

Innovations with NK Cells

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Disclosures

InnDura Therapeutics (co-founder & SAB)

Parker Institute for Cancer Immunotherapy (member/funding)

Glycostem (SAB, paid consultant)

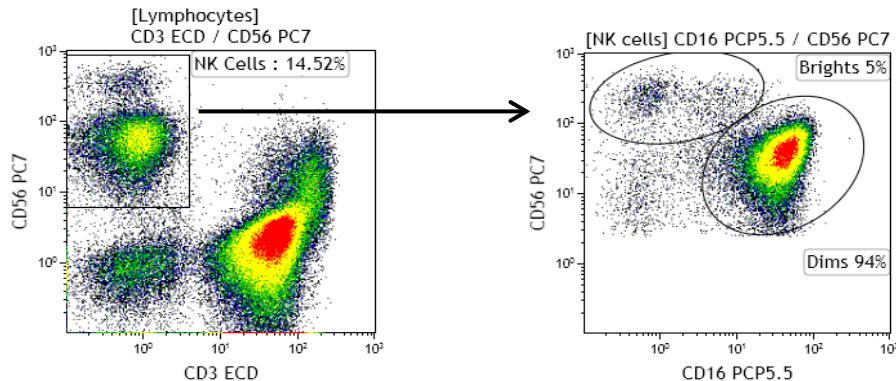
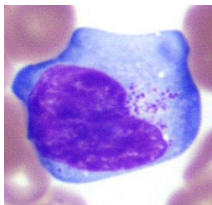
xNK Therapeutics (SAB)

Skyline Therapeutics (sponsored research)

CRISPR Therapeutics (sponsored research)

NK Cells and their Key Functions

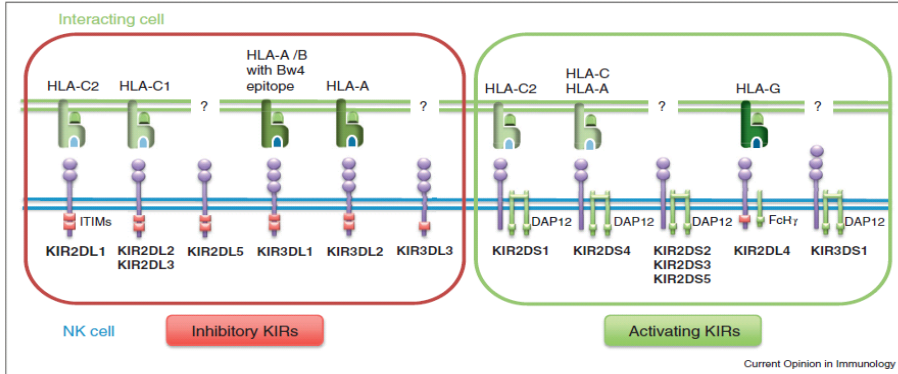
5-15% of PB lymphs



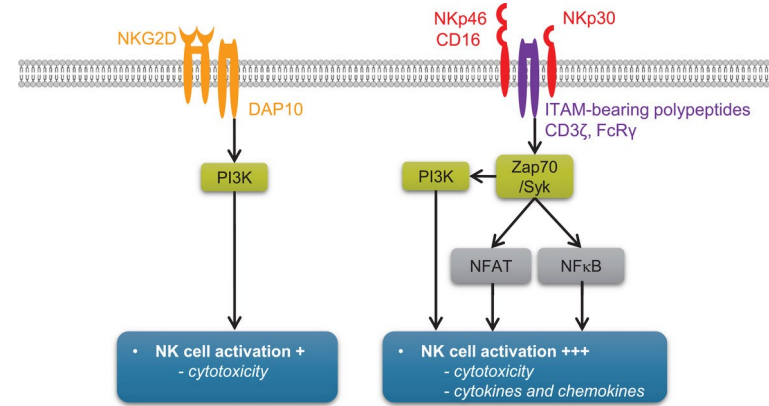
- Kill virus-infected cells
- Key contribution to human reproduction (dominant lymphocyte in pregnant decidua)
- Removal of the senescent cells
- **Exhibit anti-tumor responses**
 - Kill cancer target cells without prior sensitization
 - Key mediators of ADCC via Fc receptor (CD16a)

Regulation of NK Cell Responses

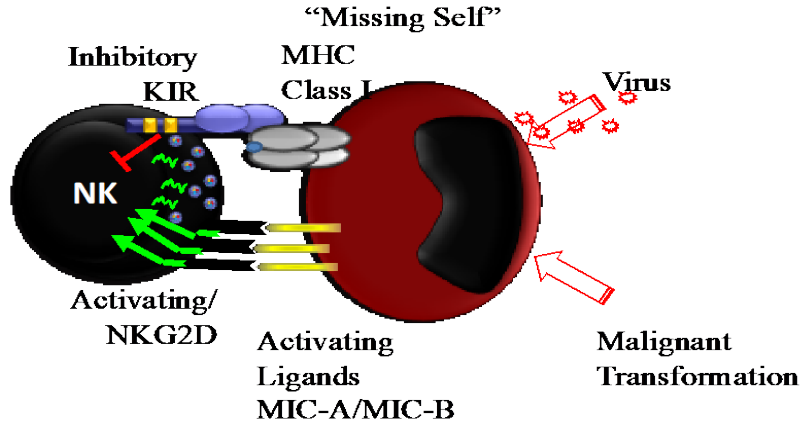
Killer immunoglobulin-like receptors (KIRs)



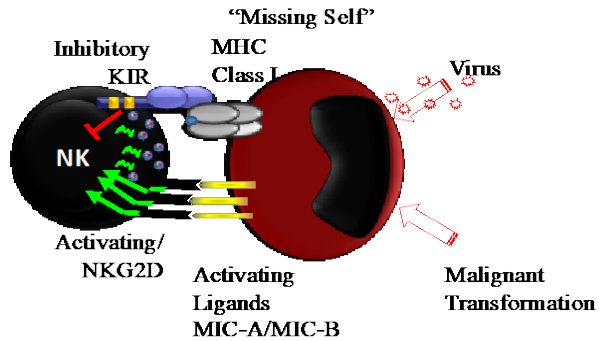
Romagnane et al, 2013



Demaria et al, Eur J Immunol 2021



KIR Ligand Mismatch and Decreased Risk of Relapse



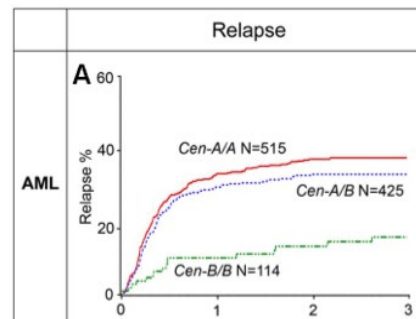
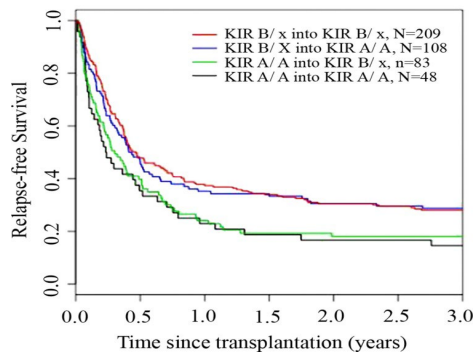
Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants

Loredana Ruggeri *et al.*
Science **295**, 2097 (2002);
 DOI: 10.1126/science.1068440

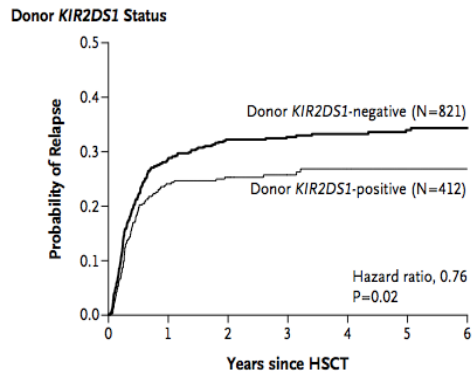
KIR ligand incompatibility in GVH direction	No	Yes
Number of transplants	58	34
Donors displaying antirecipient NK clones	1/58	34/34*
Disease		
ALL	21	14
AML	37	20
Transplantation outcomes		
Rejection	15.5%	0%*
Acute GVHD, \geq grade II	13.7%	0%*
Probability of relapse at 5 years		
ALL	90%	85%
AML	75%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

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

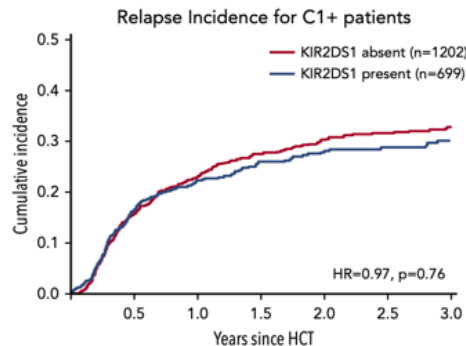
Donor KIR Haplotype and Relapse after Matched Stem Cell Transplantation



