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NOVEMBER 7-11
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Society for Immunotherapy of Cancer

SITC 2018 NOVEMBER 7–11 • WASHINGTON, D.C.

Harnessing Natural Killer cells to potentiate antitumor immunity

Amir Horowitz, PhD

Assistant Professor of Oncological Sciences

Precision Immunology Institute / Tisch Cancer Center

Icahn School of Medicine at Mount Sinai

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Presenter Disclosure Information

Amir Horowitz, PhD

The following relationships exist related to this presentation:

<No Relationships to Disclose>

Primary role of immune system:

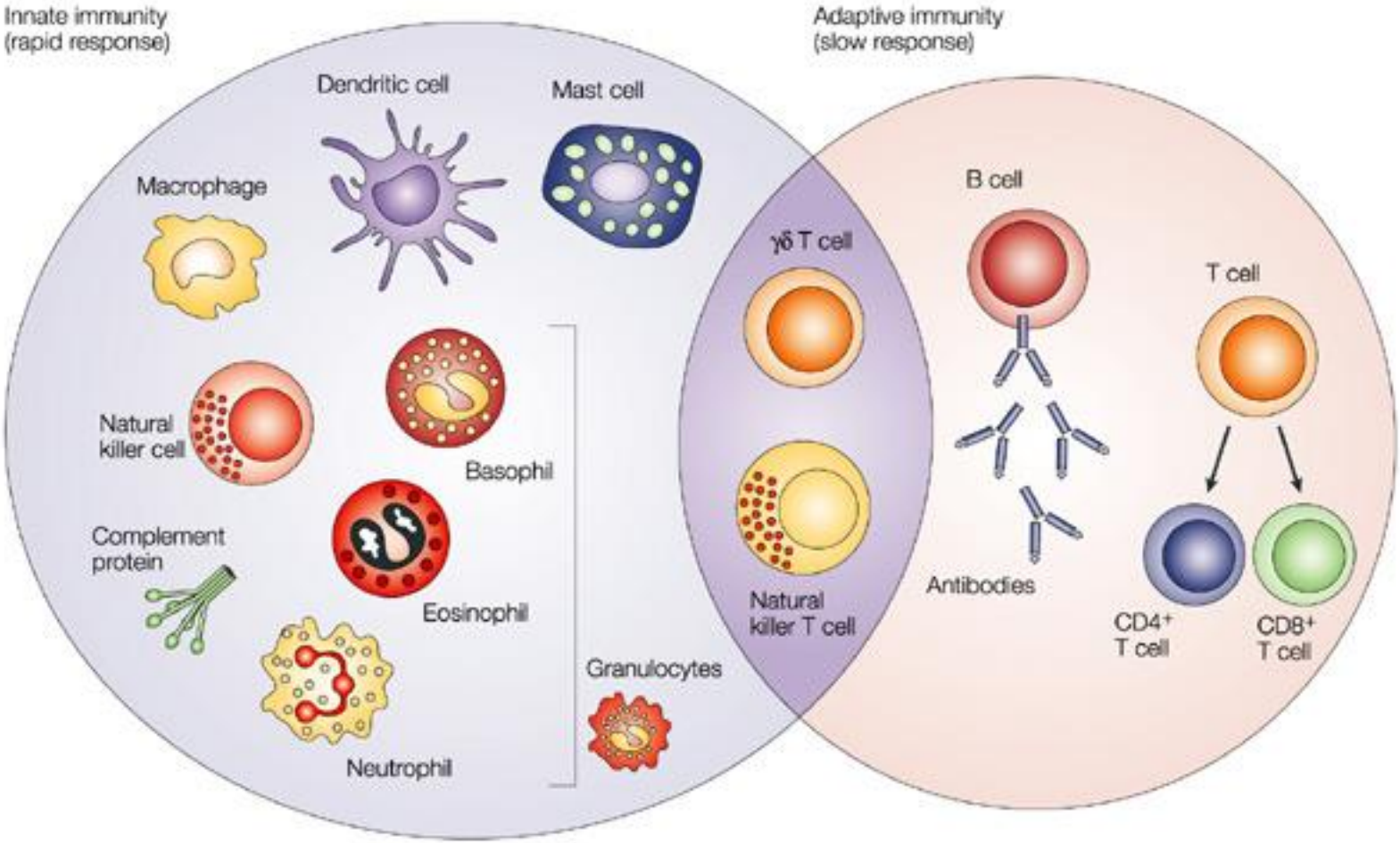
- It protects us from ~1,400 infections with:
  - Viruses
  - Bacteria
  - Fungi
  - Worms
  - parasitic protozoa
    - << 1% total microbial species on planet
- Promotes tissue cleanup, wound repair
- Eliminates abnormal cells including malignant ones
- Also promotes disease when dysregulated (allergies, autoimmunity, transplant rejection, etc.)

Innate Immunity vs. Adaptive immunity

- **Innate immunity** does not require prior sensitization, and little adaptation through life experience
- limited numbers of distinct receptors; recognize highly conserved features of classes of microbes.

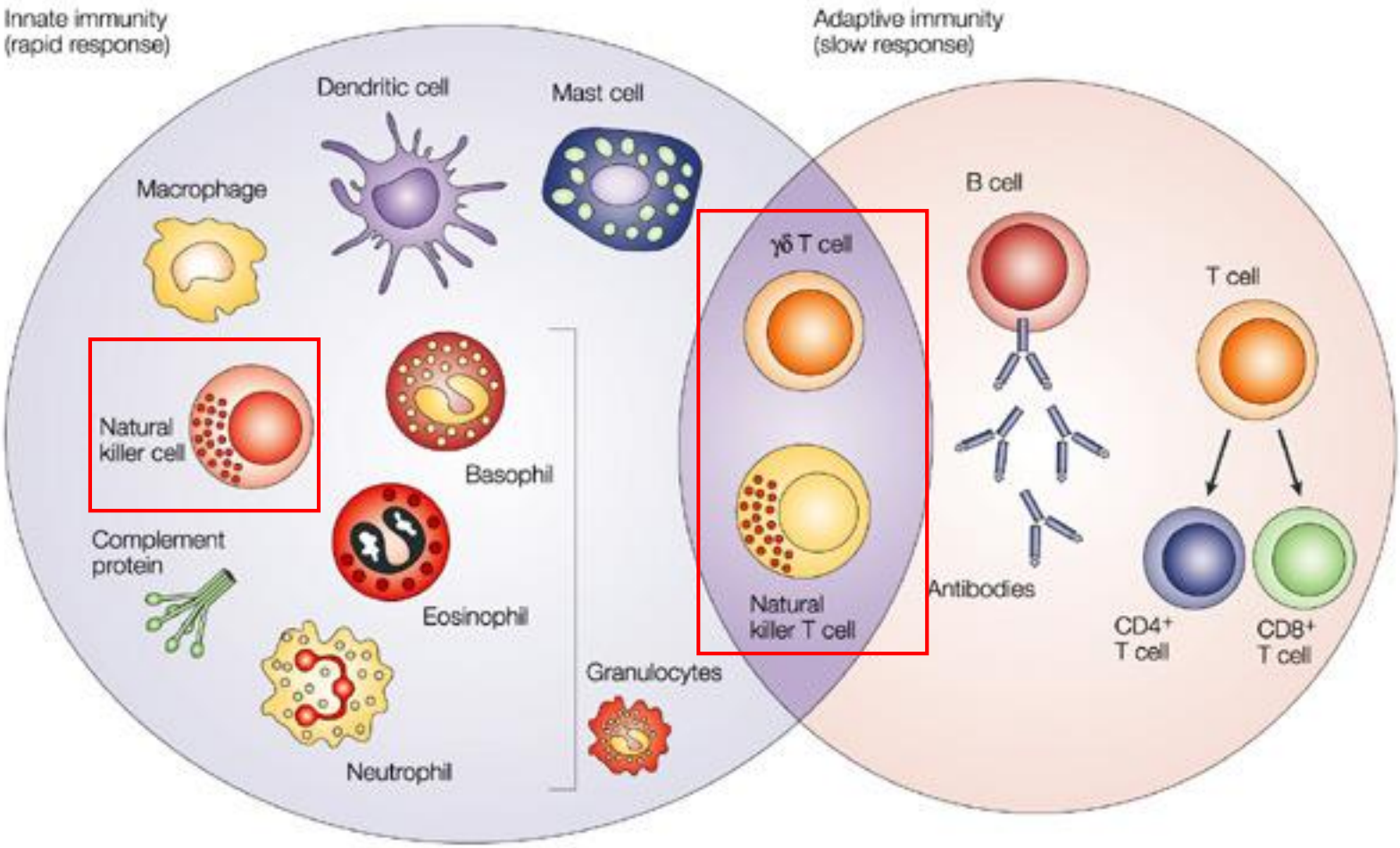
- **Adaptive immunity** adapts to previous experience; Stronger protection following secondary exposure.
- Very large number of distinct “antigen receptors” of T and B lymphocytes;
- generated by DNA rearrangement in each developing lymphocyte;
- clonal selection of lymphocytes recognizing antigen derived from microbe or self

