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NATIONAL HARBOR, MARYLAND

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Society for Immunotherapy of Cancer

Harnessing Natural Killer cells to potentiate antitumor immunity

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Precision Immunology Institute / Tisch Cancer Center

Icahn School of Medicine at Mount Sinai

Presenter Disclosure Information

Amir Horowitz, PhD

The following relationships exist related to this presentation:

HTG Molecular – Advisory Board
FATE Therapeutics – Ad hoc Advisory Board
Genentech - Consulting

Primary role of immune system:

- It protects us from ~1,400 infections with:
  - Viruses
  - Bacteria
  - Fungi
  - Worms
  - parasitic protozoa

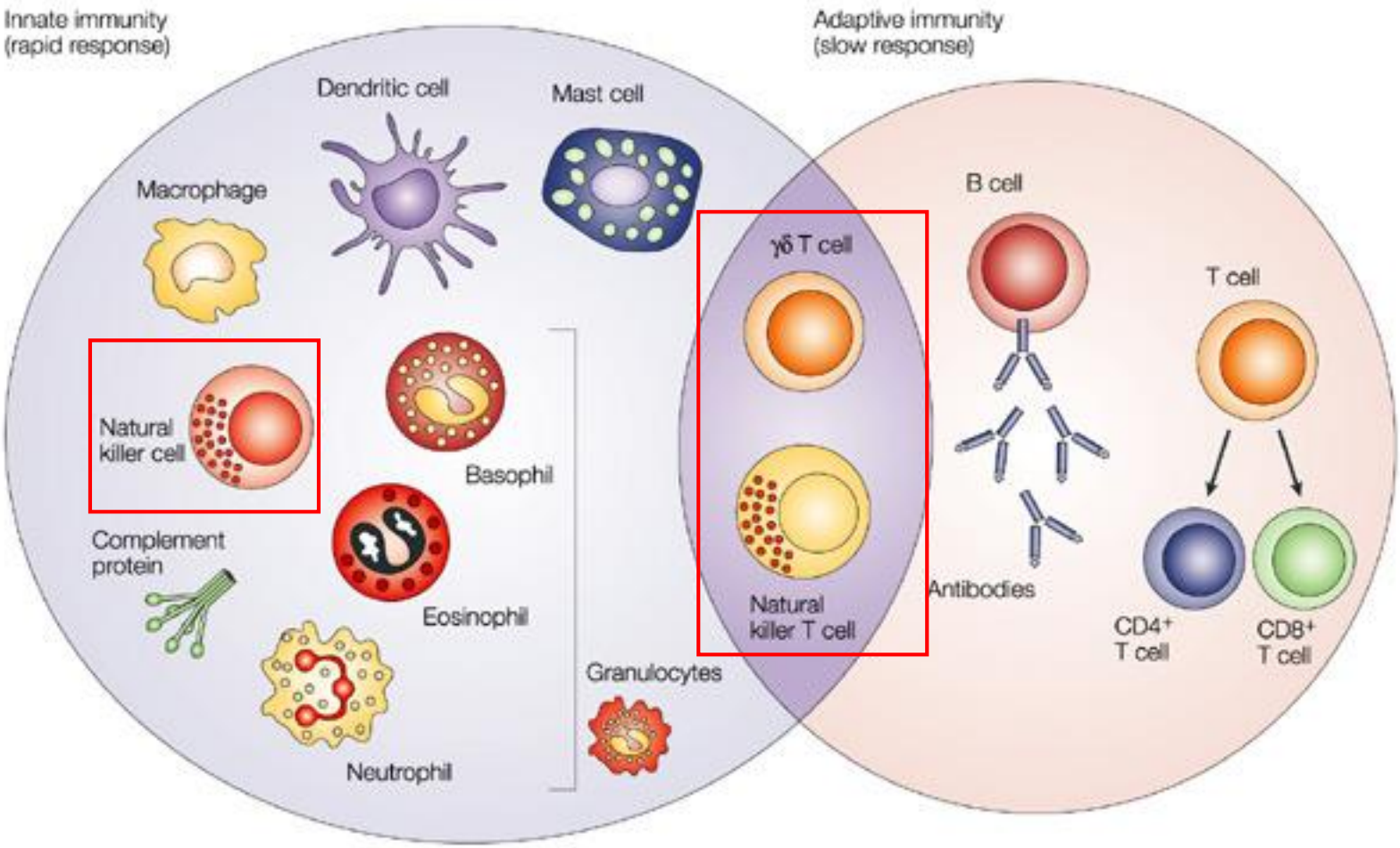
    << 1% total microbial species on planet
- Promotes tissue cleanup, wound repair
- Eliminates abnormal cells including malignant ones
- Also promotes disease when dysregulated (allergies, autoimmunity, transplant rejection, etc.)

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.

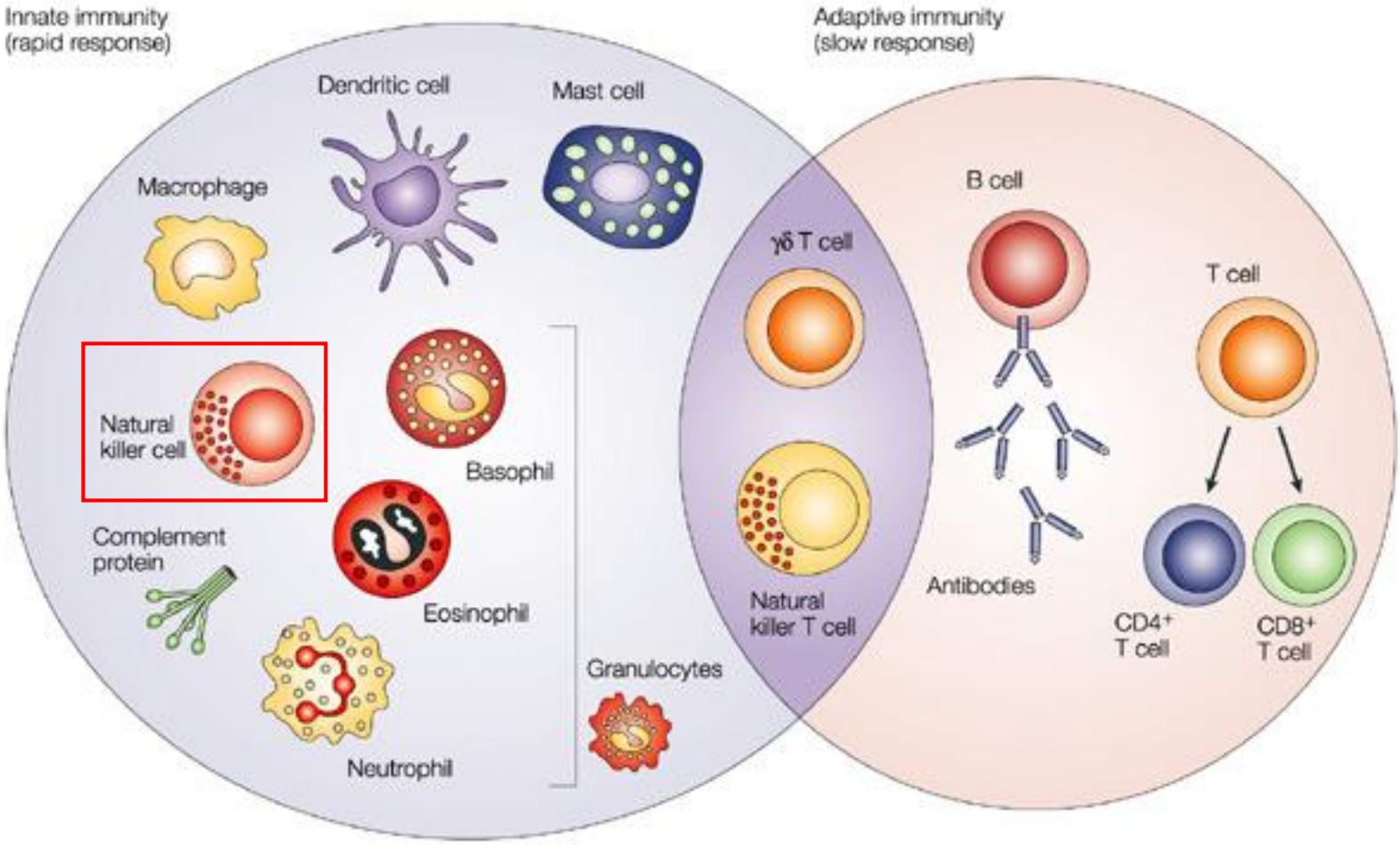
- **Adaptive immunity** adapts to previous experience; Stronger protection following secondary exposure.
- Very large number of distinct “antigen receptors” of T and B lymphocytes;
- generated by DNA rearrangement in each developing lymphocyte;
- clonal selection of lymphocytes recognizing antigen derived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

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



Nature Reviews | Cancer

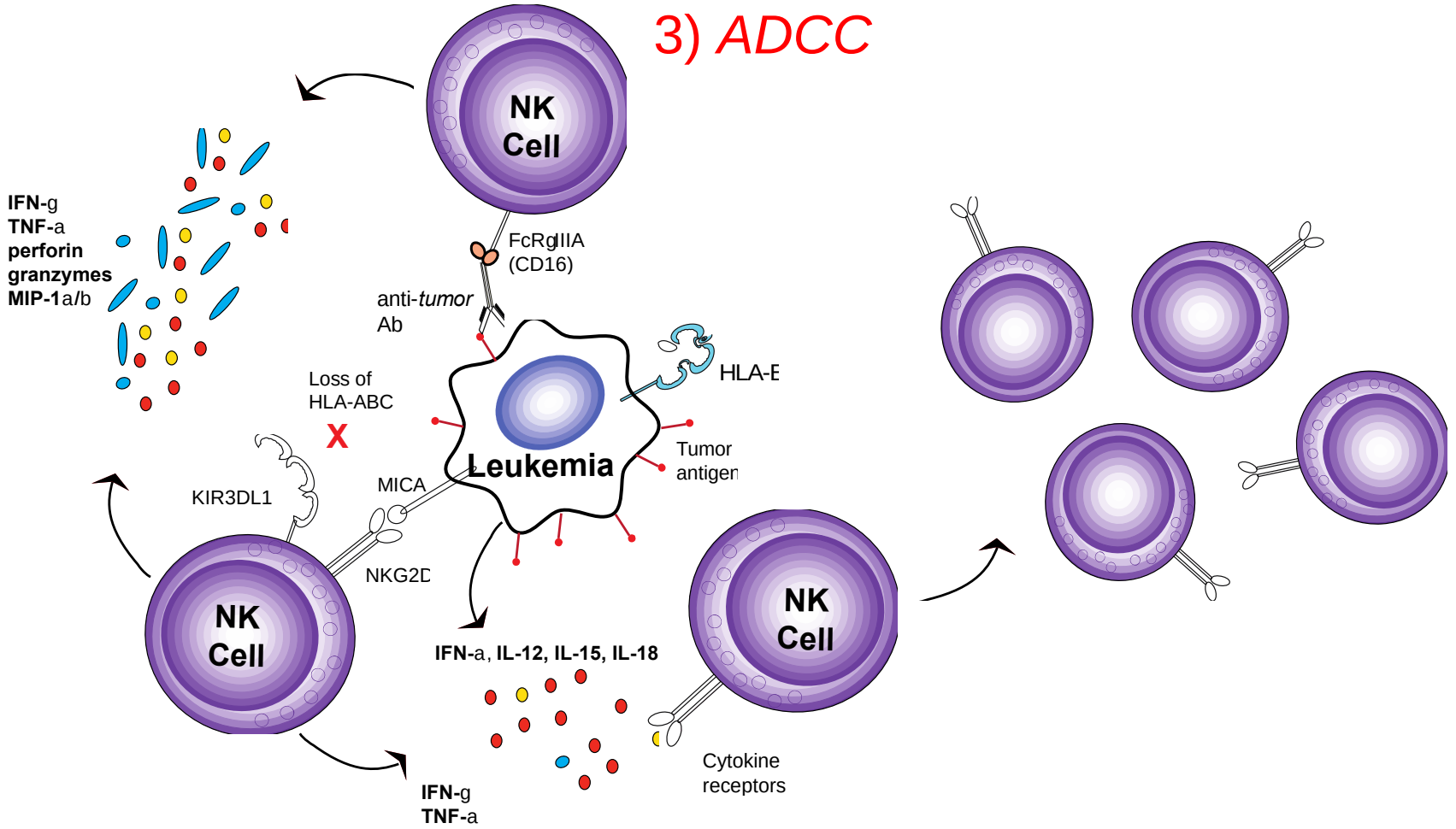
NK cells are an evolutionary predecessor to T cells



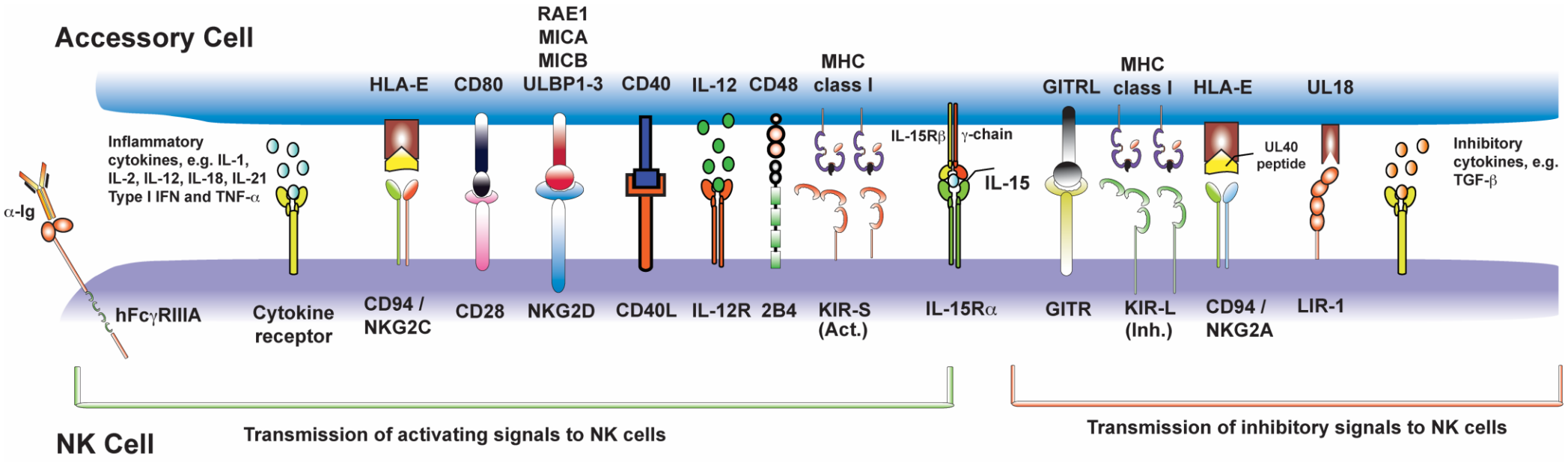
Nature Reviews | Cancer

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)