Cooley et al, Blood 2009 and 2010

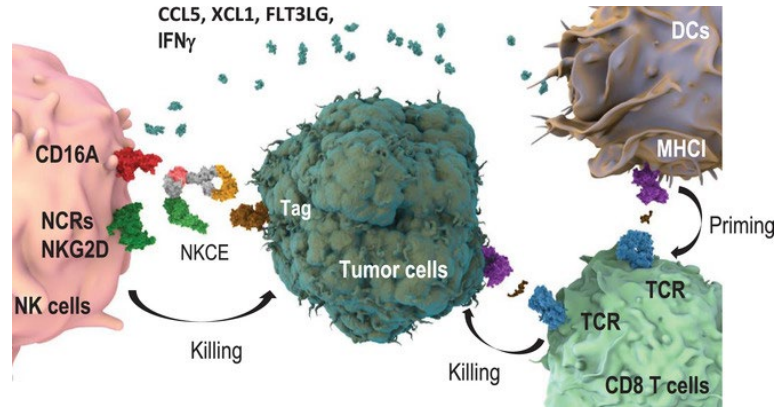


Venstrom et al, NEJM 2012



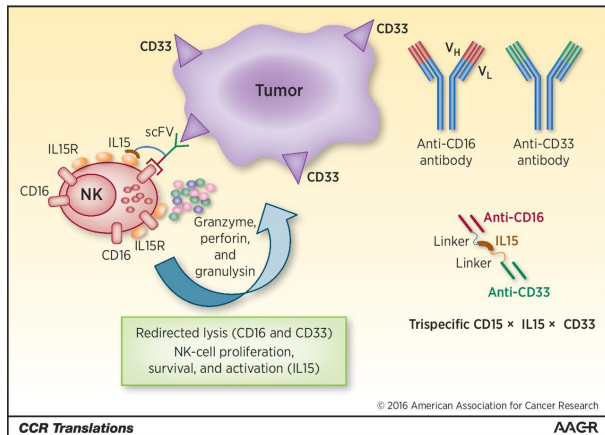
Schetelig et al, Blood 2020

NK Cell Engagers (NKCEs) in Clinical Trials



Demaria et al, Eur J Immunol 2021

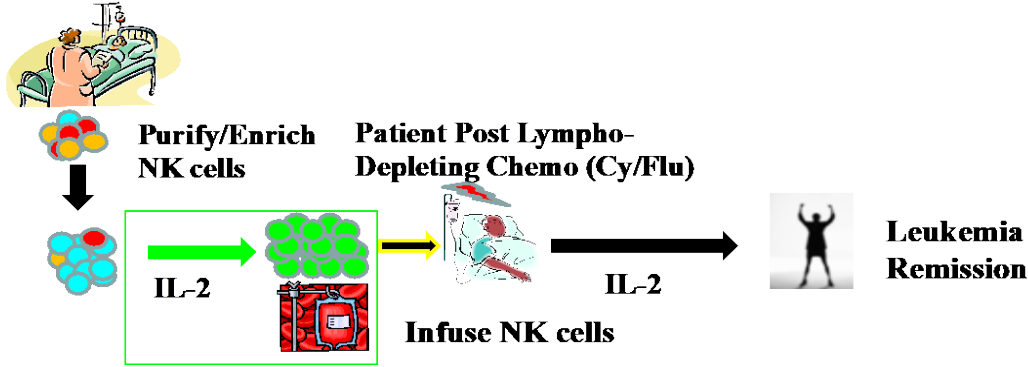
TriKE



	NK cell	Tumor cell	Killing	Cytokine production	Proliferation	
ADCC			+	+	-	
Trispecific ANKET			++	++	-	
Tetraspecific ANKET			+++	+++	+++	Innate Pharma/Vivier Group

Why Adoptive NK Cell Therapy?

**Haploidentical Donor:
leukapheresis**



Miller et al, Blood 2005
Rubnitz et al, JCO 2010
Curti et al, Blood 2011
Bachanova et al, Blood 2014
Nahi et al, Cell Rep Med 2022

Advantages

- 'Ready to Kill
- No severe CRS or neurotoxicity
- No GvHD risk, no need to TCR KO
- Propensity to target class 1- cells

Major Challenges

- Short life spans of NK cells
- Limited *in vivo* persistence
- Low NK cells numbers

NK Cell CARs Demonstrate Safety and Promising Activity

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors

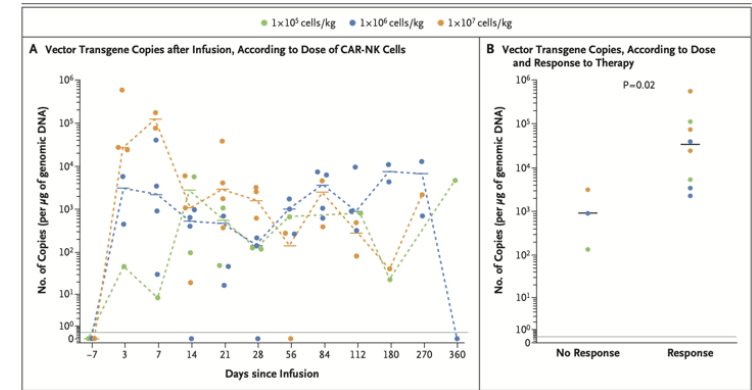
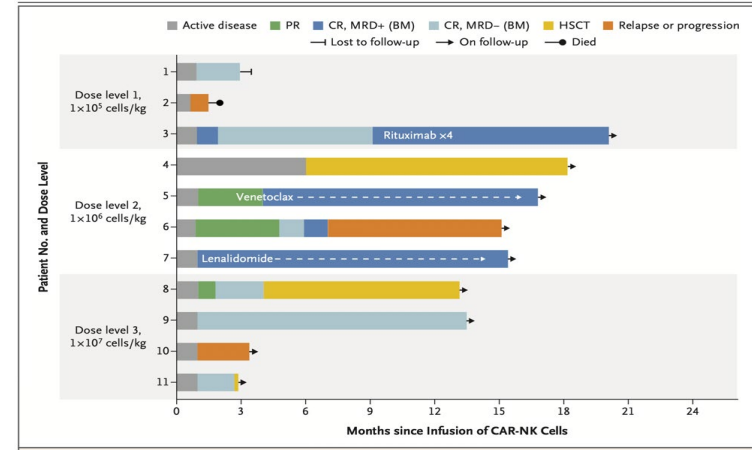
8/11 (73%) ORR, 7/11 CR

No CRS, No neurotoxicity, No GvHD

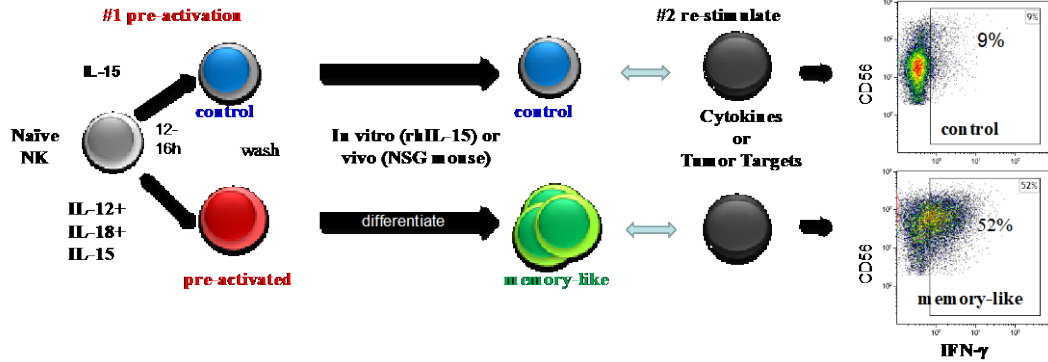
Liu et al, NEJM 2020

Trogocytosis as a novel mechanism of relapse

Li et al, Nat Med 2022

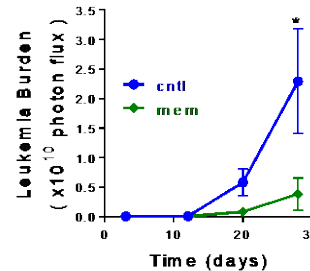
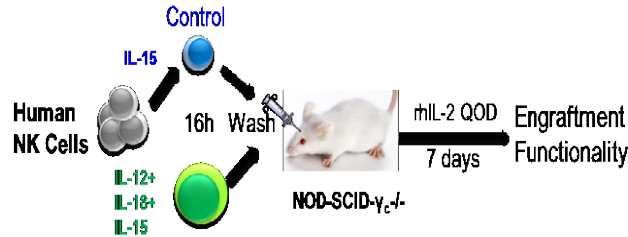


Cytokine Activation Induces Human *Memory-Like* NK Cells



Memory-Like NK Cell Properties:

- Enhanced anti-tumor responses
- Prolonged survival in vivo
- Enhanced proliferation
- Enhanced ADCC
- May inhibit GVHD
- High CD25
- Low TGFβ receptor



