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Beyond CAR T: NKT Cell Platform for Adoptive Cell Therapy

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Disclosure Information

I have the following financial relationships to disclose:

- An inventor on patents licensed by Baylor College of Medicine to Cell Medica, Ltd
- Research support from Cell Medica, Ltd

I will discuss the following investigational use in my presentation:

- Phase 1 Clinical Trial: GD2 Specific CAR and Interleukin-15 Expressing Autologous NKT Cells to Treat Children With Neuroblastoma (NCT03294954)

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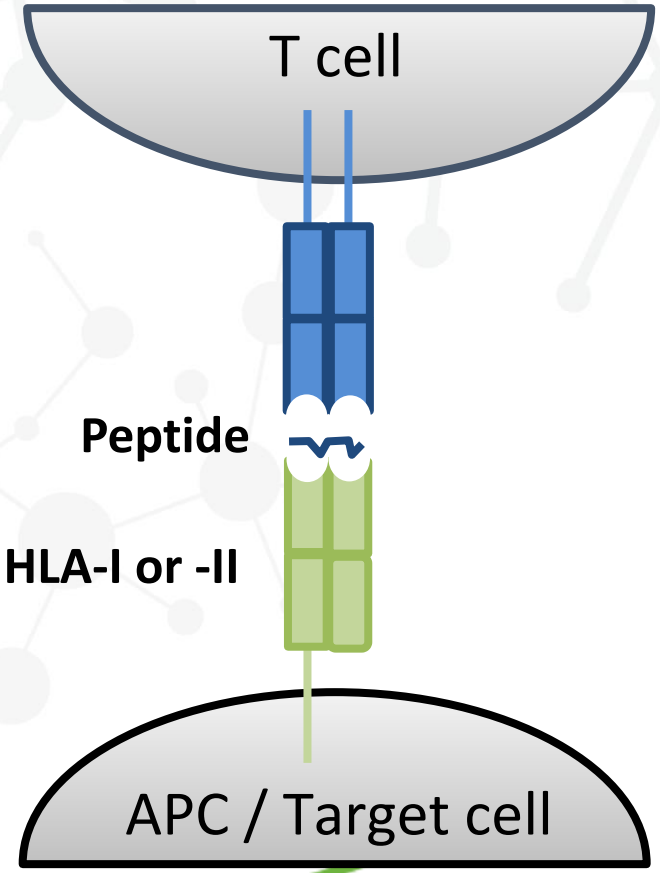
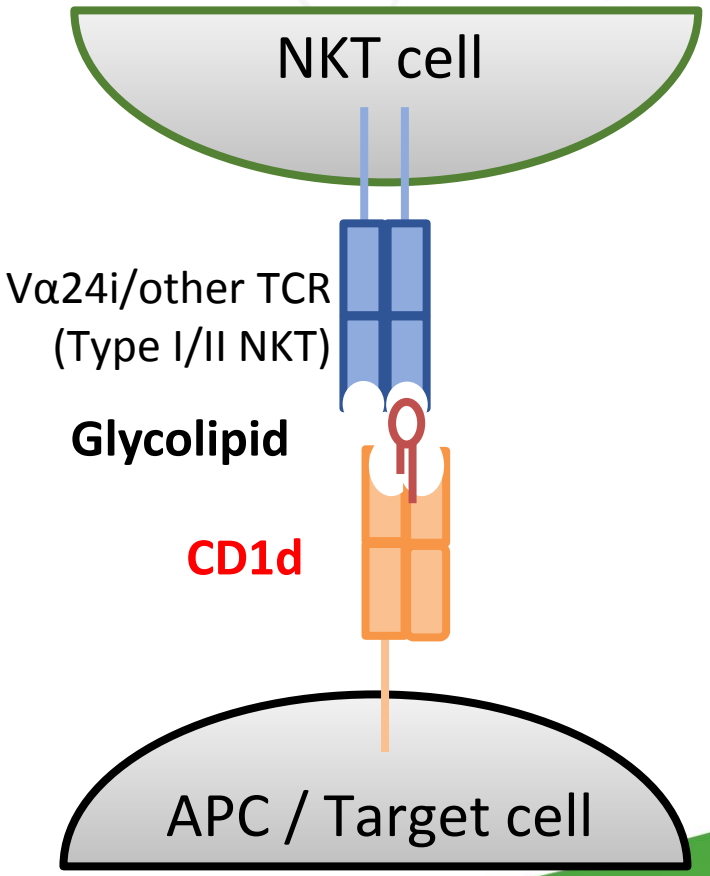
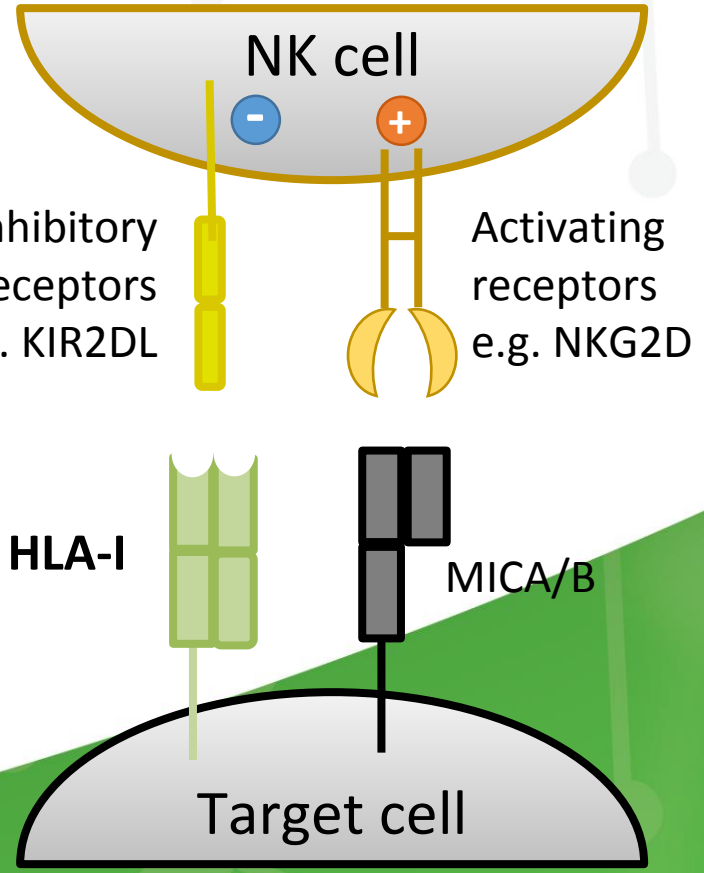
Natural Killer T (NKT) cells

- NKT cells are an evolutionarily conserved subset of PLZF-instructed innate-like lymphocytes that share properties of T and NK cells and react to self- and microbial-derived glycolipids presented by CD1d.
- Type I (invariant) (i)NKT cells;
  - Express an invariant alpha-chain Vα24-Jα18 of T cell receptor, react to both self (e.g. tumor-derived) and foreign (e.g. bacterial) CD1d-presented glycosphingolipids and phospholipids. They can be identified by reactivity to CD1d-bound α-galactosylceramide.
  - Demonstrate potent antitumor activity in murine tumor models and have been associated with favorable outcome in cancer patients.
- Type II NKT cells;
  - Express variable T cell receptor, react to CD1d-presented self-glycolipids, including sulfatide, β-glucosylceramide, or lysophosphatidylcholine. They are identified based on CD1d-dependence and the lack of reactivity to CD1d-bound α-galactosylceramide. A
  - Are associated with immunosuppression in murine tumor models. However, their role in humans remains largely unknown.