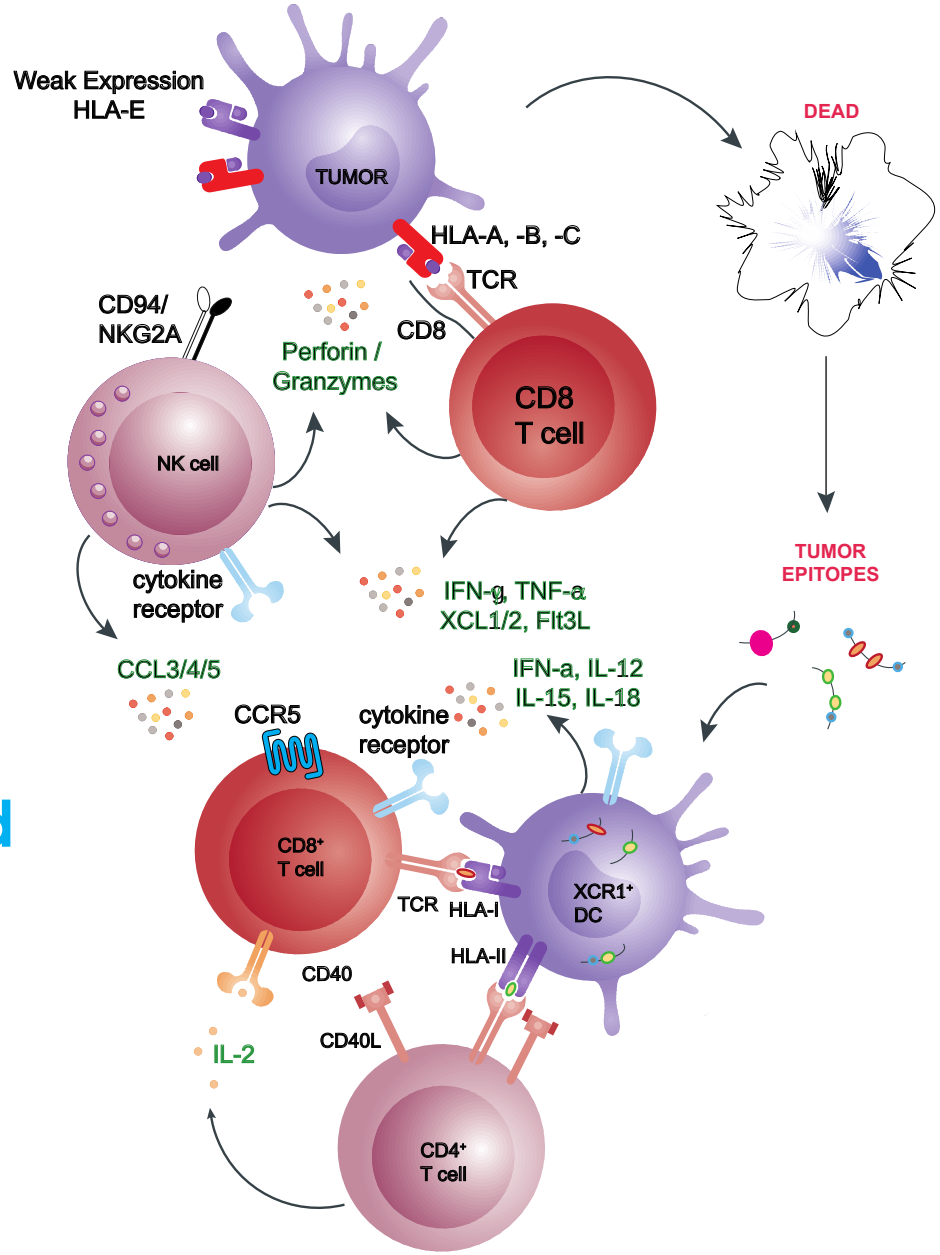


NK cell activation is regulated by the collective strength of inhibitory and activating signals



Horowitz, 2012 *Front Immunol*

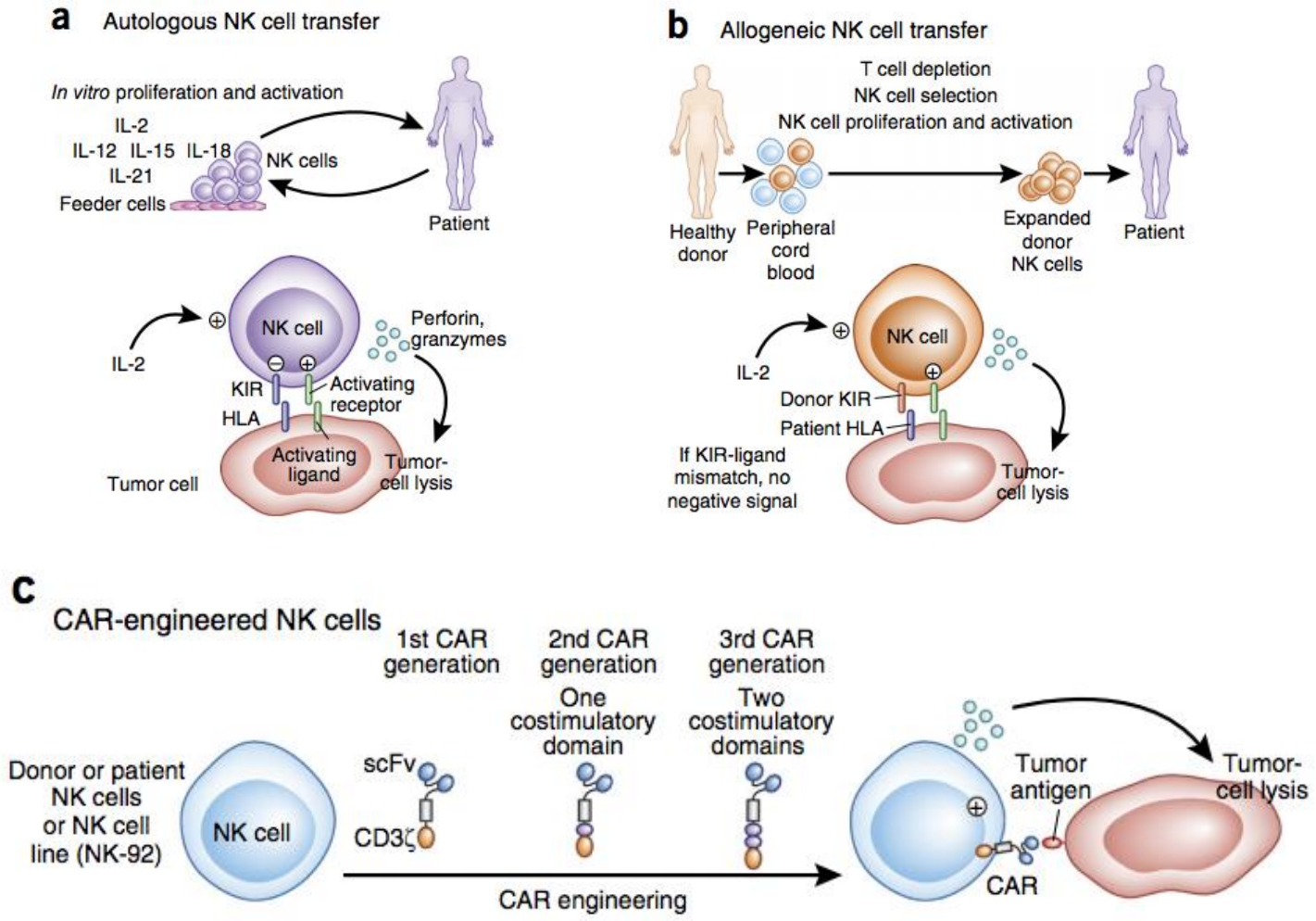
Quality of early tumor control determines availability of tumor epitopes for antigen presentation and priming of antitumor T cells



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

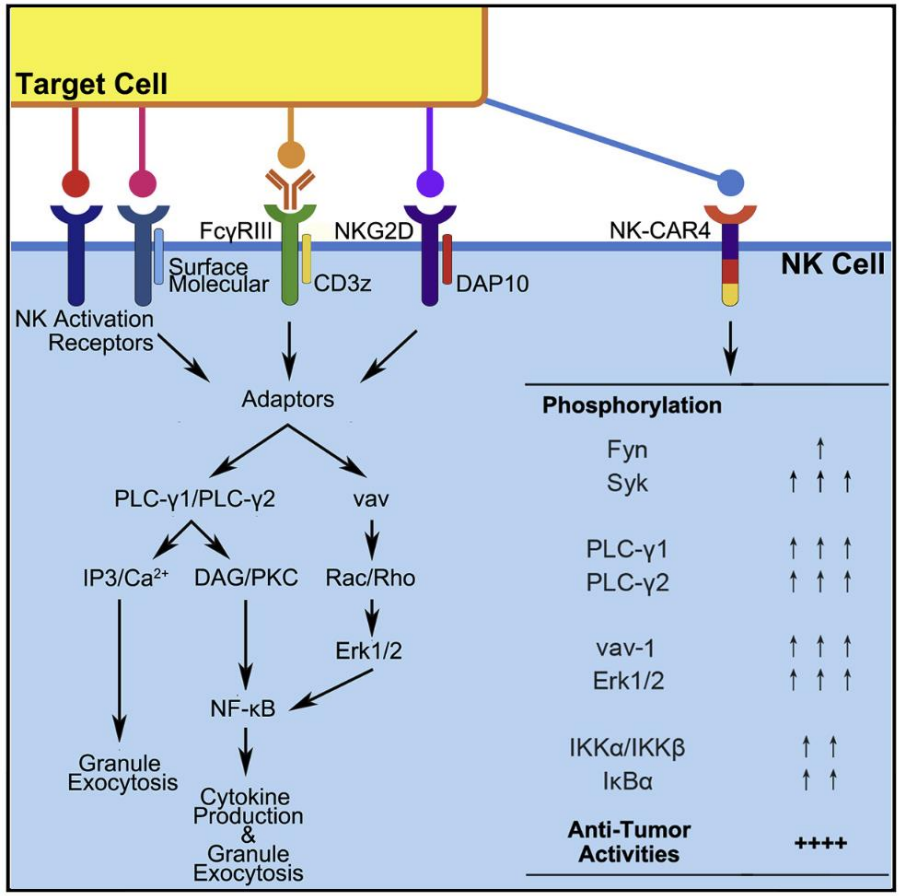
Adoptive NK cell transfer therapies



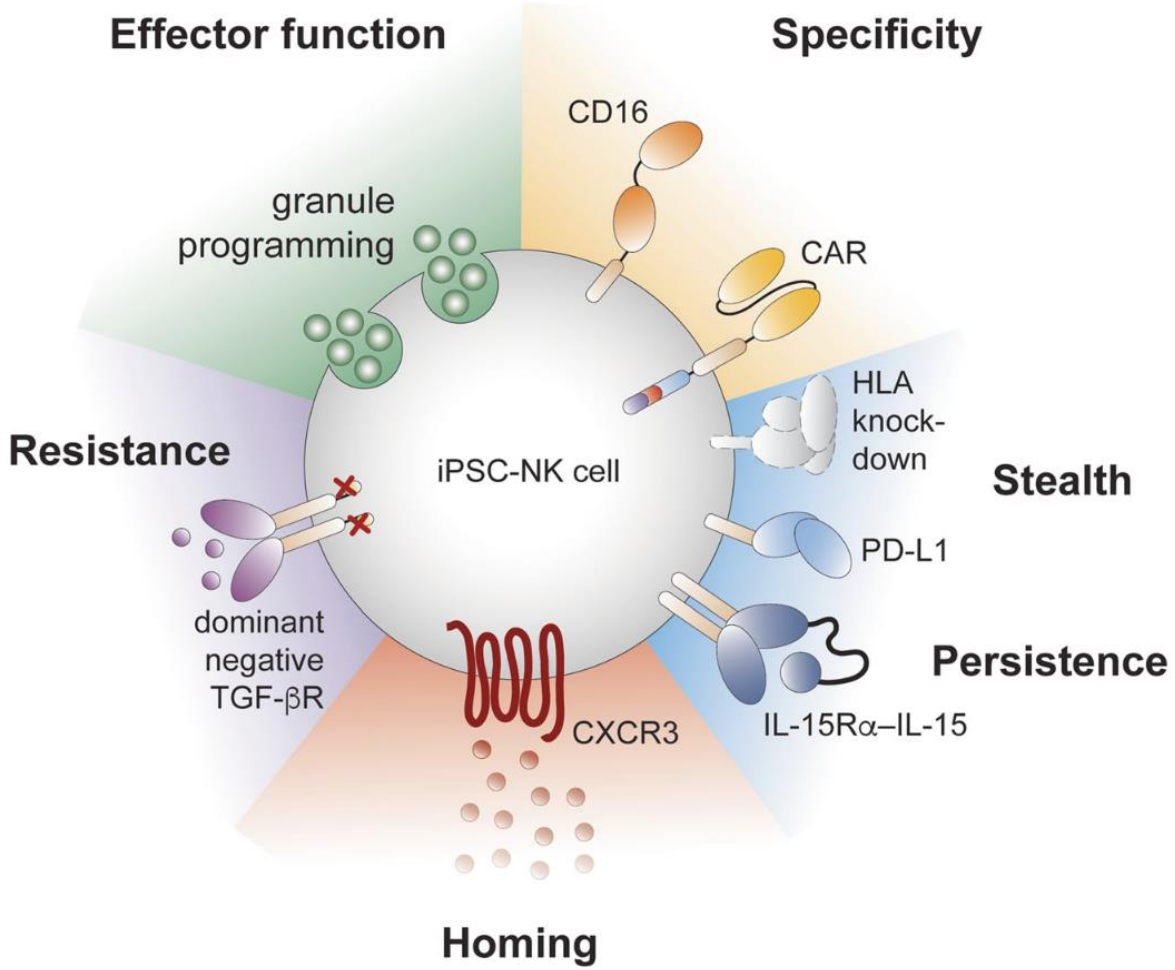
Li, 2018 *Cell Stem Cell*
NK-CAR-iPSCs-NK cells
hMesothelin
CD16, NKG2D, 2B4, CD137

