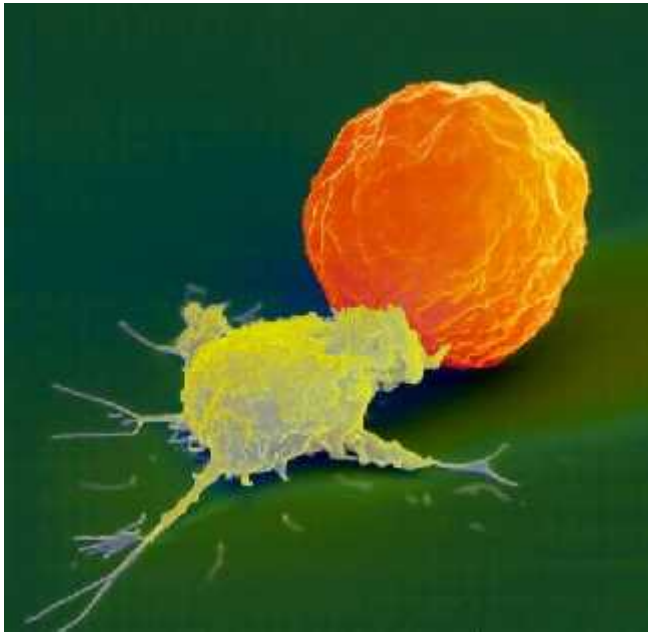
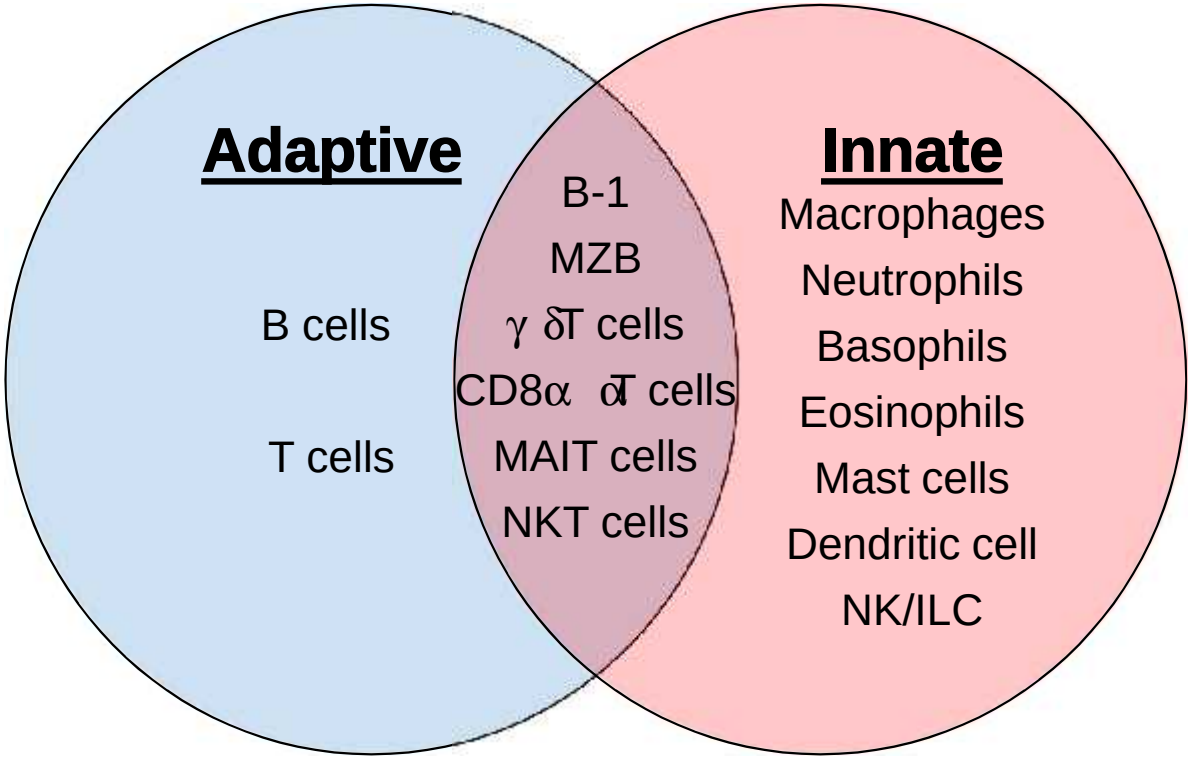
Rare immune cell types

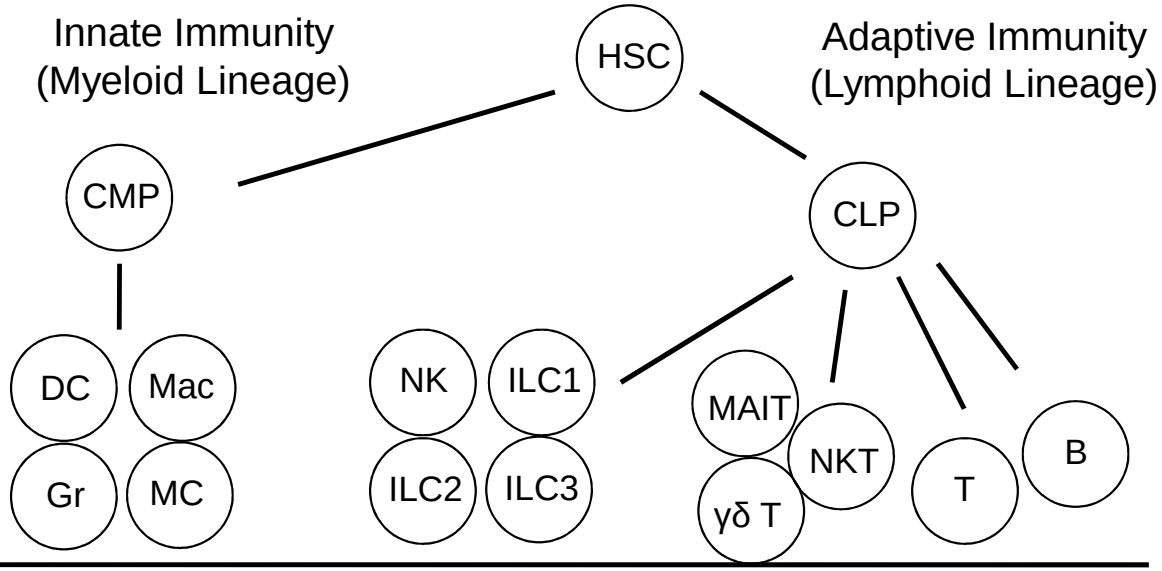


Mihalis Verykokakis, PhD
Stavros Niarchos Foundation Investigator
BSRC “Alexander Fleming”