Cooper et al, PNAS 2009

Romee et al, Blood 2012

Ni et al, J Exp Med 2012

Romee et al, Science TM 2016

Berrien-Elliott et al, Cancer Discov 2020

Terren I et al, Sci Rep, 2021

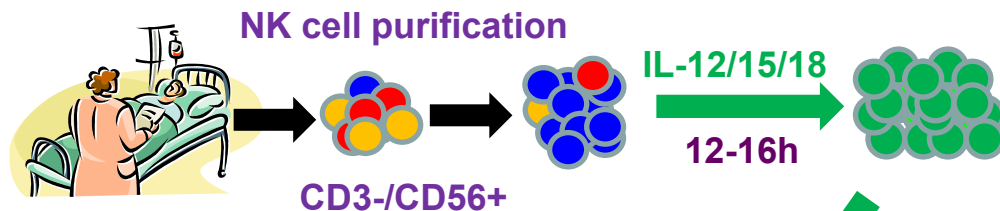
Berrien-Elliott et al, Science TM, 2022

Bednarski et al, Blood 2022

Shapiro, et, al, JCI, 2022

Phase 1 Study of CIML NK Cells in Rel / Ref AML

Day -1: Donor leukapheresis



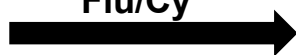
Cell dose levels:

1. $0.5 \times 10^6/\text{kg}$
2. $1.0 \times 10^6/\text{kg}$
3. Max capped at $10 \times 10^6/\text{kg}$

AML patient



Flu/Cy



Day 0



IL-2 $1\text{mIU}/\text{m}^2$
qOD x 2 weeks

Endpoints:

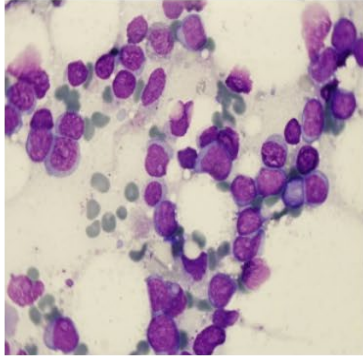
Primary:
Safety / MTD

Secondary:
Leukemia Response
In vivo biology

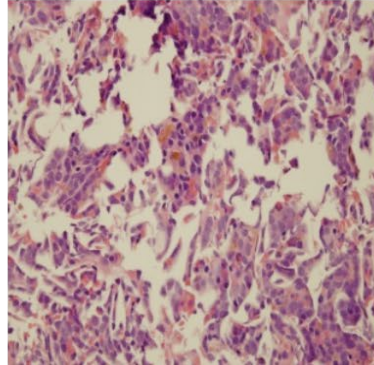
Safe and with Very Encouraging Response Rates

19 patients treated
No CRS, No GVHD
~50 CR rates

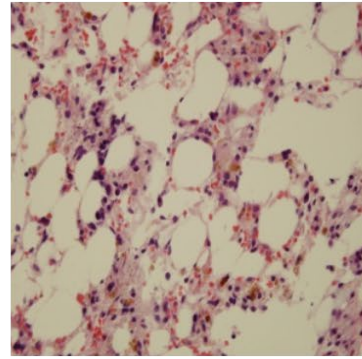
Pre-infusion Asp



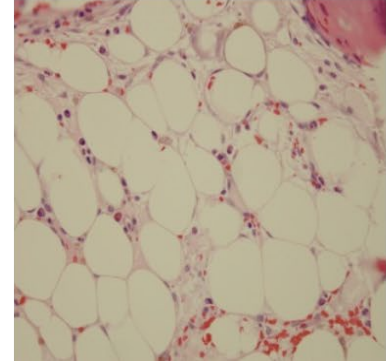
Pre-infusion Core



Day +14



Day +28



Romee et al, Science TM 2016

Dana Farber NK Cell Therapeutic Initiative

In Clinic

DFCI NK Cell Trials

1. Relapsed AML/MDS (*Enrolling*)
2. Head & Neck Ca (*Enrolling*)
3. MRD+ Myeloma (*Enrolling*)
4. Ovarian Cancer (*IND Approved*)

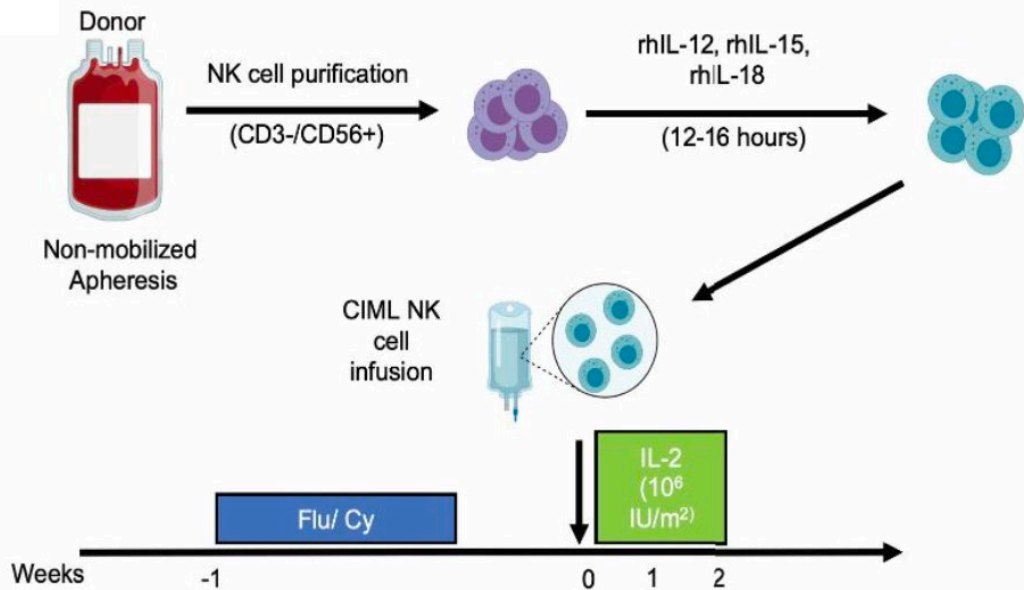
5. Kidney Cancer (*Regulatory*)
6. Pre-emptive Post HCT (*Regulatory*)
7. CD123 NK CAR (*Regulatory*)
8. NPM1c NK CAR (*Planning*)

In Lab

NK Cell Gene Manipulation Efforts

- CAR and TCR-mimetic CAR arming
- CRISPR Gene editing
- Non-CAR engineering

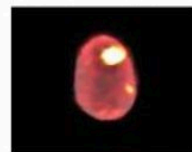
Can Memory-like NK Cells Target Relapse after Stem Cell Transplantation?



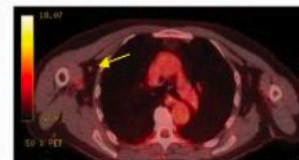
Safe, 4/6 CR/CRi

Patient 4

Pre-CIML NK



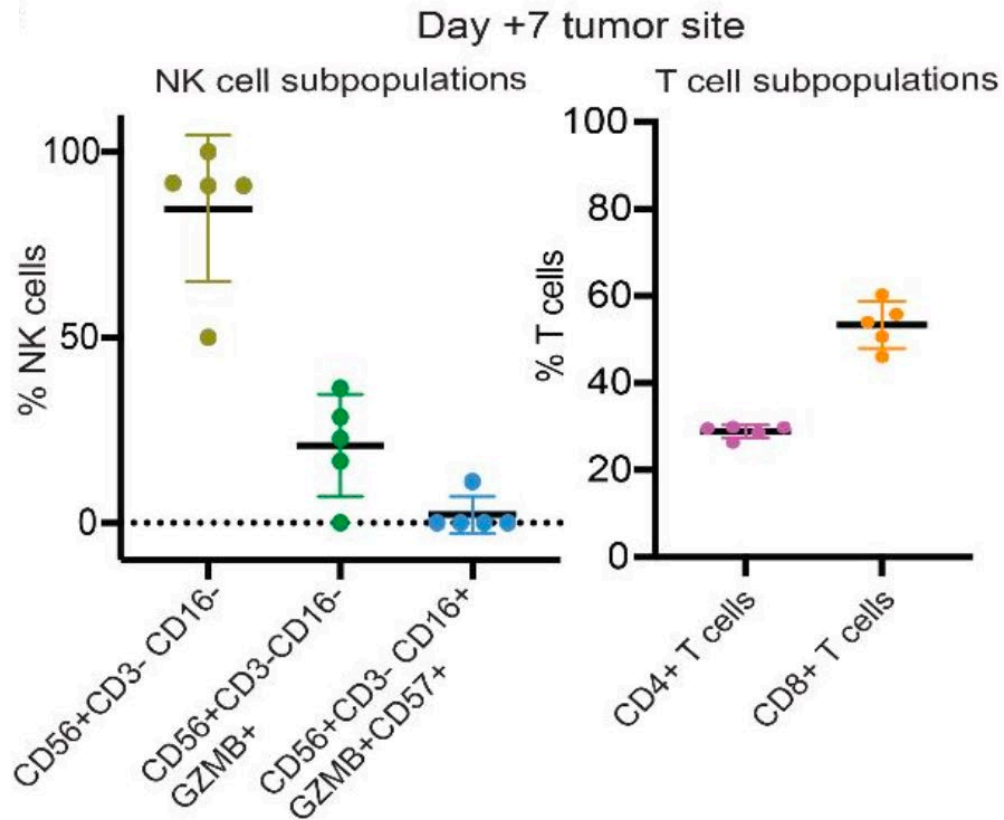
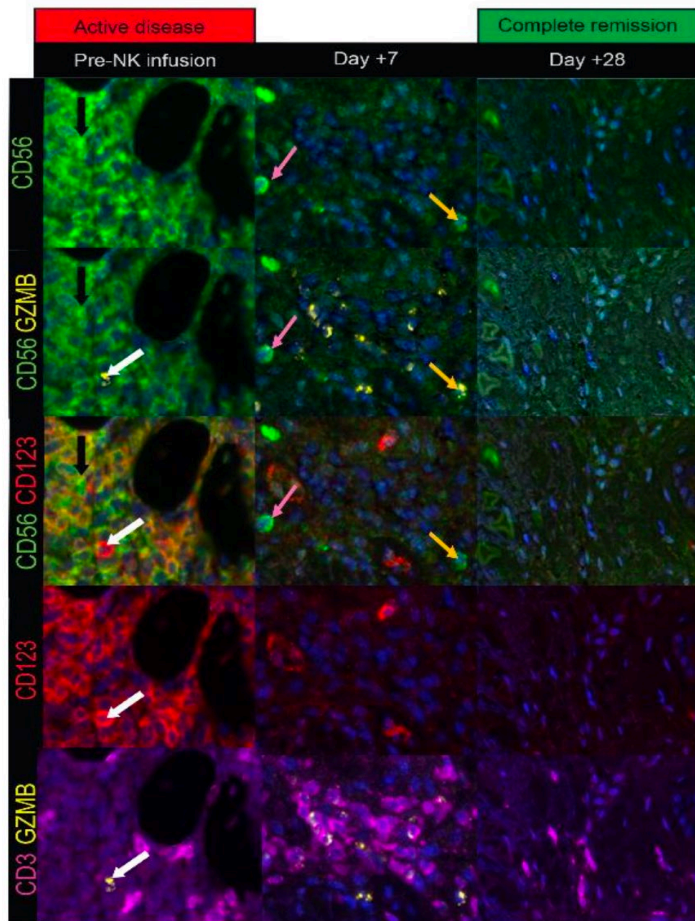
Post-CIML NK