Guillerey, 2016 *Nat Rev Immunol*

iPSC-derived NK cells can be enhanced for specific functions

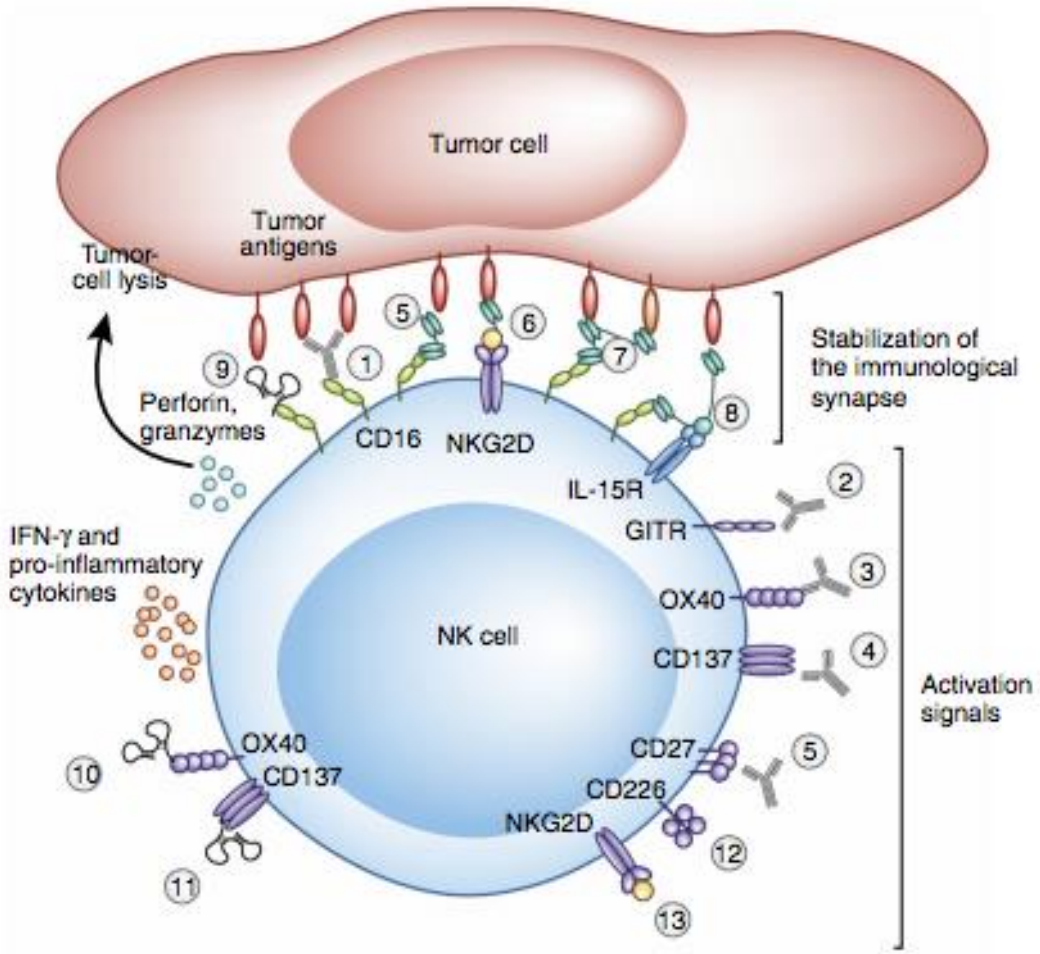


Li, 2018 *Cell Stem Cell*
NK-CAR-iPSCs-NK cells (hMesothelin)
CD16, NKG2D, 2B4, CD137



Saetersmoen, 2019 *Semin Immunopathol*

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

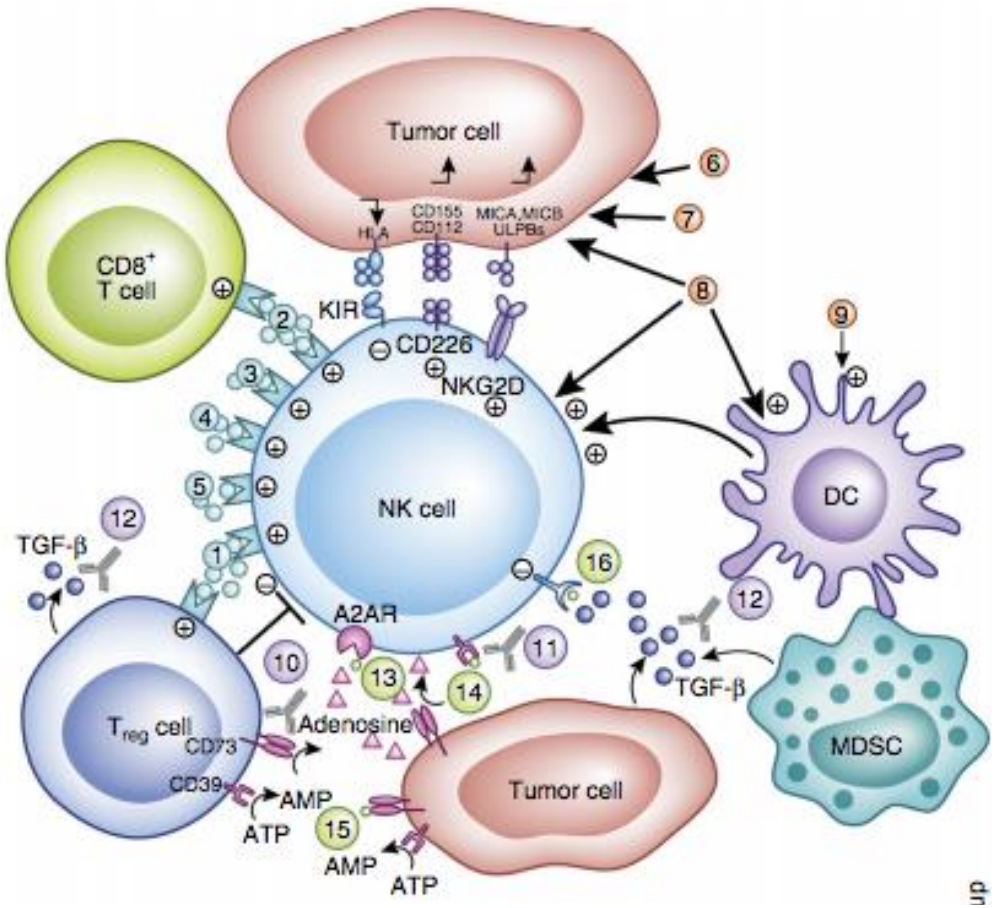
5. BiKE
6. NKG2D ligand–antitumour Fv fusion
7. TriKE that binds two different tumor antigens
8. TriKE that incorporates IL-15
9. Bispecific aptamer
10. OX40 agonistic aptamer
11. CD137 agonistic aptamer

Not developed yet

12. CD226 agonist
13. Soluble activating NKG2D ligand

Guillerey, 2016 *Nat Rev Immunol*

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

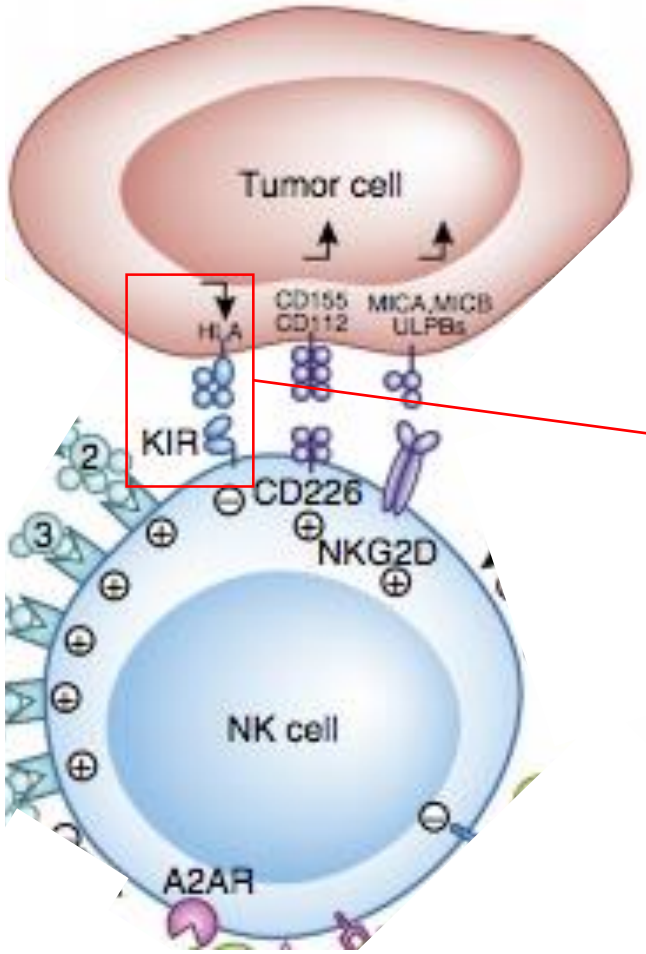
6. Genotoxic drugs (demythelating agents, histone deacetylases)
7. Proteasome inhibitors (bortezomib)
8. IMiDs (lenalidomide, pomalidomide)
9. Imatinib

Small-molecule inhibitors

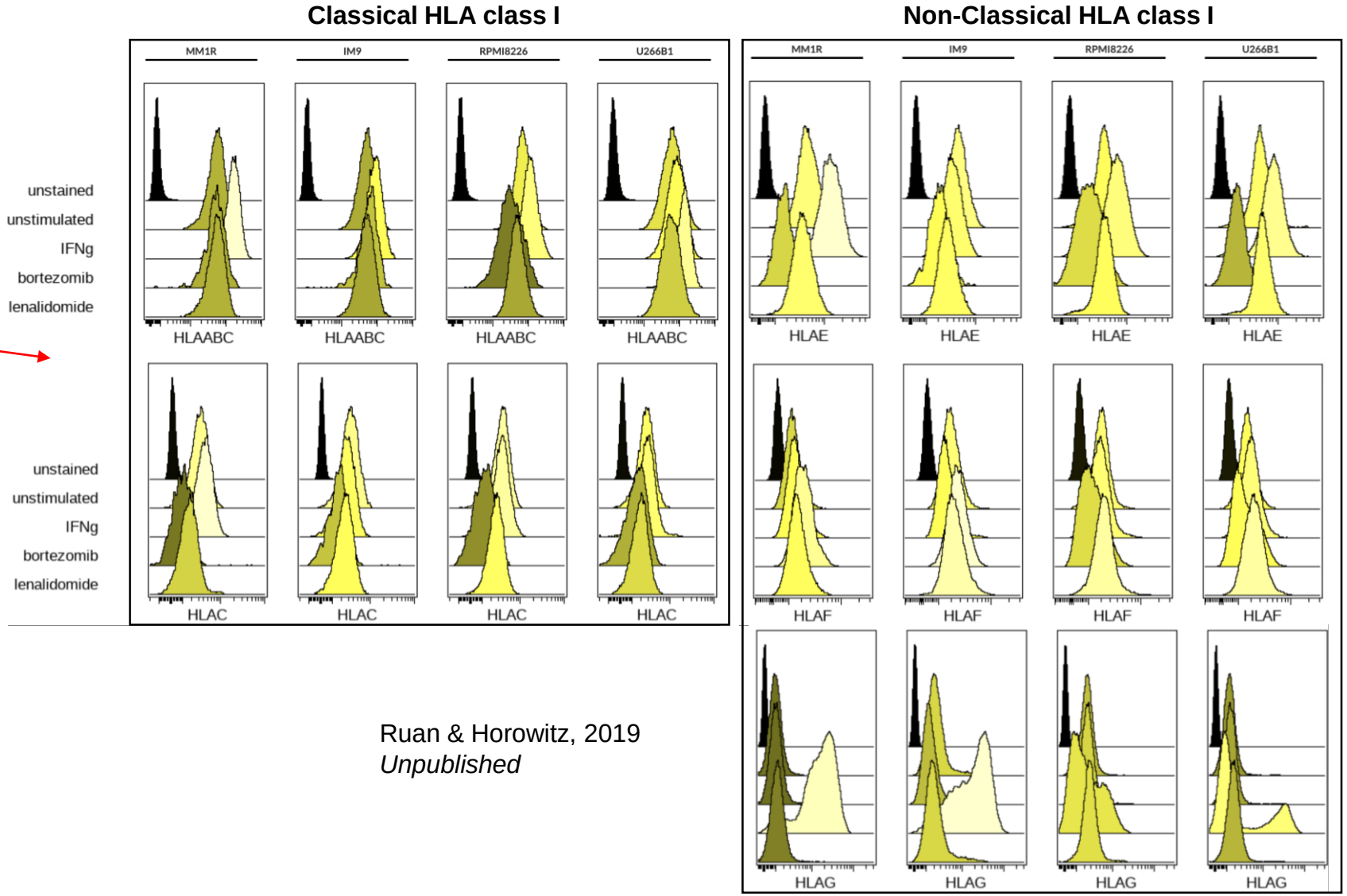
13. A2A receptor antagonist (PBF-509)
14. CD39 inhibitor (POM-1)
15. CD73 inhibitor (APCP)
16. TGF-β receptor inhibitor