2nd Immunology School for Clinicians
Heraklion, Crete
Nov 1st, 2019

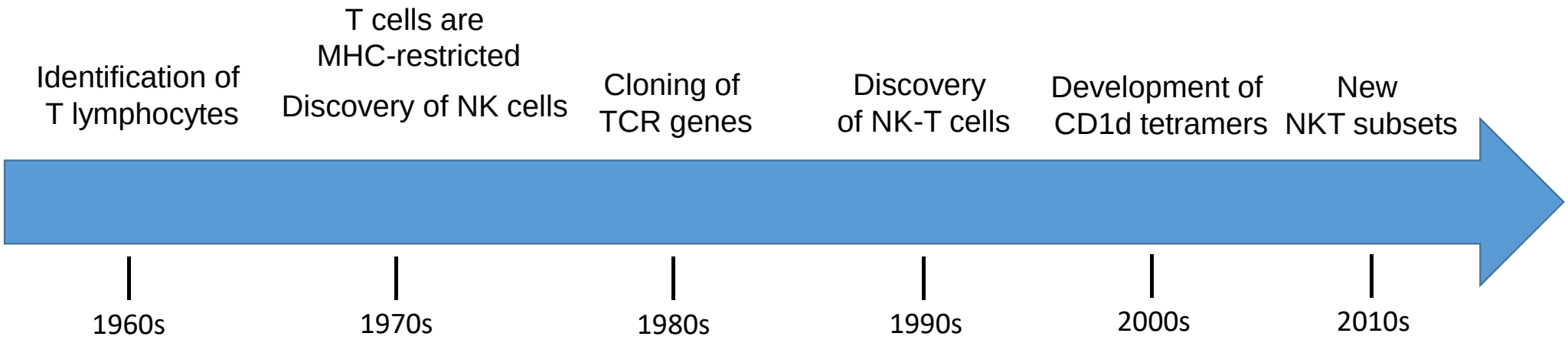
Immune system

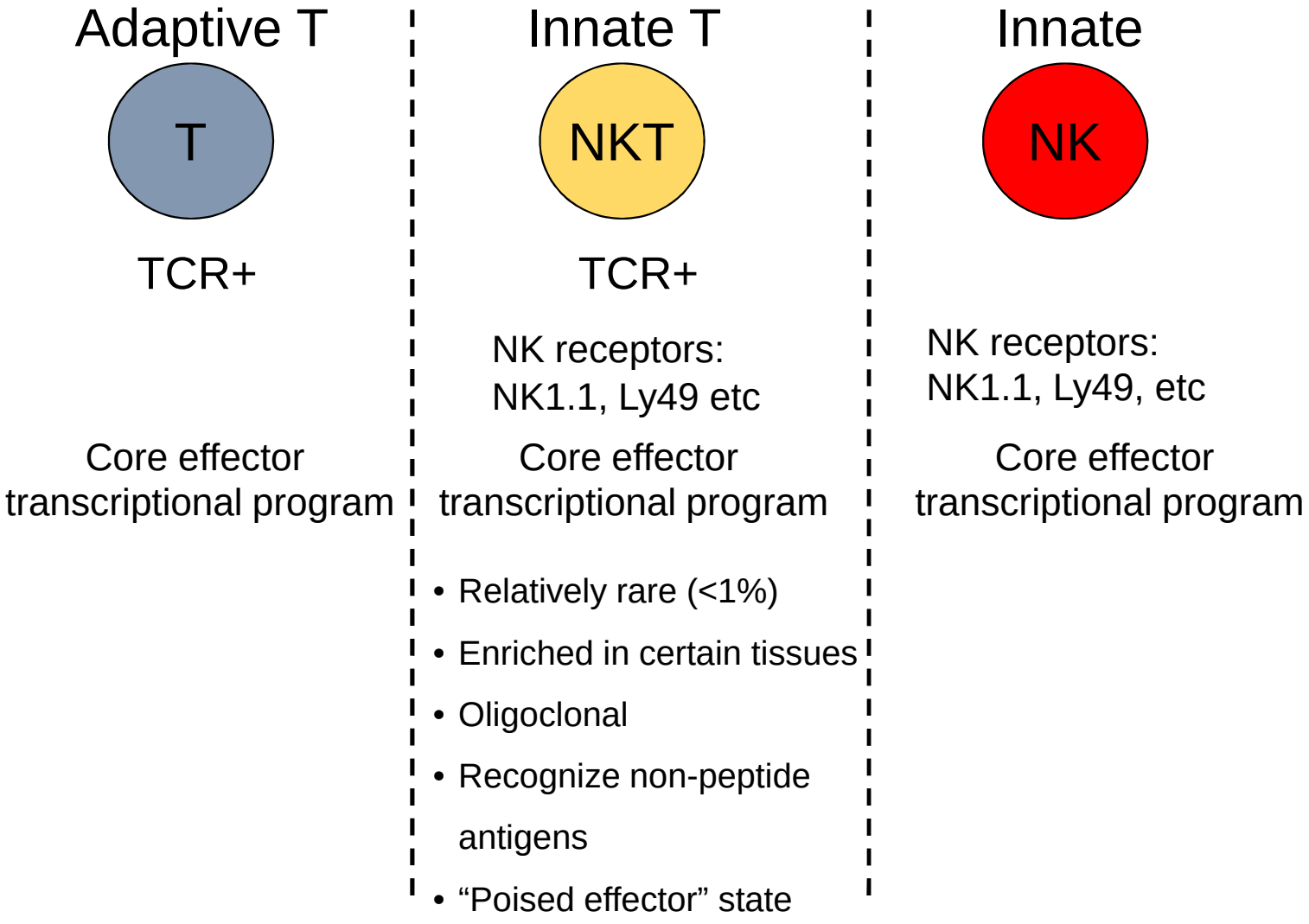




Property	Innate cells	Innate Lymphocytes	Innate T Lymphocytes	Adaptive Lymphocytes
Receptors	Germline encoded	Germline encoded	Rearranged and germline encoded	Rearranged
Recognition	Molecular patterns	Molecular patterns MHC-I	Molecular details	Molecular details
Action time	Immediate	Immediate	Immediate	Delayed
Memory	No	Yes?	No	Yes

Adaptive and Innate lymphocytes: 50+ years in the making




Adaptive T
T
TCR+
Core effector transcriptional program
Innate T
NKT
TCR+
NK receptors: NK1.1, Ly49 etc
Core effector transcriptional program
• Relatively rare (<1%)
• Enriched in certain tissues
• Oligoclonal
• Recognize non-peptide antigens
• “Poised effector” state
Innate
NK
NK receptors: NK1.1, Ly49, etc
Core effector transcriptional program

Innate-like T cells: Classification

Natural Killer T cells (NKT):

- Type I or invariant NKT
  - Invariant Vα14-Jα18 TCRα (human Vα24-Jα18) paired with limited TCRβ diversity (Vβ2, 7, 8.1-8.2, human Vβ11)
- Type II diverse NKT cells-Diverse TCRs
- Lipid reactive (ceramides, glycerols, sulfatides)
- Restricted by non-polymorphic CD1d molecules (MHC-I like)

Mucosal Associated Innate T cells (MAIT):

- Invariant TCRs (Vα19-Jα33 in mice, Vα7.2-Jα33 in humans)
- Abundant in humans
- Vitamin metabolites reactive
- MR1-restricted

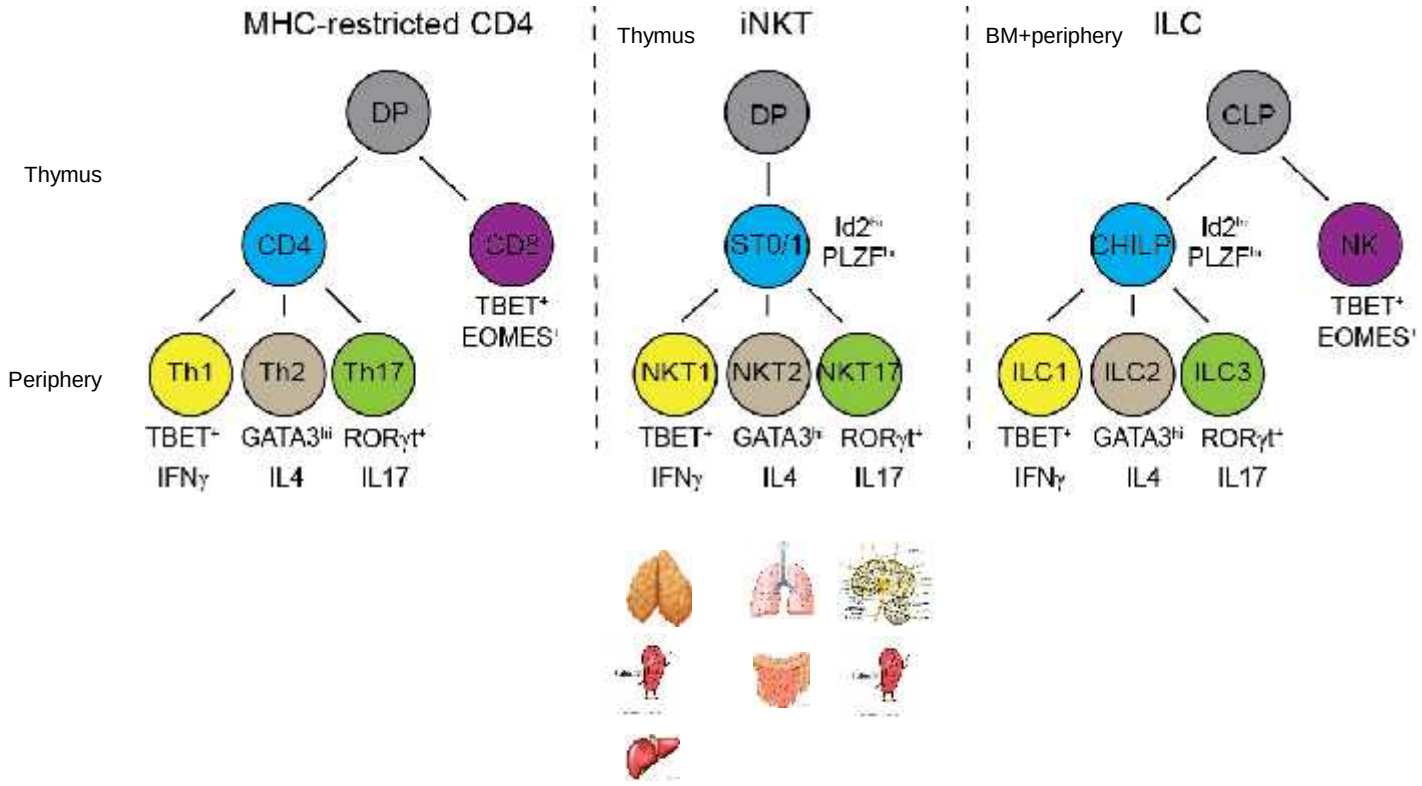
CD8αα intraepithelial T cells (CD8αα IEL):

- Diverse α βor γ δTCRs
- Unknown TCR reactivity
- ?-restricted (β2M is required)
- Intestine residents