Target recognition by NKT vs. T and NK cells

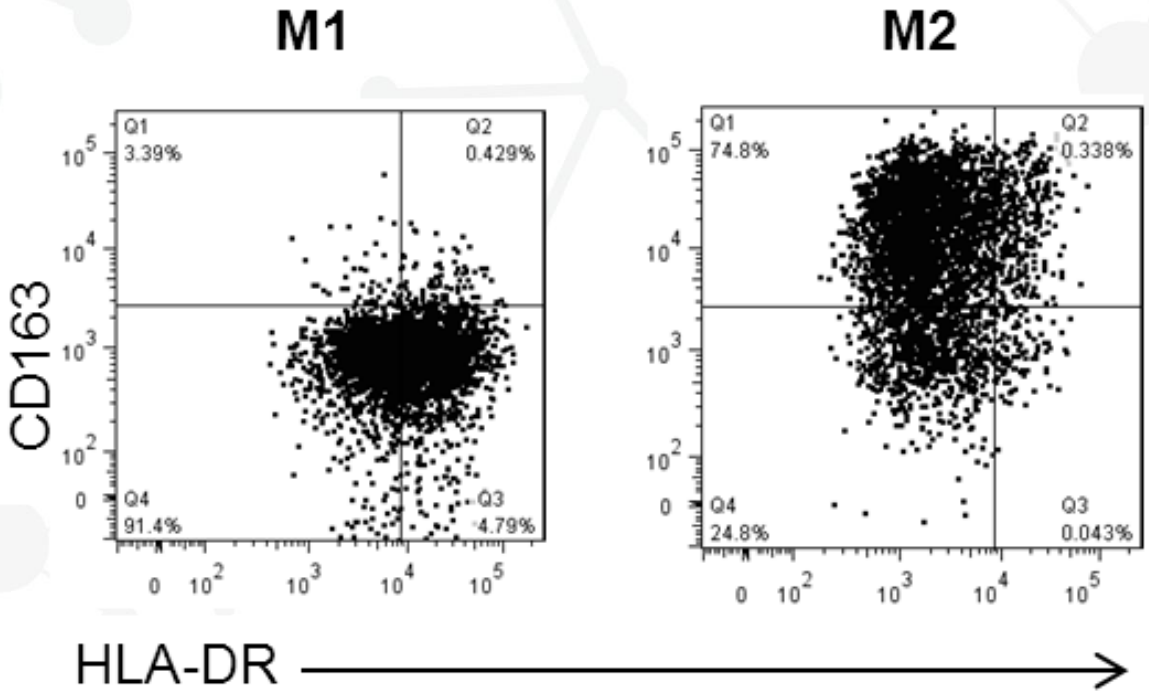


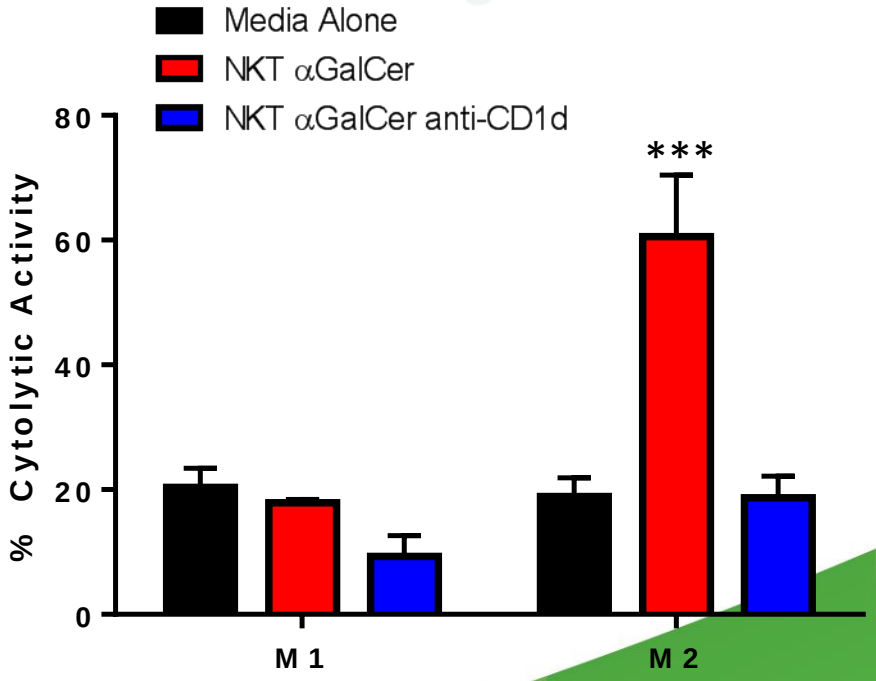




NKTs selectively and specifically kill M2 macrophages

In vitro polarized macrophages





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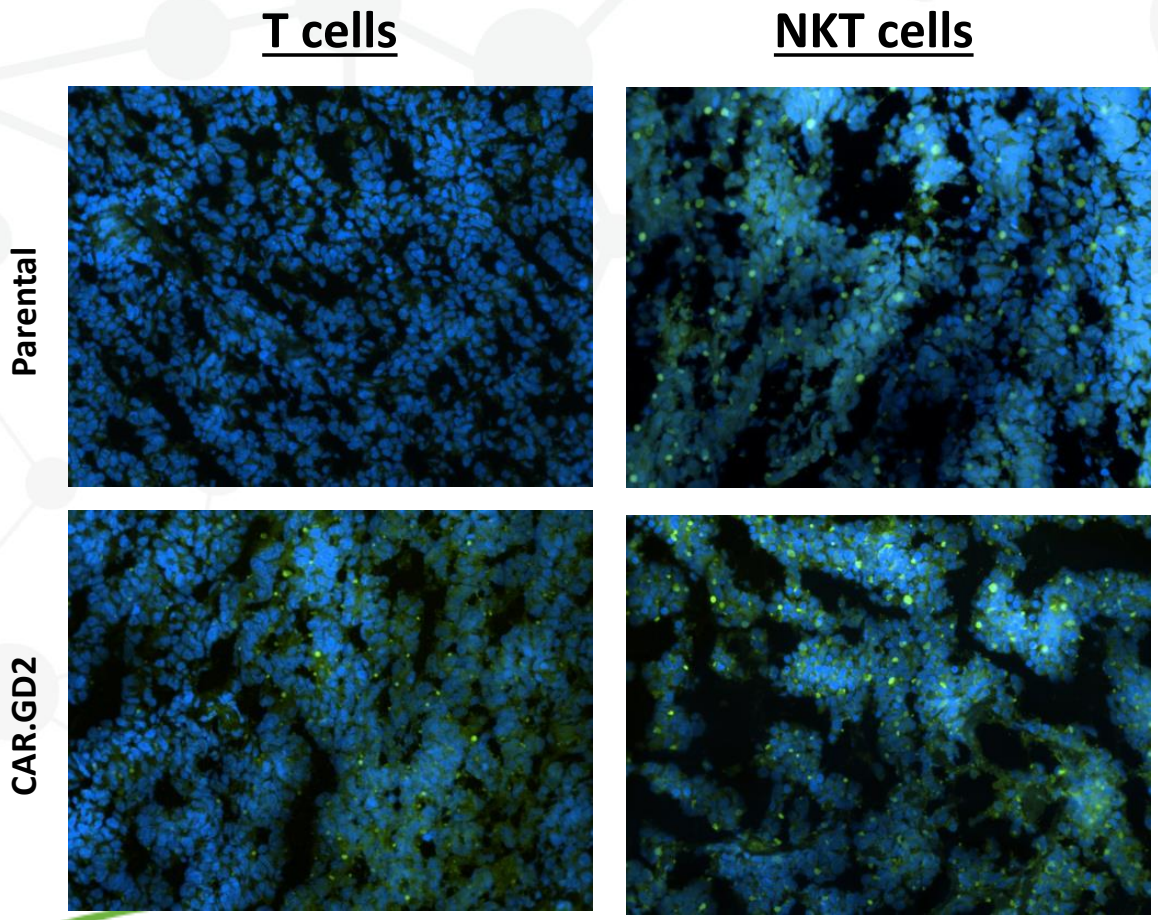
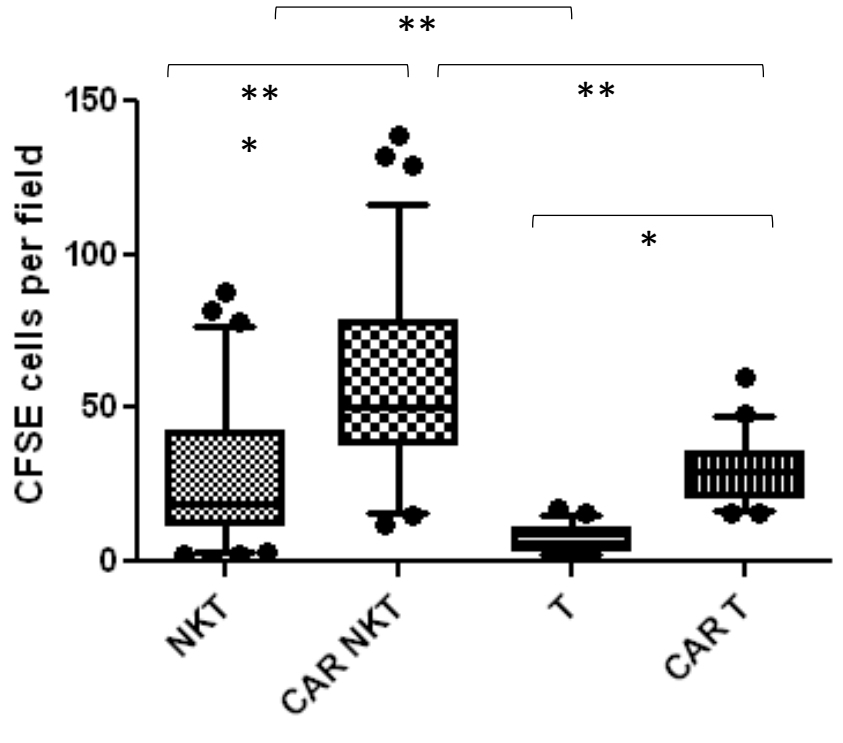
NKT and CAR-NKT traffic to NB tumor more effectively than T and CAR-T cells





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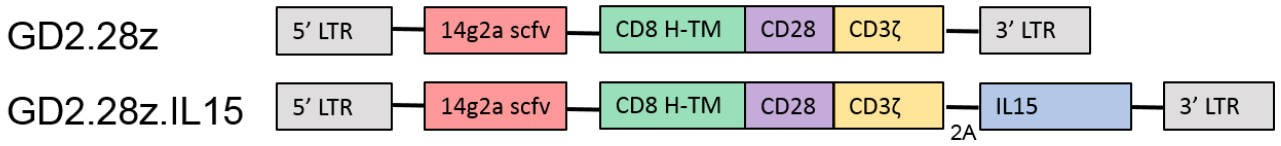
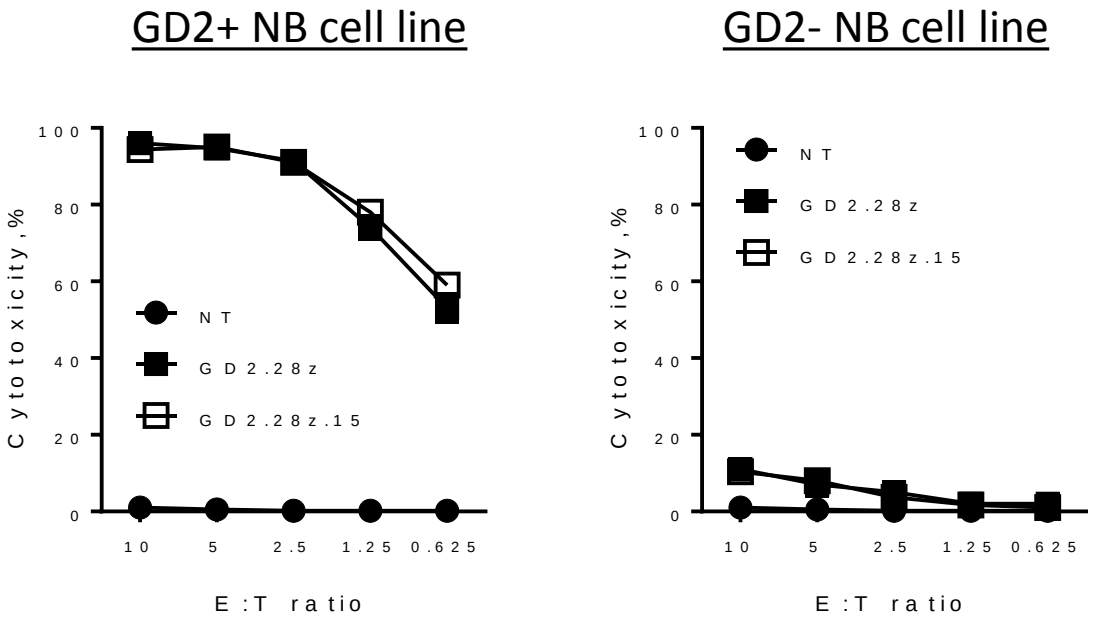
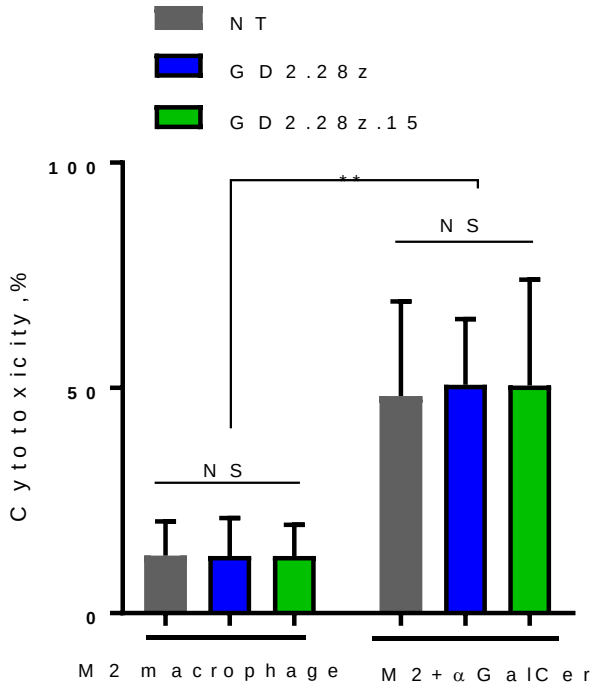
Heczey *et al. Blood*, 2014, 124:2824-33.