Guillerey, 2016 *Nat Rev Immunol*

Bortezomib and lenalidomide have strong effects on expression of HLA-C and HLA-E

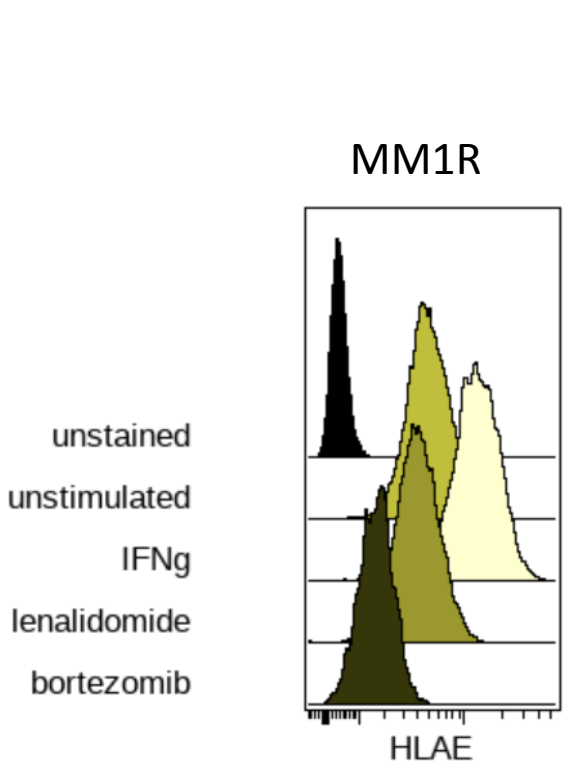


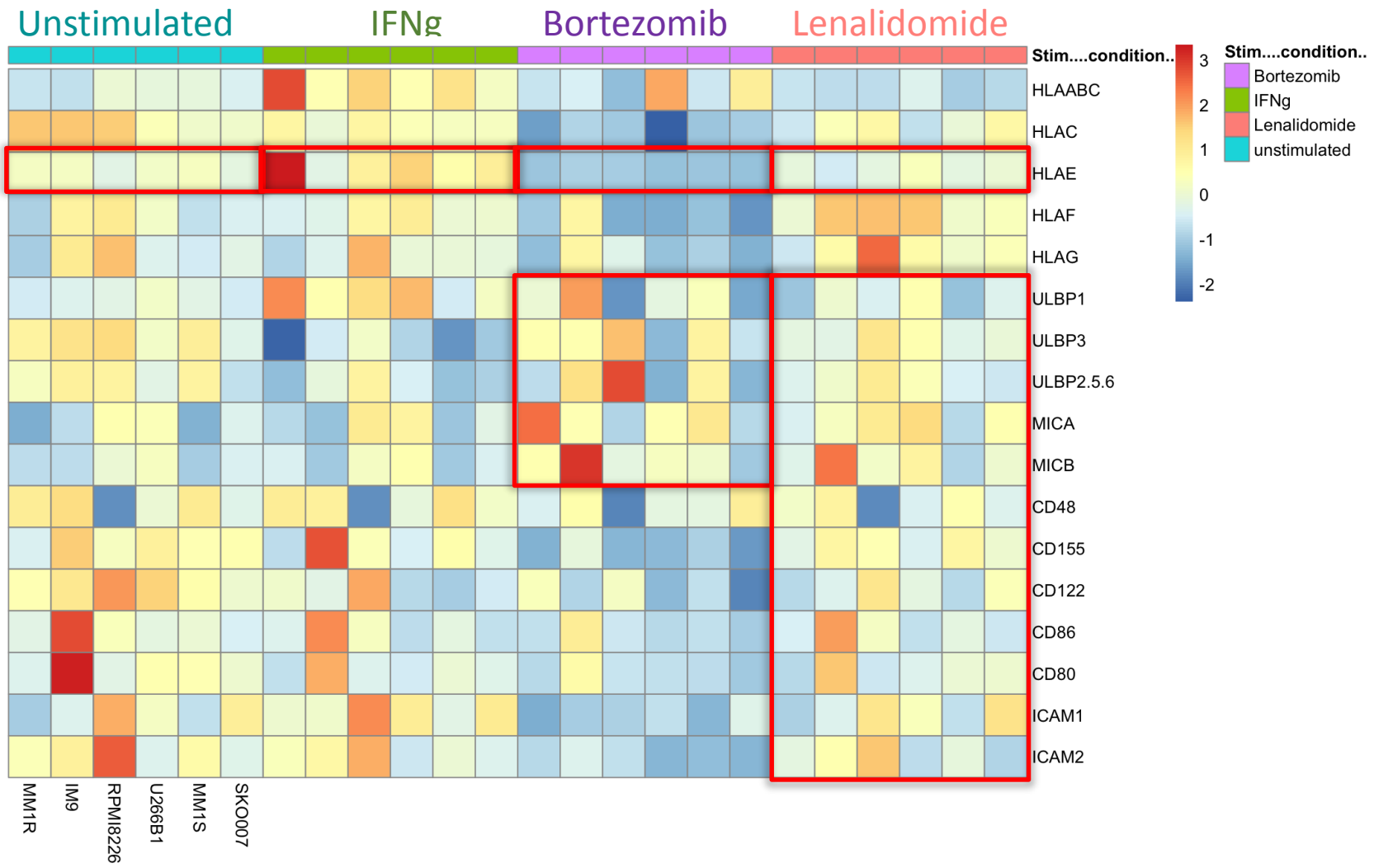
Guillerey, 2016 *Nat Rev Immunol*



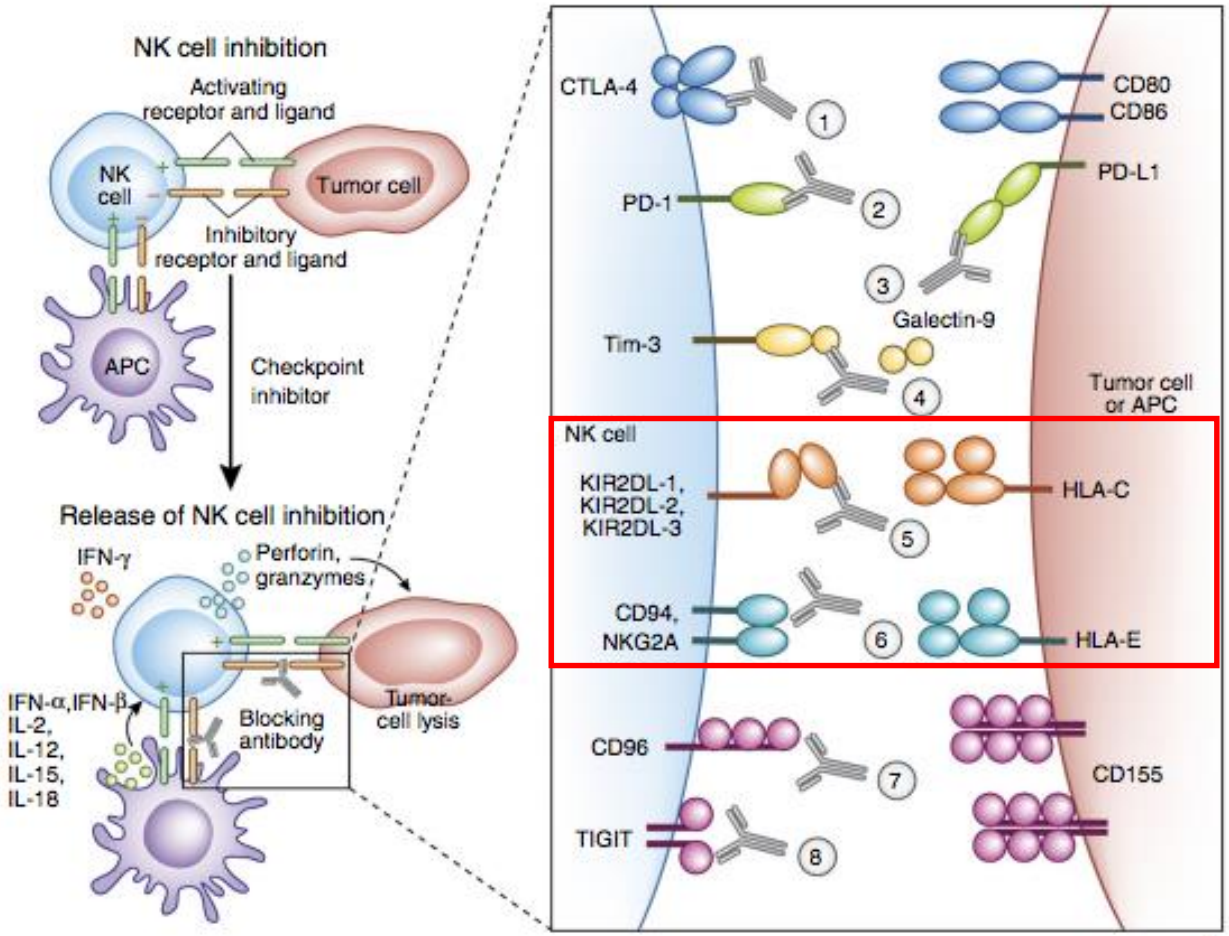
Ruan & Horowitz, 2019
Unpublished

Bortezomib and lenalidomide affect expression of activating ligands





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 Immunolc*

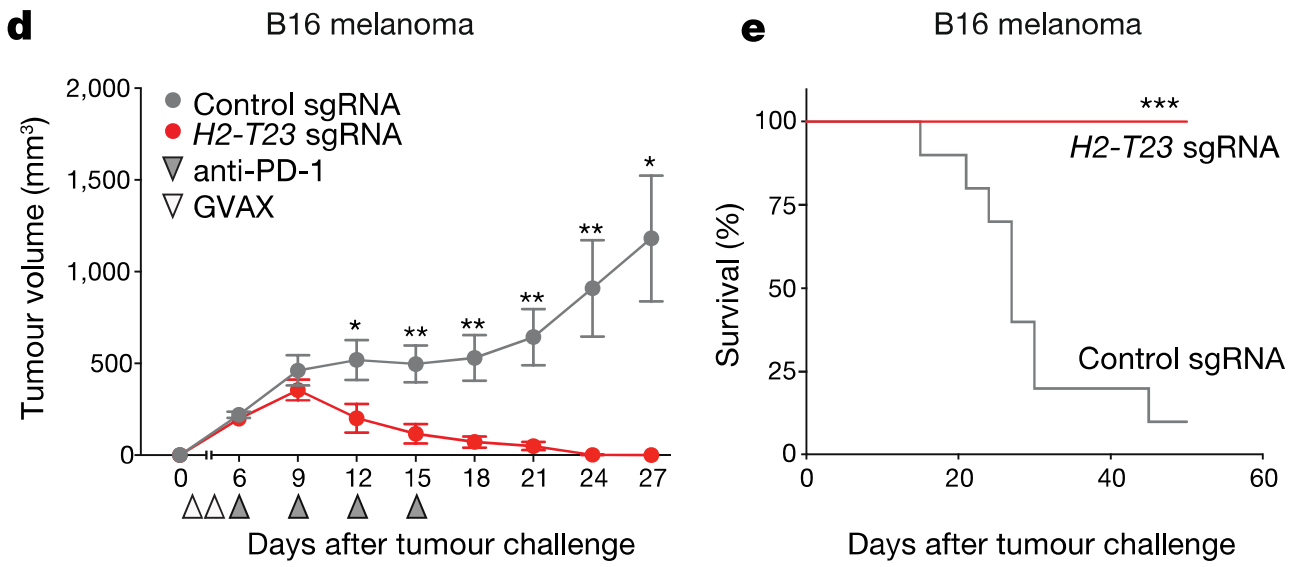
HLA-E expression on tumors may explain failure of checkpoint blockade monotherapies

ARTICLE

doi:10.1038/nature23270

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

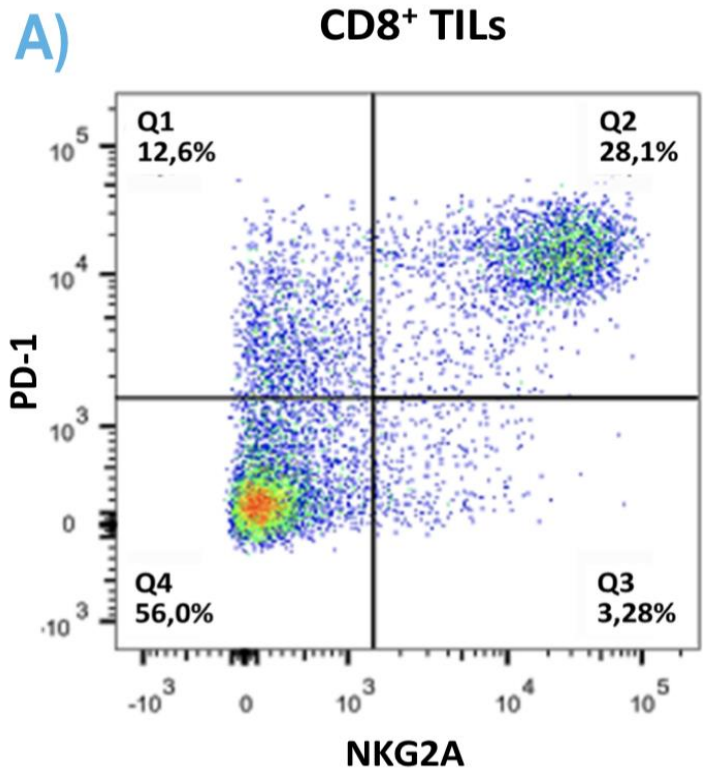
Robert T. Manguso[1,2,3], Hans W. Pope[1,3], Margaret D. Zimmer[1,3], Flavian D. Brown[1,2], Kathleen B. Yates[1,3], Brian C. Miller[1,3,4], Natalie B. Collins[1,3,5], Kevin Bi[1,3], Martin W. LaFleur[1,2], Vikram R. Juneja[6], Sarah A. Weiss[1], Jennifer Lo[7], David E. Fisher[7], Diana Miao[2,3], Eliezer 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

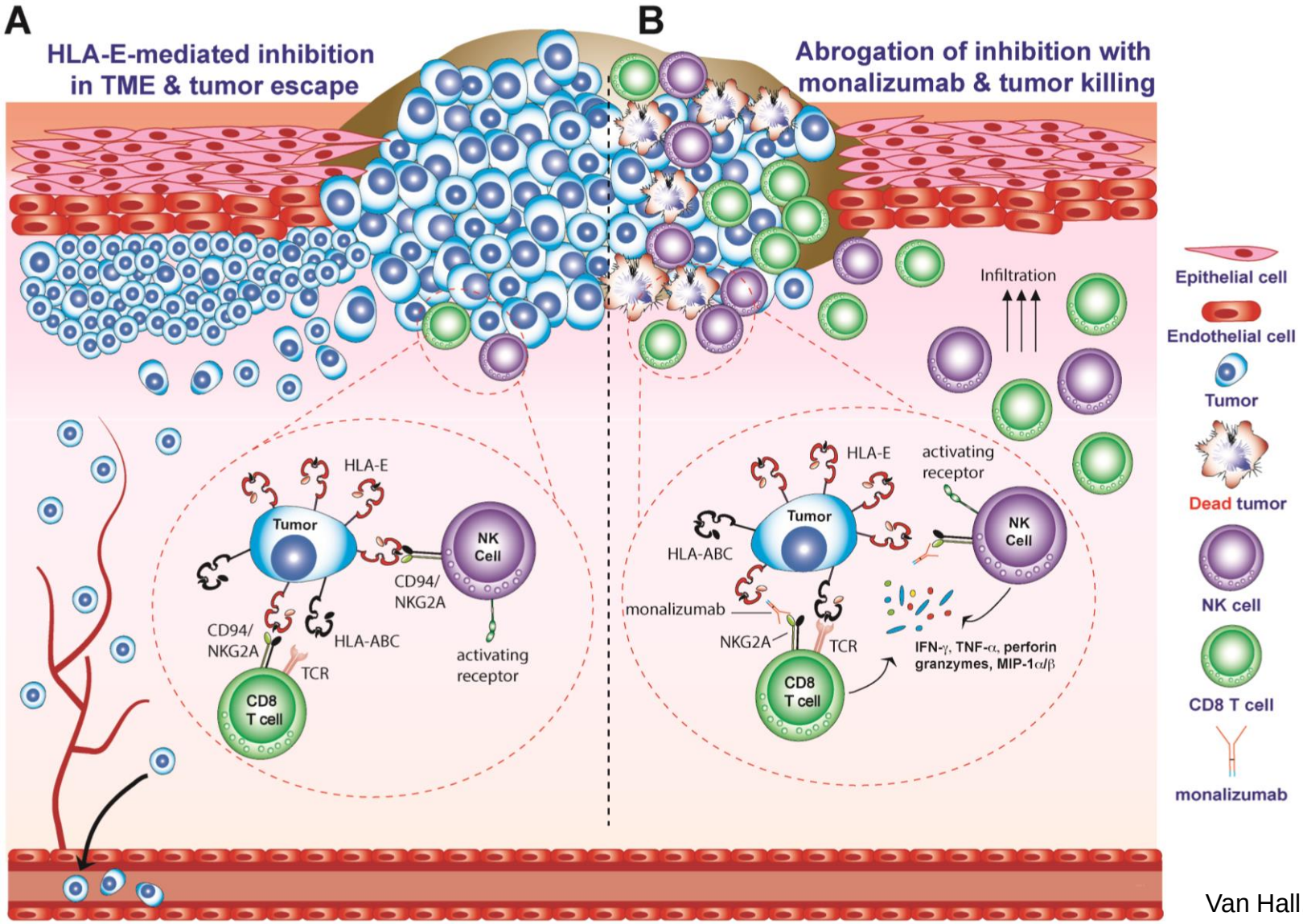
Combined blockade of PD-L1 and NKG2A checkpoints enhances anti-tumor CD8⁺ T cell response