Defining cell lineages within the immune system



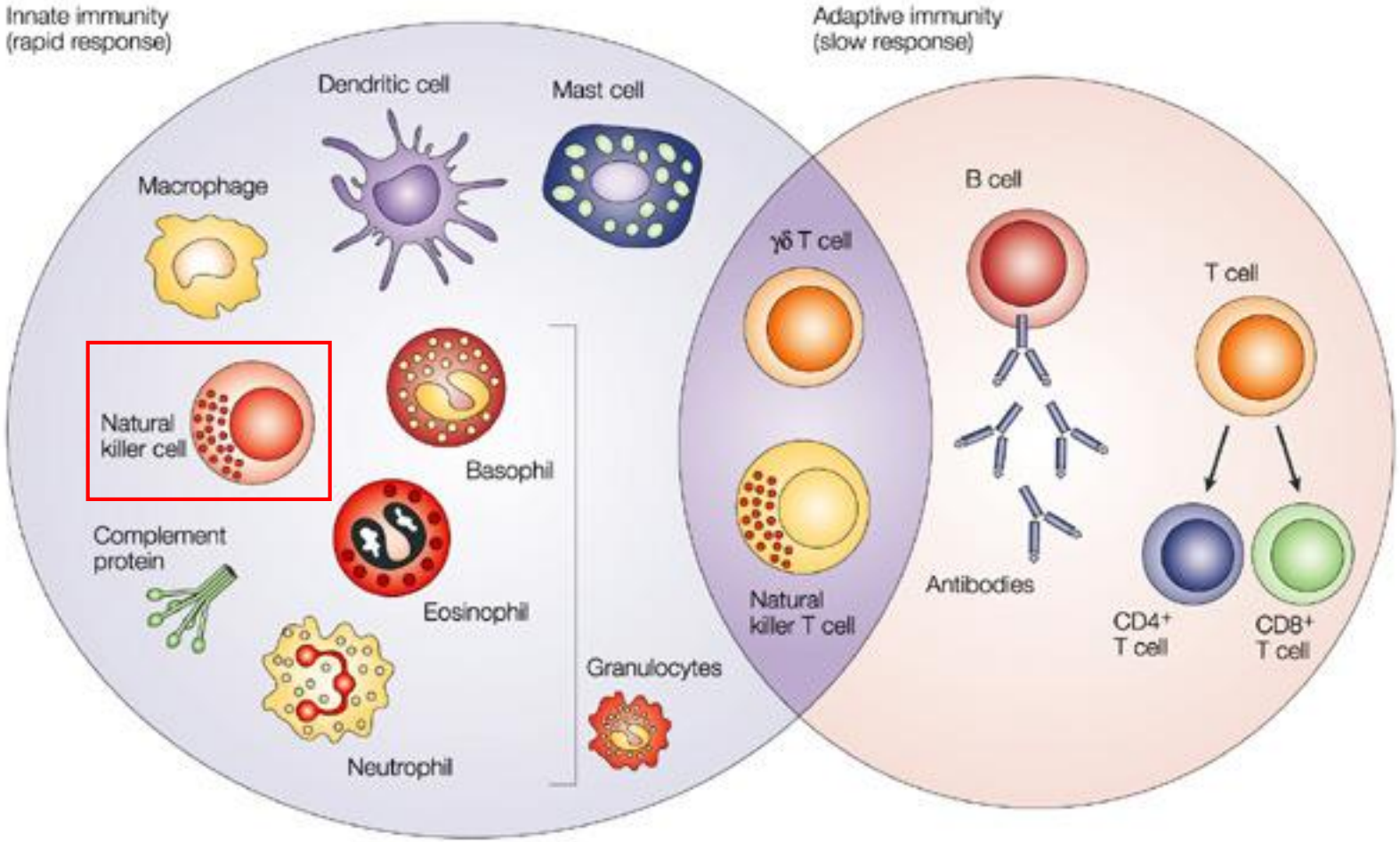
Nature Reviews | Cancer

Innate lymphocytes are comprised of NK cells, NK T cells and γδ T cells



Nature Reviews | Cancer

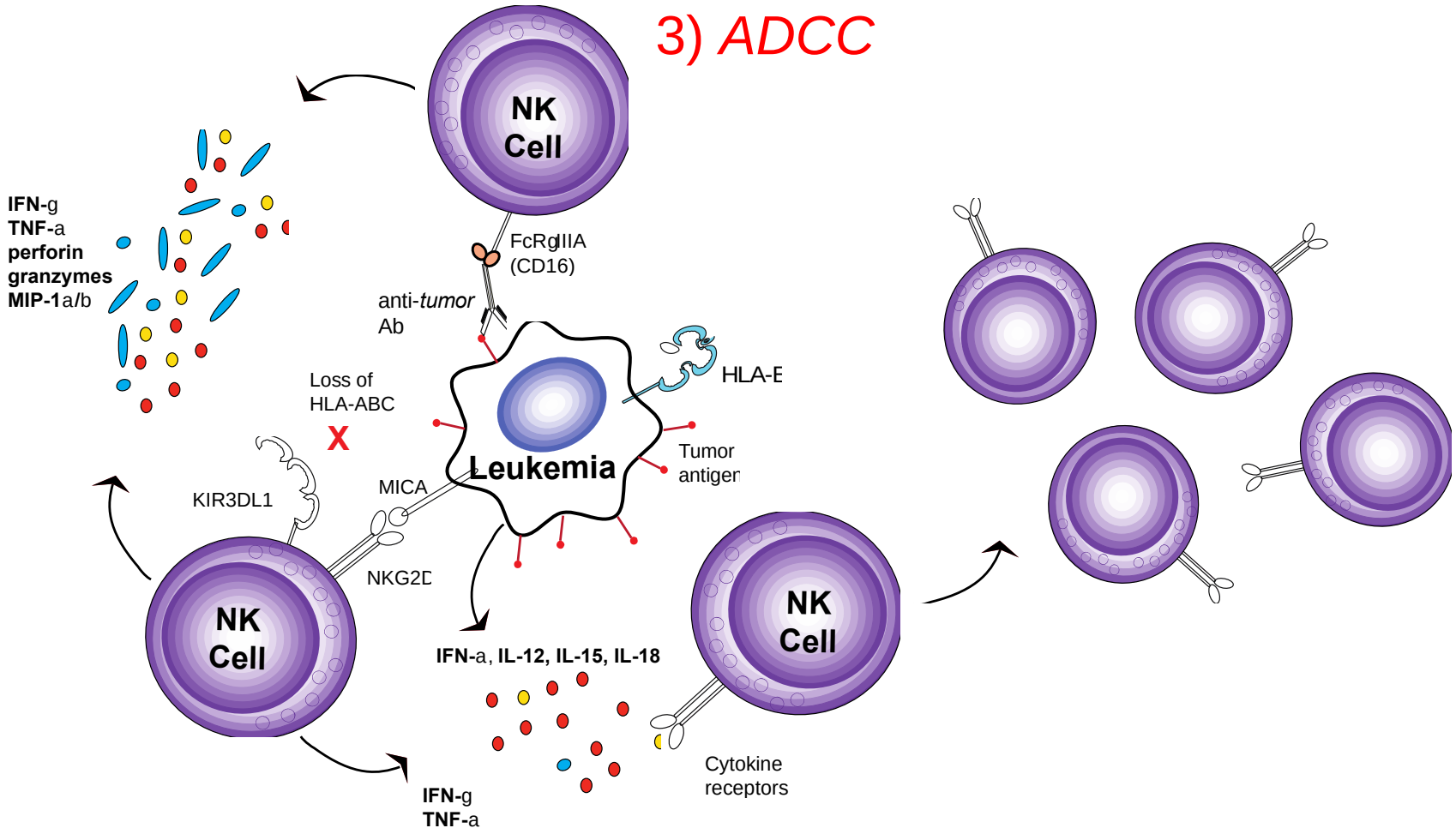
NK cells are an evolutionary predecessor to T cells



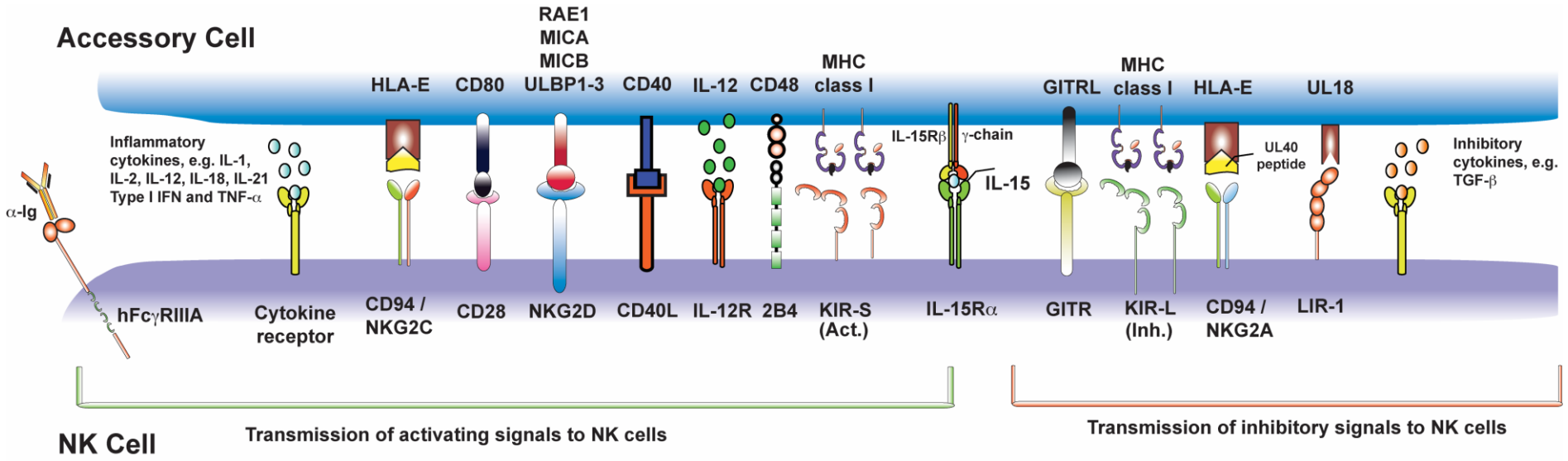
Nature Reviews | Cancer

NK cells: large, granular cells with pre-formed cytolytic vesicles; sense modulation of HLA class I as well as cytokines, chemokines and activating ligands; defend against 'all' microbes, tumors; critical for vascularization and arterial remodeling; pregnancy and promoting GVL after transplantation

NK cell functions are coordinated across specialized subsets - Example: acute myeloid leukemia (AML)

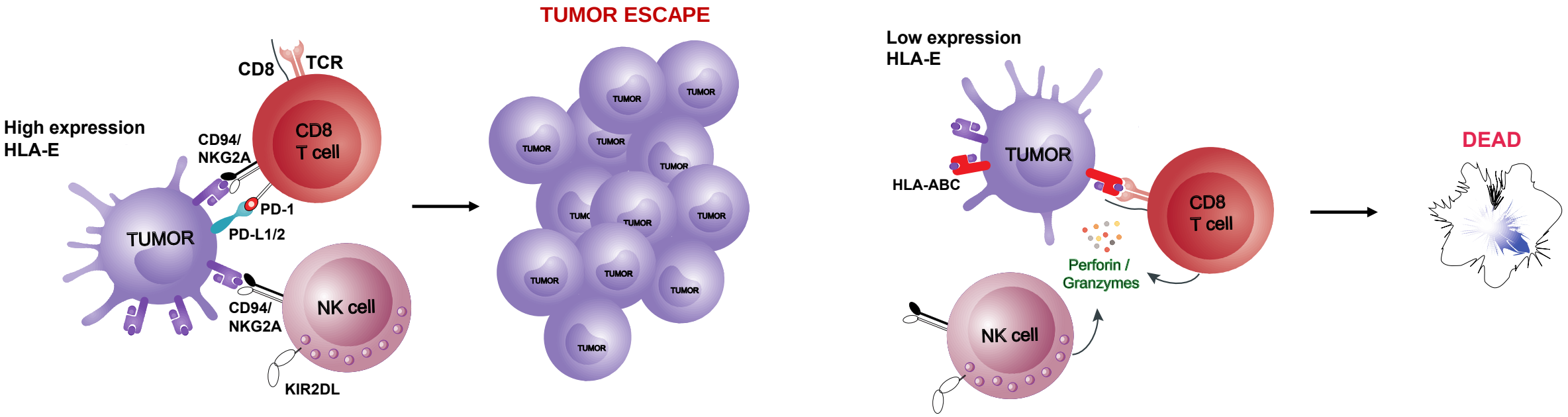


NK cell activation is regulated by the collective strength of inhibitory and activating signals