CAR.GD2 NKTs target both GD2+ Tumor Cells and CD1d+ M2

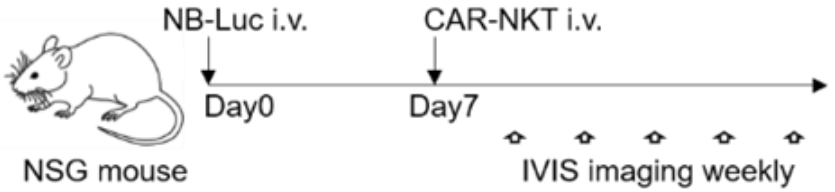


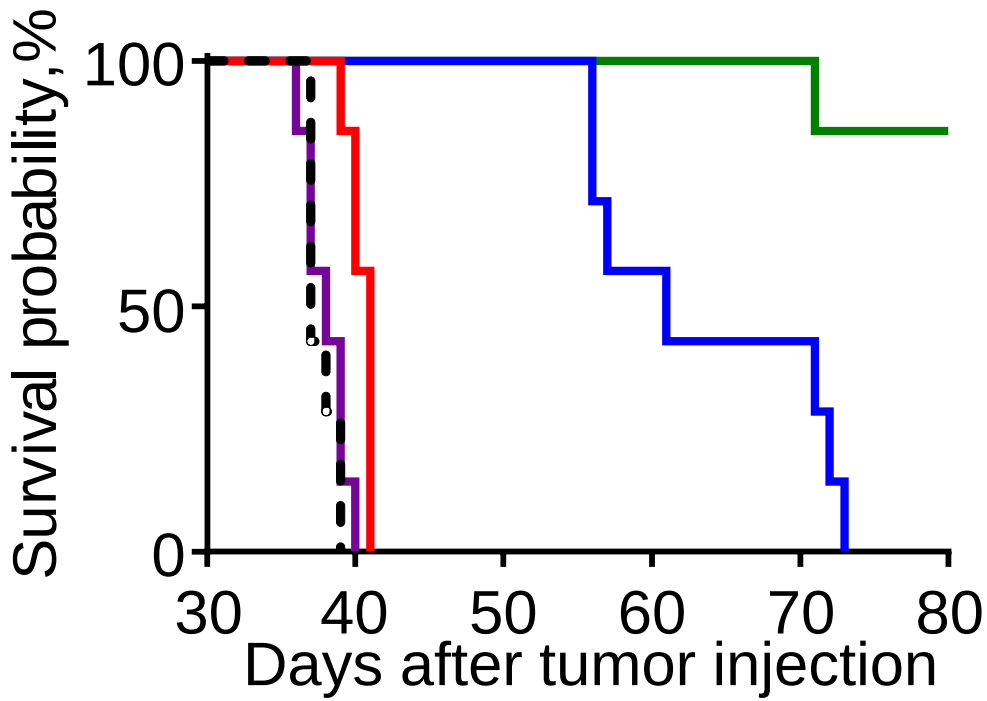


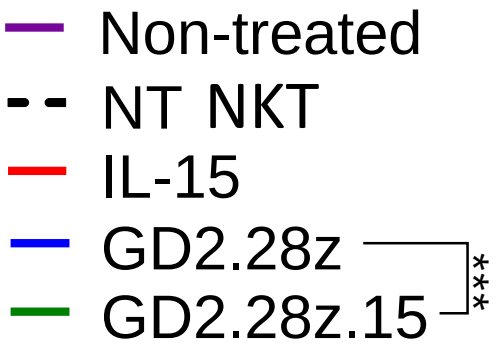


Xu X. et al. *Clinical Cancer Research,* in press

Therapeutic efficacy of GD2.CAR NKTs in the metastatic NB NSG mouse model





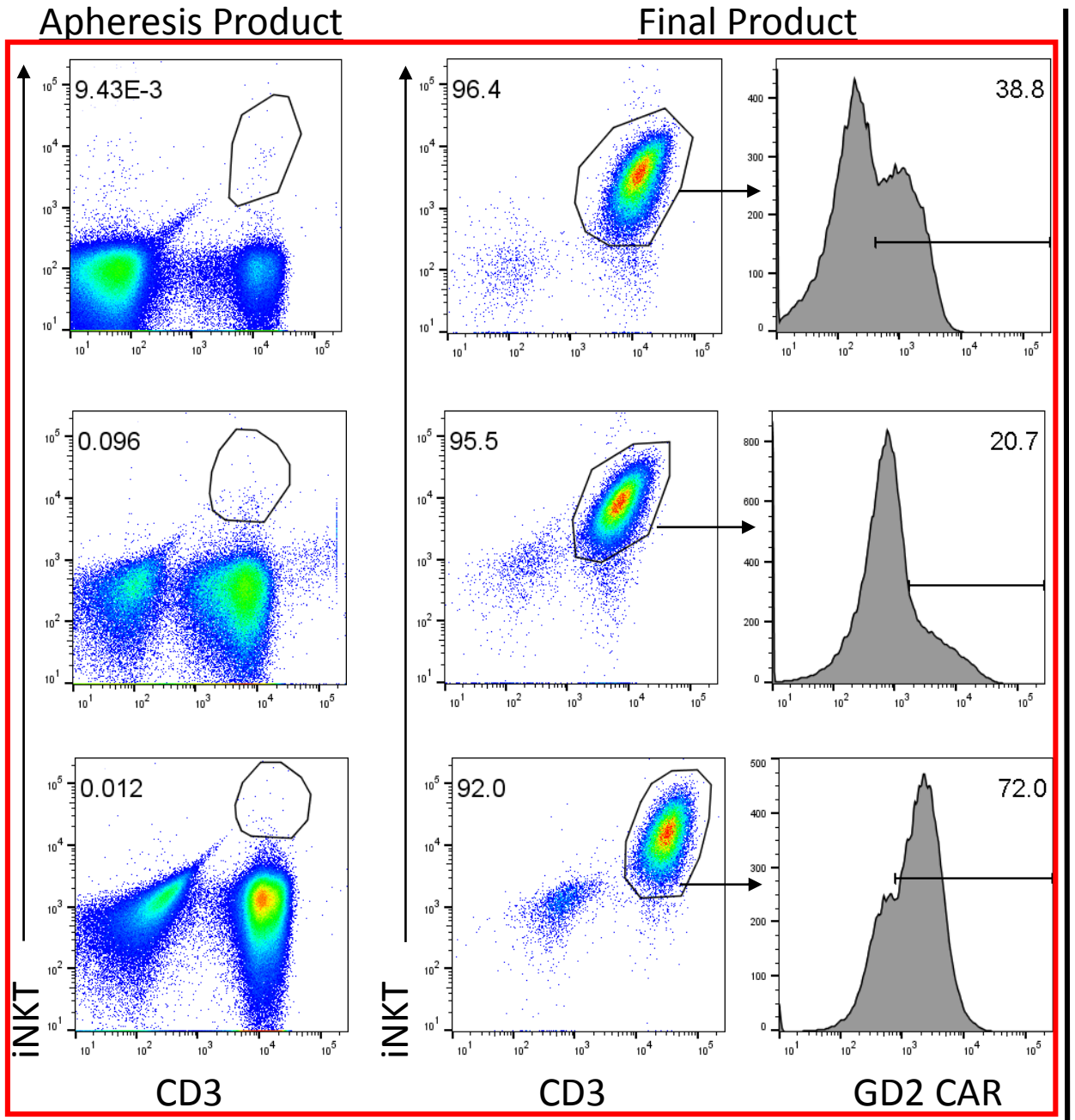


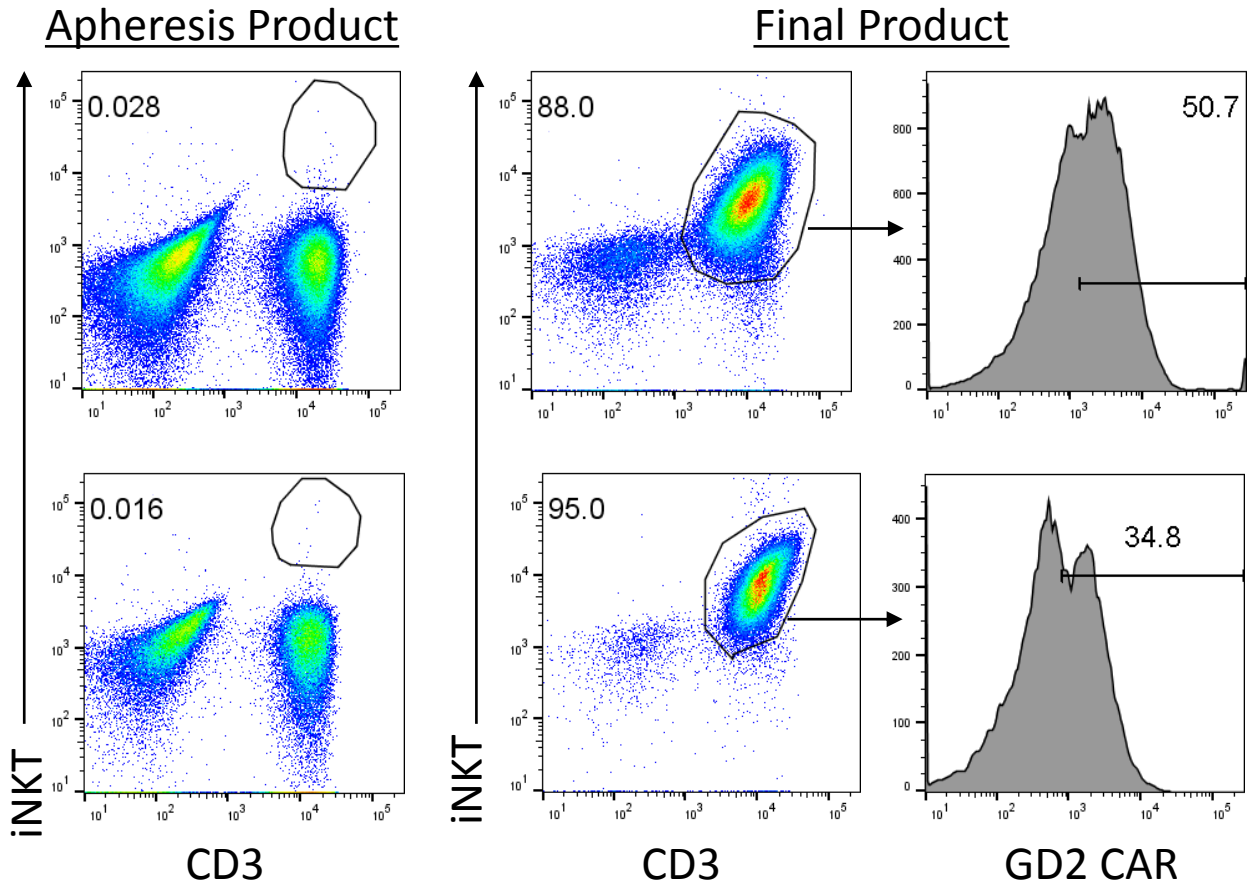
Xu X. et al. *Clinical Cancer Research,* in press

Phase 1 Clinical Trial: GD2 Specific CAR and Interleukin-15 Expressing Autologous NKT Cells to Treat Children With Neuroblastoma (GINAKIT2), NCT03294954

- R/R high-risk neuroblastoma
- Dose escalation: $3x10^6$; 10^7; $3x10^7$ and 10^8 /m^2
- Safety
- CAR NKT persistence and trafficking
- Antitumor responses