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



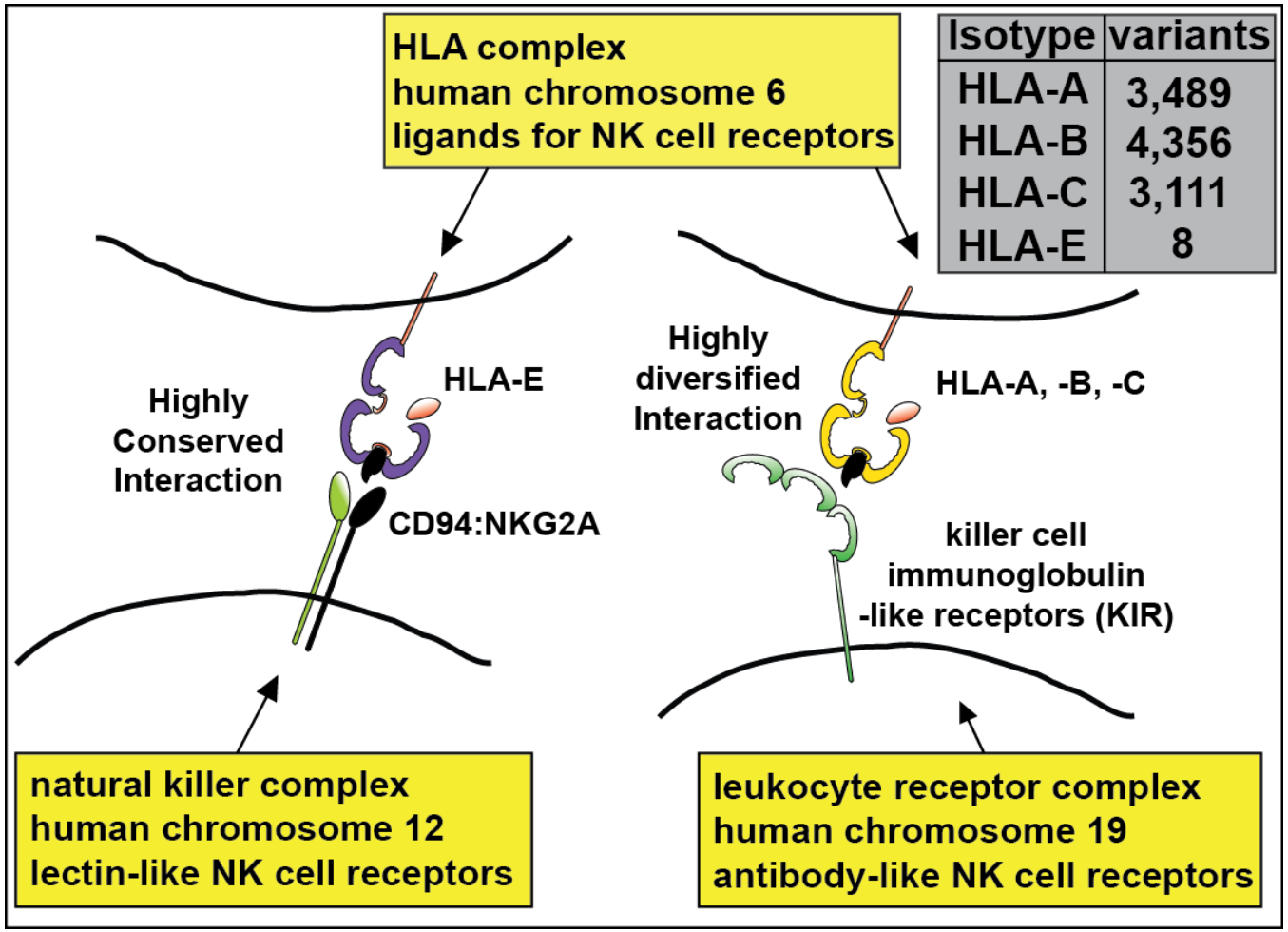
André, *Cell*, 2018

Overcoming HLA-E-mediated inhibition in the TME



Van Hall, *J Immunother Cancer 2019*

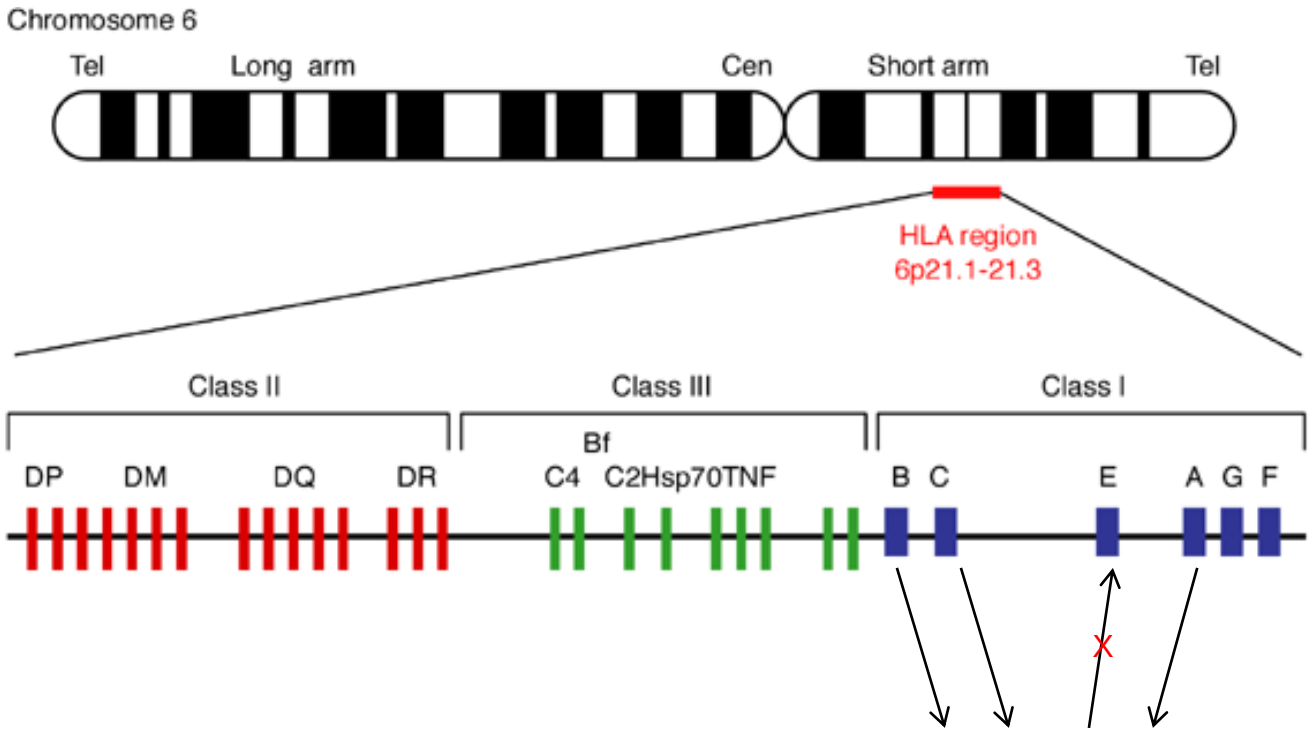
NK cells (and CD8 T cells) are regulated by system of Immunogenetics



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

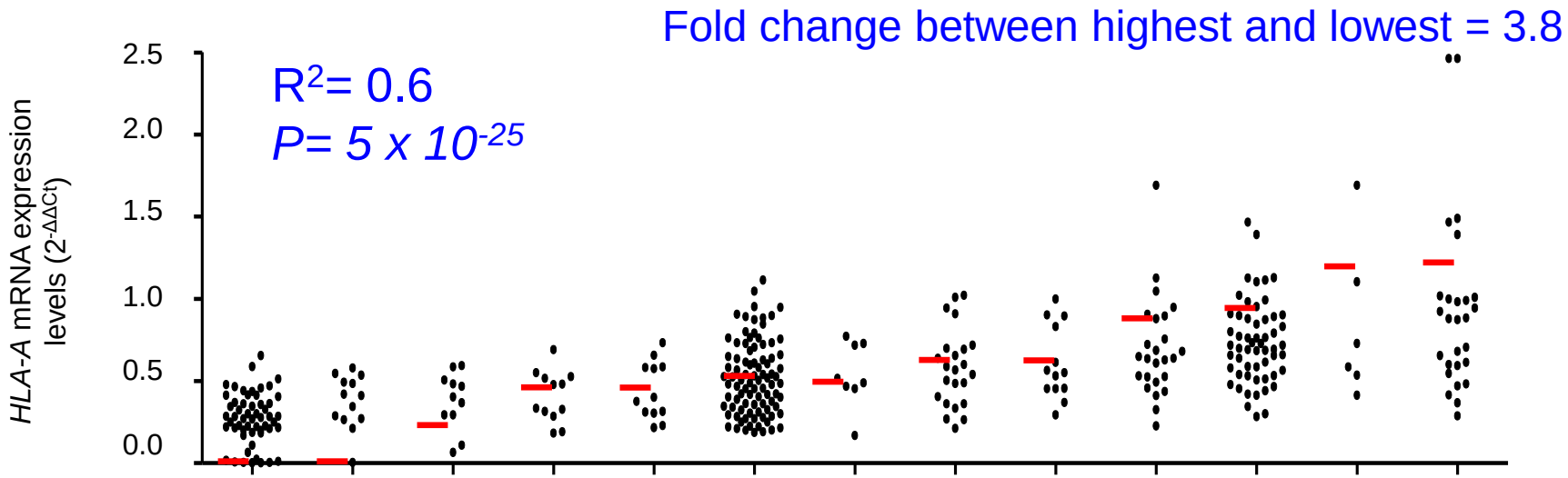
Robinson, 2017 *PLoS Genetics*
(IPD: Up to date list of HLA alleles)

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



Horowitz, 2016 *Sci Immunol*

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



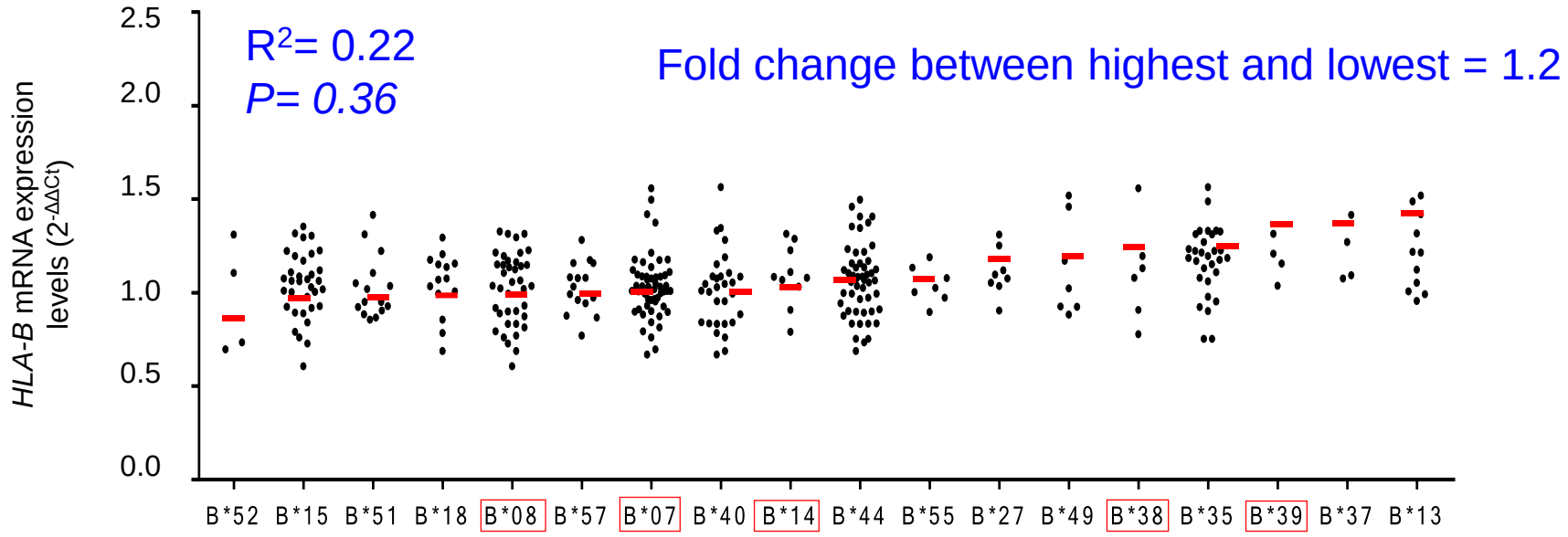
Allelic polymorphisms define broad range in transcription of HLA-A

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



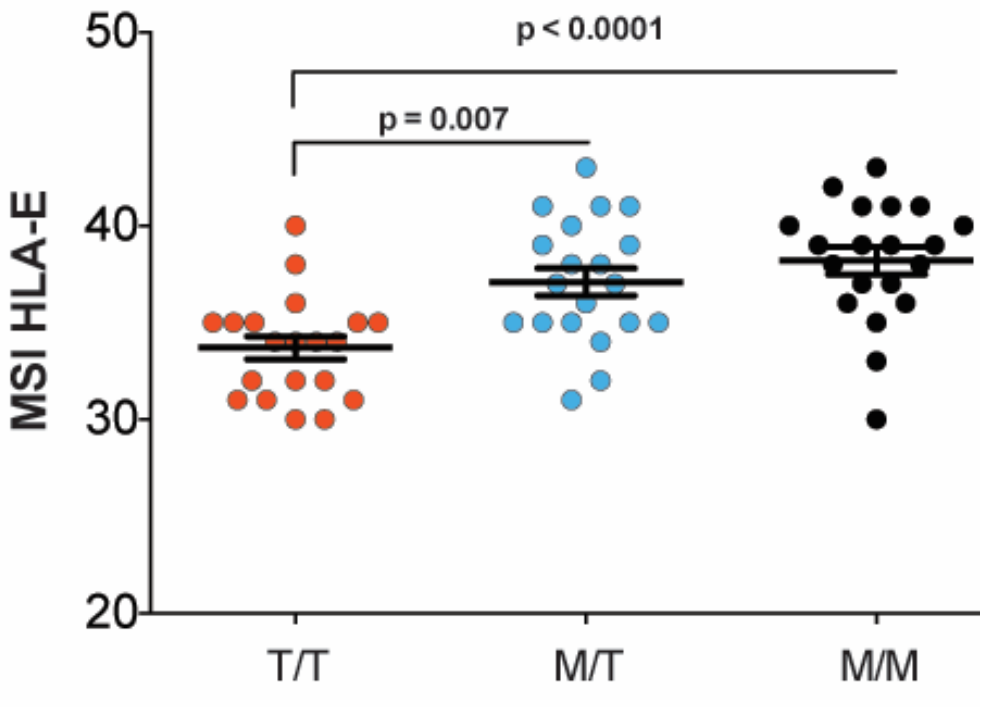
HLA-B is transcribed at very uniform levels