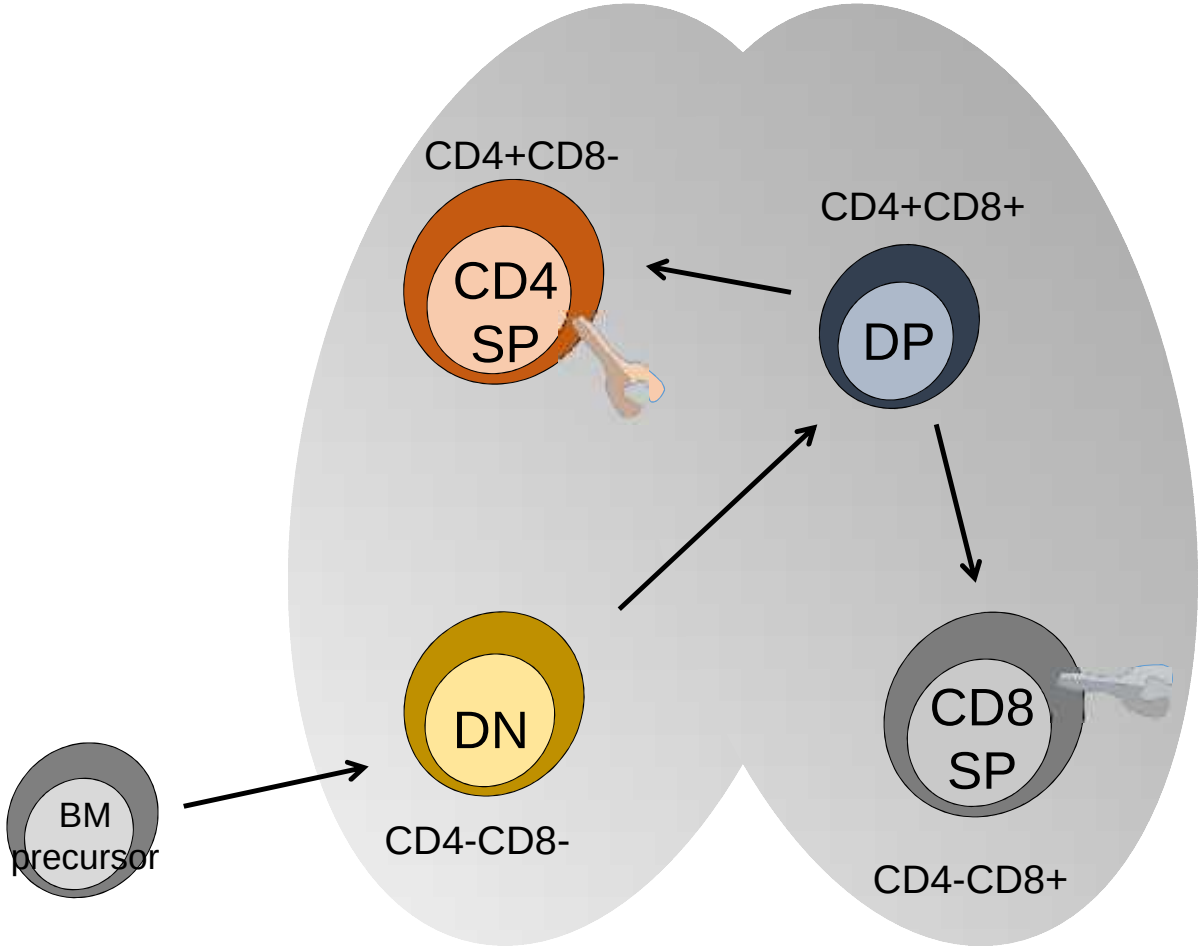
γδ T cells:

- Multiple invariant TCR
- Not CD1d-restricted
- Poorly defined reactivities

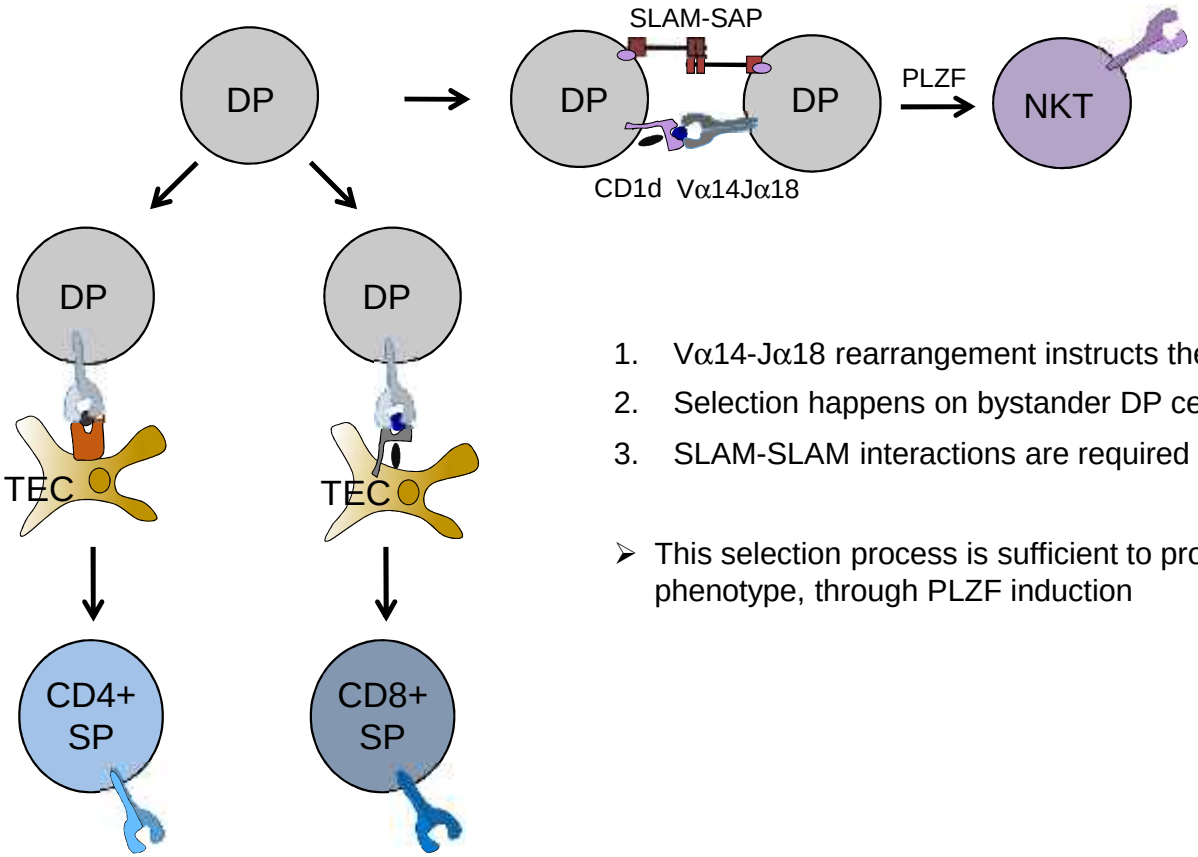
iNKT cells: the Usual Subsets



T cell lineage development happens in the thymus



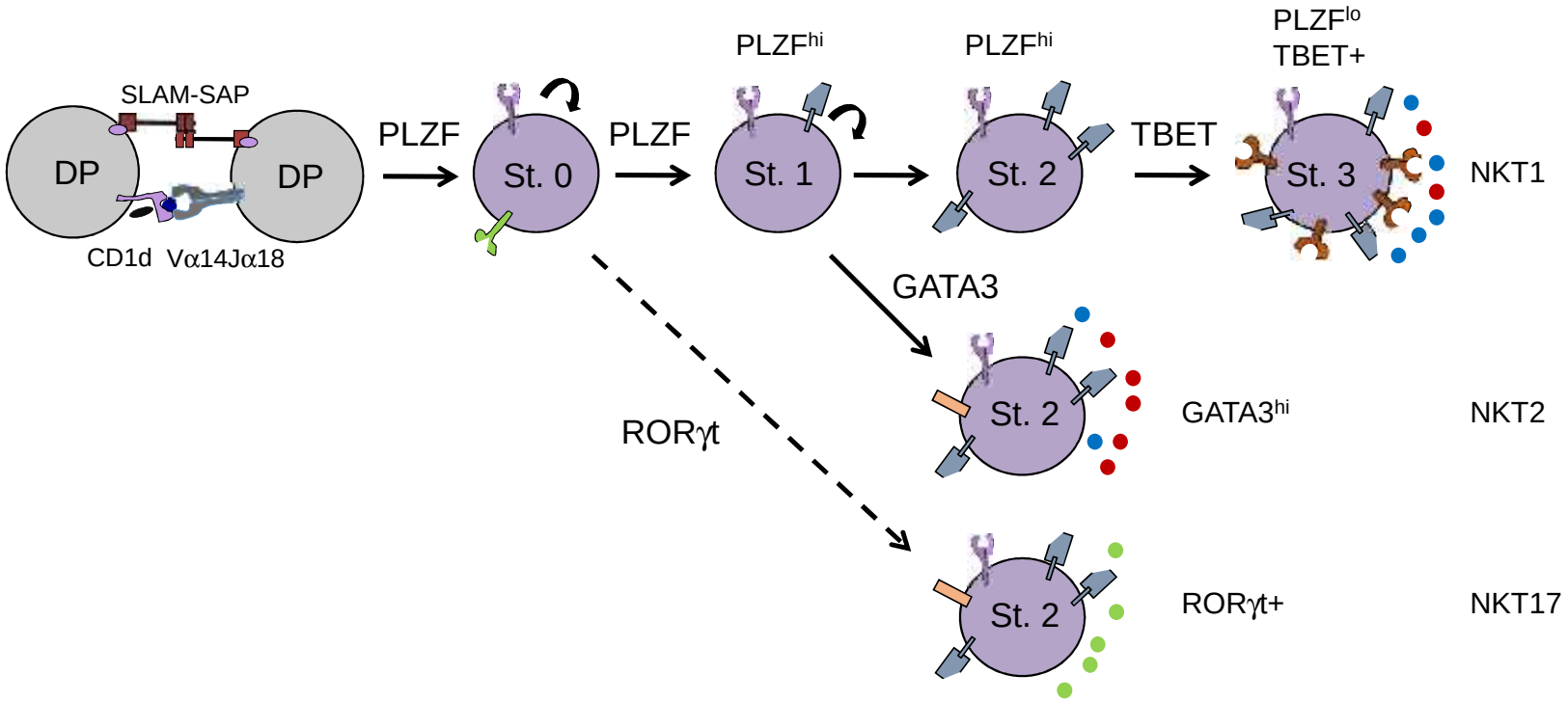
NKT cells: Distinct Thymic Selection



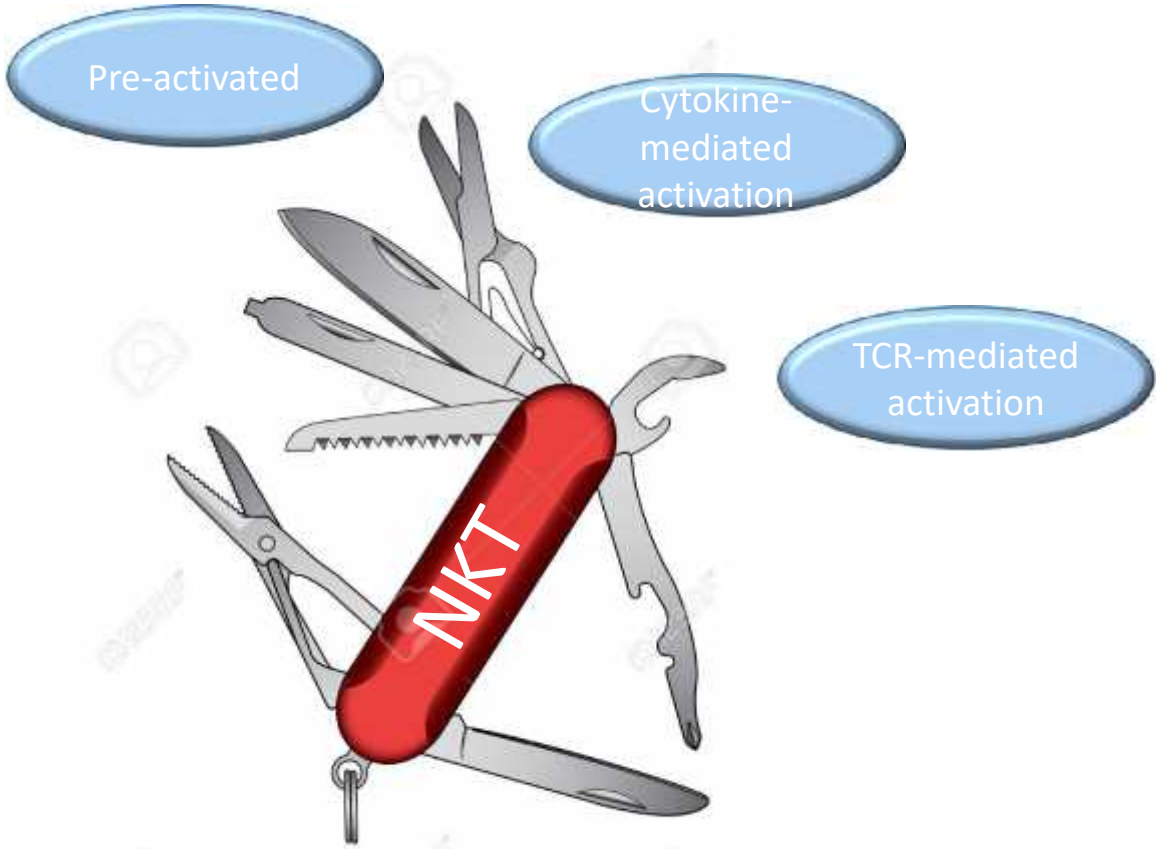
1. Vα14-Jα18 rearrangement instructs the NKT cell fate
2. Selection happens on bystander DP cells, through CD1D
3. SLAM-SLAM interactions are required

- This selection process is sufficient to promote the innate phenotype, through PLZF induction