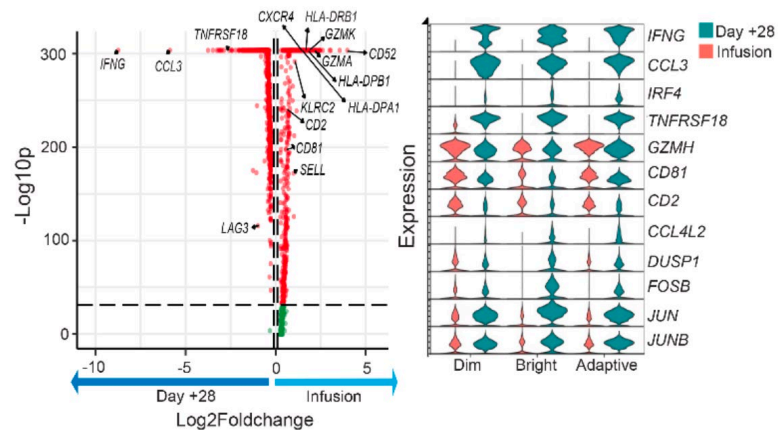
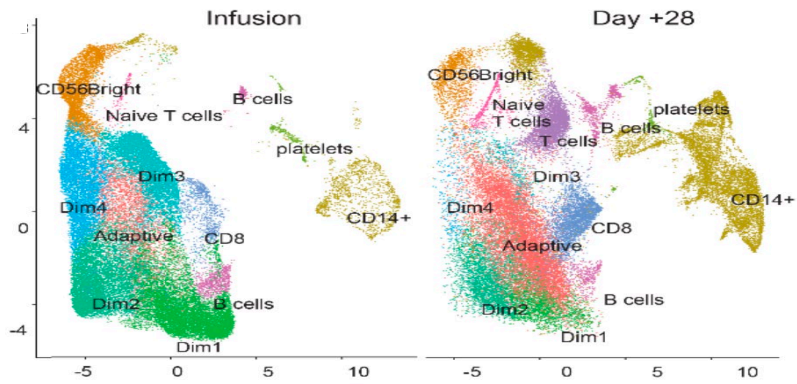
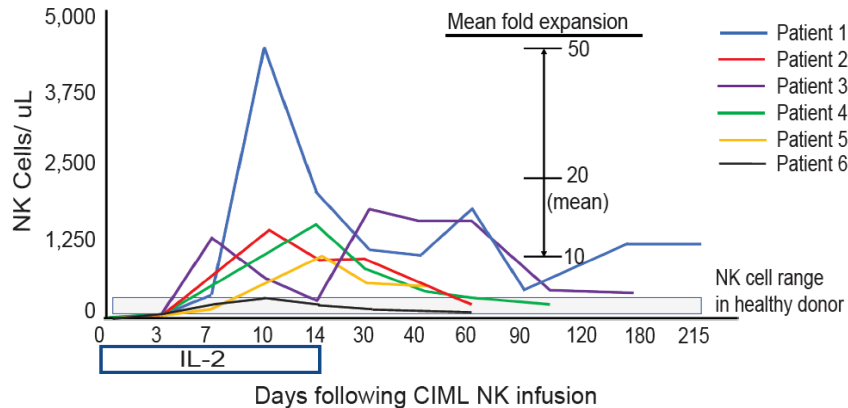
PET-CT