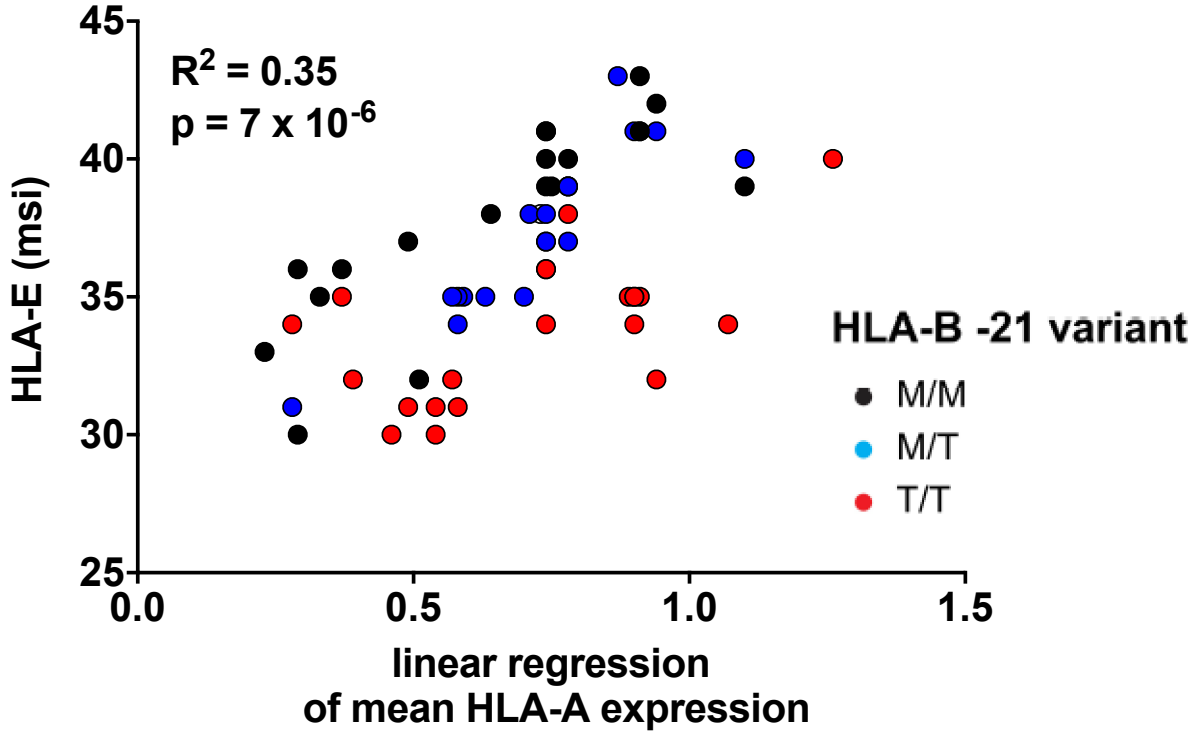
80% of alleles encode unsuitable HLA-E binding peptide: V**T**APRTLLL

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

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

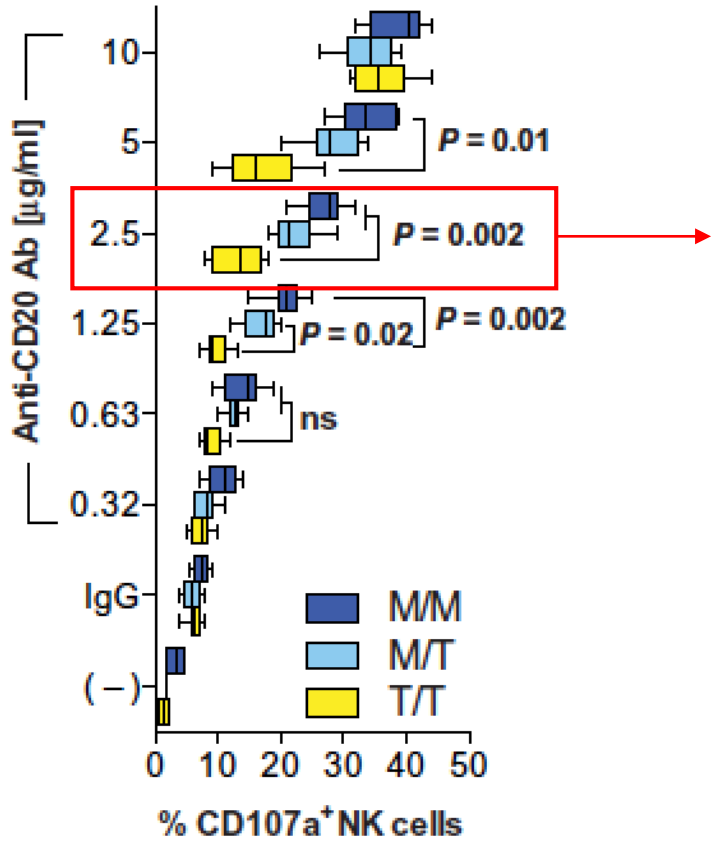
HLA-A and *HLA-B* cooperate to regulate expression of HLA-E