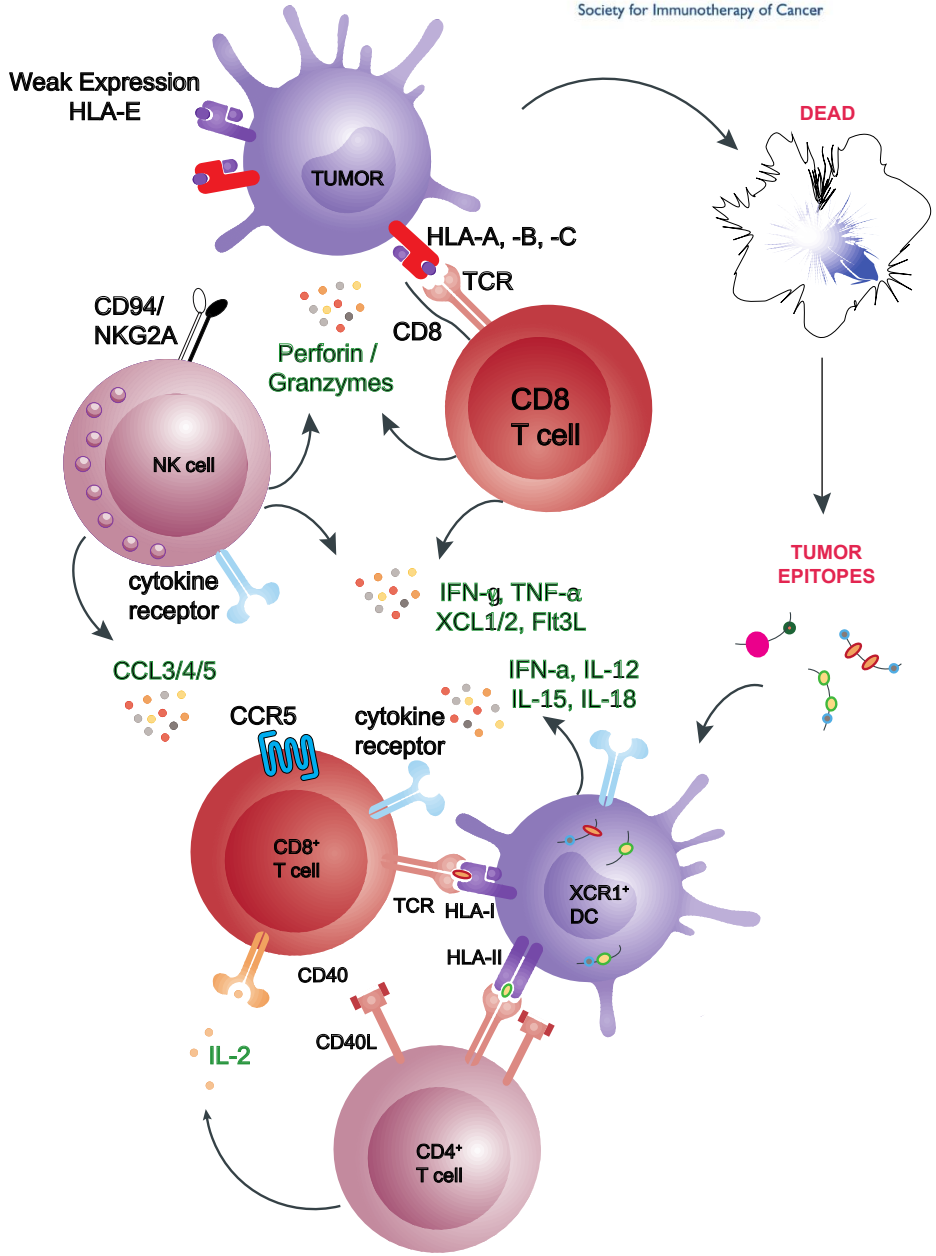


Horowitz, 2012 *Front Immunol*

Hypothesis: Expression of HLA-ABC and HLA-E on tumor cells will determine the capacity for NK cell and CD8 T cell reactivity to tumors



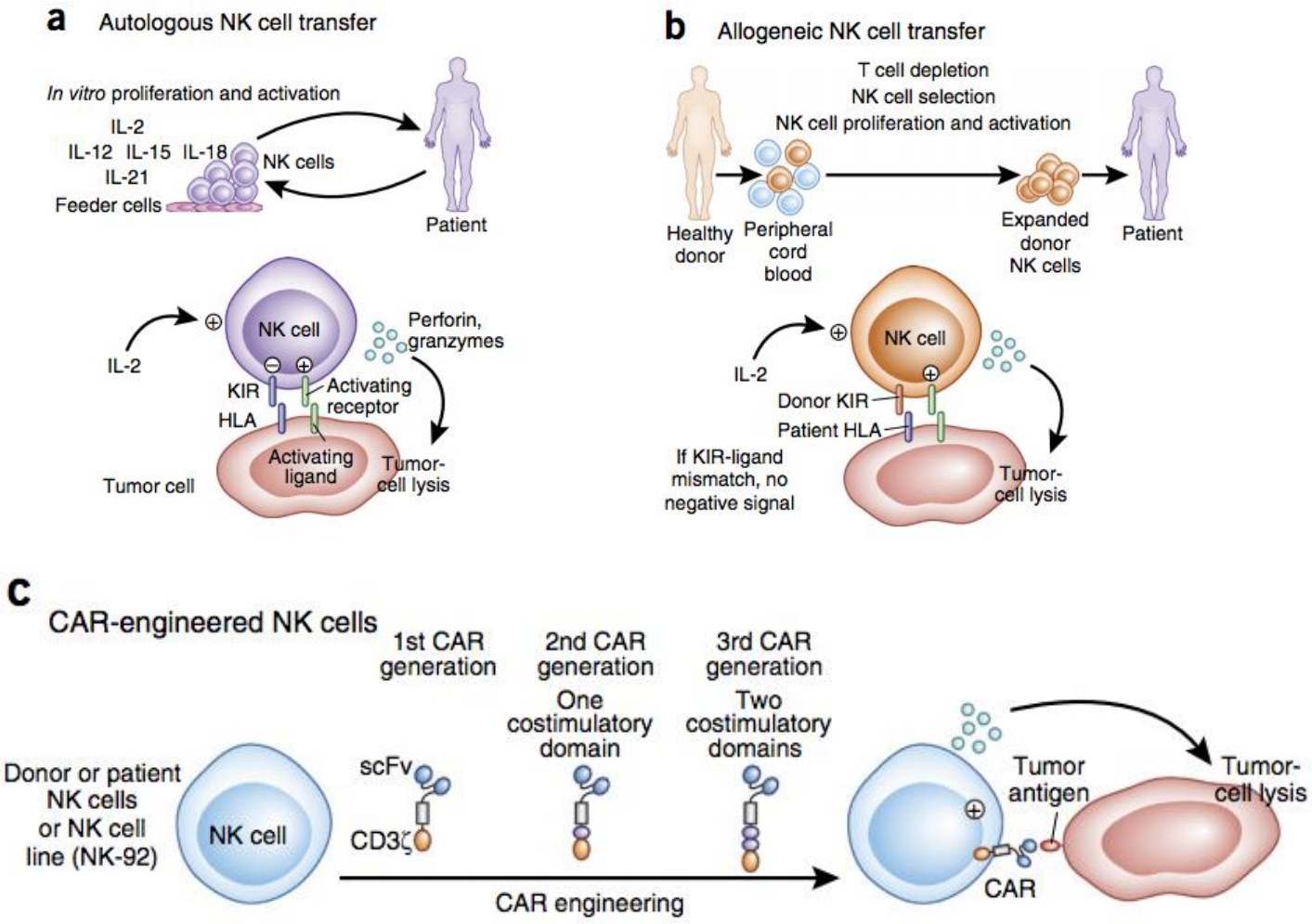
Quality of early tumor control determines availability of tumor epitopes for antigen presentation and priming of antitumor T cells



How can NK cells be harnessed for treatment against cancers?

- **Adoptive cell transfer**: autologous; allogeneic; NK cell lines; CAR NK cells
- **Cytokines**: IL-2; IL-15; IL-15SA-IL-15Rα-Su-Fc (ALT-803)
- **Anti-cancer agents**: IMiDs; Bortezomib and genotoxic agents; GSK3 inhibitors
- **Targeting immune-suppressive pathways**: Treg depletion; TGF-β blockade
- **Agonists of NK-cell activating receptors**: tumor-targeting mAbs; BiKEs and TriKEs; mAbs to CD137
- **Checkpoint inhibition**: mAbs to KIRs (IPH2101 and Lirilumab); mAbs to NKG2A (monalizumab), TIGIT, Tim-3

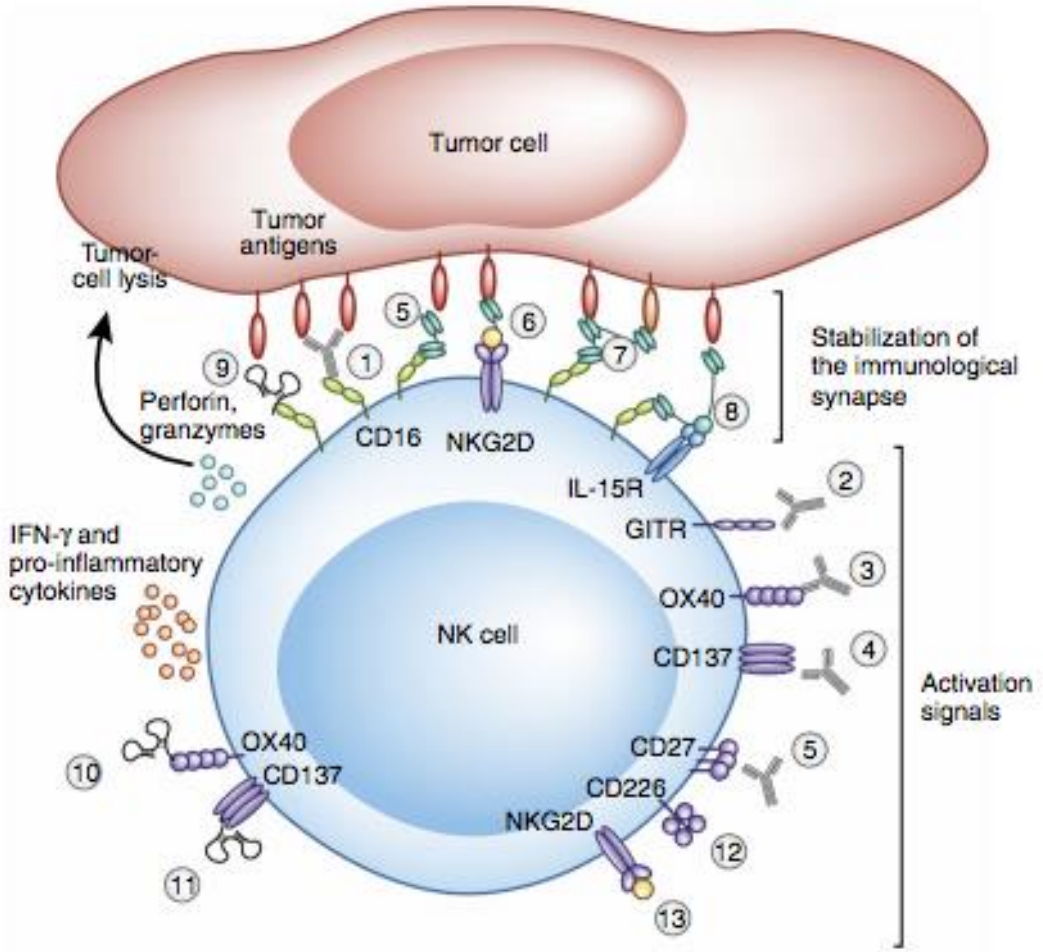
Adoptive NK cell transfer therapies



Li, 2018 *Cell Stem Cell*
NK-CAR-iPSCs-NK cells
hMesothelin
CD16, NKG2D, 2B4, CD137