PI: Dr. Roman Shapiro
NCT04024761

Mem-like NK Cells Traffic to the Tumor Site

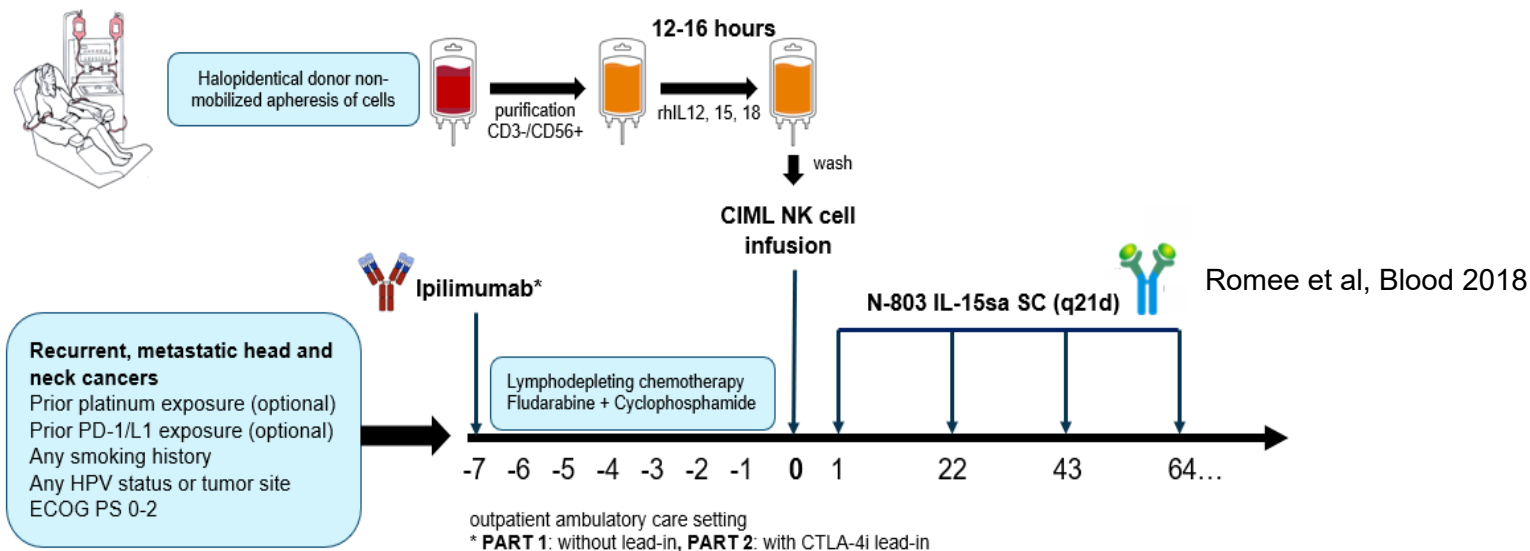


Mem-like NK Cells Traffic & 'Evolve' *in vivo*



Allogeneic Memory-like NK Cells Plus CTLA-4 Blockade in Head & Neck Cancer

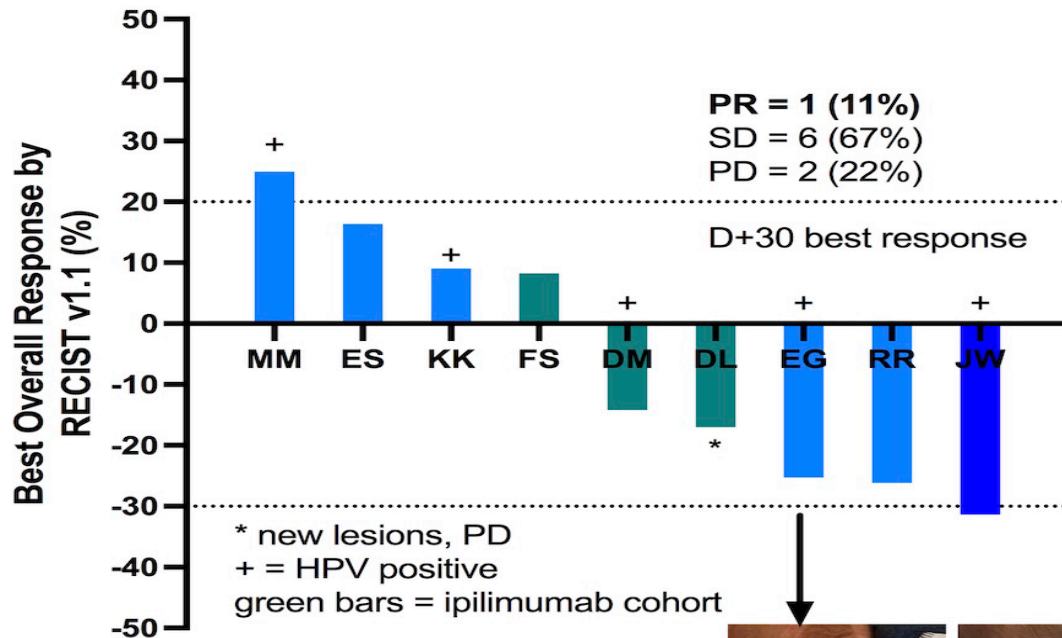
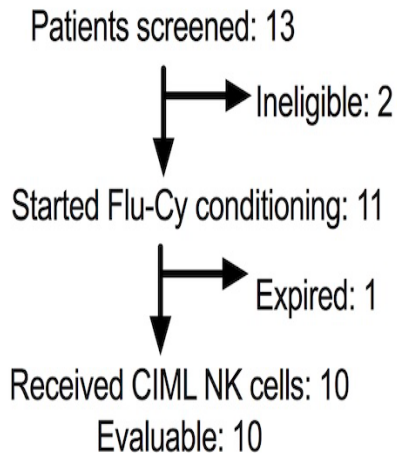
Anti-CTLA-4 antibody mediated Treg depletion to facilitate NK cell activity
NK cell infiltration associated with favorable outcomes in H&N ca patients



PI: Dr. Glenn Hanna

[NCT04290546](#)

Allogeneic Memory-like NK Cells Plus CTLA-4 Blockade in Head & Neck Cancer



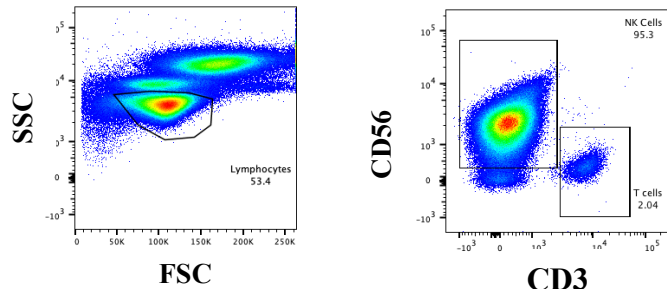
Gr 1-2 CRS in 6 patients

Median PFS of 3.43 months

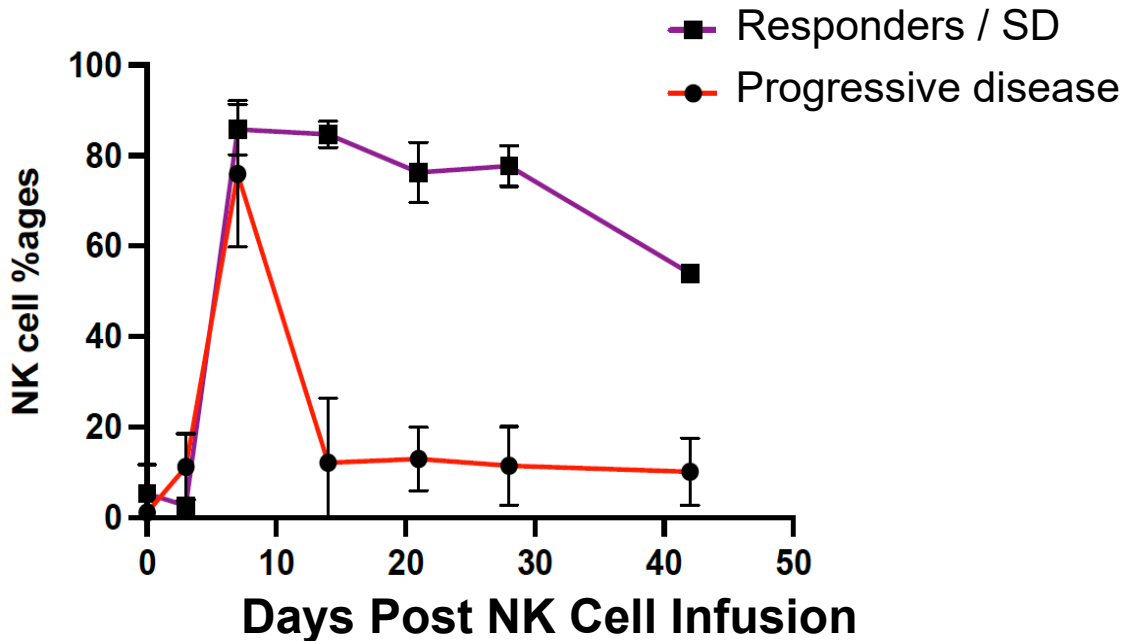
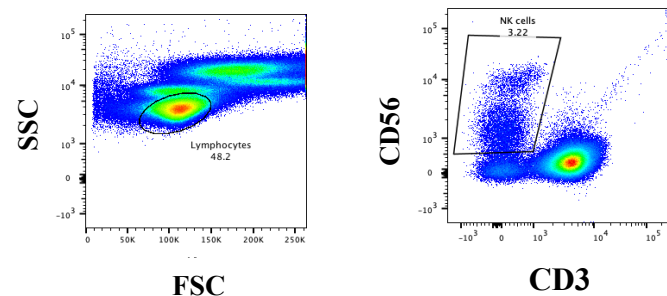


Disease Response and *in vivo* NK Cell Expansion

Responder

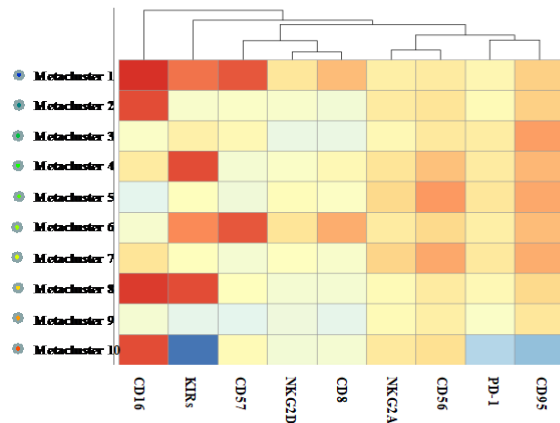
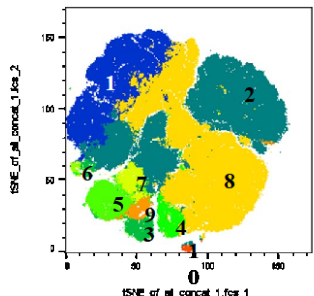


Progressive disease

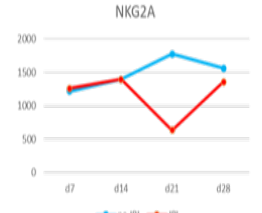
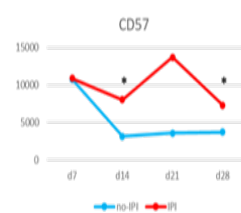
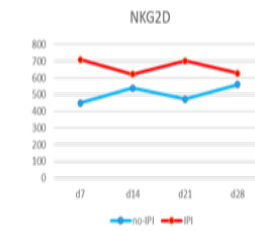
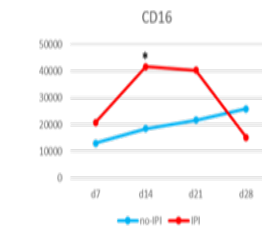
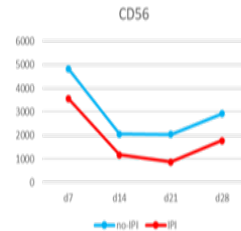
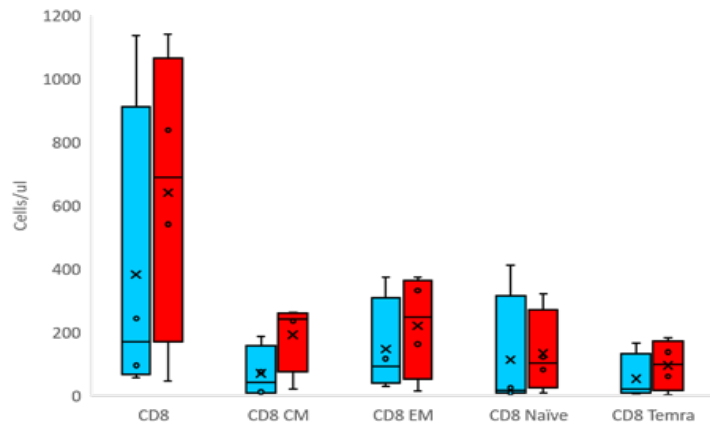


CTLA-4 Blockade Impacts NK Cell Phenotype?