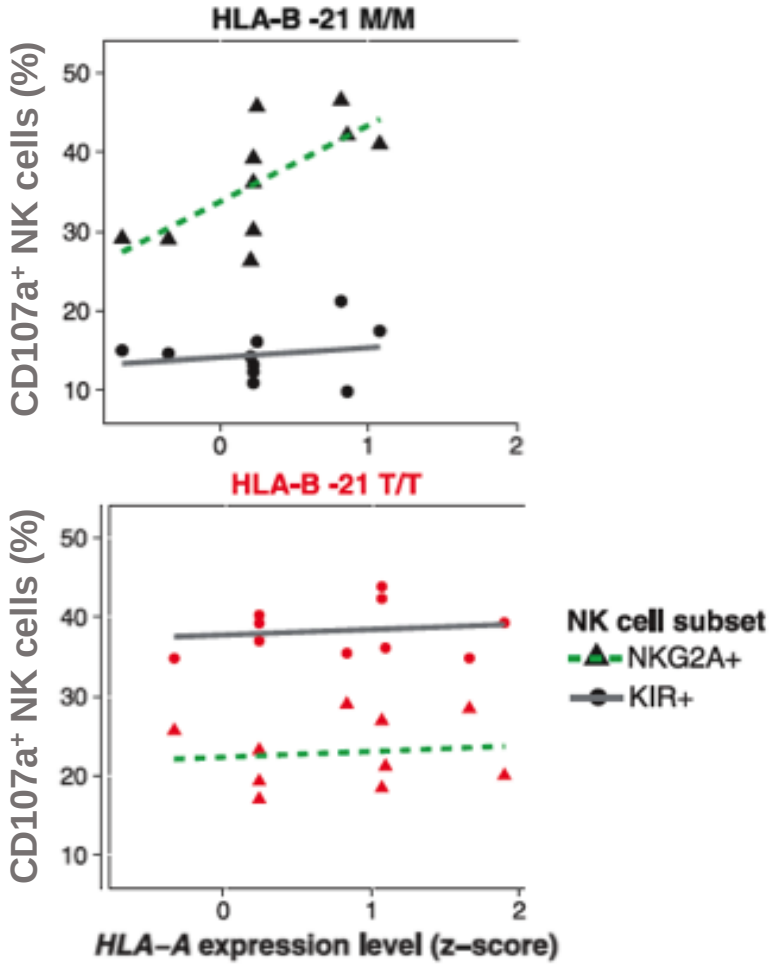




Horowitz, 2016 *Sci Immunol*

ADCC-activated NK cells mirror direction of education

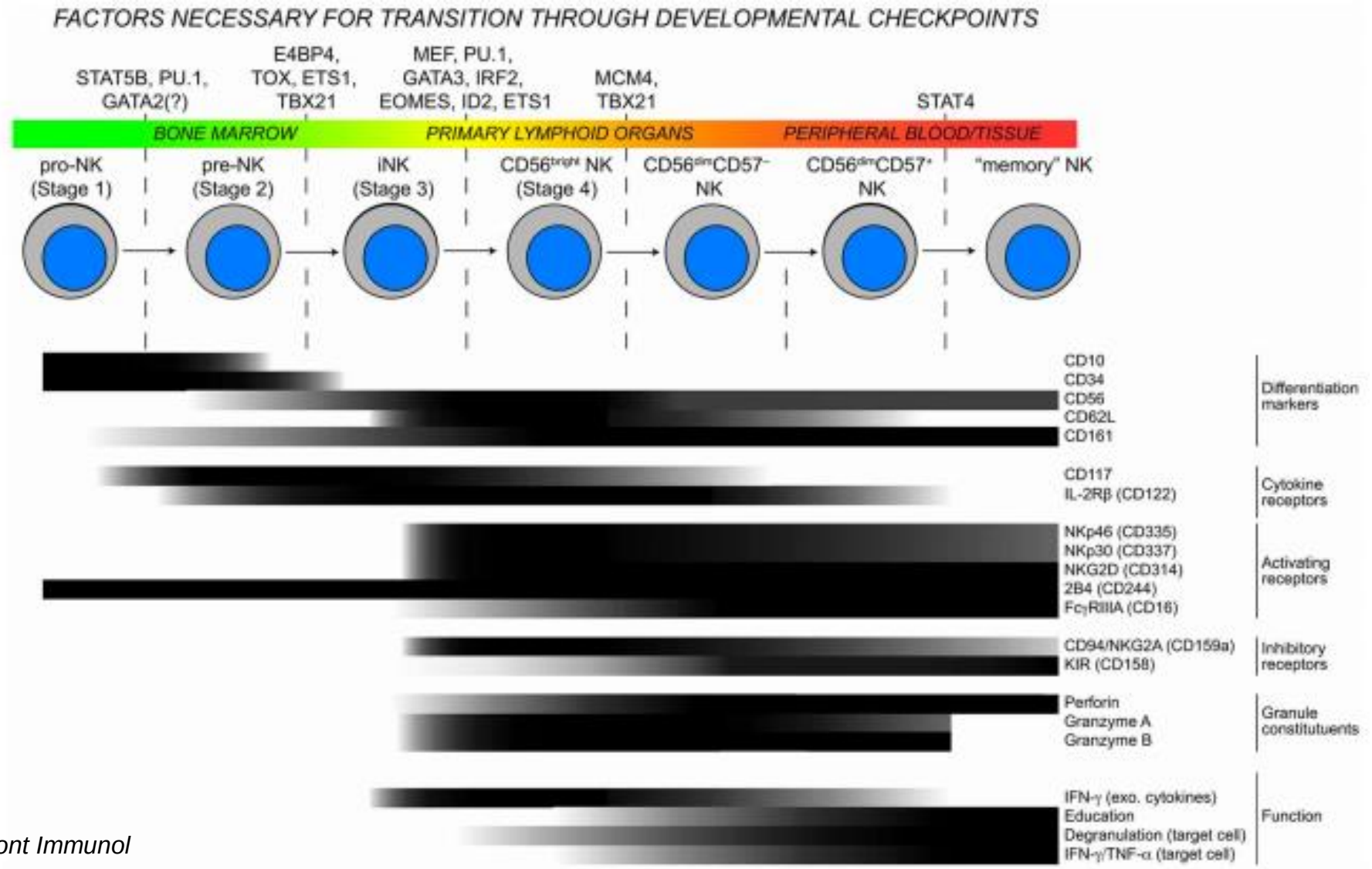




Horowitz, 2016 *Sci Immunol*

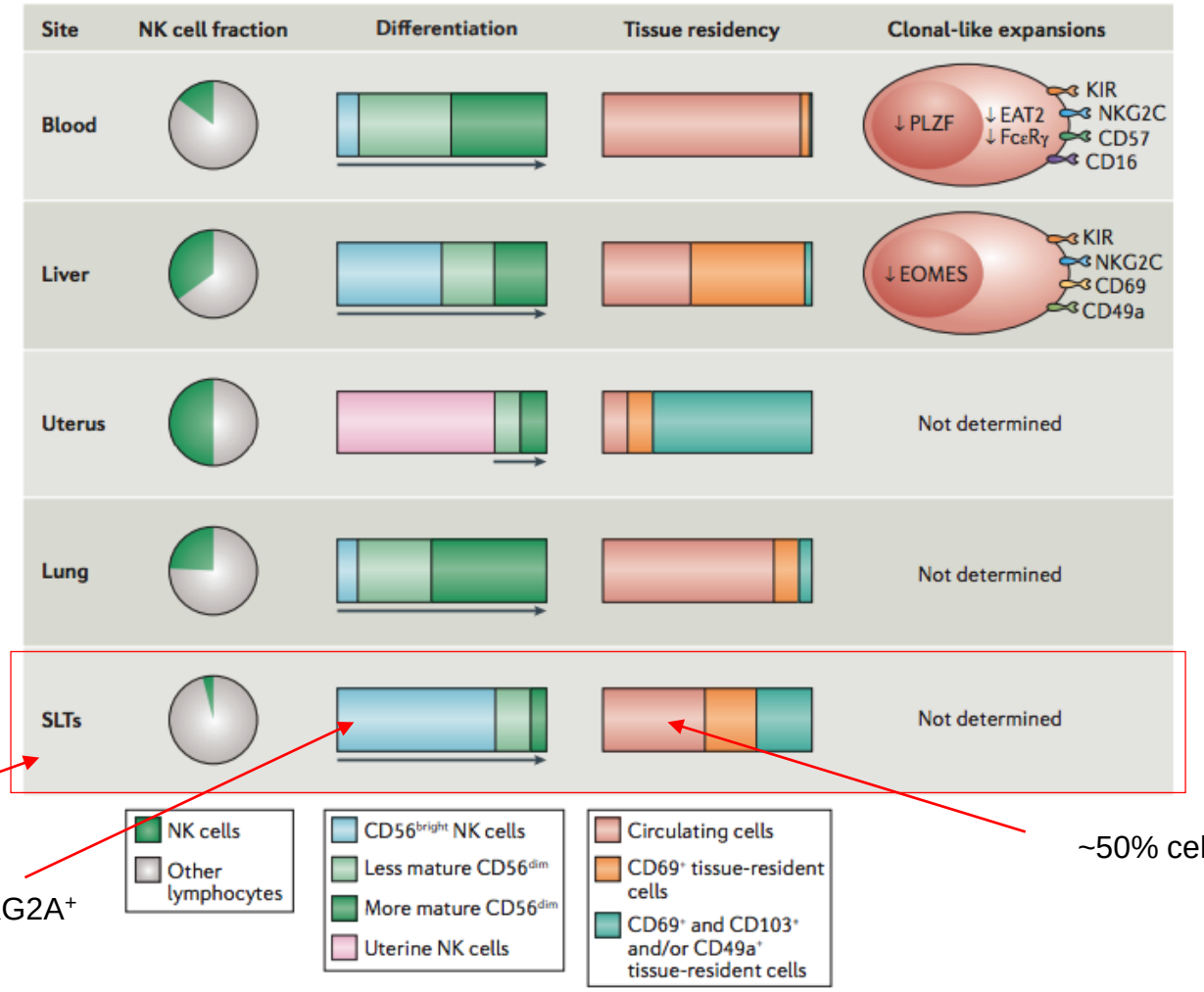
Ramsuran, *Science*, 2018

NK cell functions are acquired and regulated as NK cells mature



Cichocki, 2013 *Front Immunol*

Different distribution of NK cells in peripheral blood and tissues



Similar composition
In lymph nodes

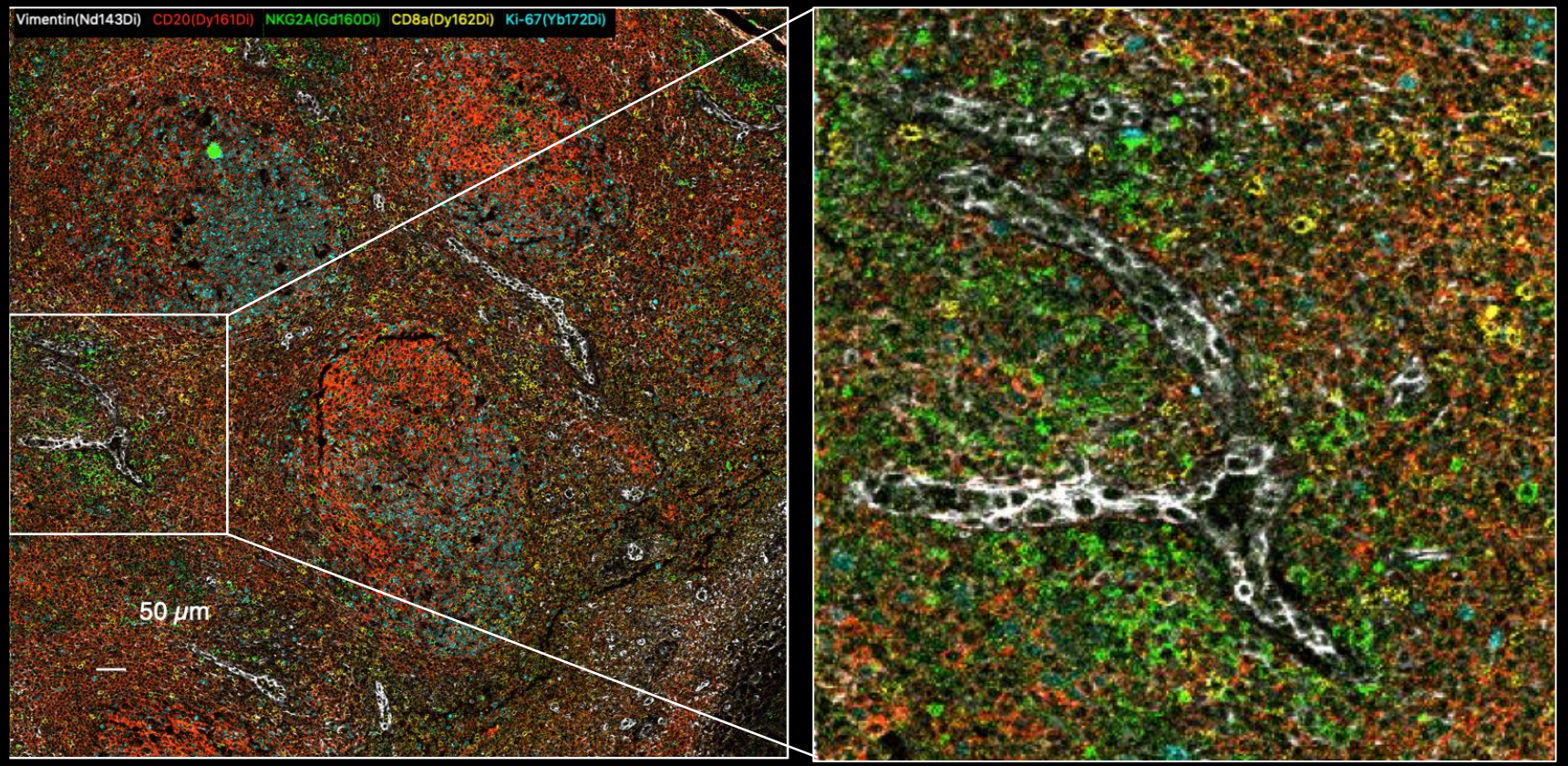
100% NKG2A^{+}

~50% cells represent circulating/non-resident cells

Bjorkstrom, 2016, *Nat rev Immunol*

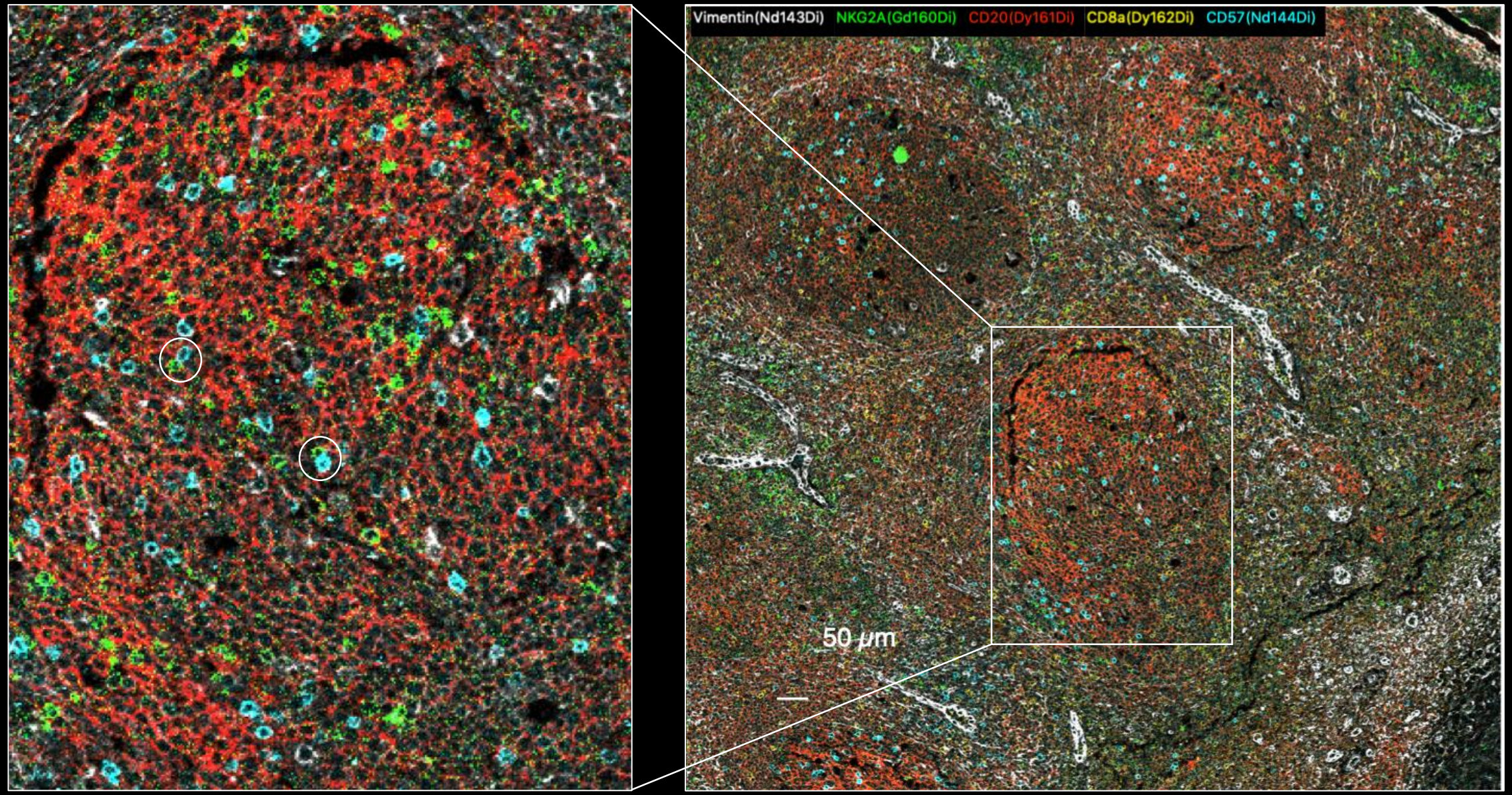
Profiling NK cells in human tissues revealed by Imaging Mass Cytometry

Metal	Marker	Function
141Pr	alpha-SMA	Structure
142Nd	CD5	T cells
143Nd	Vimentin	Structure
144Nd	CD57	NK terminal maturation
145Nd	Tbet	Transcription factor
146Nd	CD16	NK terminal maturation
147Sm	CR1	Dendritic cells
148Nd	Pan keratin	Structure
149Sm	CD200R	ILC1
150Nd	NKG2C	Adaptive NK cells
151Eu	CD31	Structure
152Sm	CD45	Lymphocytes
153Eu	GATA3	Transcription factor
154Sm	CD11c	Dendritic cells
155Gd	HLA-C	MHC
156Gd	HLA-E	MHC
158Gd	NKp46	NK cells
159Tb	CD56	NK cells/stroma
160Gd	NKG2A	NK cells
161Dy	CD20	B cells
162Dy	CD8a	T cells
163Dy	CD94	NK cells
164Dy	CD117	NK precursors
165Ho	CXCR6	Tissue resident NK
166Er	CD68	Monocytes/macrophages
167Er	Granzyme B	Mature NK cells
168Er	CD127	NK cell precursors
169Tm	Collagen type I	Structure
170Er	Eomes	Transcription factor
171Yb	RORgt	Transcription factor
172Yb	KI67	Cell proliferation
173Yb	CD122	NK cell precursors
174Yb	NKp80	NK cells
175Lu	CD34	NK cell precursors
176Yb	Mast cell tryptase	Mast cells



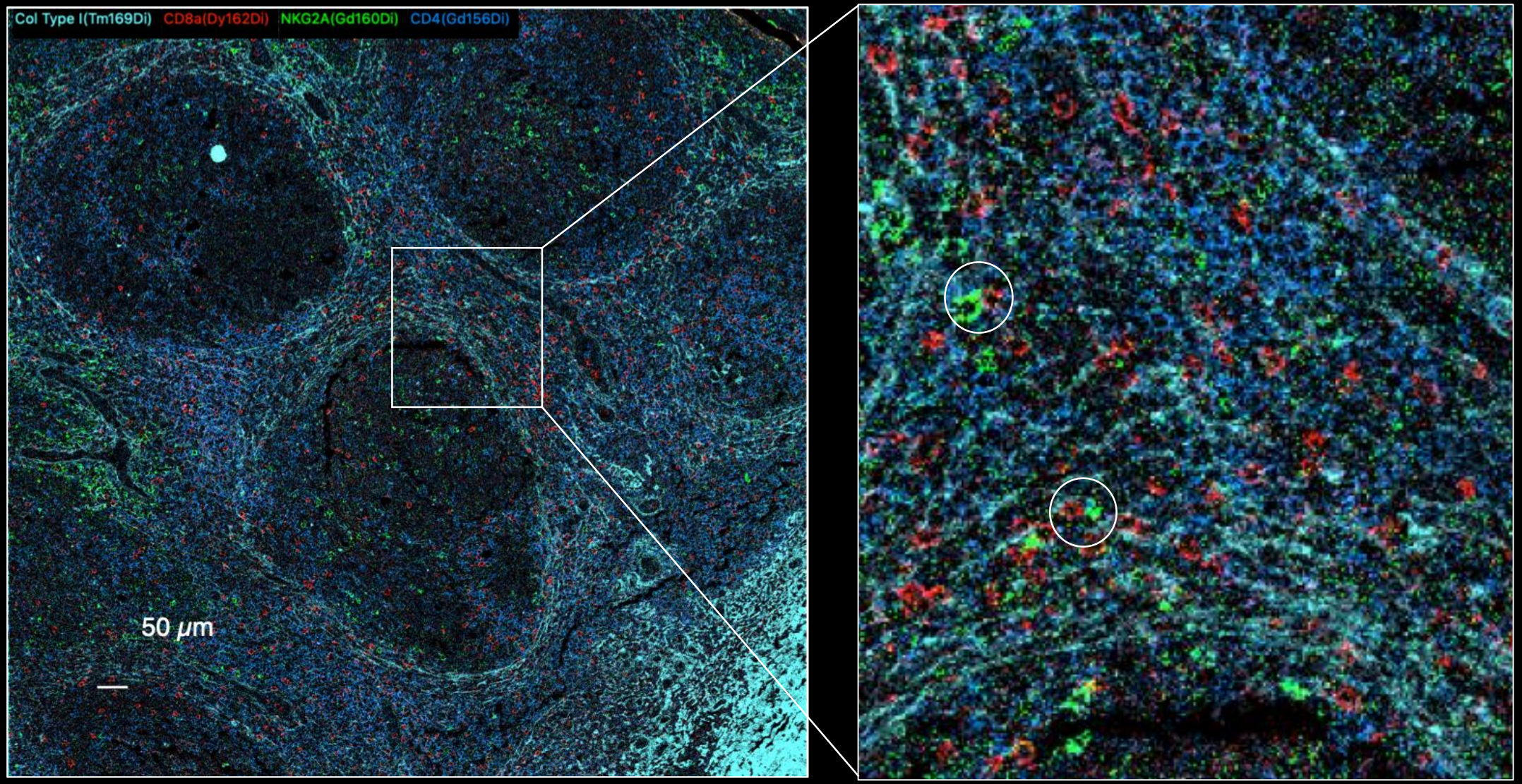
Elemento, Mace & Horowitz, *Unpublished*

NK cells are evenly distributed in a germinal center and interact with neighboring lymphocytes