5 Patient Products manufactured on GINAKIT2 protocol

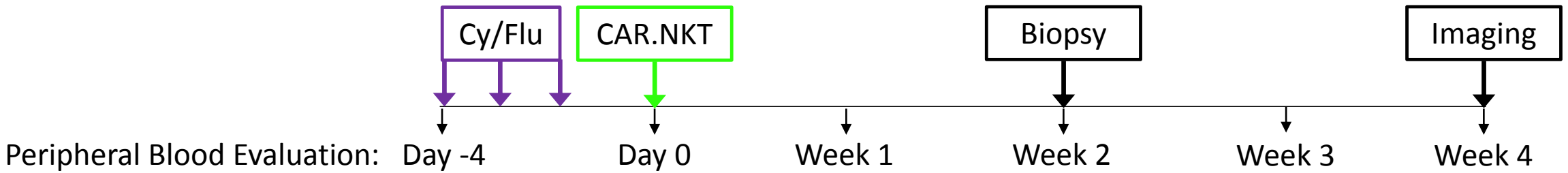




Number of NKTs cryopreserved:
Mean = 1.75×10^9
Range = $1.9 \times 10^8 - 3.4 \times 10^9$

Cells cryopreserved after 9-15 days in culture

3 Patients treated on GINAKIT2 protocol



N	Age, Years	Gender	INSS*	Involved Sites	Cy/Flu**	Dose, CAR NKT cells per m^2	Response*
1	12	M	4	Multifocal bone and bone marrow, soft tissues and paraspinal masses	Yes	3×10^6	Stable Disease
2	12	M	4	Multifocal bone	Yes	3×10^6	Partial Response
3	6	M	4	Multifocal bone	Yes	3×10^6	Stable Disease

* Response criteria is determined by revised international neuroblastoma response criteria, PR = elimination of 50% of bone mets

** Cyclophosphamide 500 mg/m^2 IV on Days -4 and -3 and Fludarabine 30 mg/m^2/dose IV on Days -4 to -2

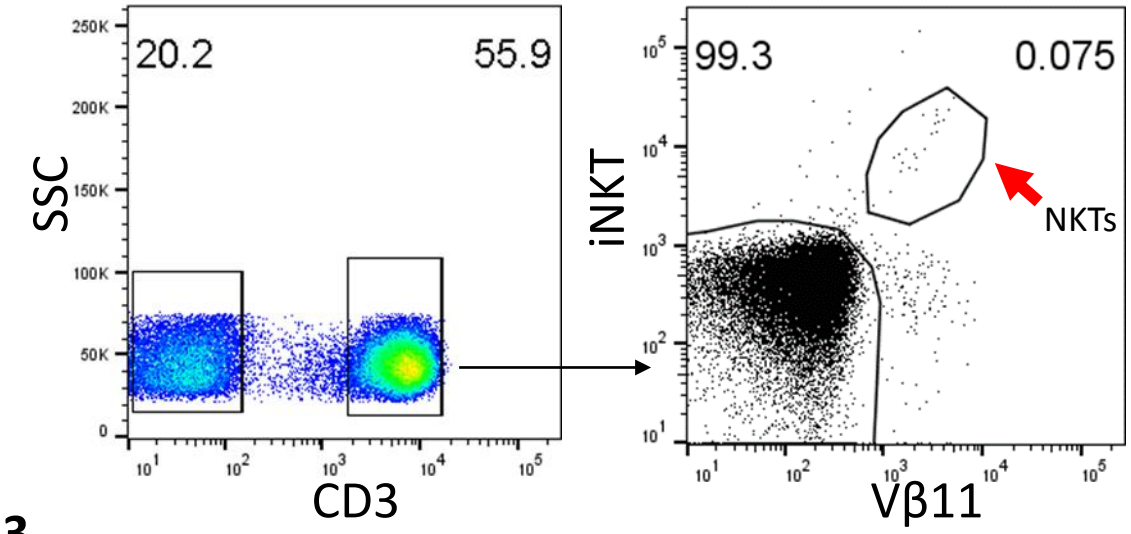
CAR NKT infusions are safe

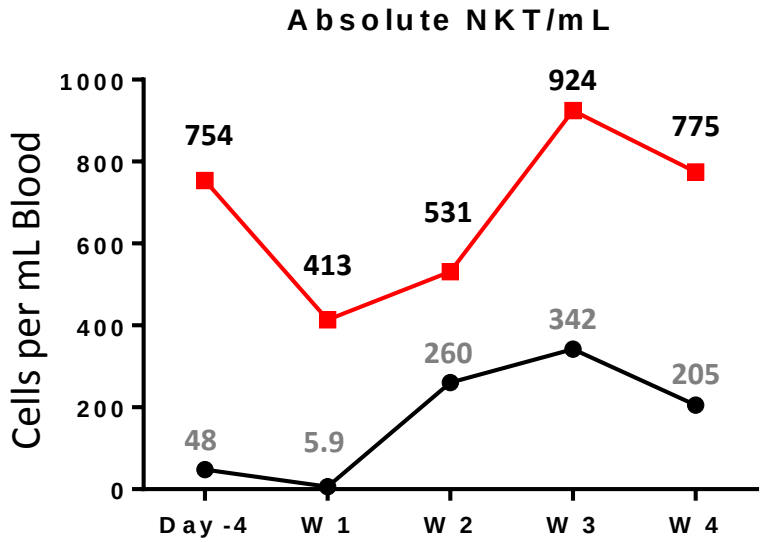
Body System	Event	Grade
Blood and lymphatic system disorders	Anemia	3
	Lymphocyte count decreased	4
	Neutrophil count decreased	4
	Platelet count decreased	4
	White blood cell decreased	4
Metabolism and nutrition disorders	Anorexia	1
	Dehydration	1
	Hyperglycemia	1
	Hypermagnesemia	1
	Hyponatremia	1
	AST increased	1
Musculoskeletal and connective tissue disorders	Back pain	1
Respiratory, thoracic and mediastinal disorders	Epistaxis	1