Guillerey, 2016 *Nat Rev Immunol*

Therapies targeting activating NK receptors



FDA approved

1. Tumor-antigen-specific mAb

Clinical trials

2. mAb to GITR (TRX518)
3. mAb to OX40 (MEDI6469, MEDI6383, MOXR0916)
4. mAb to CD137 (urelumab, PFZ-05082566)
5. mAb to CD27 (varlilumab)

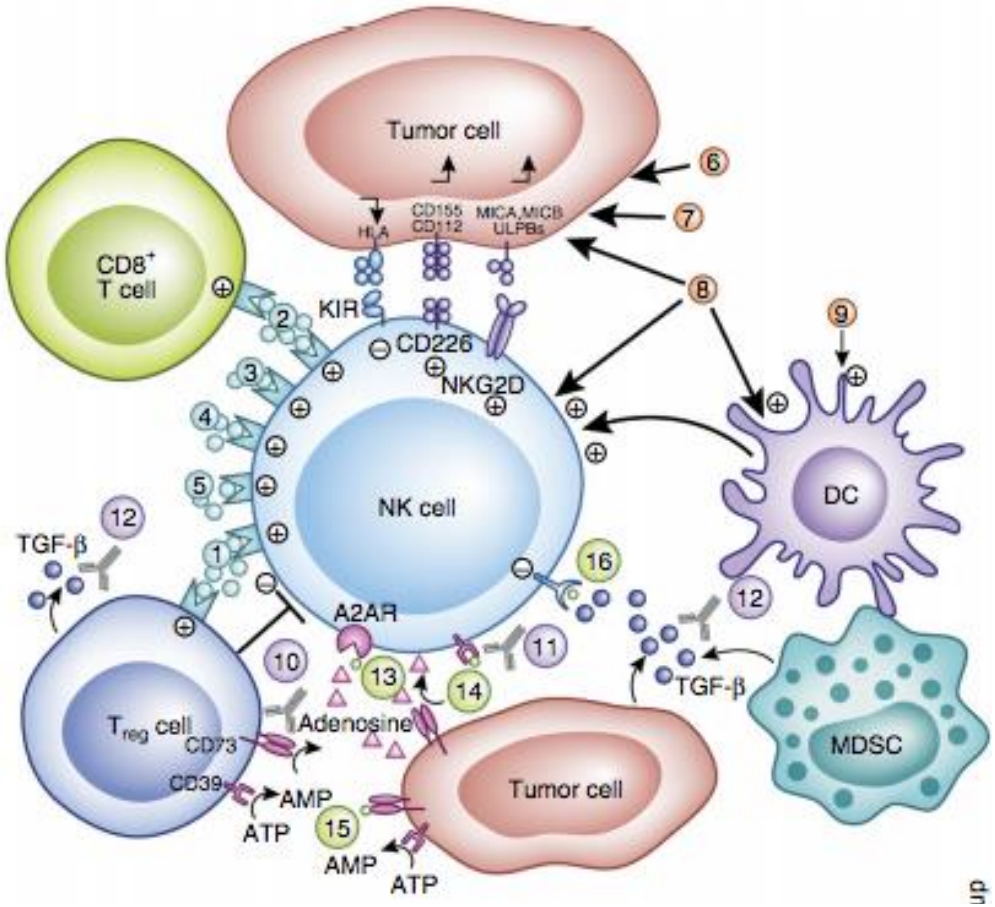
Preclinical development

5. BiKE
6. NKG2D ligand–antitumour Fv fusion
7. TriKE that binds two different tumor antigens
8. TriKE that incorporates IL-15
9. Bispecific aptamer
10. OX40 agonistic aptamer
11. CD137 agonistic aptamer

Not developed yet

12. CD226 agonist
13. Soluble activating NKG2D ligand

Therapies targeting activating cytokines, chemotaxic agents and Abs abrogating inhibitory signals



Activating cytokines

1. IL-2
2. IL-15
3. IL-12
4. IL-18
5. IL-21

Blocking antibodies

10. mAb to CD73
11. mAb to CD39
12. TGF-β-neutralizing mAb

Chemotherapeutics

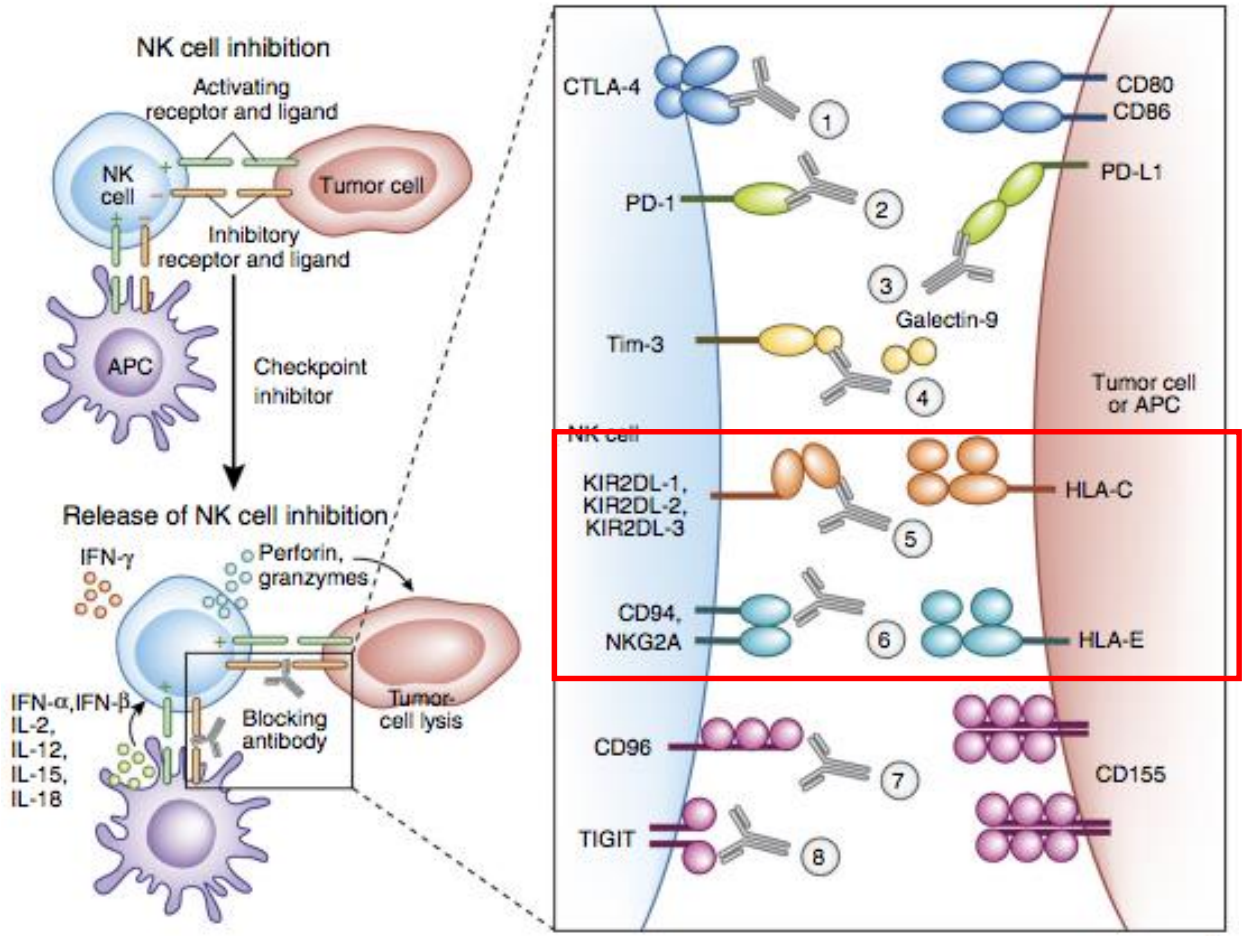
6. Genotoxic drugs (demythelating agents, histone deacetylases)
7. Proteasome inhibitors (bortezomib)
8. IMiDs (lenalidomide, pomalidomide)
9. Imatinib

Small-molecule inhibitors

13. A2A receptor antagonist (PBF-509)
14. CD39 inhibitor (POM-1)
15. CD73 inhibitor (APCP)
16. TGF-β receptor inhibitor

Guillerey, 2016 *Nat Rev Immunol*

Therapies targeting checkpoint inhibitors



FDA approved

1. mAb to CTLA-4 (ipilimumab)
2. mAb to PD-1 (nivolumab or pembrolizumab)

Clinical trials

3. mAb to PD-L1 (atezolizumab)
4. mAb to Tim-3 (MBG453)
5. mAb to a KIR (1-7F9 (IPH2101) or lirilumab (IPH2102))
6. Anti-NKG2A (monalizumab (IPH2201))

Preclinical development

7. mAb to CD96
8. mAb to TIGIT

Guillerey, 2016 *Nat Rev Immunol*

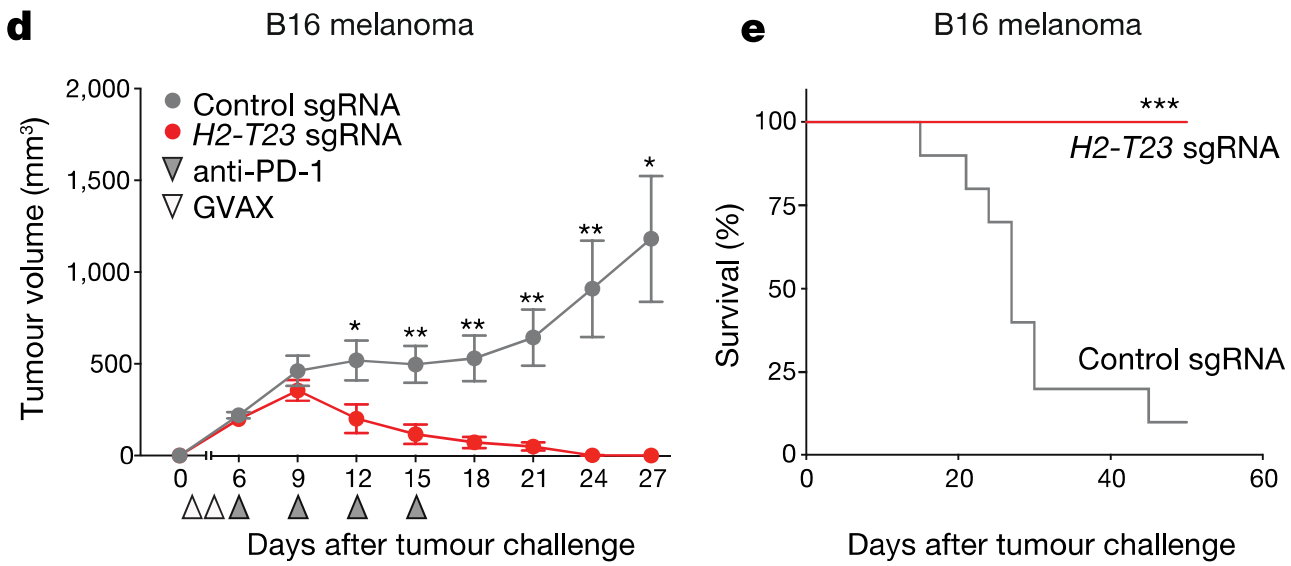
HLA-E expression on tumors may explain failure of checkpoint blockade monotherapies