iNKT cells: Step-wise thymic development



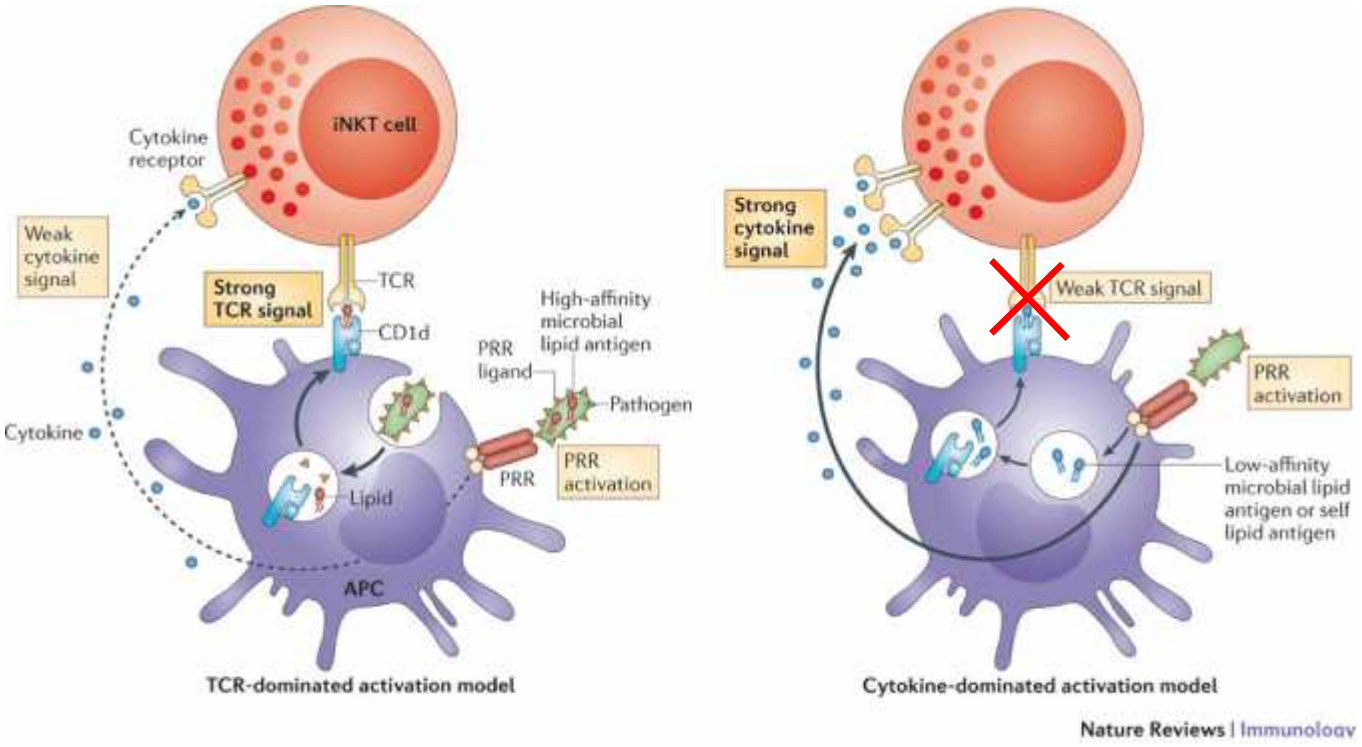
NKT cells: Unique properties



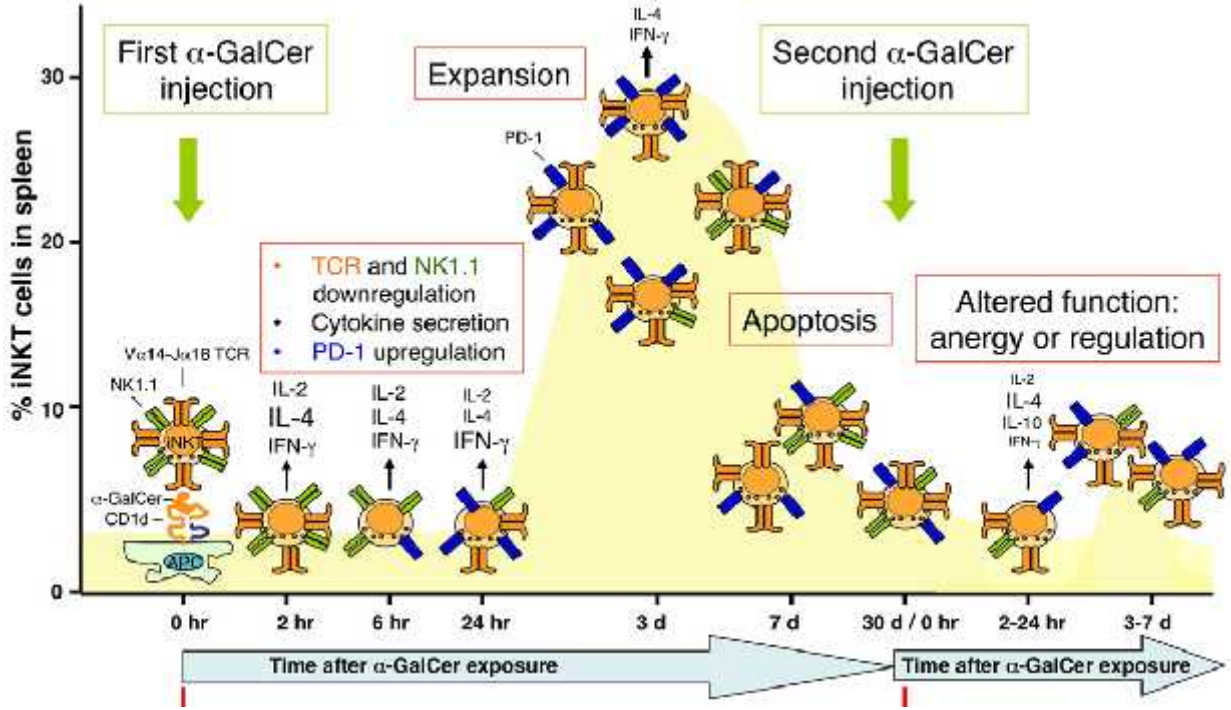
NKT cells: Modes of activation

Recognition of
foreign lipid antigens

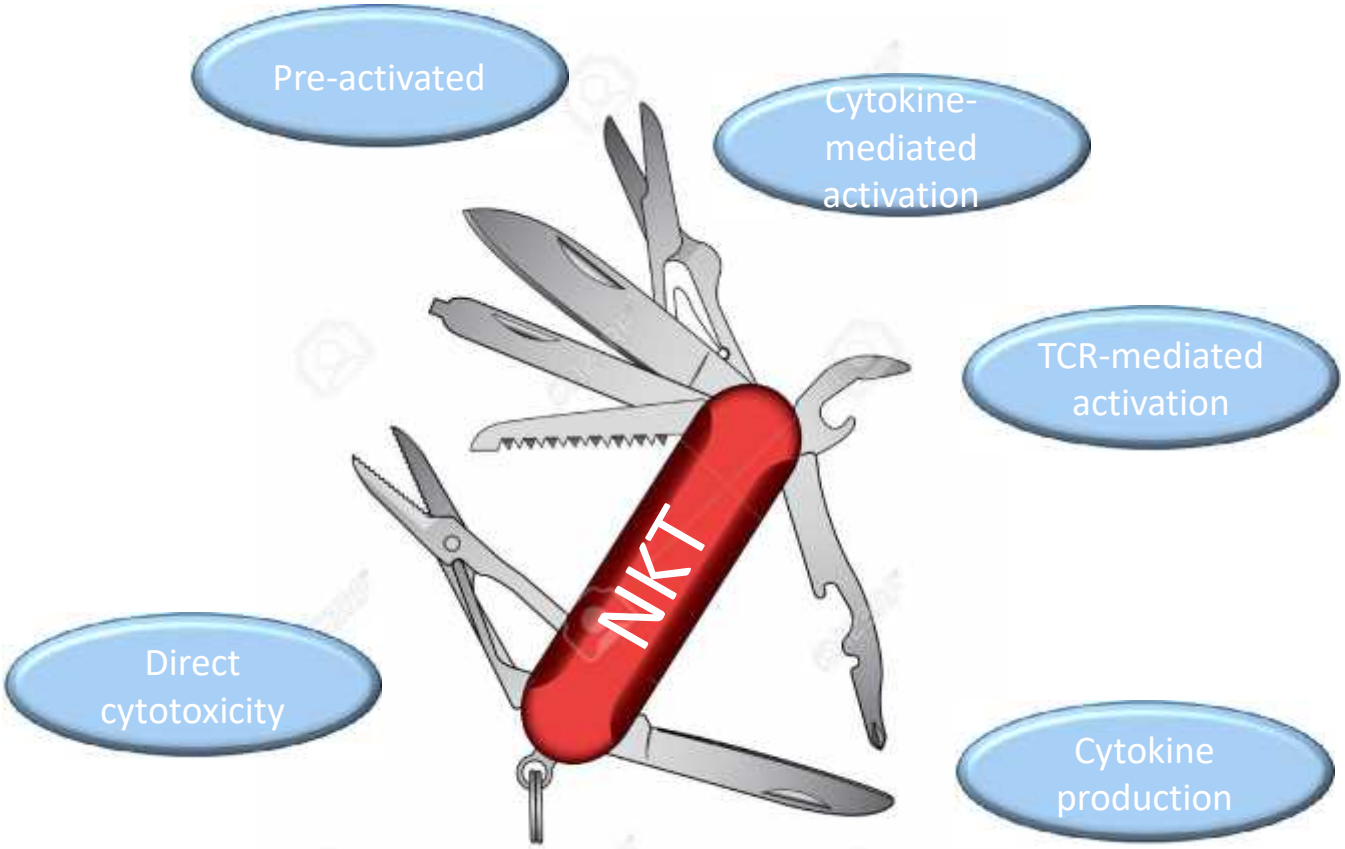
Recognition of
endogenous lipid antigens



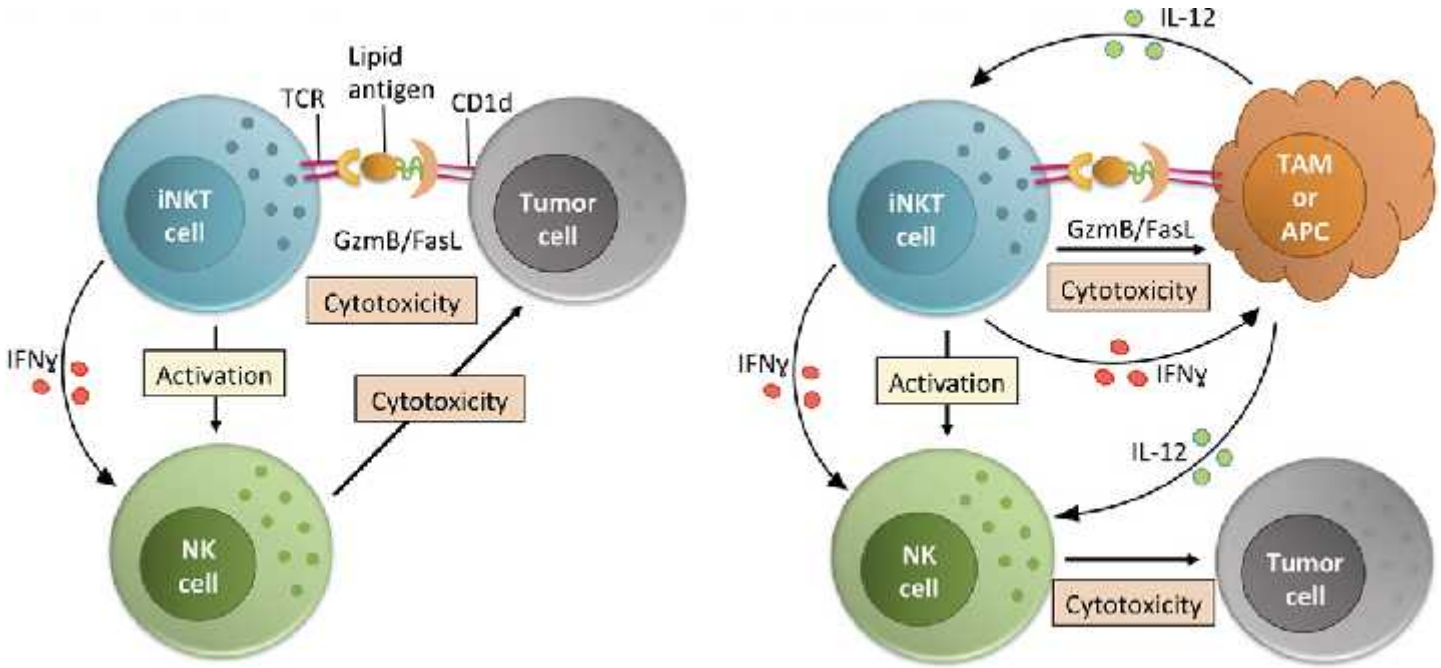
NKT cells become anergic after initial activation



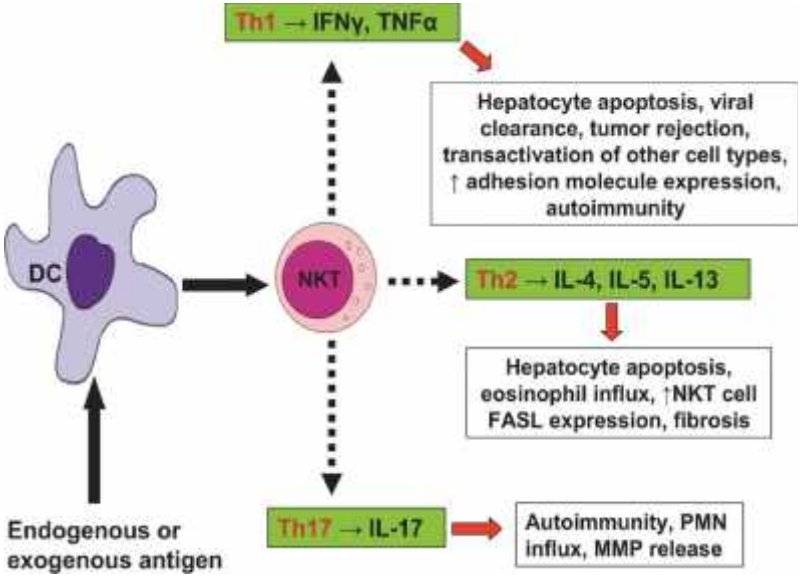
NKT cells: Unique properties

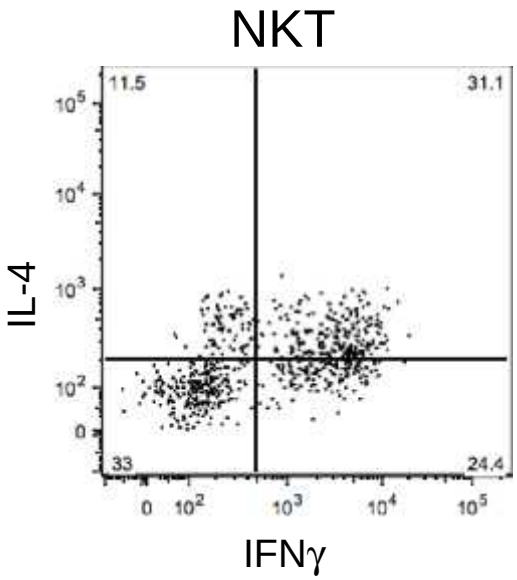


Direct killing by NKT cells

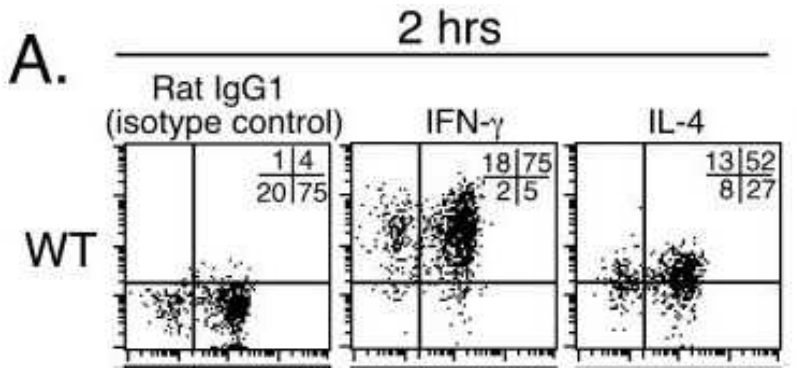


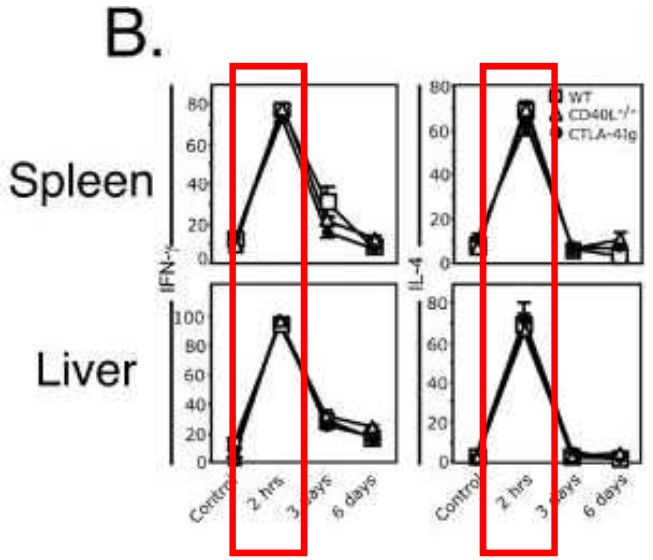
NKT cells produce a vast array of cytokines





