tr>
<tr>
<td>Aspartic acid (D)</td>
<td>Threonine (T)</td>
</tr>
<tr>
<td>Serine (S)</td>
<td>Threonine (T)</td>
</tr>
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</table>
Some KIR Bind HLA Subsets

<table>
<thead>
<tr>
<th>KIR</th>
<th>HLA</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2DL1</td>
<td>HLA-C (group 2)</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>2DL2</td>
<td>HLA-C (group 1, low g2)</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>2DL3</td>
<td>HLA-C (group 1)</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>3DL1</td>
<td>HLA-Bw4</td>
<td>Inhibitory</td>
</tr>
</tbody>
</table>

- Function of other iKIR proteins (2DL4, 2DL5, 3DL2, 3DL3) is less clear
- Some stimulatory KIR bind HLA but ligands not well understood
Transplant Implications
Absence of Specific HLA Types in Recipient May Lead to NK Activation

GvL

Donor NK cell 2DL3+

Recipient Tumor Cell HLA-Cg1- HLA-Cg2+

2DL3 binds Cg1

Licensing of NK Cells

Instagram

KIR

1 like (my mom)

KIR I have a lot in common with James Bond but I think I am better looking.
**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

*GvL?*

- **Donor Cell**
  - HLA-Cg2-
  - HLA-Cg1+
- **Donor NK cell**
  - 2DL1+
- **Recipient Tumor Cell**
  - Class I – or HLA-Cg2-

Not licensed, hyporeactive in donor, tolerant, no killing

*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

CMV, cytomegalovirus
2DL2 ligand is Cg1

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
# Alleles of KIR

I want to be like HLA—allelopalooza! #Confusing

<table>
<thead>
<tr>
<th>Number</th>
<th>Gene</th>
<th>Nomenclature</th>
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<tbody>
<tr>
<td>2DL1</td>
<td></td>
<td>2DL1*001</td>
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<tr>
<td>2DL2</td>
<td></td>
<td>2DL1*0020101</td>
</tr>
<tr>
<td>2DL3</td>
<td></td>
<td>2DL1*0020102</td>
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<tr>
<td>3DL1</td>
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<td>2DL1*00301</td>
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<td></td>
<td></td>
<td>2DL1*0030201</td>
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<tr>
<td></td>
<td></td>
<td>2DL1*004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2DL1*005</td>
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<tr>
<td></td>
<td></td>
<td>etc</td>
</tr>
</tbody>
</table>

IPD-KIR database: www.ebi.ac.uk/ipd/kir/
2DL2 & 2DL3 Allelic Products Bind to C*03:04 (g1) With Different “Strengths”

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
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?

KIR & Hematopoietic Progenitor Cell Transplantation
1st Clinical Study – KIR Ligand Incompatibility Reduces Relapse

Donor NK cell \( \xrightarrow{\text{Kill}} \) Patient tumor cell

**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


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Tool Predicts KIR Ligand Incompatibility

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

<table>
<thead>
<tr>
<th>Predicted Ligands for Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td>Ligand</td>
</tr>
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</table>

**Exceptions**

<table>
<thead>
<tr>
<th>Predicted Ligands for Donor</th>
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</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td>Ligand</td>
</tr>
</tbody>
</table>

**Mismatching in the GvH Direction**
- HLA-B: KIR ligands are matched
- HLA-C: KIR ligands are (mis)matched in the GvH direction (G2)

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

<table>
<thead>
<tr>
<th></th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>HLA-DRB1</th>
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<tbody>
<tr>
<td>Recipient</td>
<td>02:01, 11:01</td>
<td>27:05, 44:03</td>
<td>01:02, 16:01</td>
<td>14:01, 13:02</td>
</tr>
<tr>
<td>Donor 1</td>
<td>02:01, 11:01</td>
<td>27:05, 44:03</td>
<td>01:02, 16:01</td>
<td>14:01, 13:02</td>
</tr>
<tr>
<td>Donor 2</td>
<td>02:01, 11:01</td>
<td>27:05, 44:03</td>
<td>02:02, 16:01</td>
<td>14:01, 11:01</td>
</tr>
<tr>
<td>Donor 3</td>
<td>02:01, 11:01</td>
<td>18:01, 44:03</td>
<td>01:02, 16:01</td>
<td>14:01, 13:02</td>
</tr>
<tr>
<td>Donor 4</td>
<td>02:01, 11:01</td>
<td>27:05, 44:03</td>
<td>02:02, 16:01</td>
<td>14:01, 13:02</td>
</tr>
</tbody>
</table>

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

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)
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?

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

### Deciphering KIR Laboratory Reports - CenB Haplotype Donor

<table>
<thead>
<tr>
<th></th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
<th>Donor 4</th>
</tr>
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<tbody>
<tr>
<td>2DL1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2DL2</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>2DL3</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>2DL4</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>2DL5</td>
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<tr>
<td>2DS1</td>
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<td>2DS2</td>
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<tr>
<td>3DP1</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
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</tbody>
</table>

### 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

<table>
<thead>
<tr>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
<th>Donor 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>cA01/cA01</td>
<td>cA01/cA01</td>
<td>cB01/cB02</td>
<td>cA01/cB01</td>
</tr>
<tr>
<td>tA01/tA01</td>
<td>tB01/tB01</td>
<td>tA01/tB01</td>
<td>tA01/tB01</td>
</tr>
<tr>
<td>Neutral</td>
<td>Better</td>
<td>Best</td>
<td>Better</td>
</tr>
</tbody>
</table>

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
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

Friending KIR