Skewing towards CD16⁺CD57⁺ NK cells



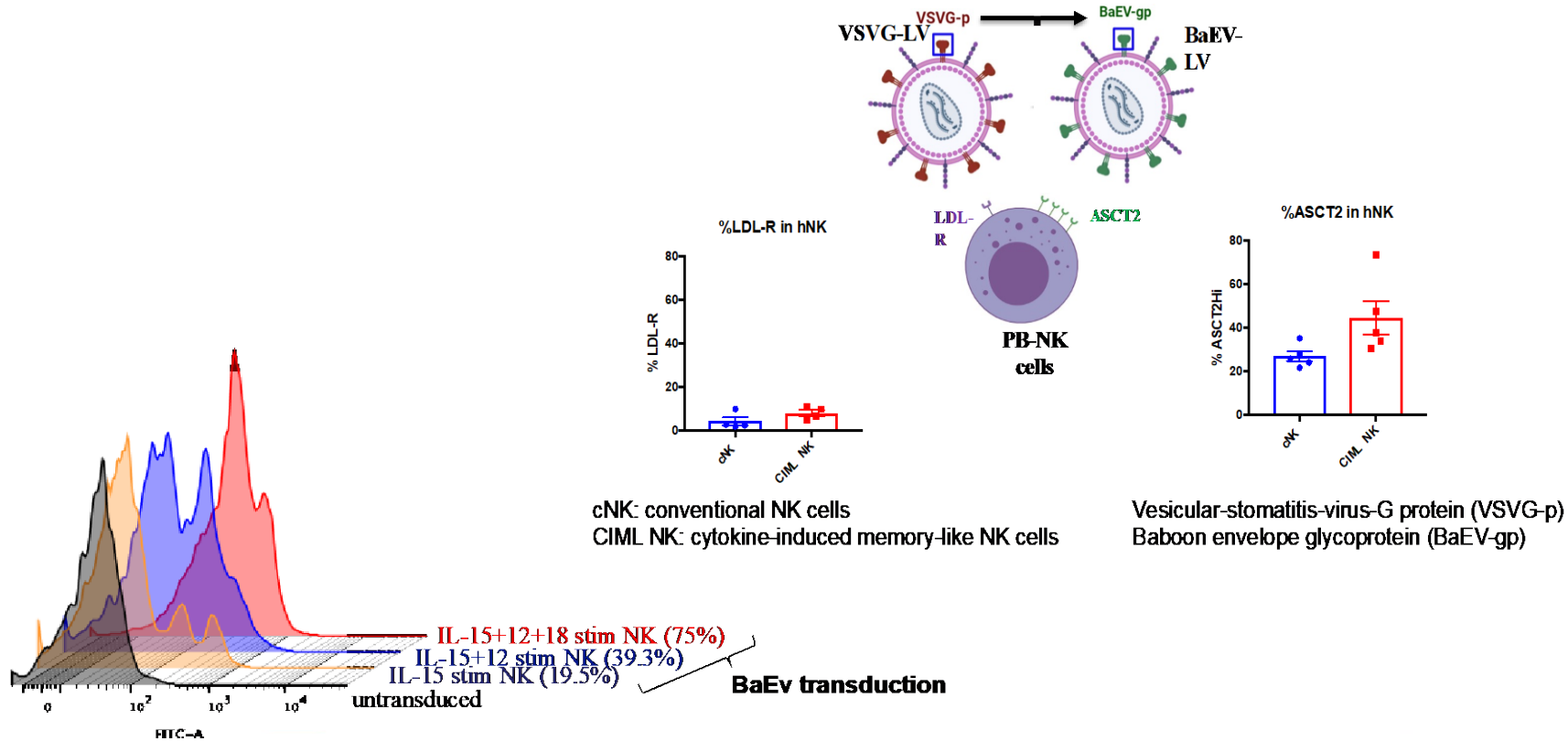
CD8 T cells at d+28



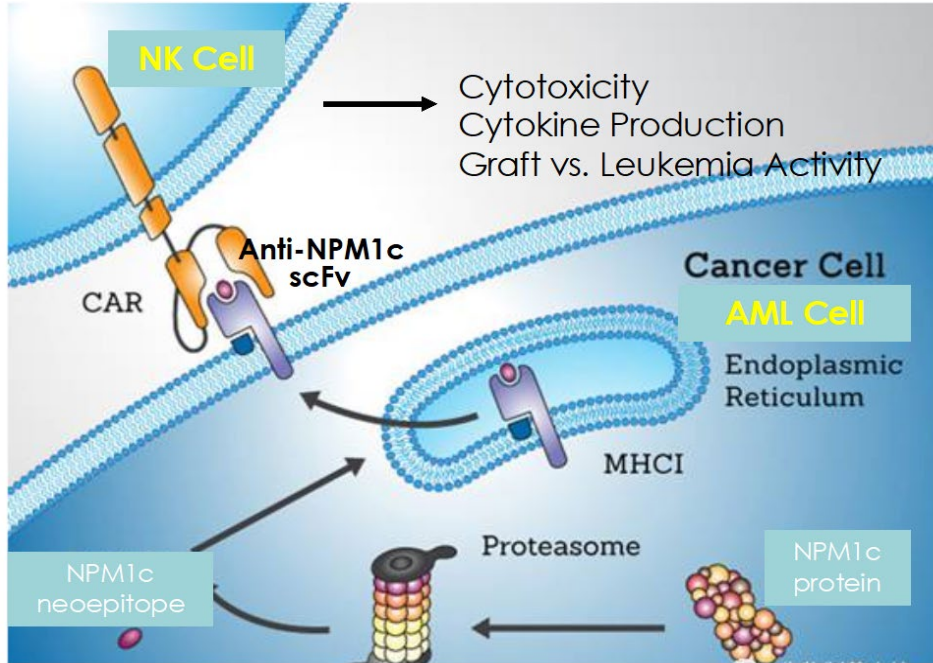
■ No Ipilimumab
■ Ipilimumab

Can we Arm Memory-Like NK Cells?

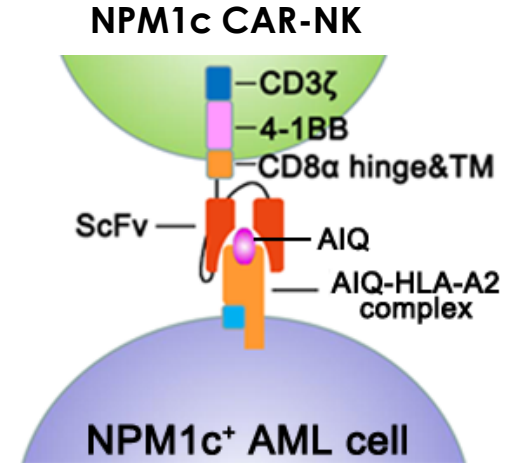
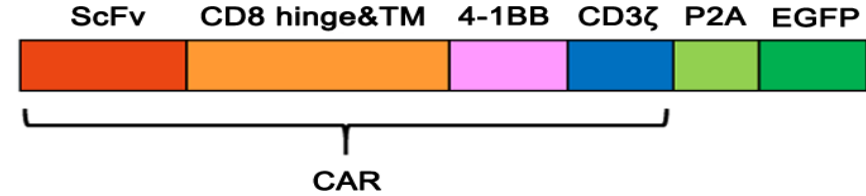
BaLV allows High Transduction Efficiency of Mem-like NK Cells



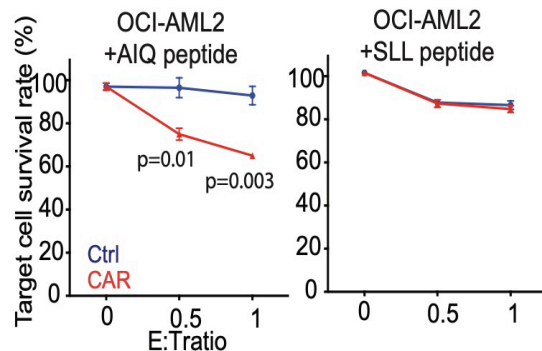
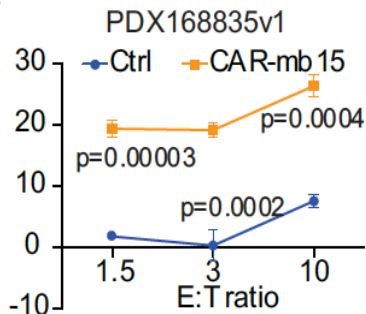
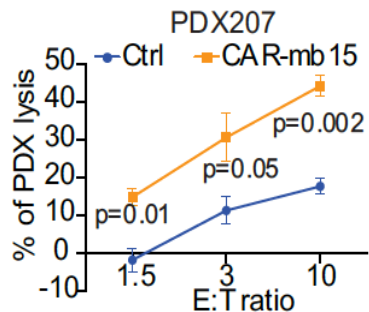
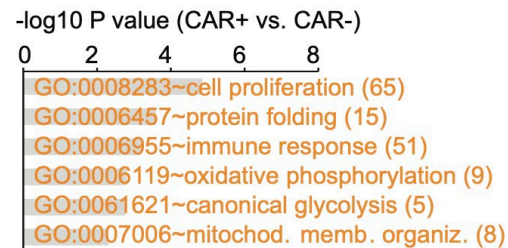
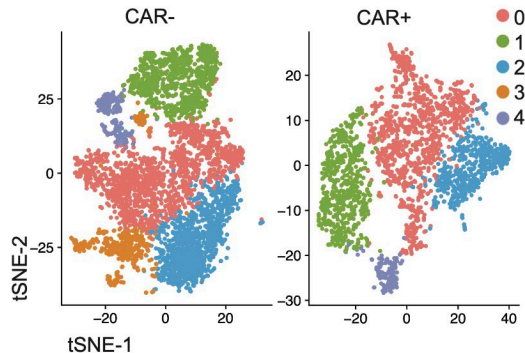
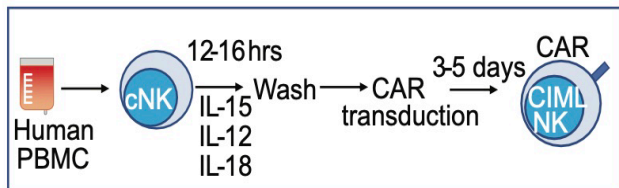
Generation of CAR-NK Cells Specific for AIQ-HLA-A2 Complex