Evidence of CAR-NKT *in vivo* expansion after infusion

Day -4

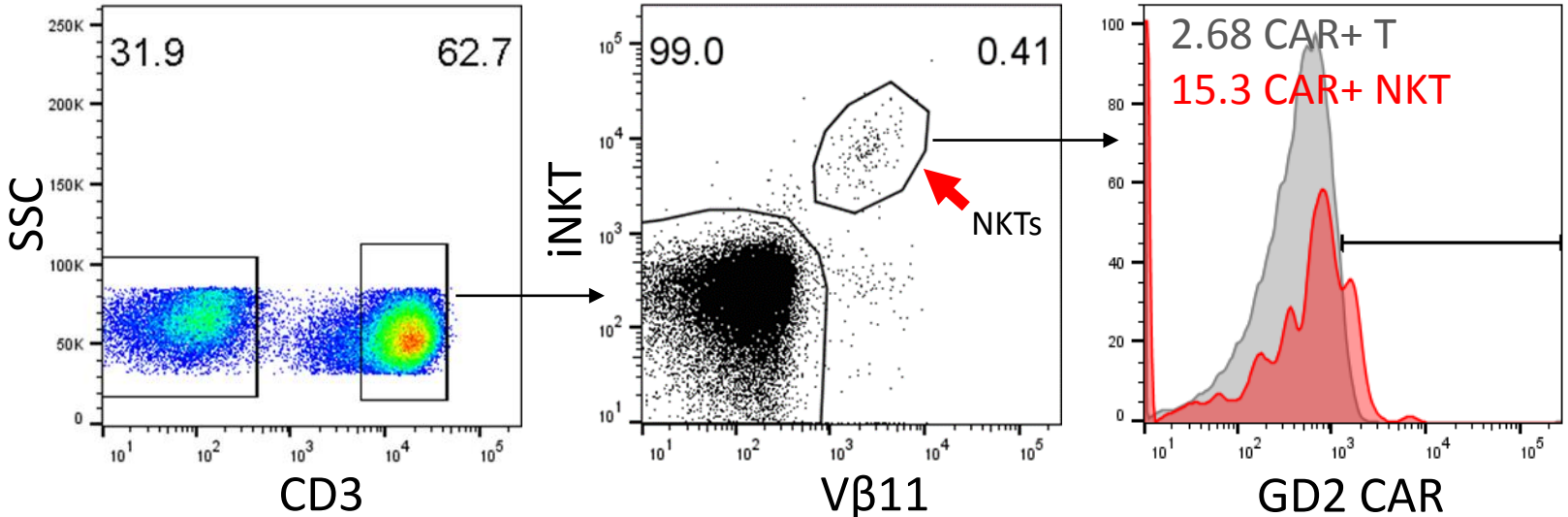
Prior to NKT infusion

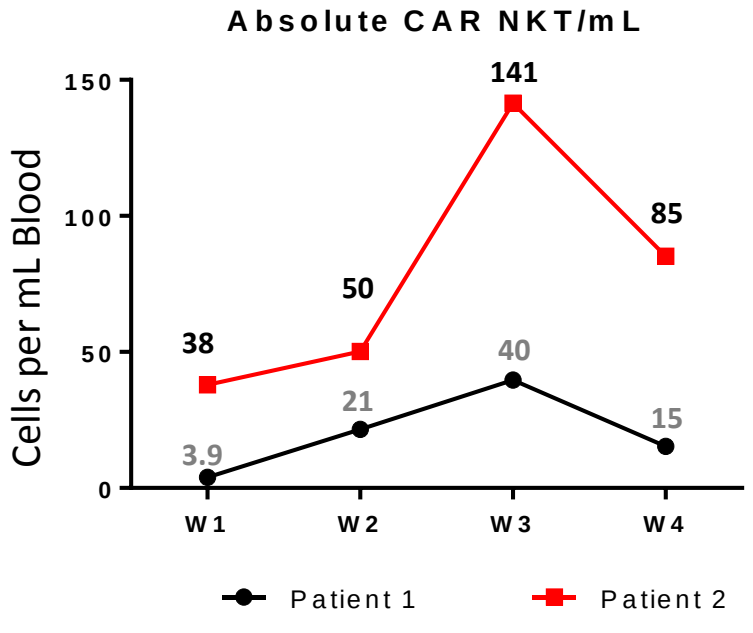




Week 3

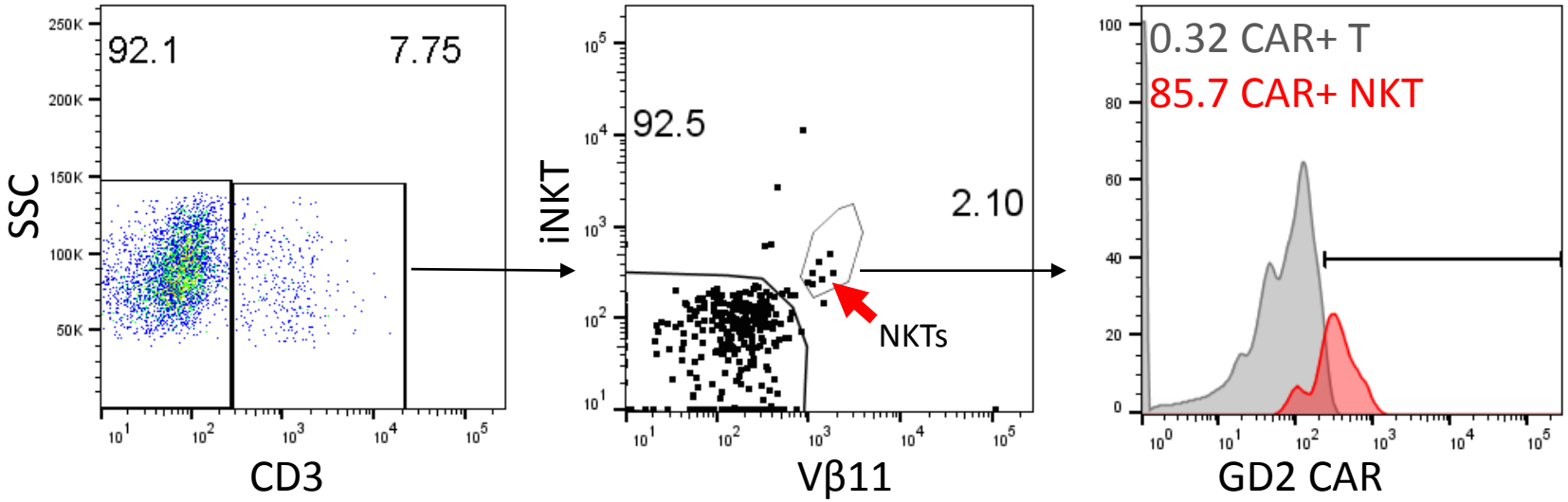
Post NKT infusion



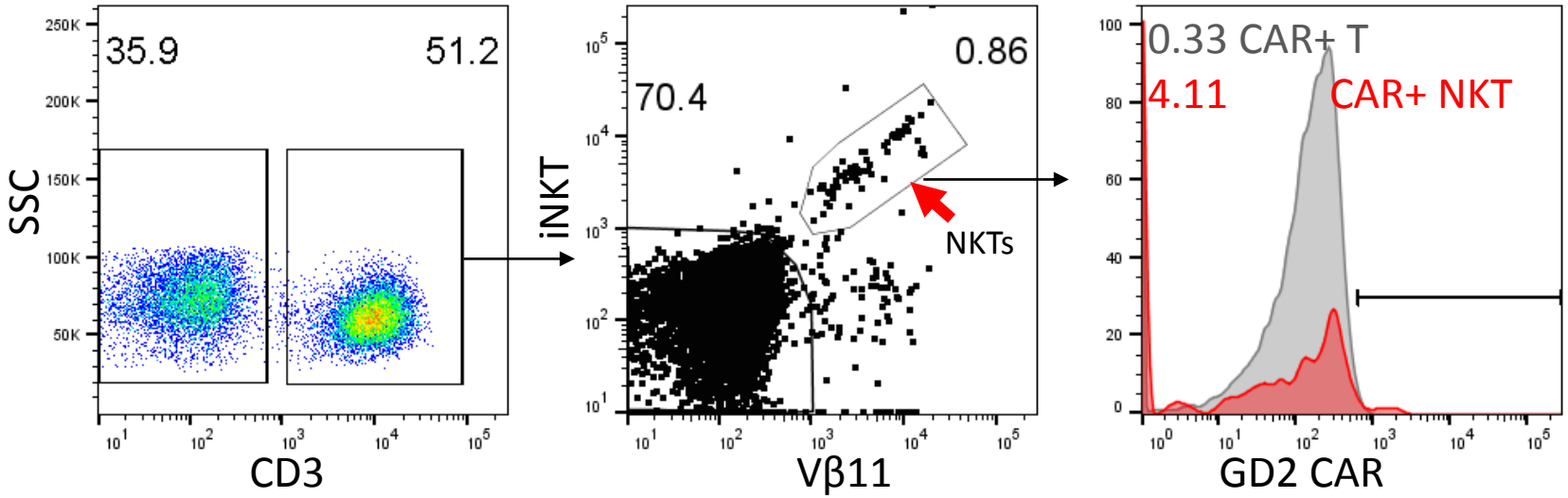


Evidence of CAR-NKT cell infiltration into solid tumor mass and bone marrow

Week 2 post-infusion
Tumor Biopsy



Week 4 post-infusion
Bone Marrow Aspirate



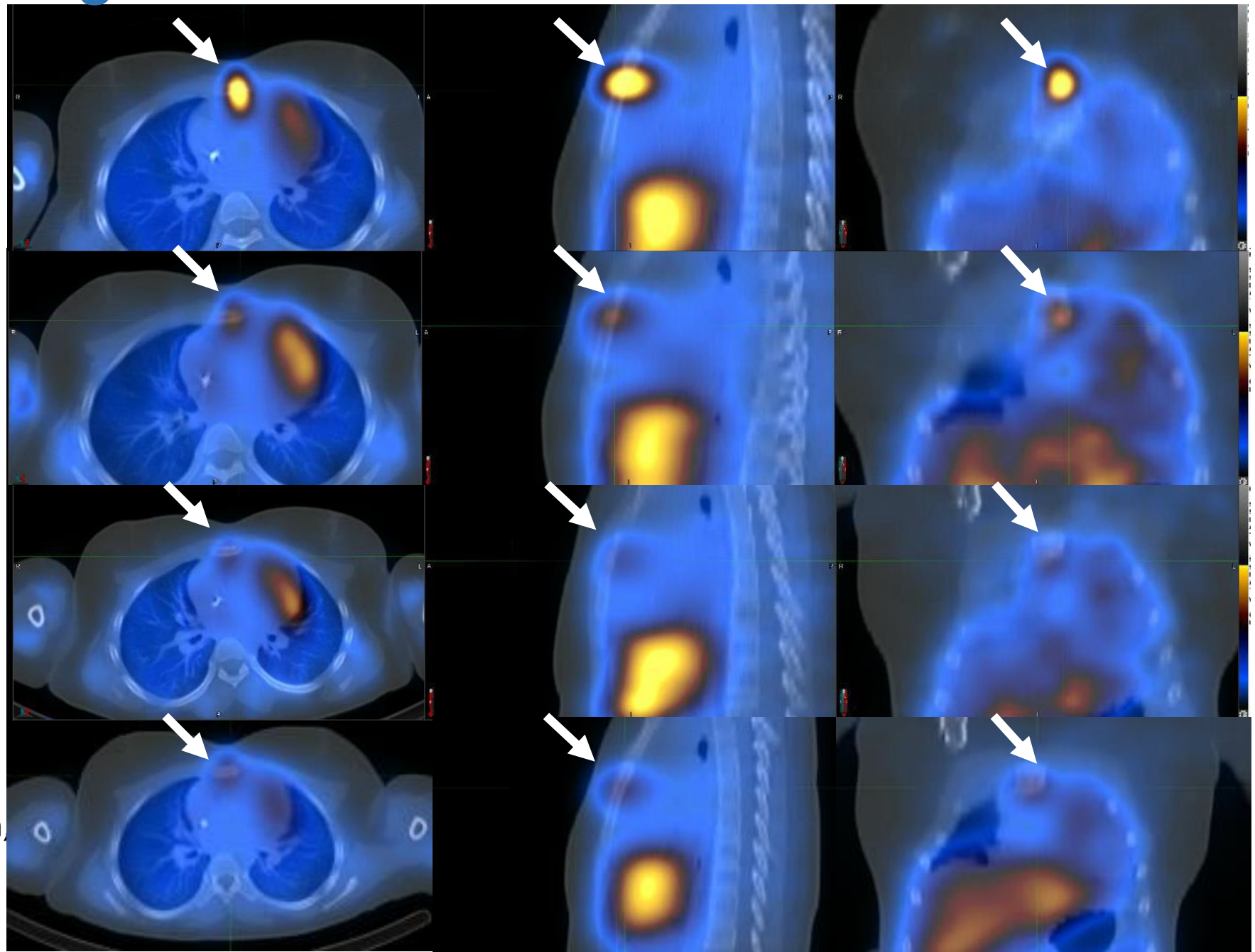
Sustained regression of thoracic tumor in 2nd Patient

Pre-infusion

Post-infusion,
4 weeks

Post-infusion,
8 weeks

Post-2nd infusion,
4 weeks



Part I Conclusions

- Tumor-infiltrating macrophages (TAMs), predictive of poor outcome in NB patients, can be specifically recognized and targeted by NKTs.
- CAR.GD2 NKTs exhibit dual specificity with high cytotoxic potential against GD2+ NB cells and CD1d+ M2 macrophages.
- CAR-NKT cells localize to the tumor site more effectively than CAR-T cells.
- A combination of CD28 and IL-15 within the CAR.GD2 construct enables *in vivo* expansion of CAR-NKTs, their accumulation at tumor sites, and long-term tumor control of $GD2^{high}$ NB xenografts in mice without significant toxicity.
- CAR-NKTs can be effectively manufactured to clinical scale according to cGMP standards and used for cancer immunotherapy.
- Initial clinical evaluation of CAR-NKTs in three patients with stage 4 R/R neuroblastoma showed minimal therapy-related toxicity, evidence of *in vivo* expansion, localization to metastatic sites, and a sustained near-complete tumor regression in one patient.

Next Step:

Use of allogeneic NKT cells as a platform for off-the-shelf cancer immunotherapy

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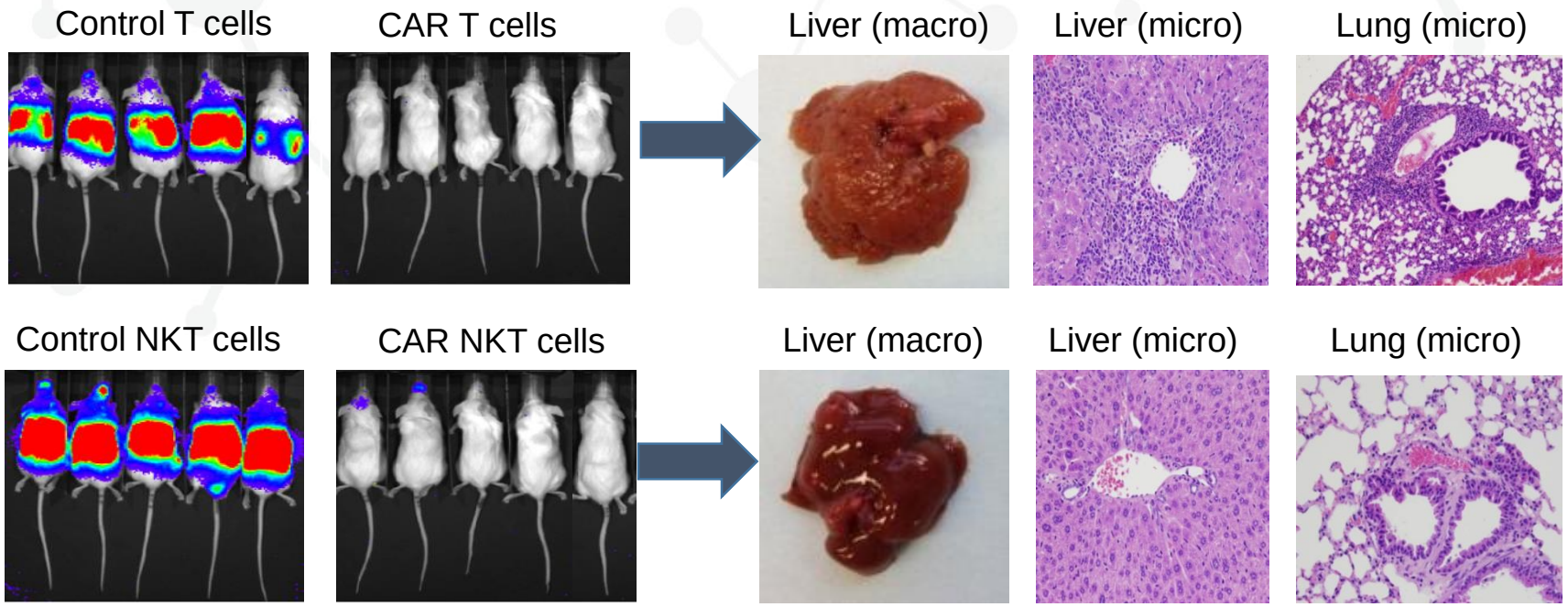
Compared to CAR.GD2 T cells, CAR.GD2 NKTs do not damage normal tissues in a xenogenic host