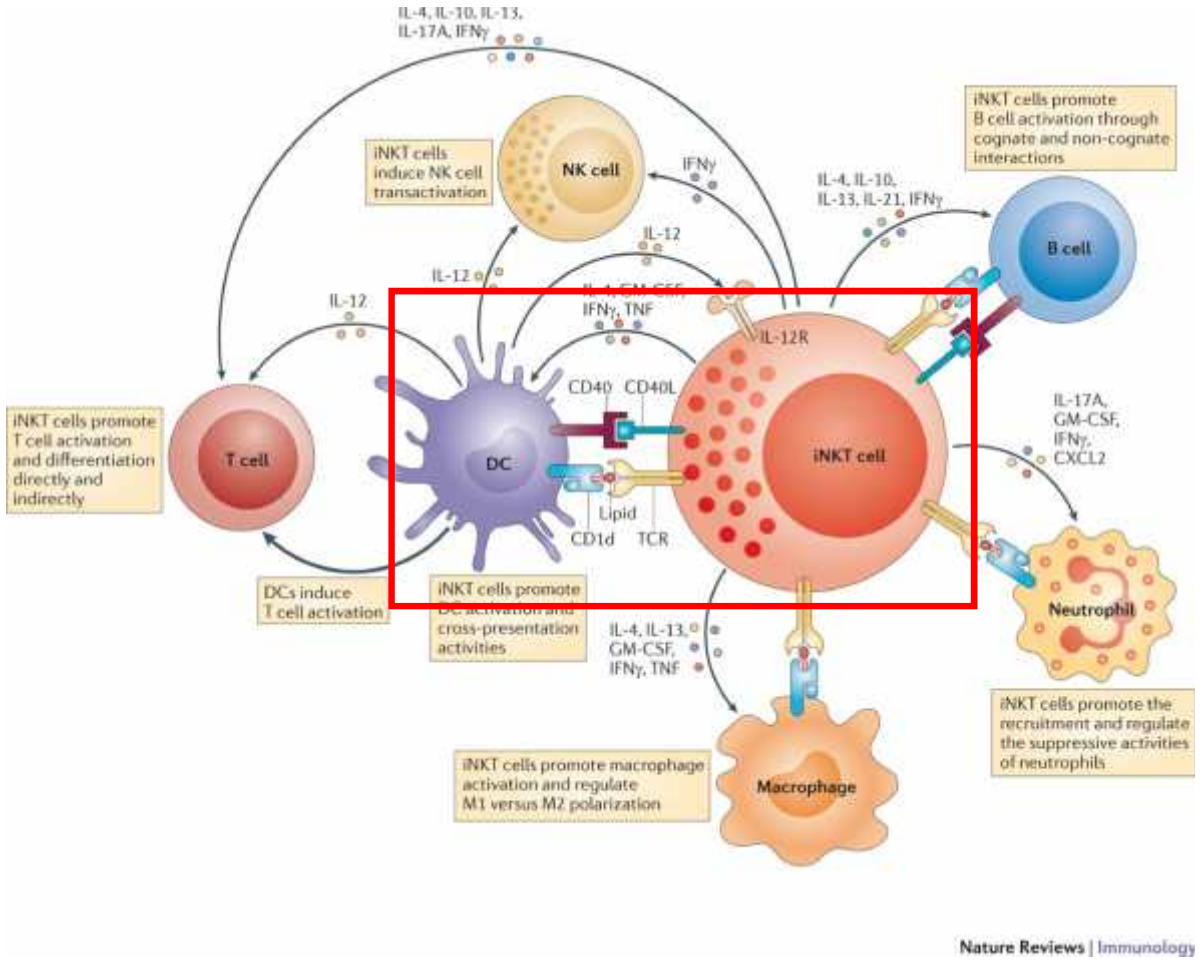
NKT cells produce a vast array of cytokines (fast)