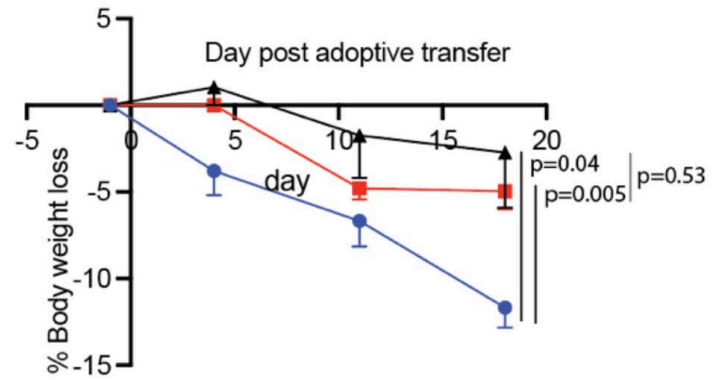
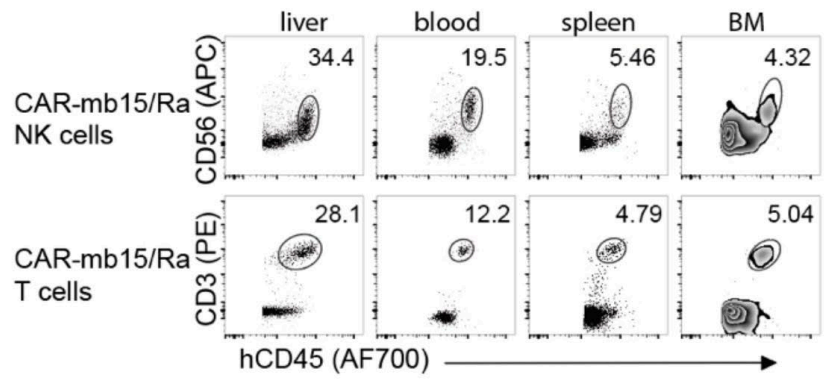
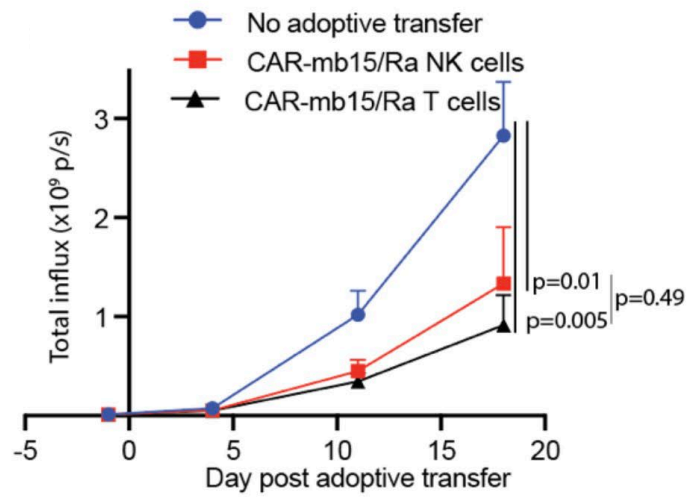
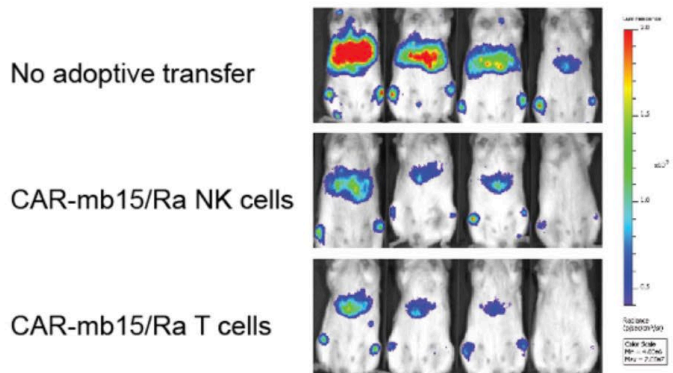
CAR targeting a neopeptide derived from NPM1c protein



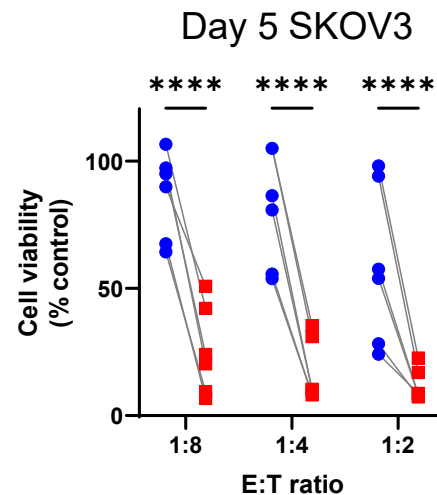
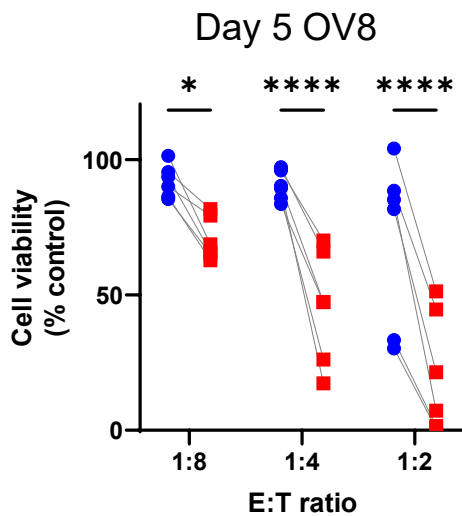
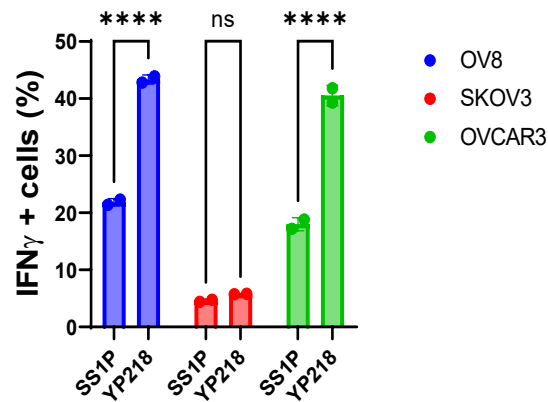
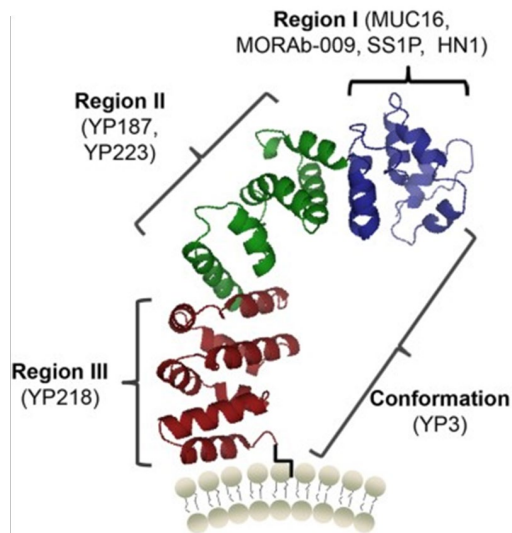
Gene Expression and Specificity of NPM1c ML-CAR



Are CAR NK & CAR T Cells Similar in Efficacy?