Tumor cells labeled with firefly luciferase

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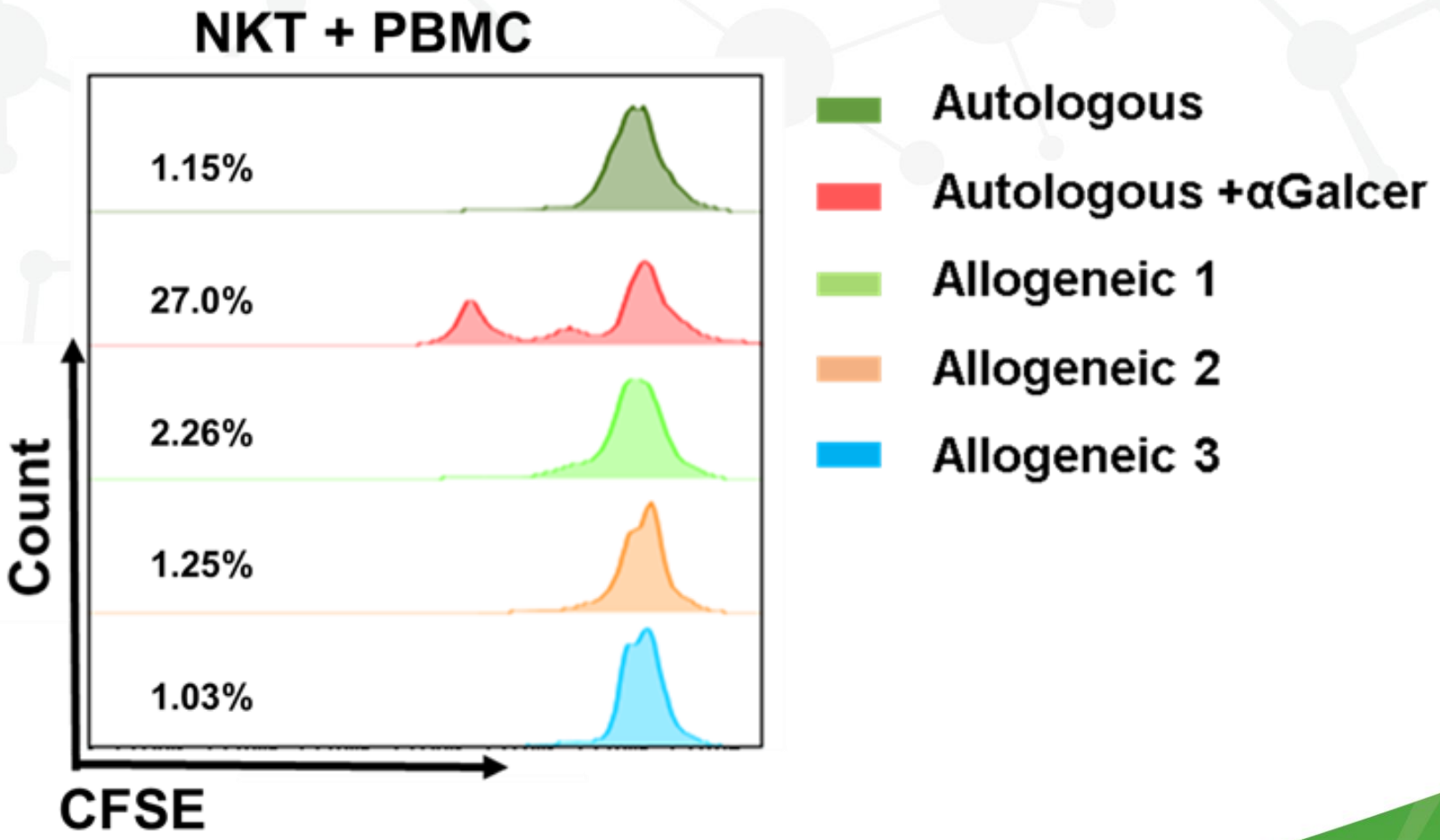
A. Heczey *et al, Blood*, 2014; 124:2824-33.

NKTs do not proliferate in the presence of allogeneic PBMCs



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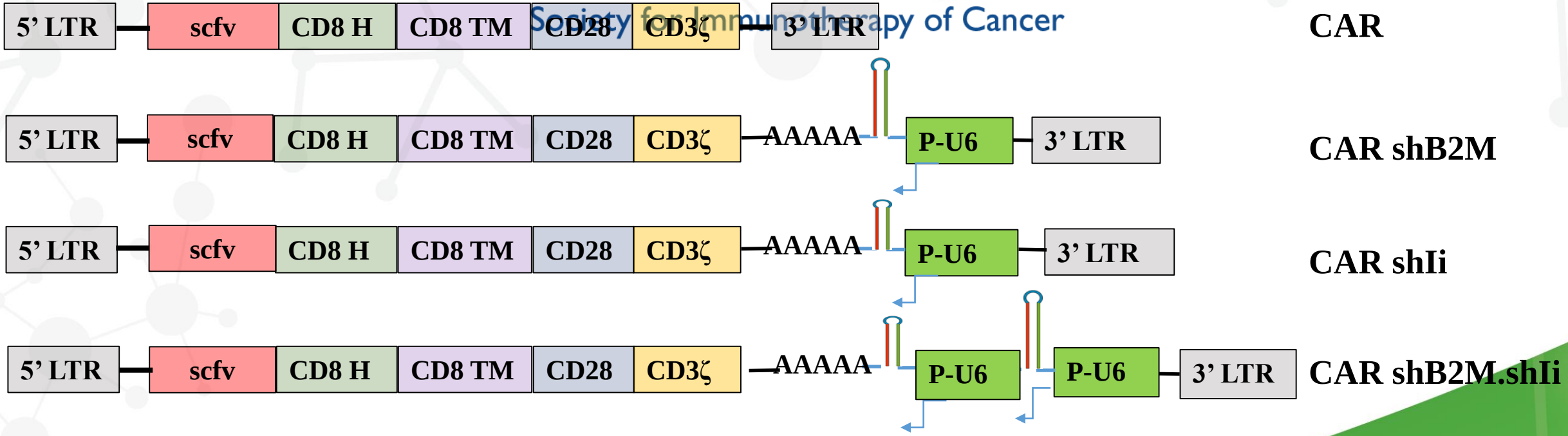
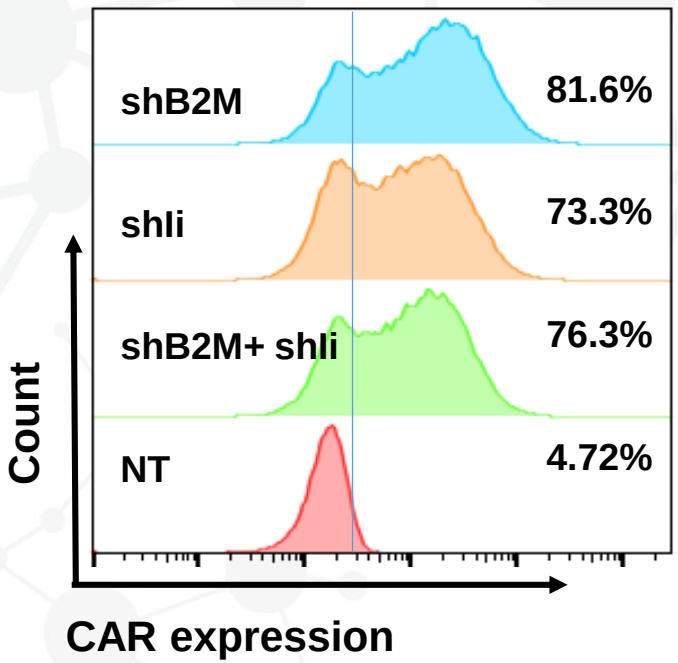
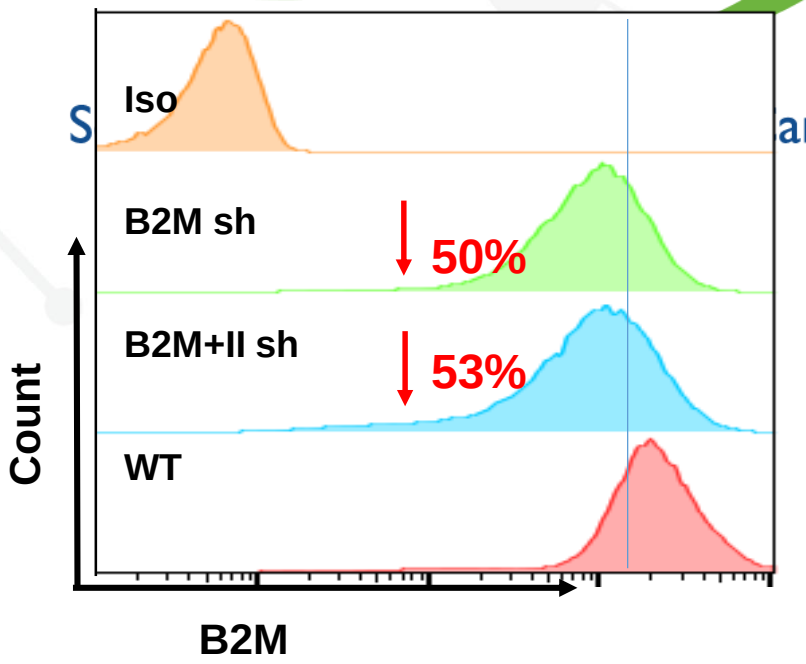
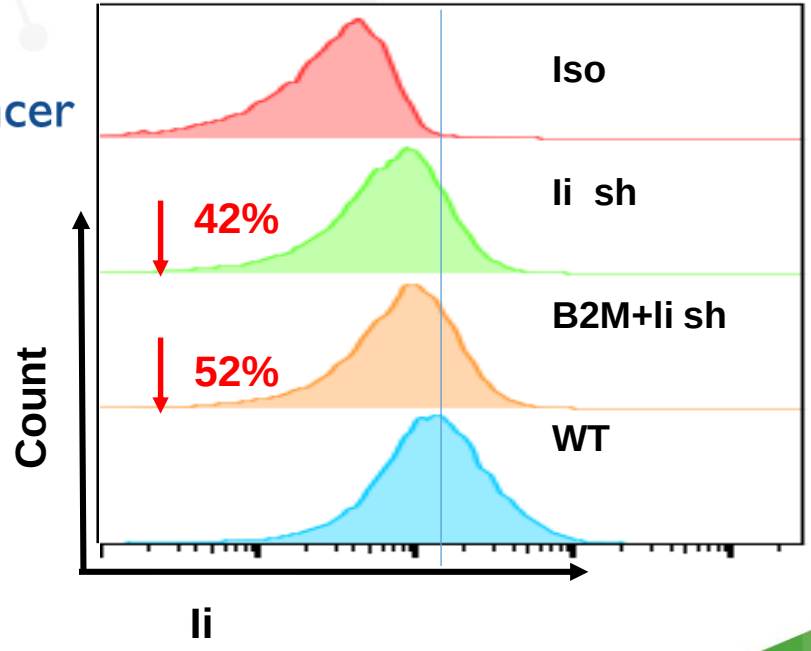
Generation of universally tolerated $^{(UT)}$NKTs co-expressing a CD19 CAR and shRNAs targeting B2M and Ii