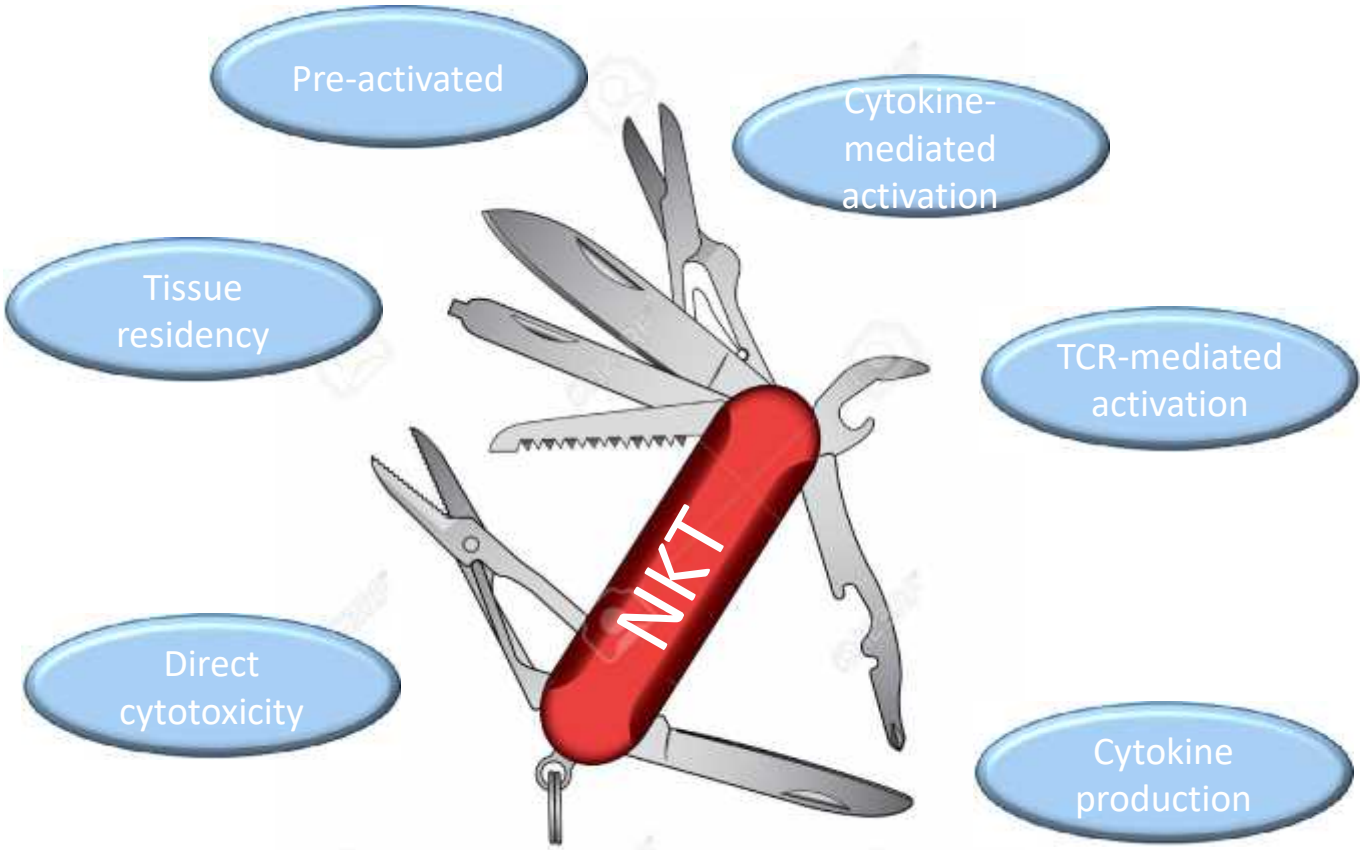


Uldrich et al, J Immunol 2005

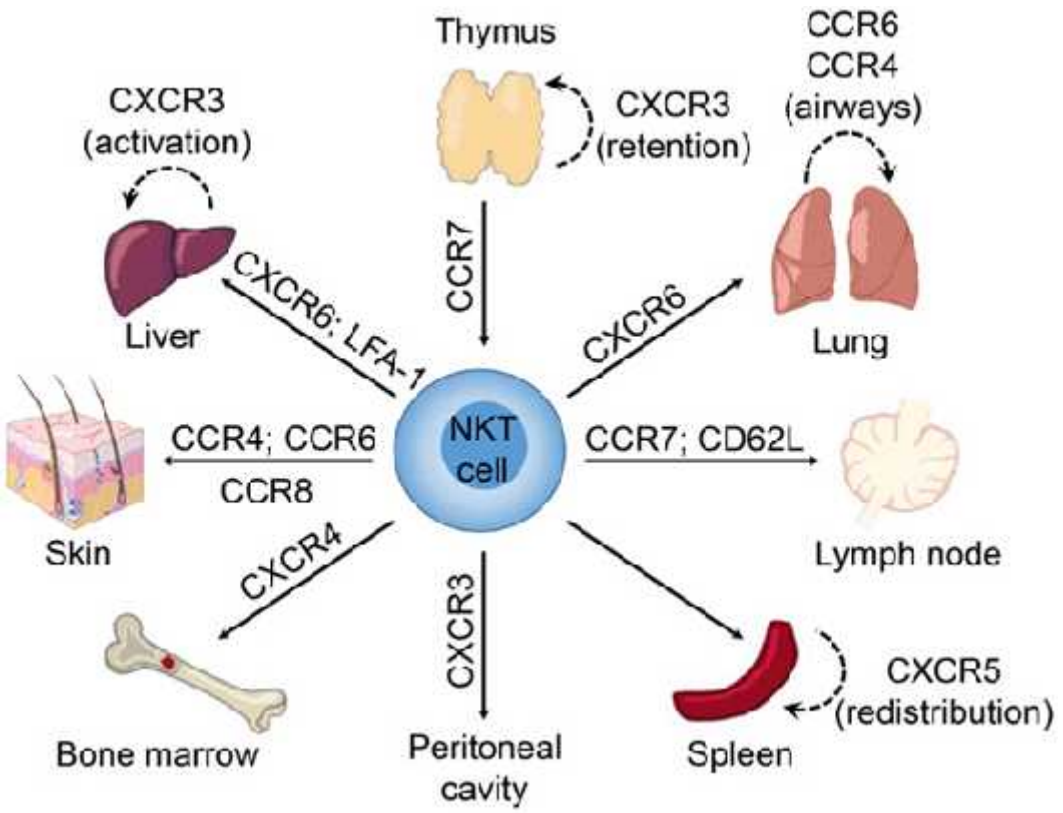
NKT cells: Interactions with immune cells



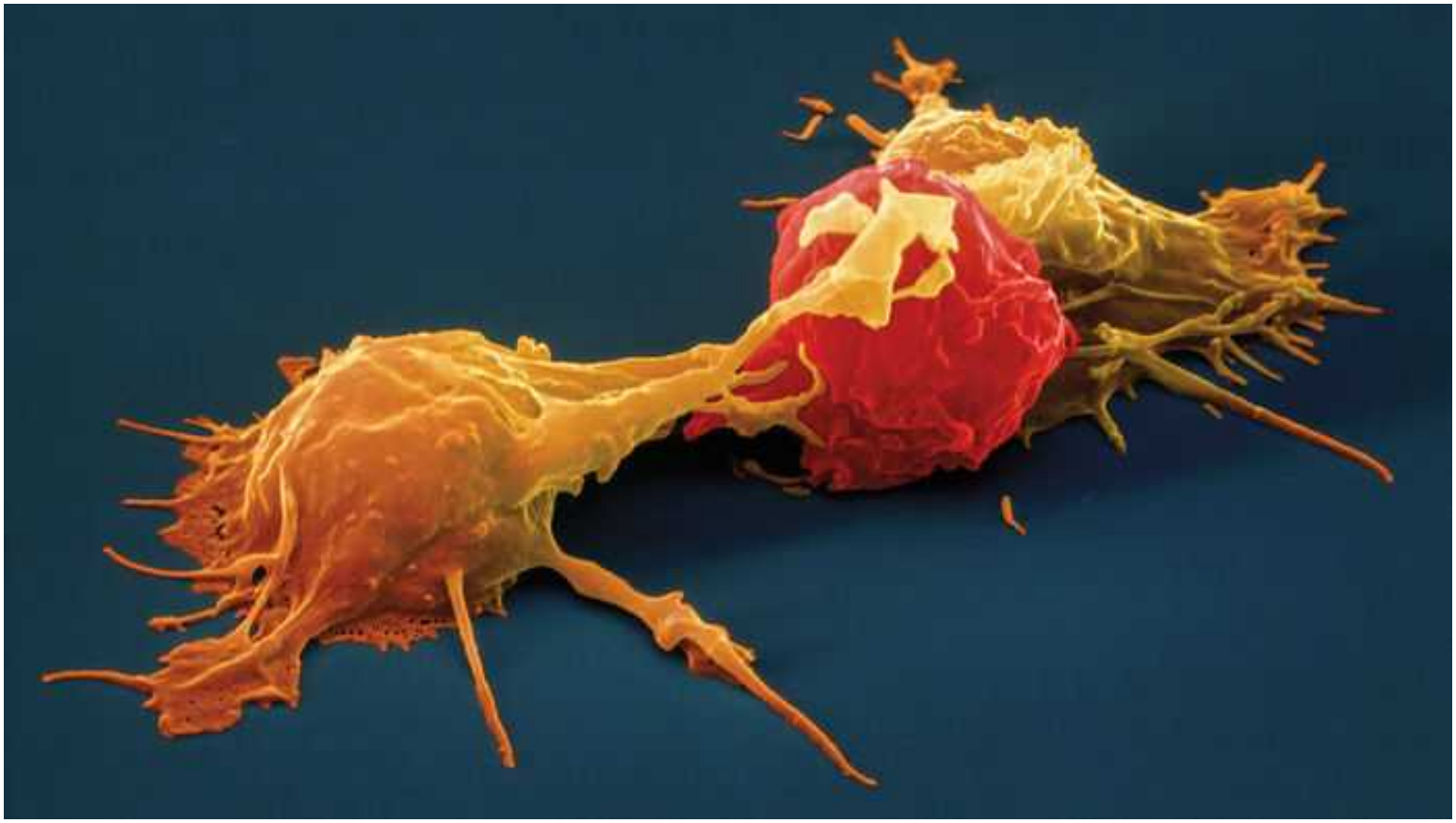
NKT cells: Unique properties



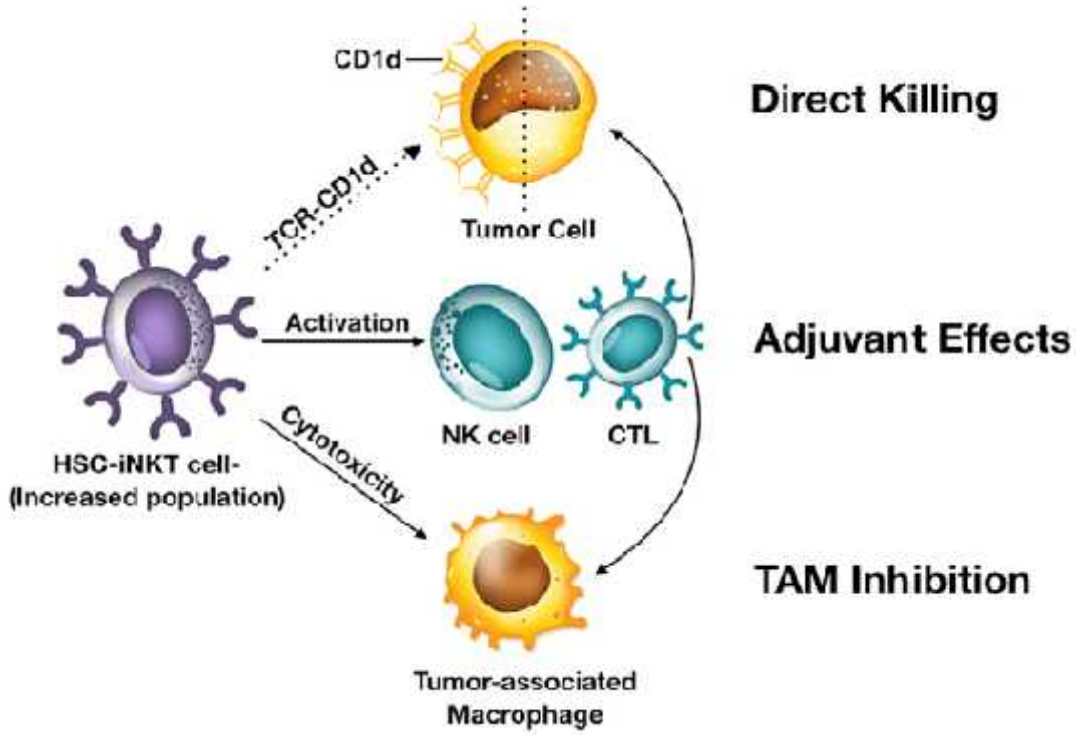
NKT cells are tissue-resident lymphocytes