ARTICLE

doi:10.1038/nature23270

In vivo CRISPR screening identifies *Ptpn2* as a cancer immunotherapy target

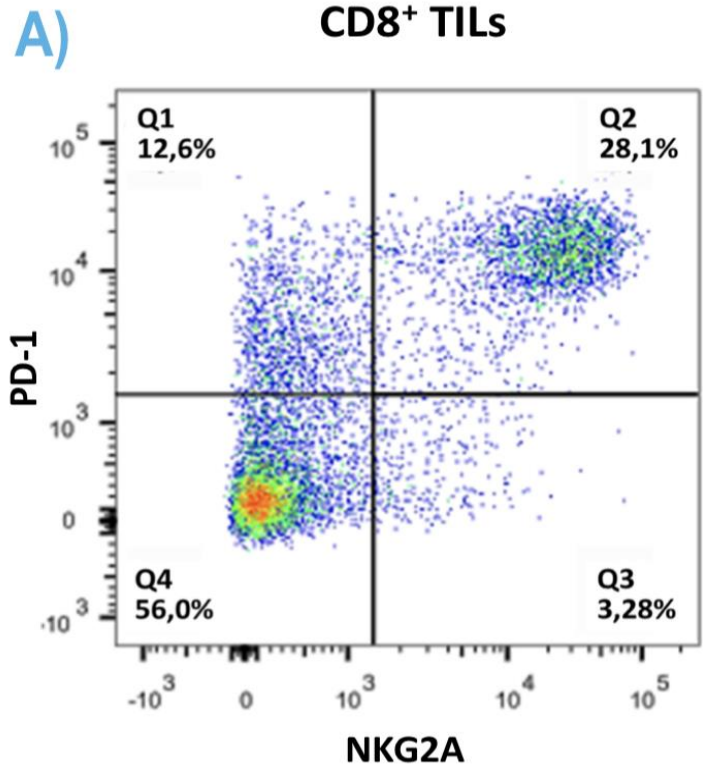
Robert T. Manguso[1,2,3], Hans W. Pope[1,3], Margaret D. Zimmer[1,3], Flavian D. Brown[1,2], Kathleen B. Yates[1,3], Brian C. Miller[1,3,4], Natalie B. Collins[1,3,5], Kevin Bi[1,3], Martin W. LaFleur[1,2], Vikram R. Juneja[6], Sarah A. Weiss[1], Jennifer Lo[7], David E. Fisher[7], Diana Miao[2,3], Eliezer Van Allen[2,3], David E. Root[3], Arlene H. Sharpe[5,8], John G. Doench[3] & W. Nicholas Haining[1,3,5]



Manguso, *Nature,* 2017

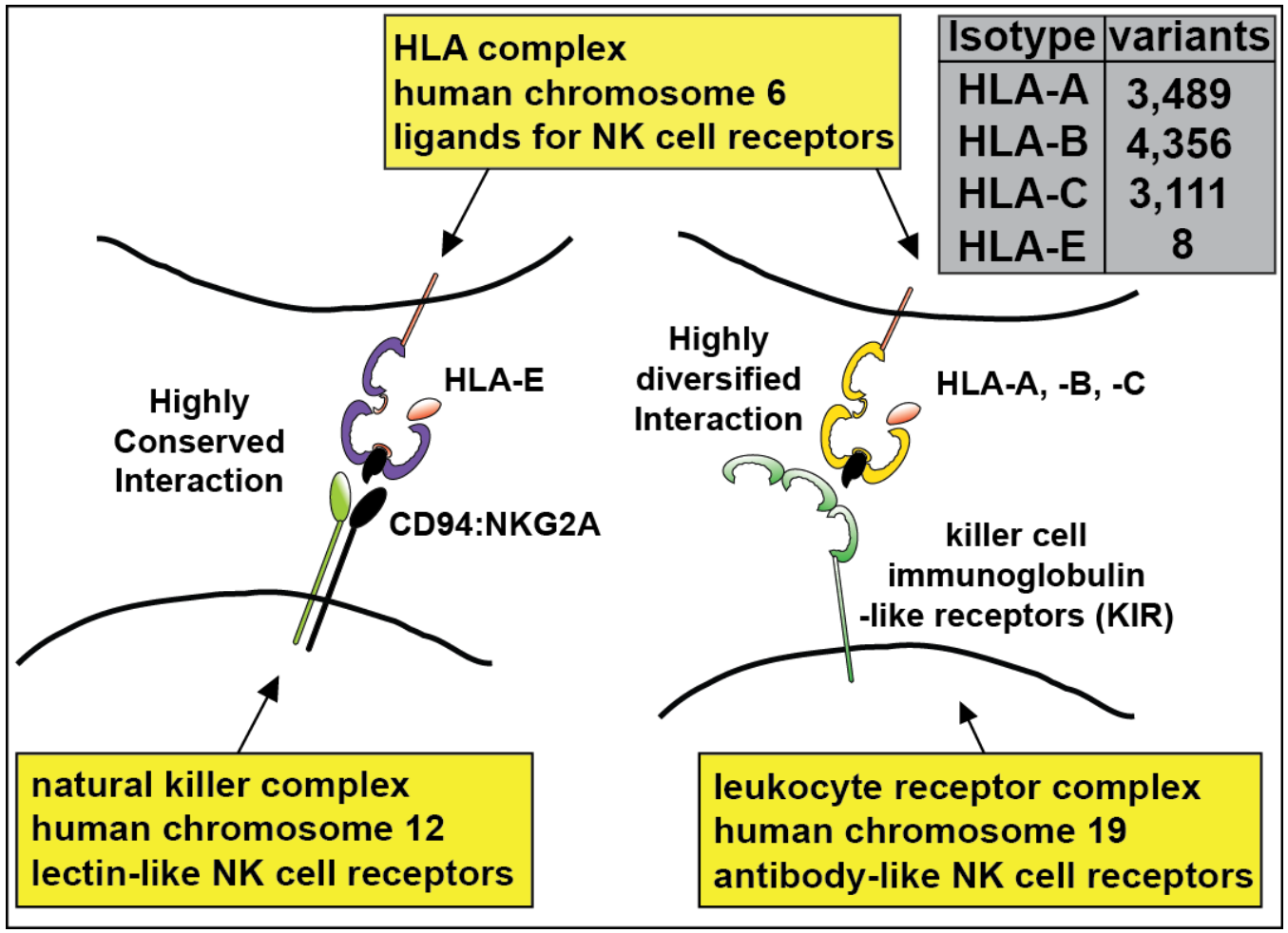
Combined blockade of PD-L1 and NKG2A checkpoints enhances anti-tumor CD8⁺ T cell response

Caroline Denis, Vedran Brezar, Thomas Arnoux, Julie Lopez, Clarisse Caillet, Fabien Chanuc, Nicolas Fuseri, Nicolai Wagtmann, Pascale André, Caroline Soulas - Innate Pharma, 117 Avenue de Luminy, 13009 Marseille, France



Innate Pharma, 2018

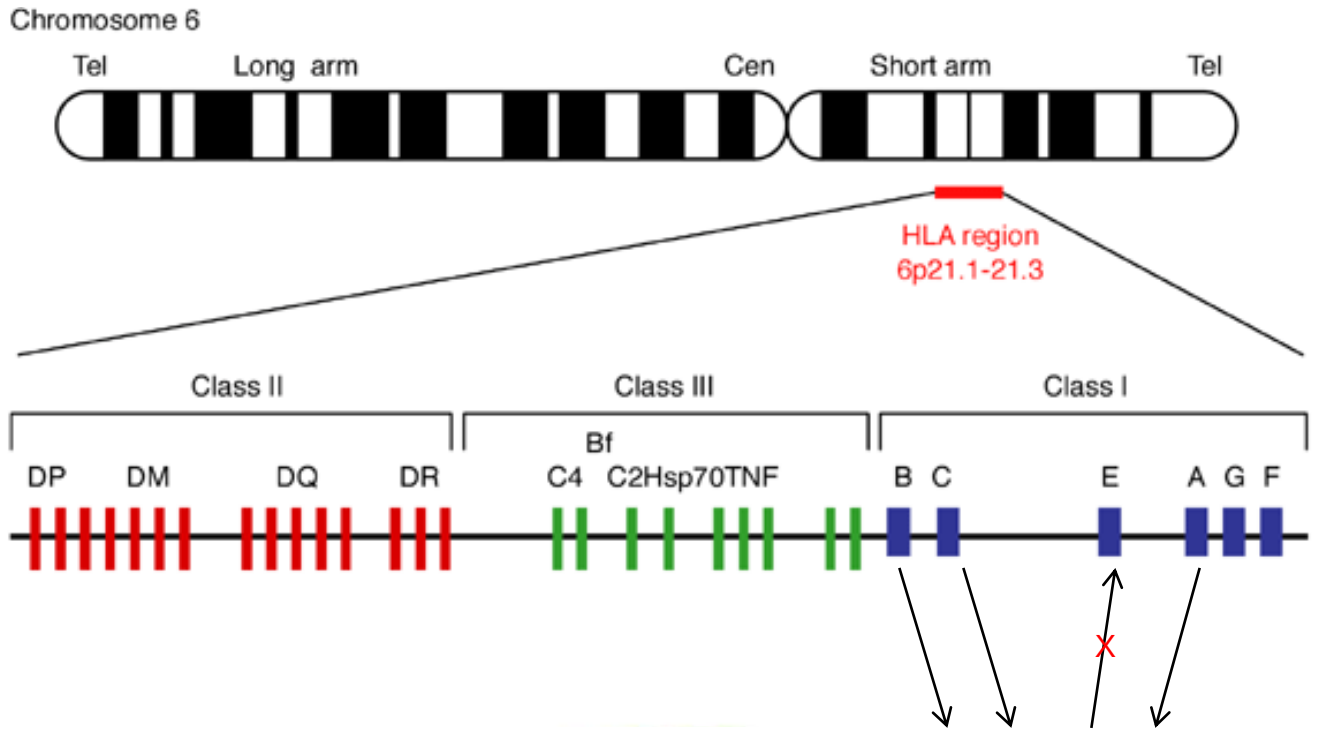
NK cells (and CD8 T cells) are regulated by system of Immunogenetics



Adapted from: Parham, 2012 *Phil. Trans. R. Soc. B;*
Horowitz, 2016, *Science Immunology*

Robinson, 2017 *PLoS Genetics*
(IPD: Up to date list of HLA alleles)