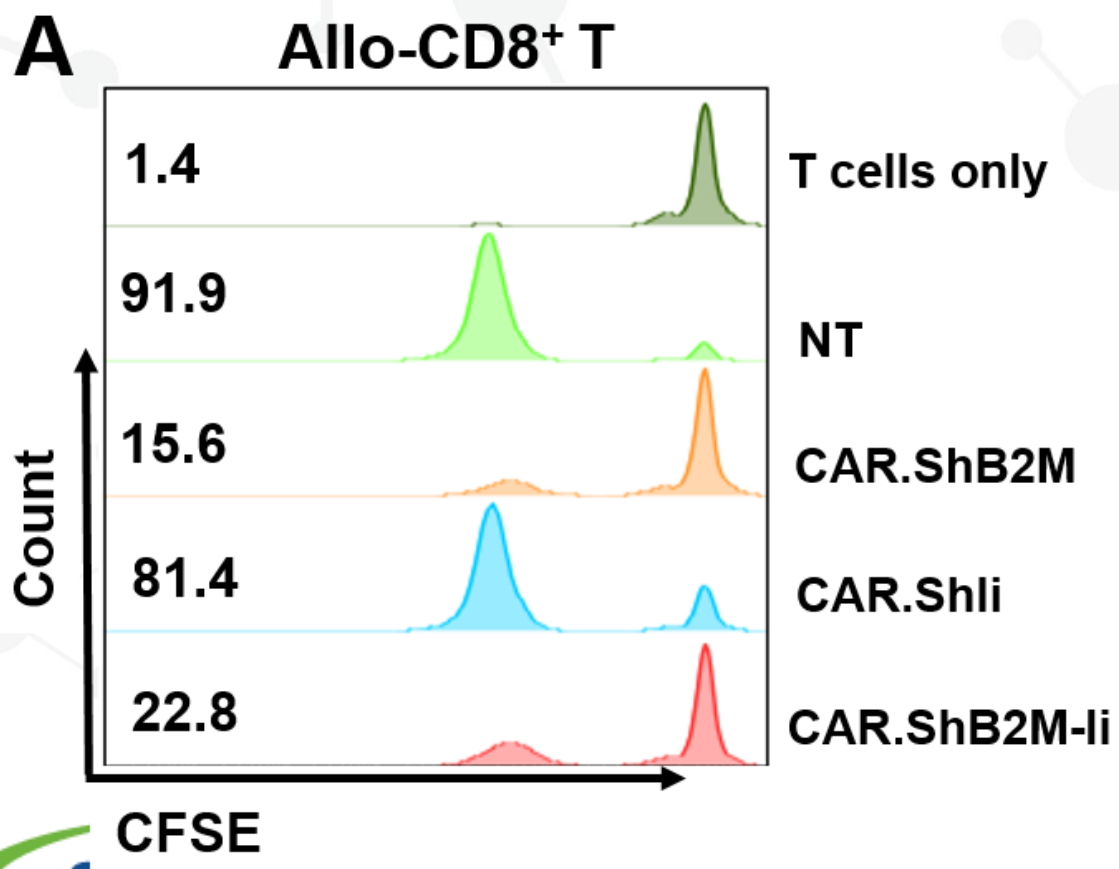
Effective Co-expression of CAR and shRNA in ^{UT}NKTs

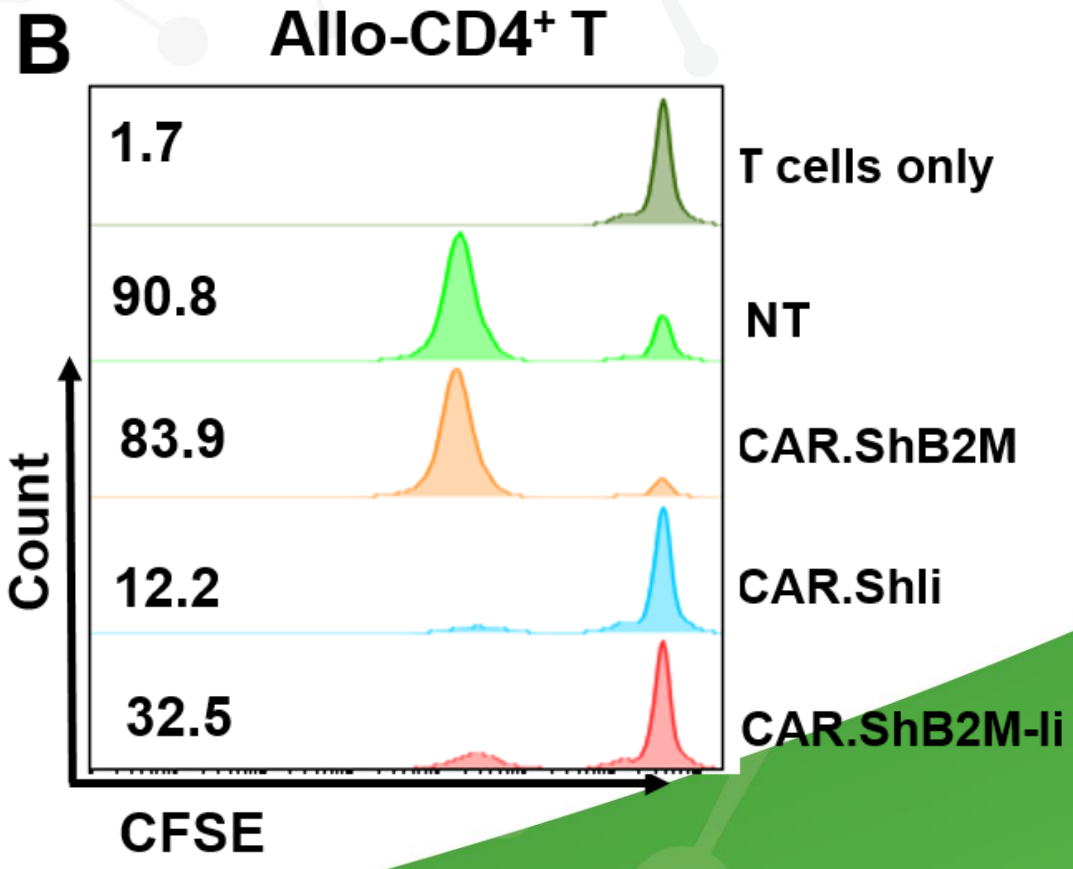






Allogeneic CD8 and CD4 T cells show diminished alloreactivity to CD19 CAR ^{UT}NKT cells in MLR assay





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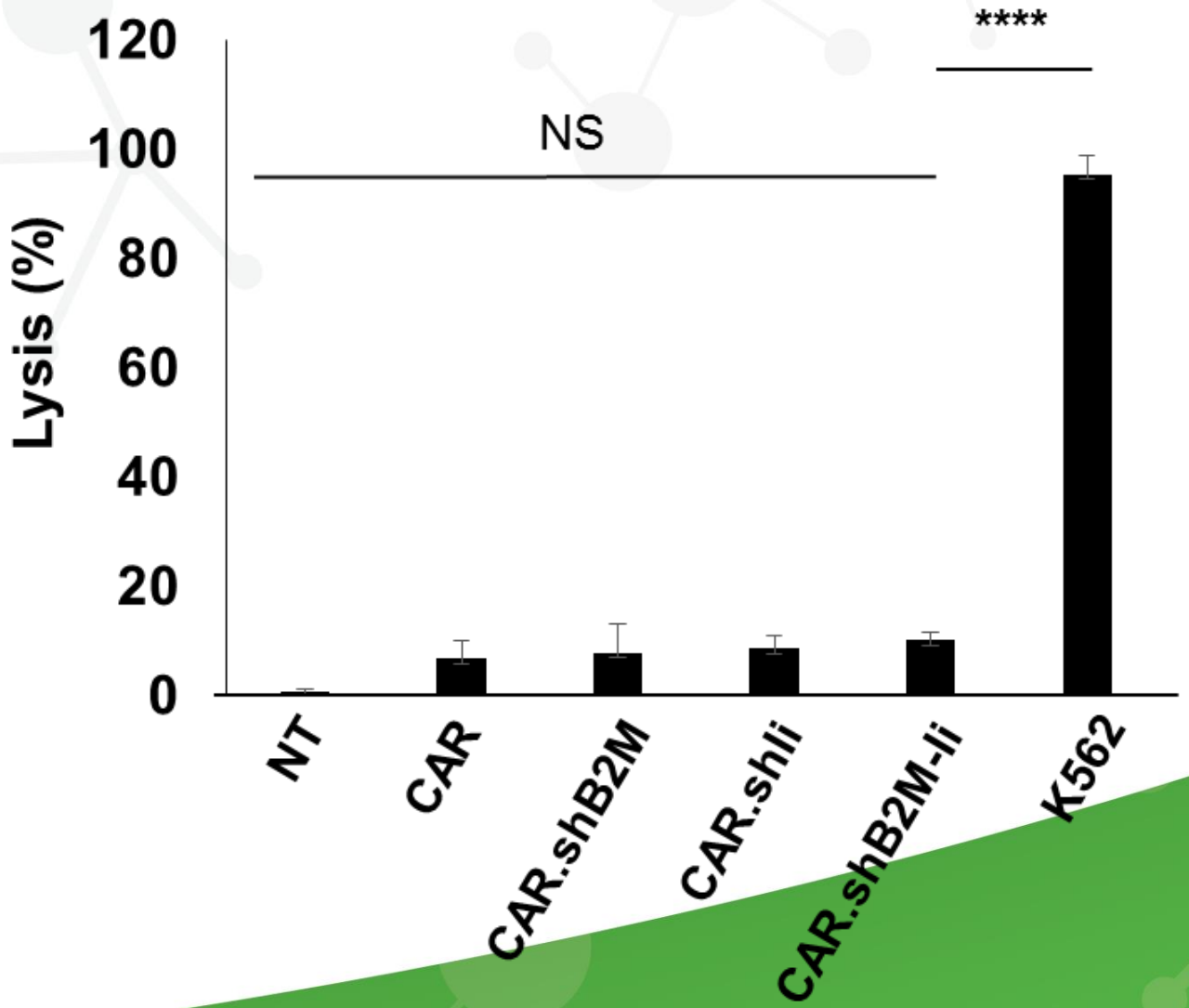
CD19 CAR ^{UT}NKT cells are minimally susceptible to NK cell cytotoxicity