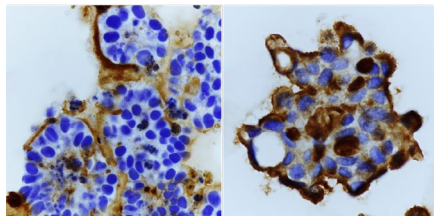


CIML NK CAR Targeting Membrane Proximal Domain



MSLN CIML NK CARs Demonstrate activity against PDOTs & TTFs

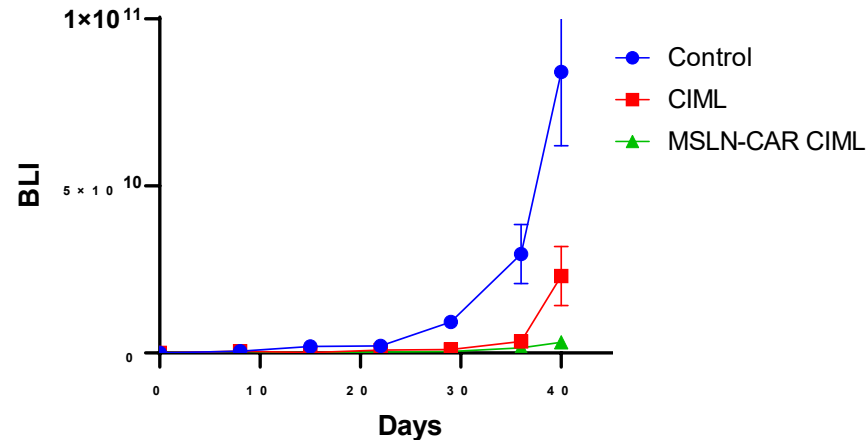
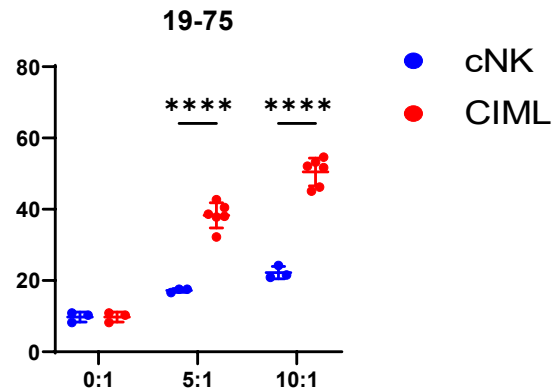
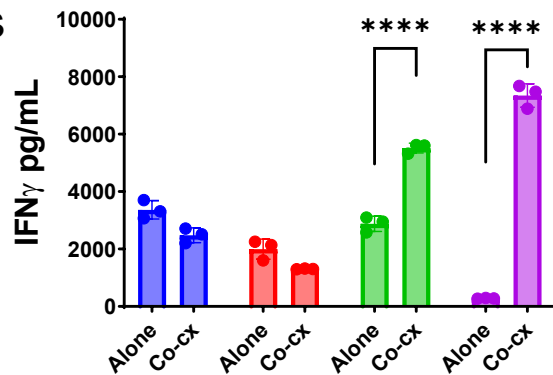
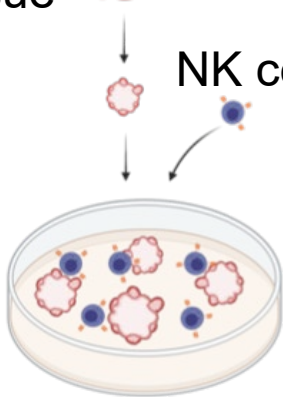
Meso⁺
PDOTs



Tumor
tissue

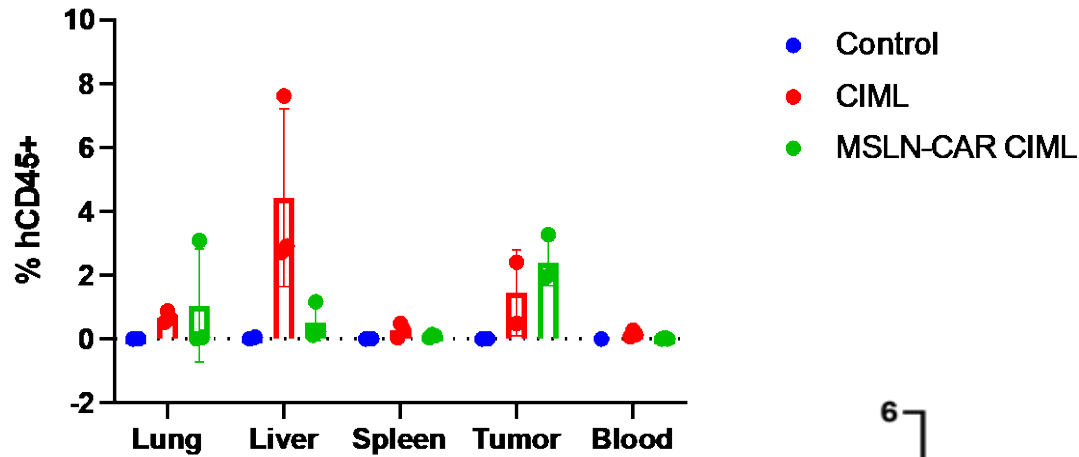


NK cells

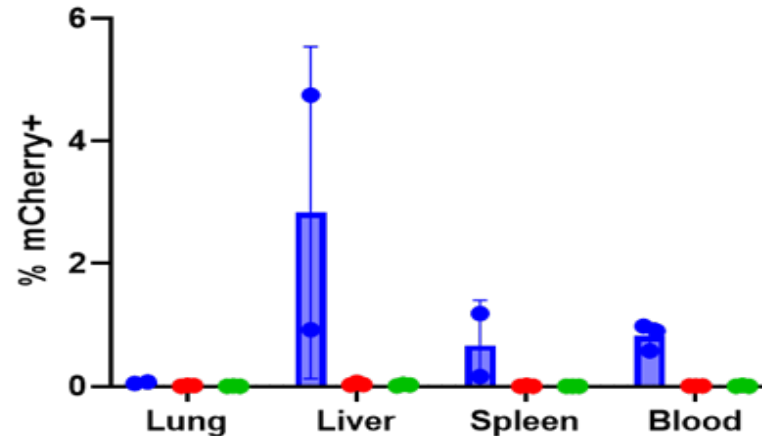


Do NK Cell CARs Traffic Out of Peritoneum?

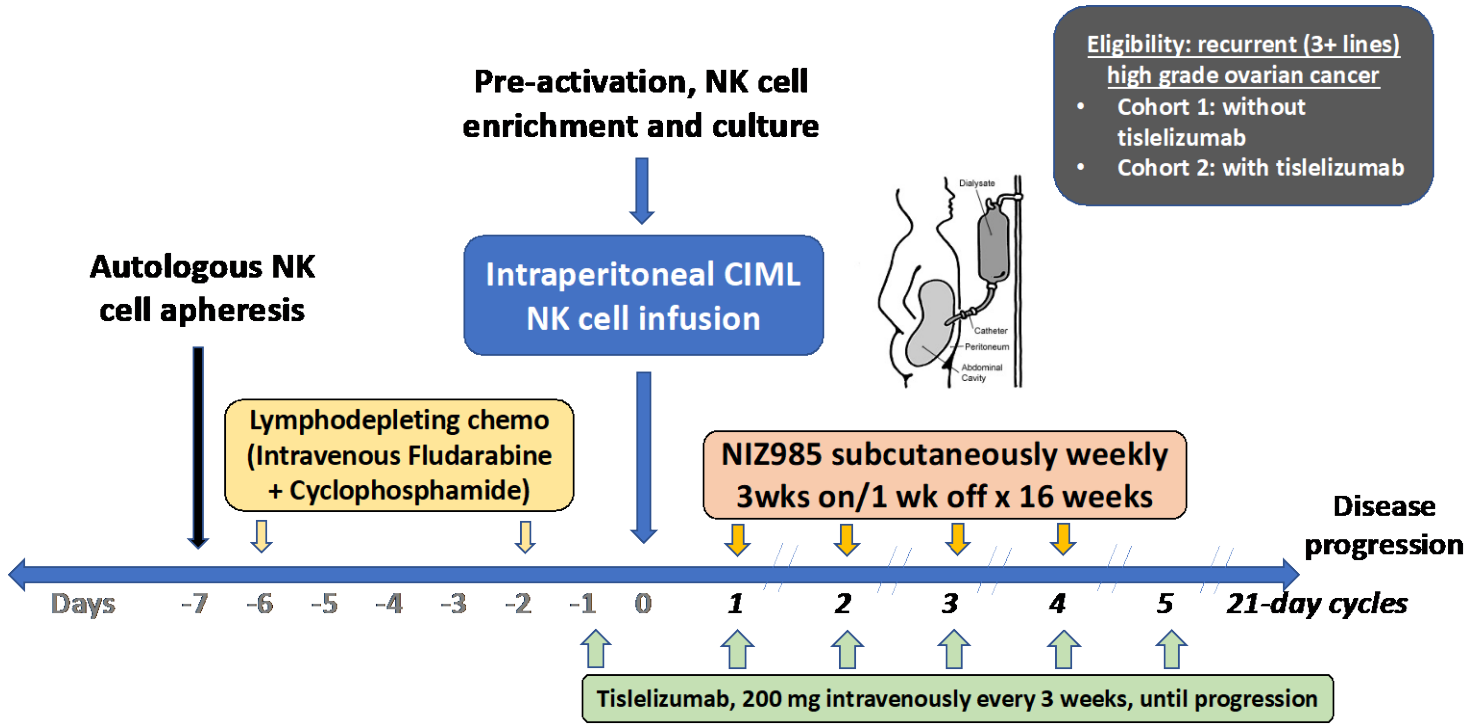
NK Cell Trafficking



Tumor Spread



Phase 1 Trial of Autologous CIML NK Cells with PD1 Blockade & IL-15



IND Approved

Summary

- NK cells play an important role in mediating graft versus tumor effect after stem cell transplantation
- Lack of severe CRS, neurotoxicity, GvHD, and intrinsic tumor targeting mechanisms are some of the major advantages of NK cells
- Memory-like NK cells with enhanced proliferation, expansion and *in vivo* persistence make them an attractive platform for NK cell-based therapies
- Novel CAR and non-CAR gene editing approaches are leading the 'revolution' in the NK cell immunotherapy field

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