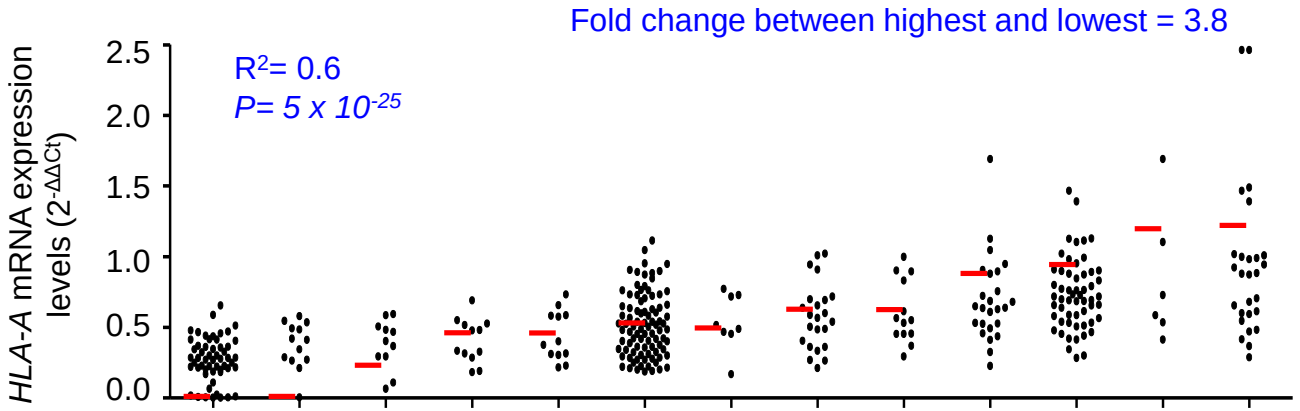
HLA-A, -B and –C contribute leader sequence-derived peptides to HLA-E



VMAPRTLLL (~80% of HLA-B alleles)

Horowitz, 2016 *Sci Immunol*

Inference of HLA-E expression from *HLA-A* and *HLA-B* genotypes



Allelic polymorphisms define broad range in transcription of HLA-A

All alleles encode suitable HLA-E binding peptide: V**M**APRTLLL

HLA-A alleles vary the amount of available peptide

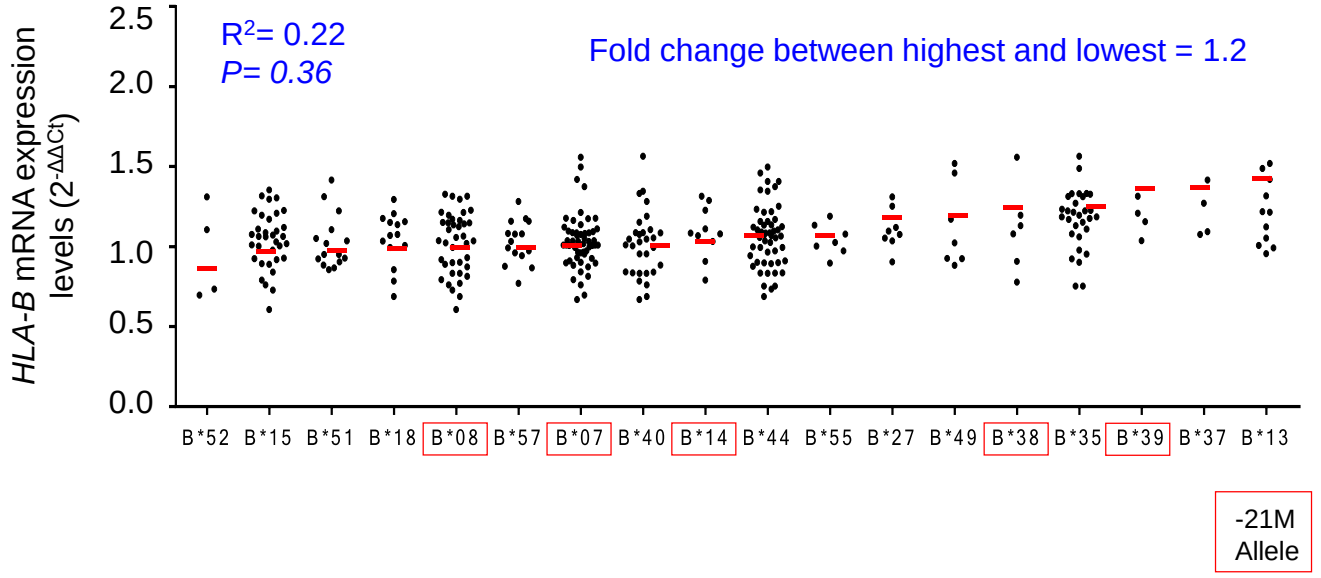
Ramsuran, 2015 *Am J Hum Genet*
Horowitz, 2016 *Sci immunol*

Inference of HLA-E expression from *HLA-A* and *HLA-B* genotypes

HLA-B is transcribed at very uniform levels

80% of alleles encode unsuitable HLA-E binding peptide: V**T**APRTLLL

HLA-B alleles vary the availability of peptide as “yes” or “no”



Horowitz, 2016 *Sci Immunol*
Ramsuran, 2017 *J Immunol*

Pipeline for examining prognostic effects of tumor HLA-E expression on survival

HLA-A

Logistic regression analysis of mean *HLA-A* mRNA previously determined in healthy and HIV-infected individuals

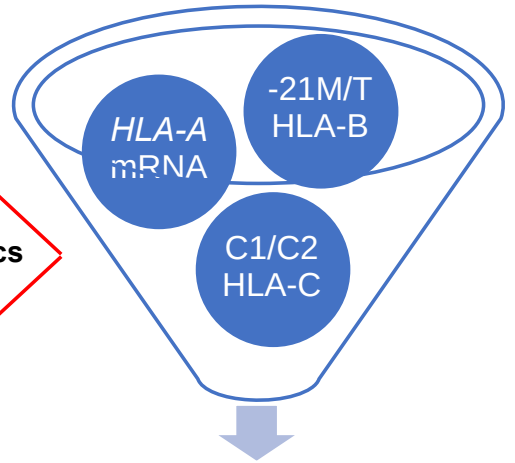
HLA-B

-21M/T HLA-B leader peptide dimorphism:
-21M peptide promotes high HLA-E expression
-21T peptide promotes low HLA-E expression

HLA-C

80N/K HLA-C KIR2DL/S allele determining residue:
80N = C1 epitope = Ligand for KIR2DL2, KIR2DL3
80K = C2 epitope = Ligand for KIR2DL1, KIR2DS1

HLA class I Bioinformatics Pipeline



= Inferred HLA-E expression and NKG2A vs KIR education

Horowitz, 2016 *Sci Immunol*
Ramsuran, 2018 *Science*

MMRF CoMMpass study: treatment-naïve multiple myeloma patients

CoMMpass cohort: 1,150 treatment-naïve patients

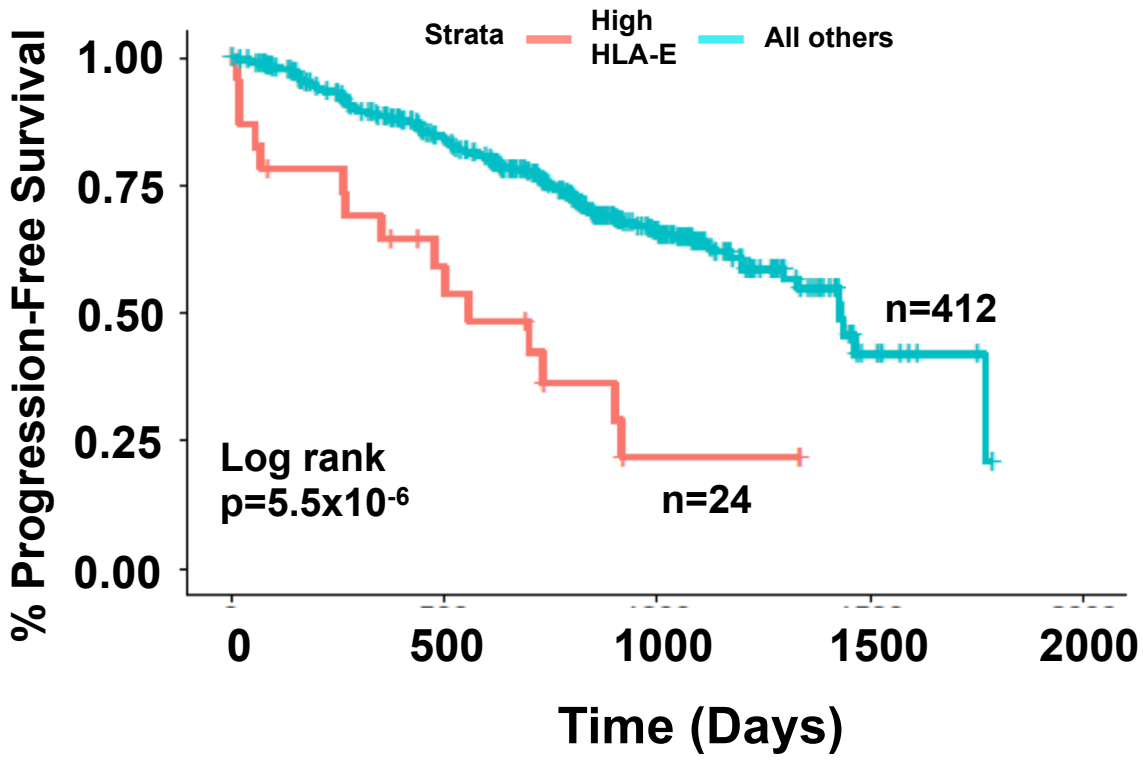
- HLA class I genotype
- Clinical data

Predict HLA-E and NK 'education'

Survival analysis

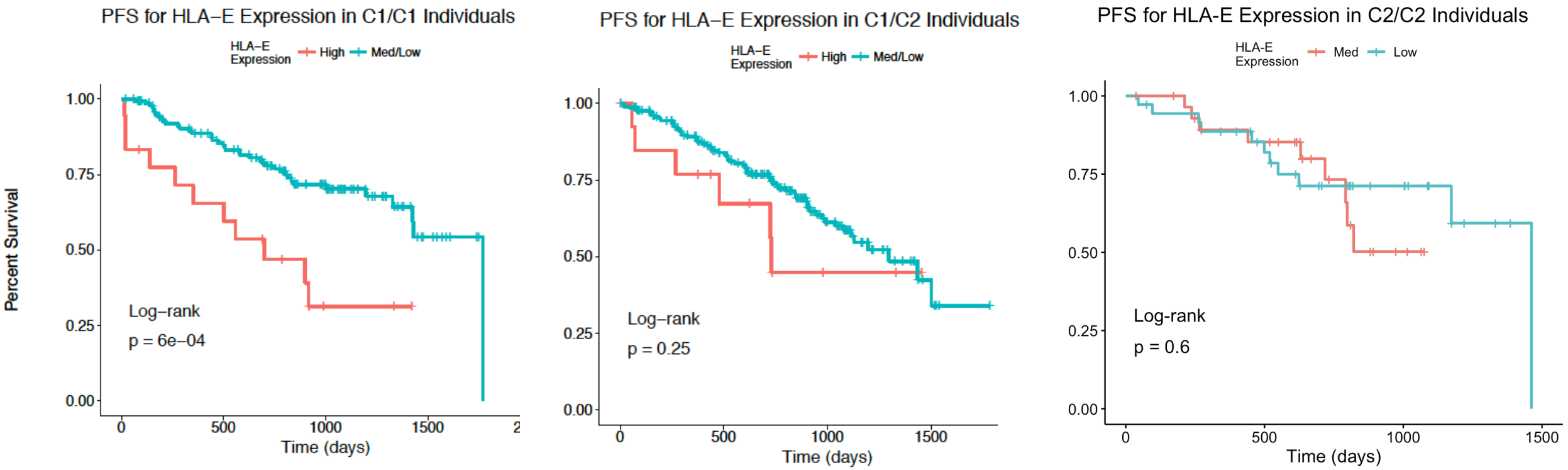
Microenvironment CyTOF

Inferring HLA-E expression	*HLA-B* leader peptide (-21M/T)		
HLA-A transcription	M/M	M/T	T/T
High	High	High	Medium
Med./High	High	Medium	Low
Med./Low	Medium	Medium	Low
Low	Medium	Low	Low