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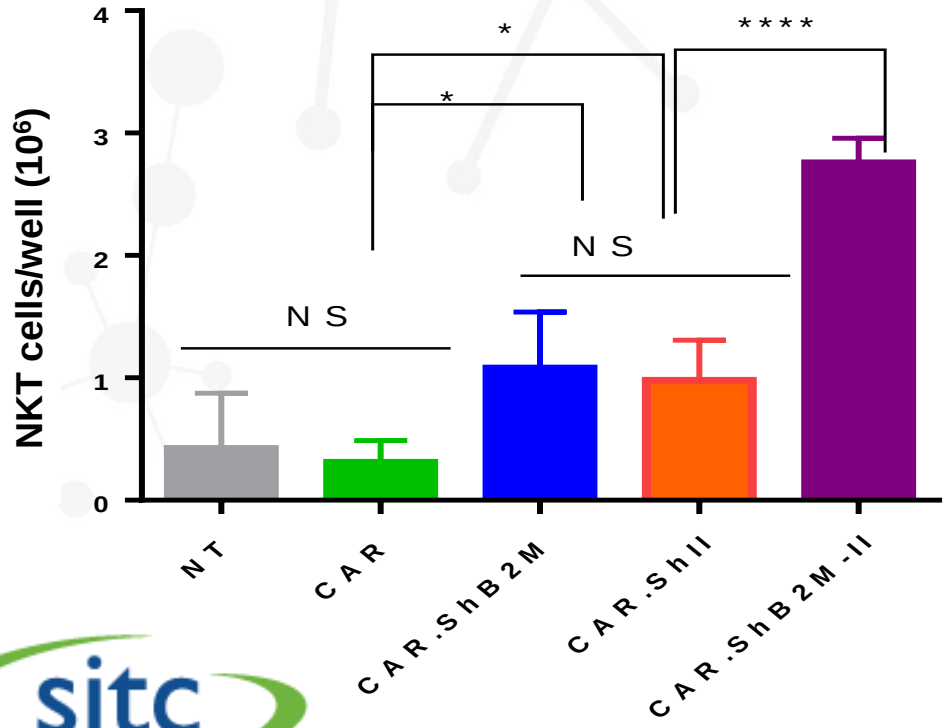
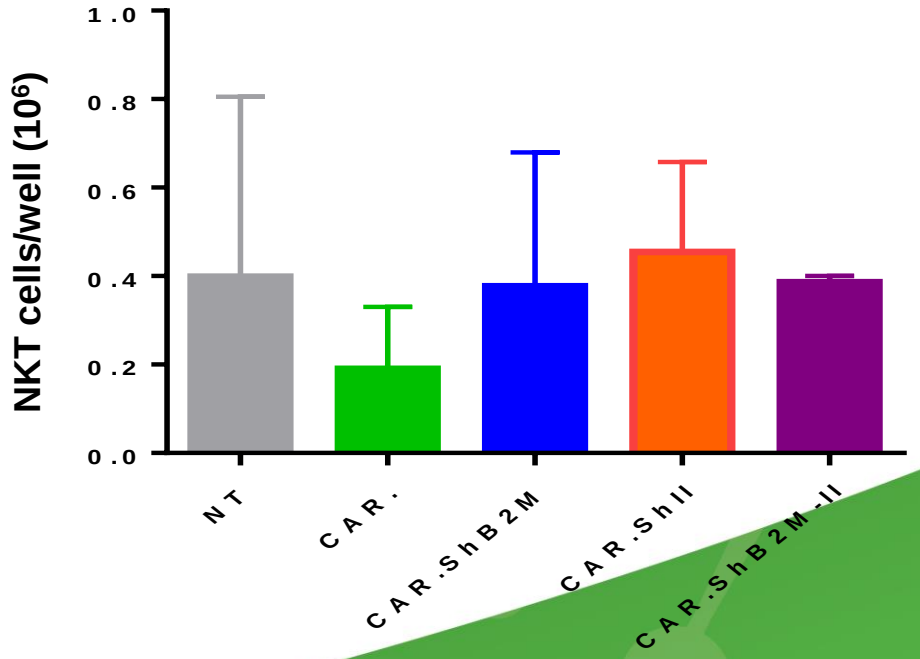
CD19 CAR ^{UT}NKT cells are selectively protected in a 4-day culture with allogeneic PBMC

CAR+ NKT cells



CAR- NKT cells



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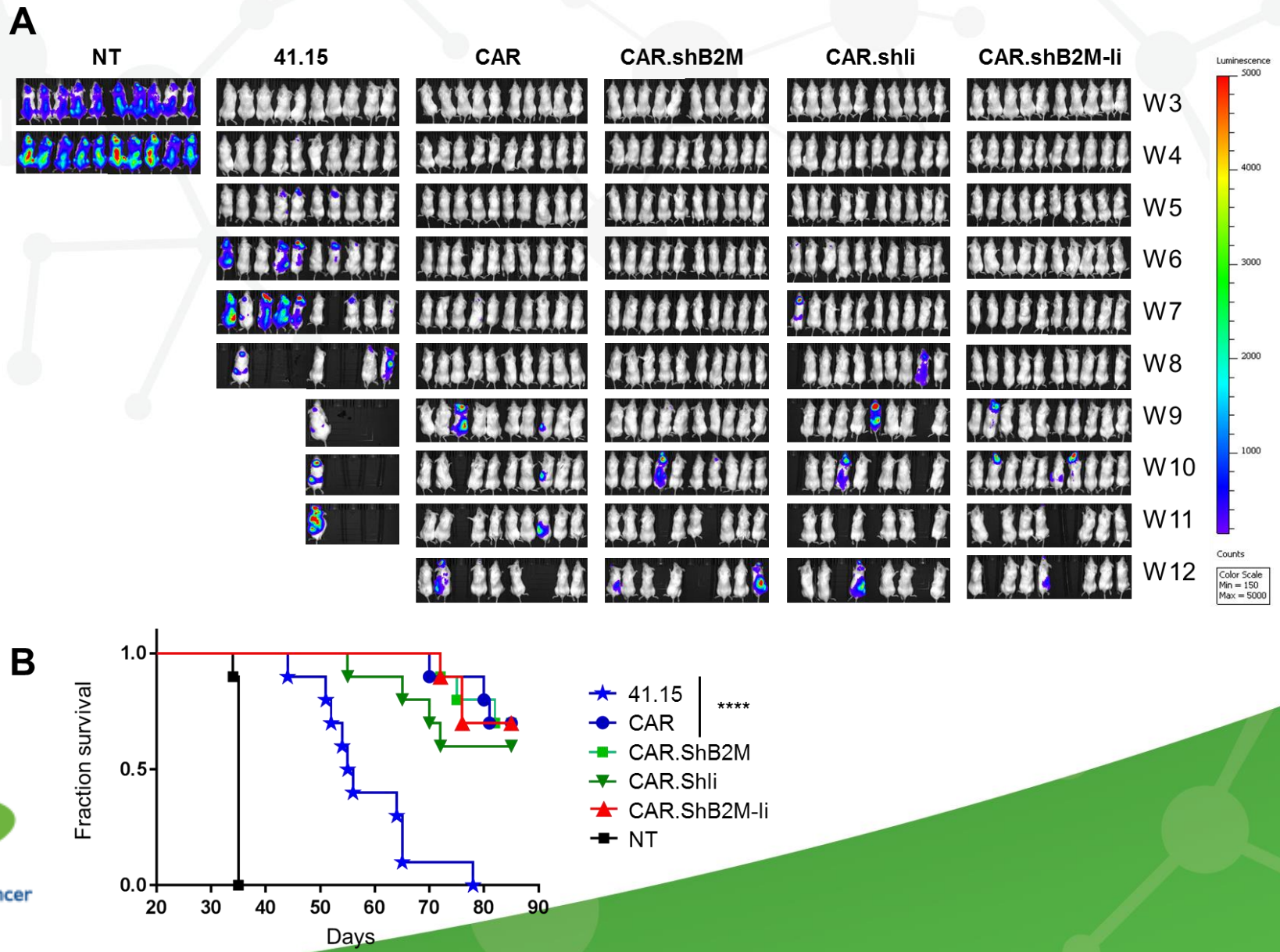
In vivo antitumor activity of CD19 CAR ^{UT}NKTs in NSG mice injected with Ffluc-labeled Daudi lymphoma cells



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Phase 1 Clinical Trial: CD19.CAR Allogeneic NKT for Patients With Relapsed or Refractory B-Cell Malignancies (ANCHOR)

NCT03774654

- R/R high-risk B-cell malignancies
- Dose escalation: 10^7; $3x10^7$, and $10^8/m^2$
- Lympho-depletion regimen:
  - Cyclophosphamide 500 mg/m^2/dose on days -4, -3, and -2 and fludarabine 30 mg/m^2/dose on days -4 and -3 intravenously
- Safety
- CAR NKT cell persistence and trafficking
- Antitumor responses

Part II Conclusions

- NKT cells are not alloreactive and can be used for off-the-shelf therapy without matching
- Universally tolerated NKT cells (^{UT}NKT) can be generated via shRNA targeting of B2M and Ii and thereby HLA class-I and class-II expression
- ^{UT}NKTs have reduced stimulatory activity for allogenic T cells and are minimally susceptible to NK cell cytotoxicity

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Anupama Gopisetty
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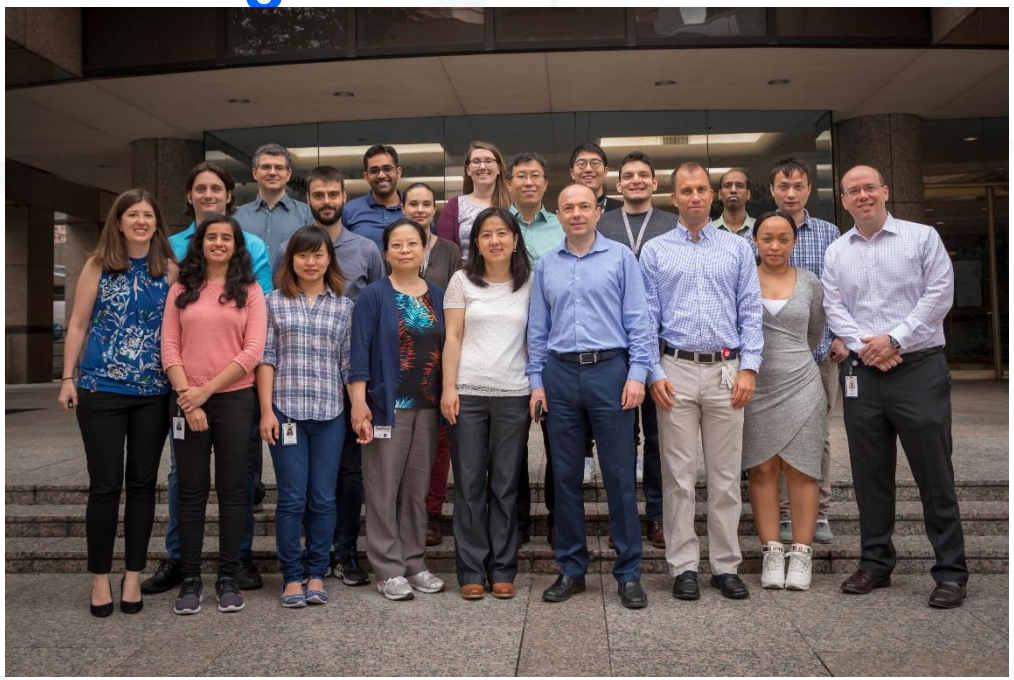

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Texas Children's Cancer Center

Center for CAGT Cell and Gene Therapy
BCM Methodist Texas Children's Hospital

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