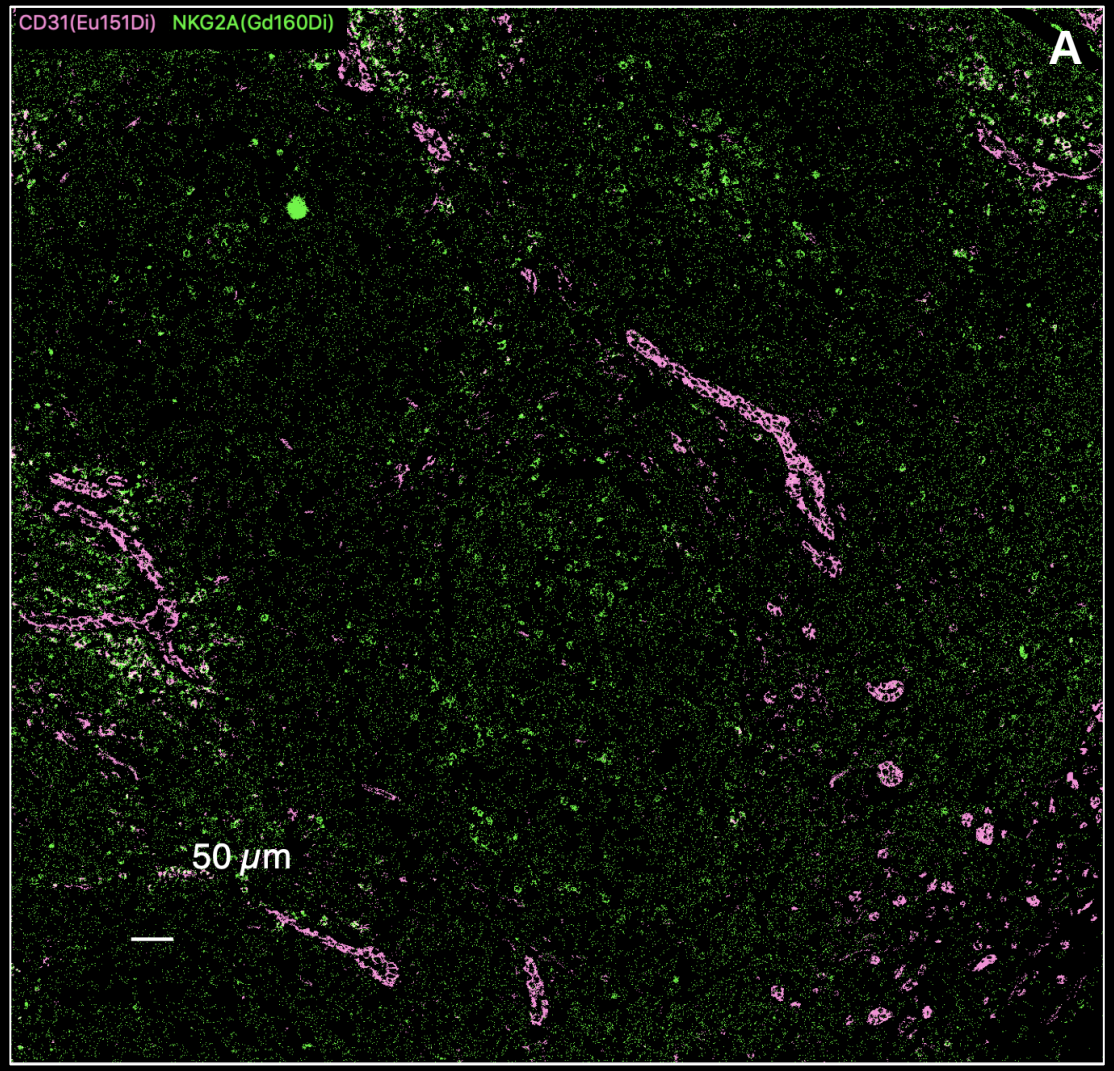
Elemento, Mace & Horowitz, *Unpublished*

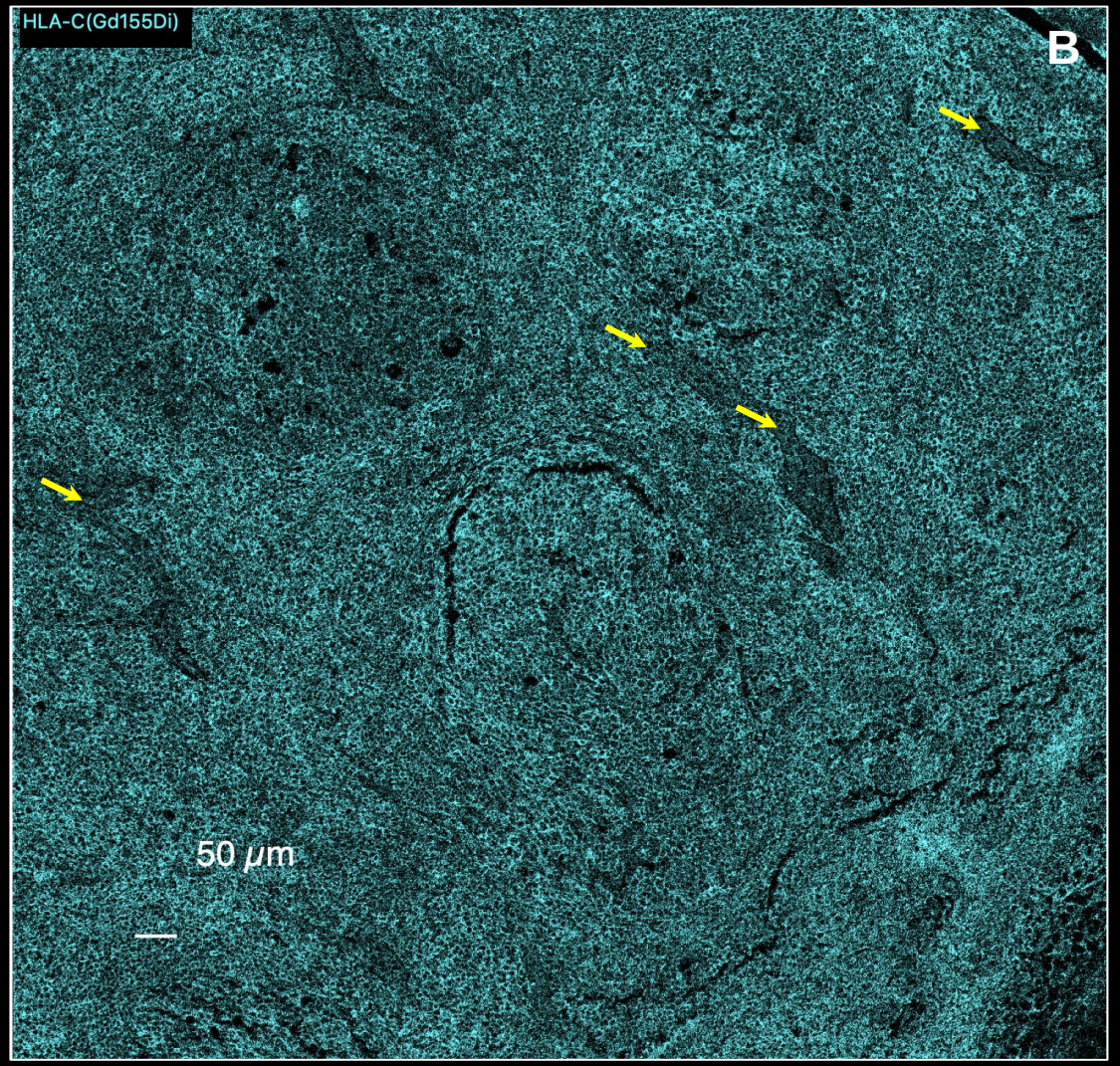
NKG2A expression is restricted to NK cells in healthy tonsil tissue



Elemento, Mace & Horowitz, *Unpublished*

Accumulation of NK cells by endothelial cells lacking expression of HLA-C



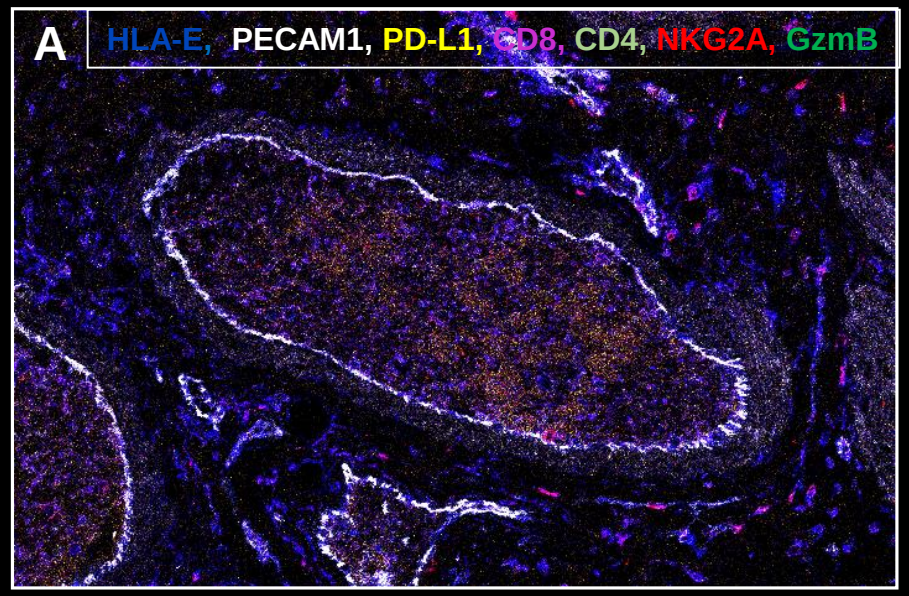


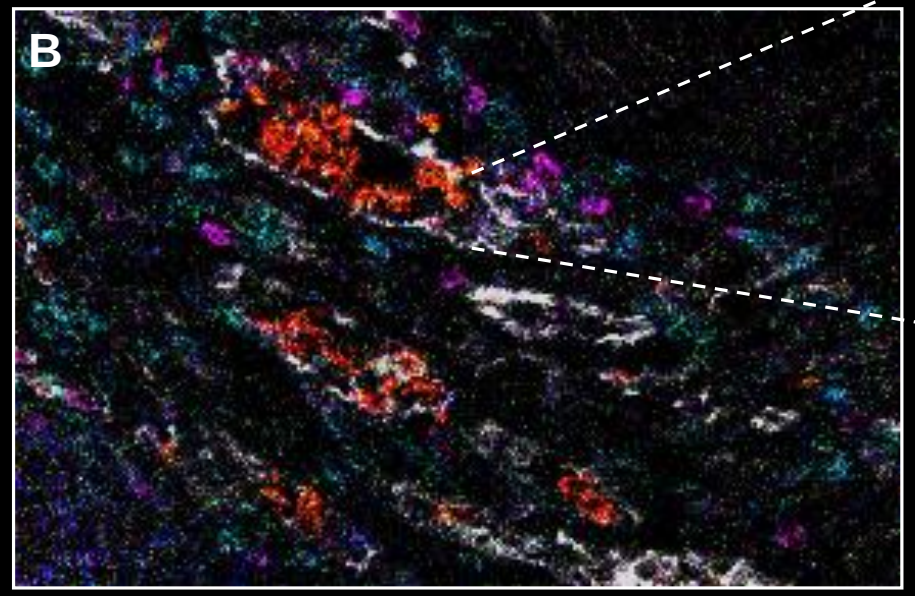
Elemento, Mace & Horowitz, *Unpublished*

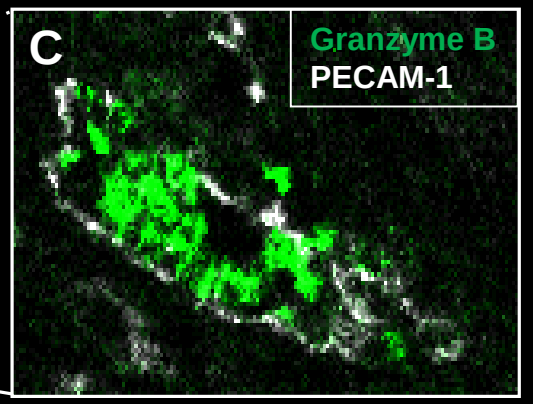
HLA-E expression correlates with degree of NK cell infiltration in the TME

High HLA-E expression

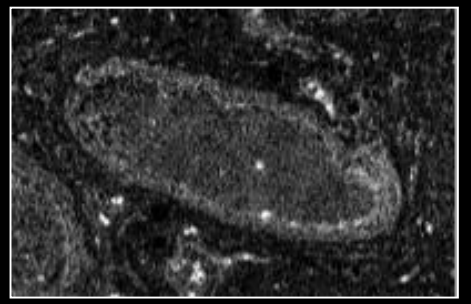
Low HLA-E expression



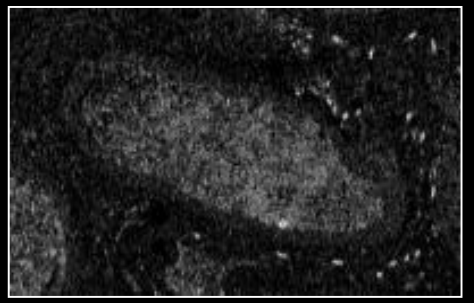




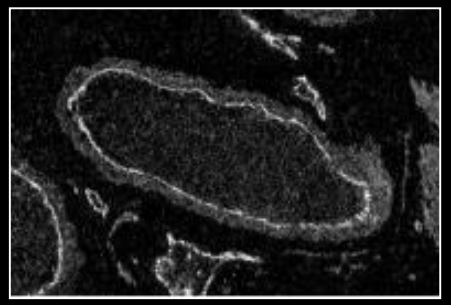
Granzyme B



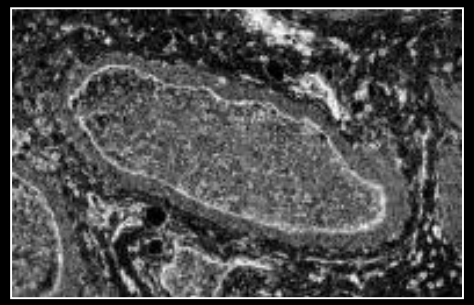
CD8



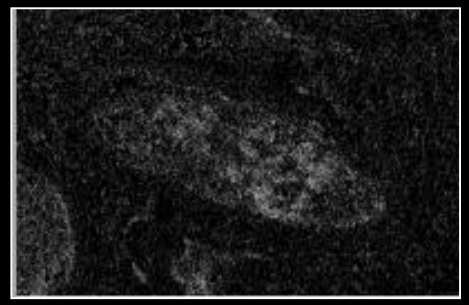
PECAM-1



HLA-E



PDL-1



Cytokeratin-7

Treatment-naive, muscle-invasive bladder cancer patients before nivolumab therapy

Elemento, Mace & Horowitz, *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