Unpublished

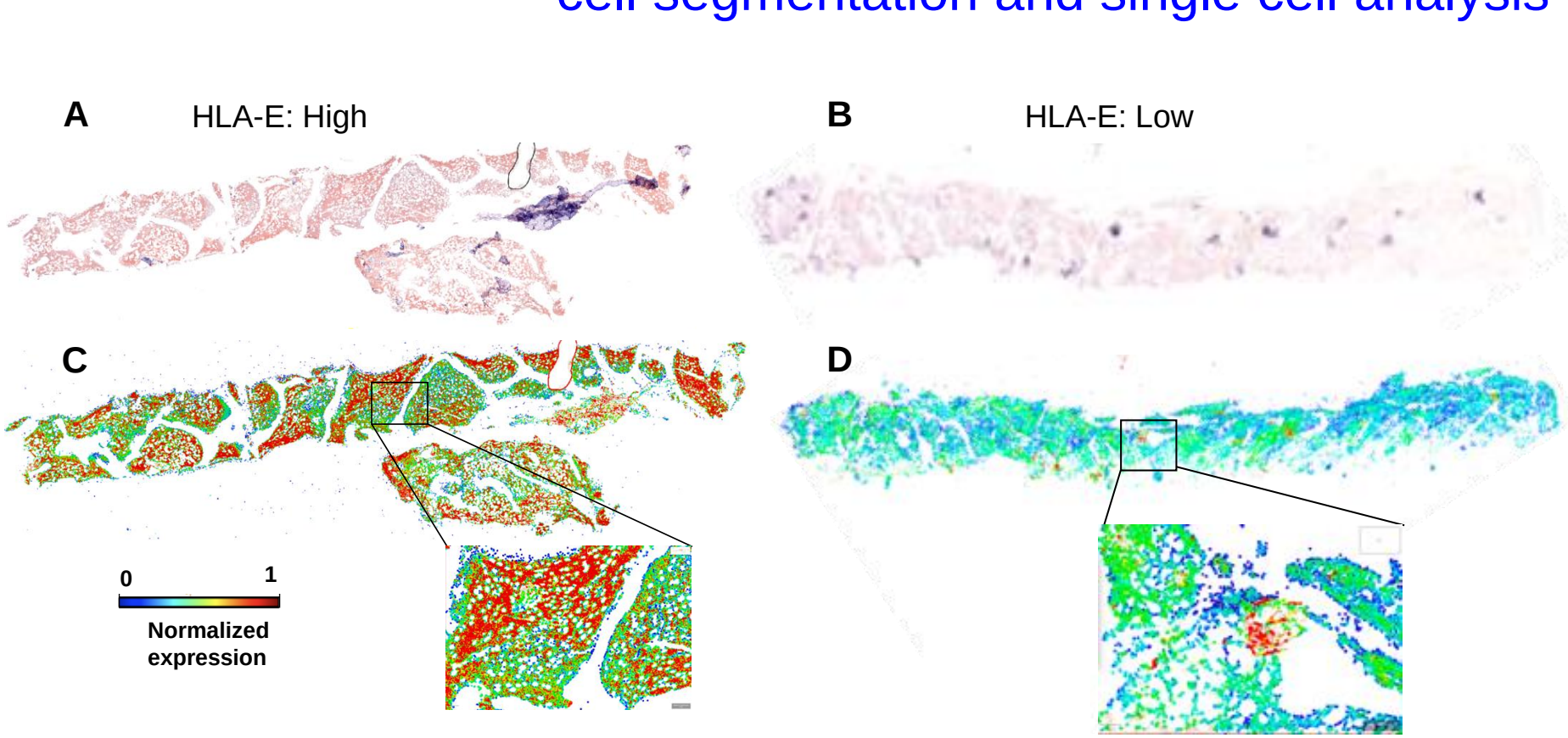
MMRF CoMMpass study: treatment-naïve multiple myeloma patients

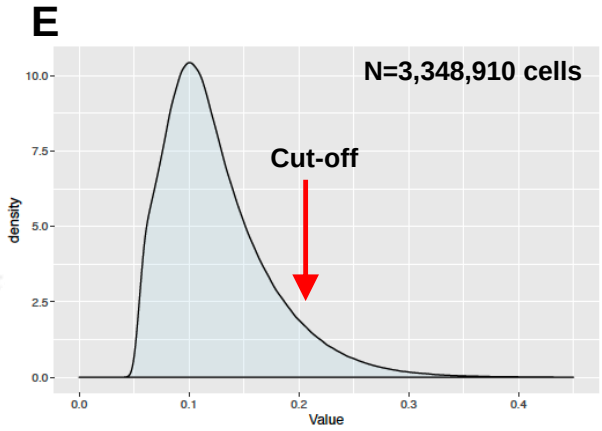


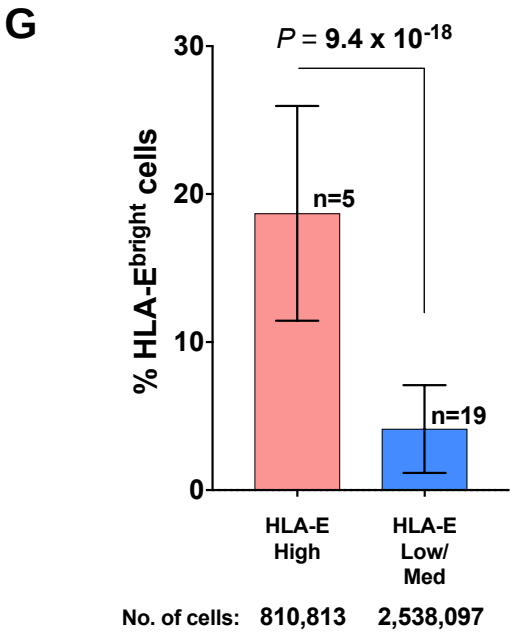
$C2^+$ *HLA-C* alleles are in strong linkage disequilibrium with *HLA-A* and *–B* alleles promoting weak cell-surface expression of HLA-E

Unpublished

Confirming HLA-E expression by IHC on bone marrow core biopsies by cell segmentation and single cell analysis



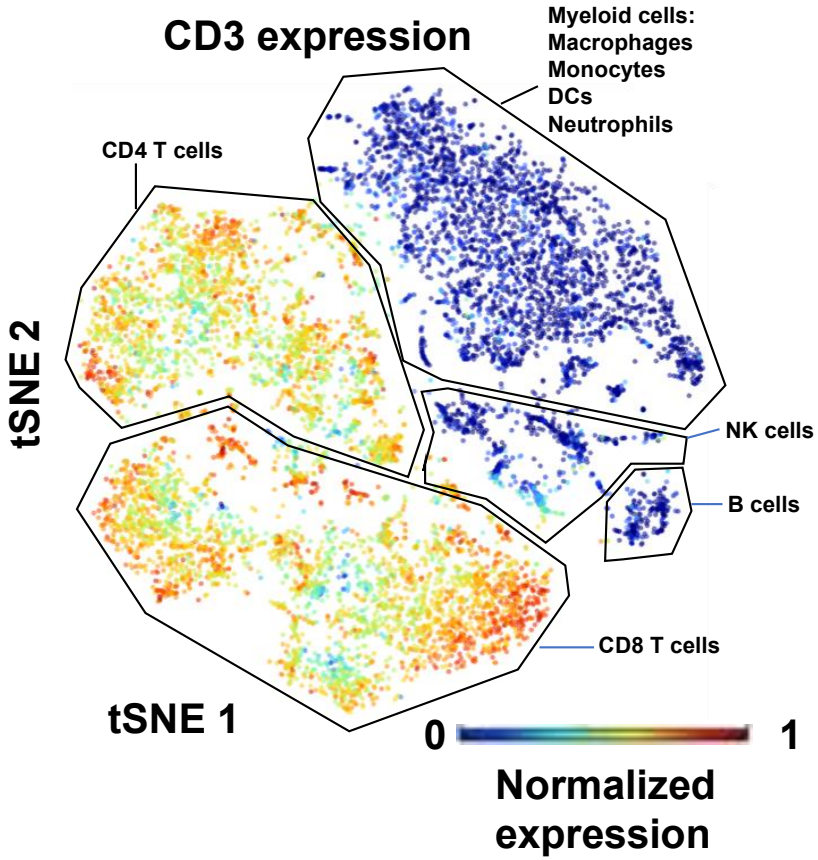


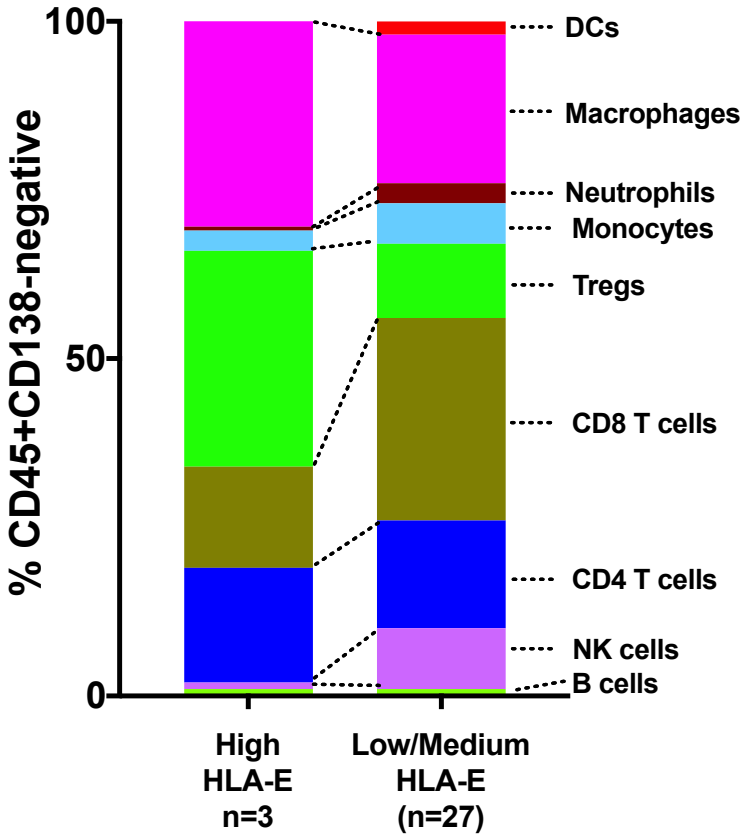


Park, unpublished

Mass cytometric analysis of 30 CoMMpass patients: Recruited for hypermutation vs non-hypermutation

40 antibodies targeting major immune Cell lineages in Bone Marrow aspirate





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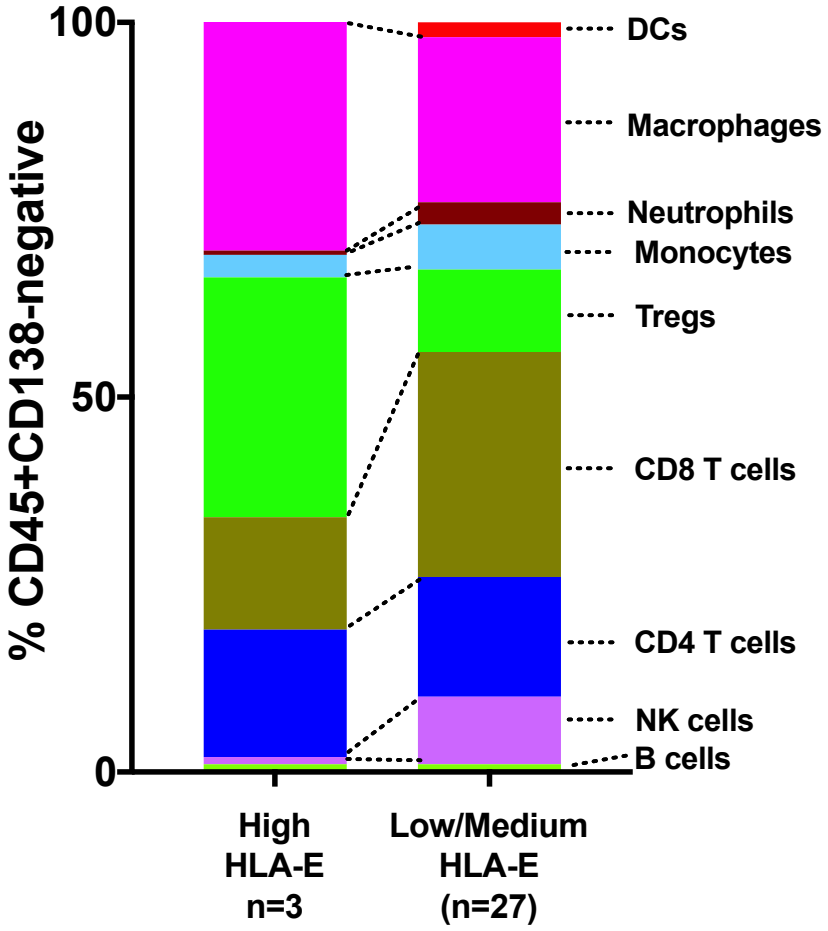
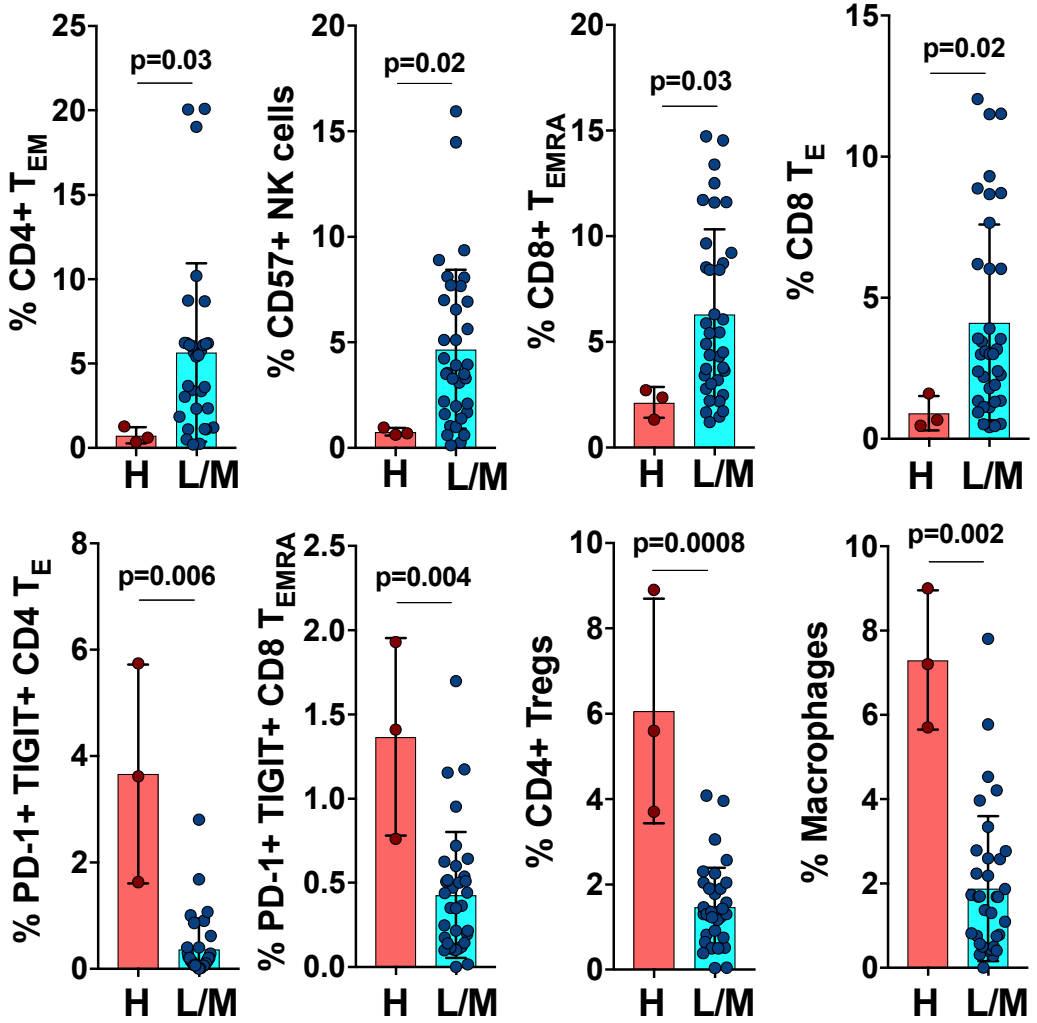
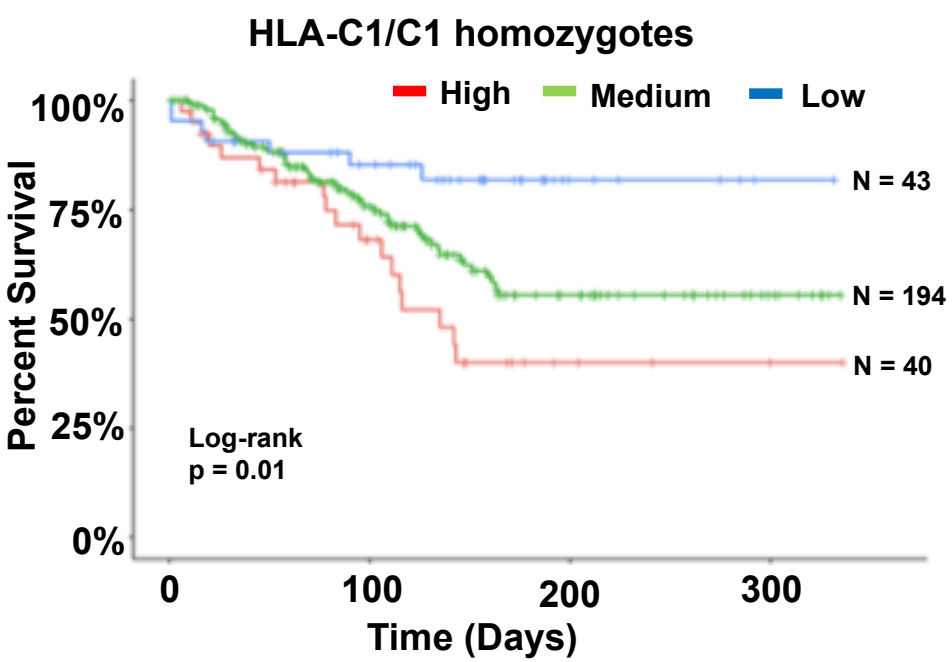


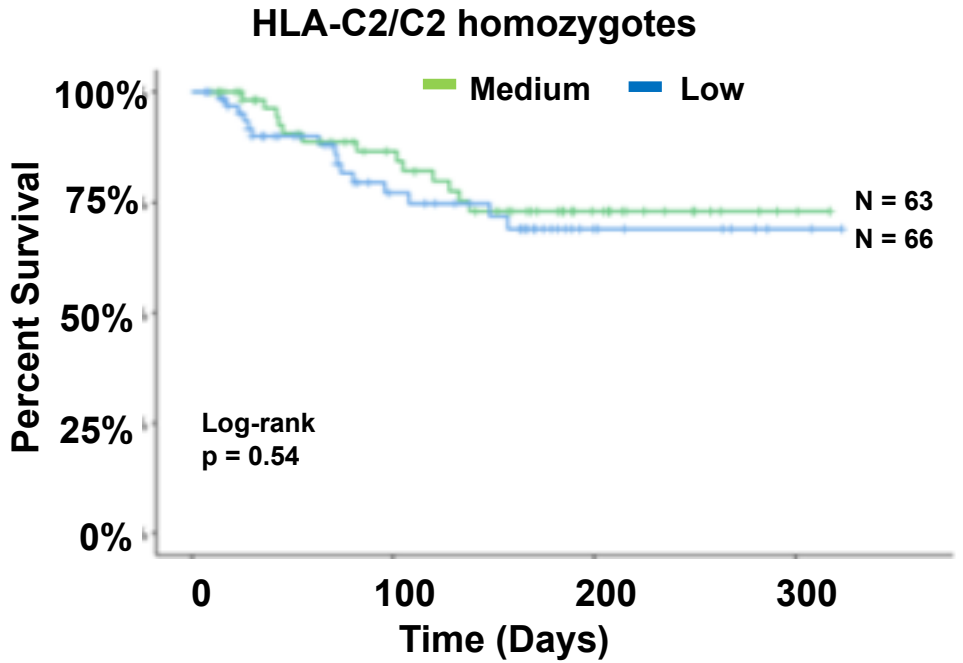
Phenotypes associated with Th1 effector response



Phenotypes associated with exhaustion and immune suppression

TCGA Analysis of Genitourinary cancers: Bladder Urothelial Carcinoma and Clear Cell Renal Cell Carcinoma





Unpublished

Lessons and Take Home Messages

- Innate lymphocytes bridge the innate and adaptive immune responses
- Collectively survey environment for cell-surface bound and soluble stimuli as well as for modulation of HLA class I molecules
- NK cells display broad range of effector functions that are mediated by specialized subsets
- NK cell activation is determined through the collective strength of activating and inhibitory signals but tightly regulated through HLA class I
- Innate lymphocytes are critical for amplifying and sustaining inflammation until antigen-specific T cells and B cells expand to sufficient numbers
- Innate lymphocytes are increasing focus for immunotherapies as strategy for tumor killing and potentiating memory T cells and B cells