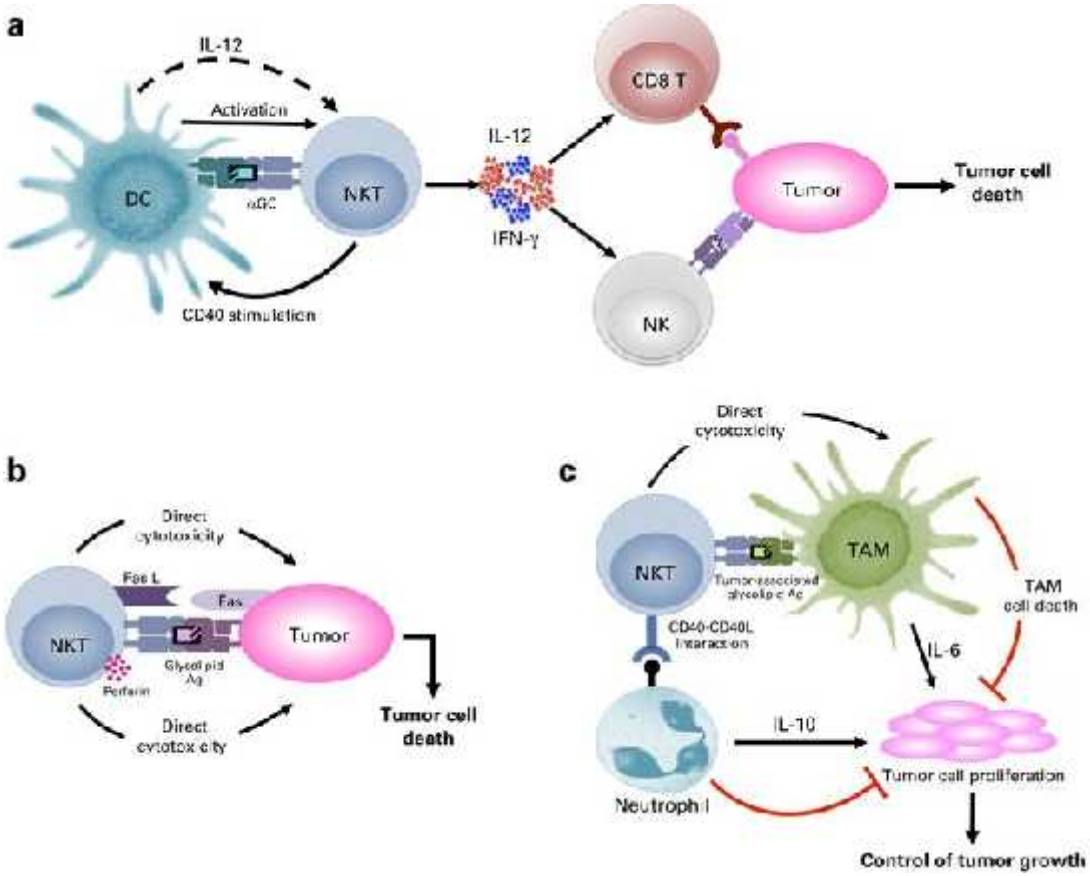
How NKT cells may fight tumors



How NKT cells may fight tumors



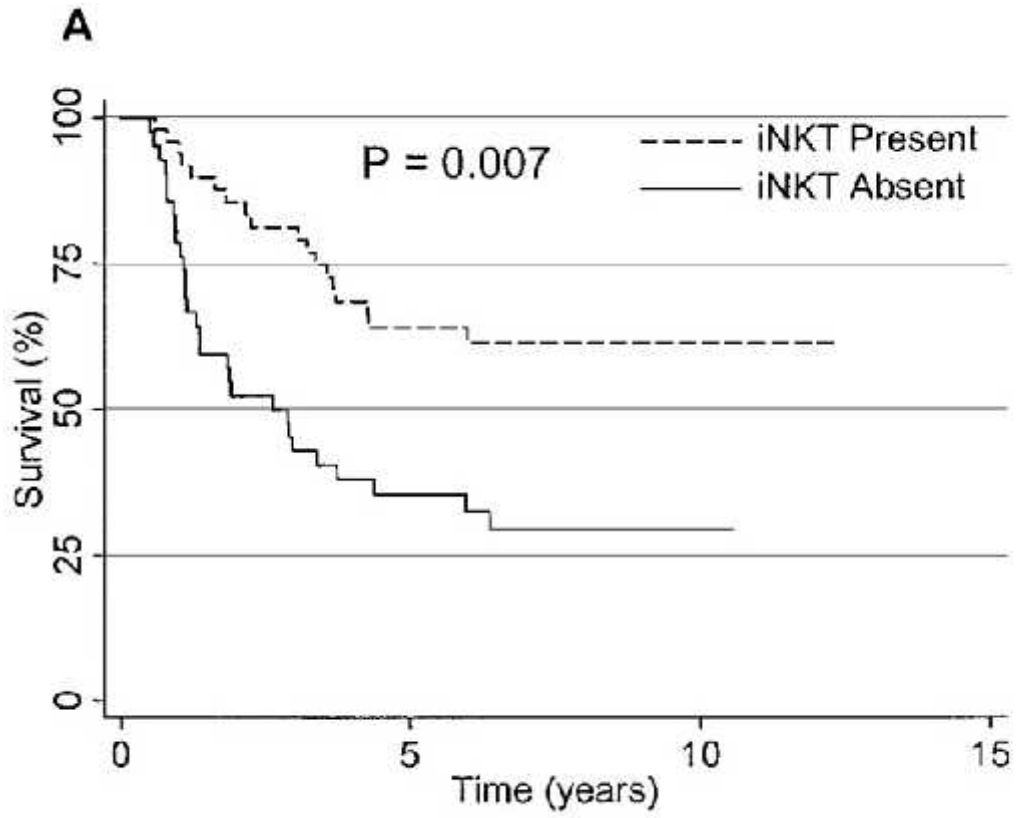
How NKT cells may fight tumors



Why NKT cells?

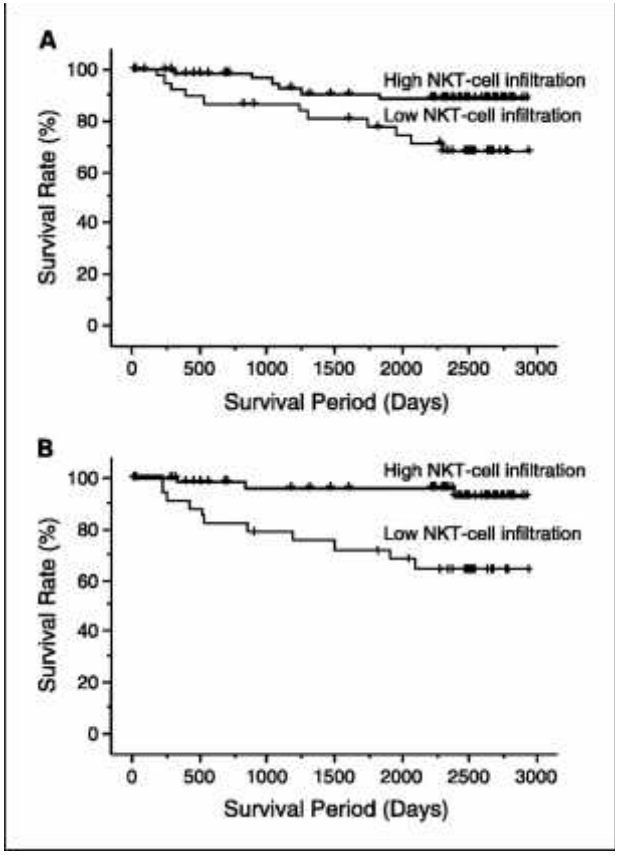
- Better outcomes in patients with high NKT tumor infiltrates

NKT cells as prognostic factors in cancer



Metelitsa et al, JEM 2004

Intratumor NKT cells as a prognostic factor in colorectal carcinoma patients.



Tsuyoshi Tachibana et al. Clin Cancer Res 2005;11:7322-7327

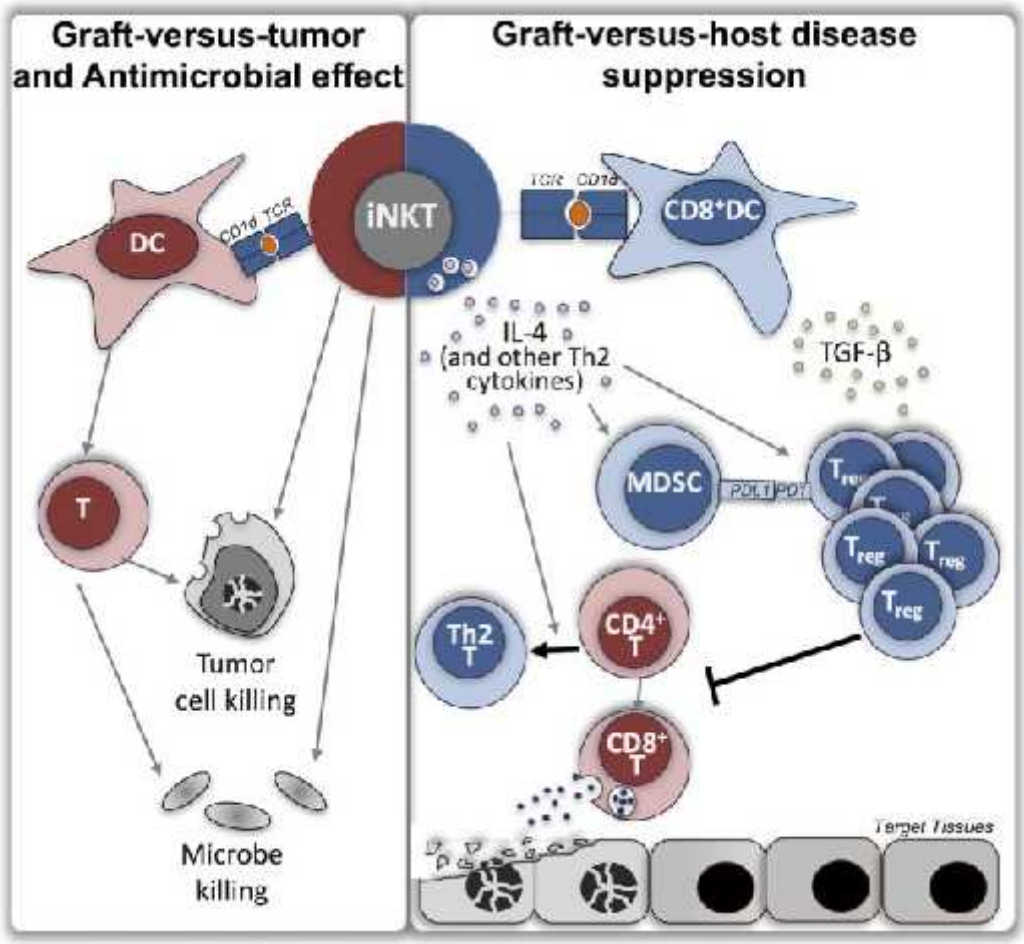
Clinical Cancer Research
AACR

Why NKT cells?

- Better outcomes in patients with high NKT tumor infiltrates
- Ameliorate GvHD

NKT cells suppress GvHD

- Host NKT cells protect from GvHD
- Activation of NKT cells protects from GvHD
- Adoptive transfer of NKT cells protects from GvHD

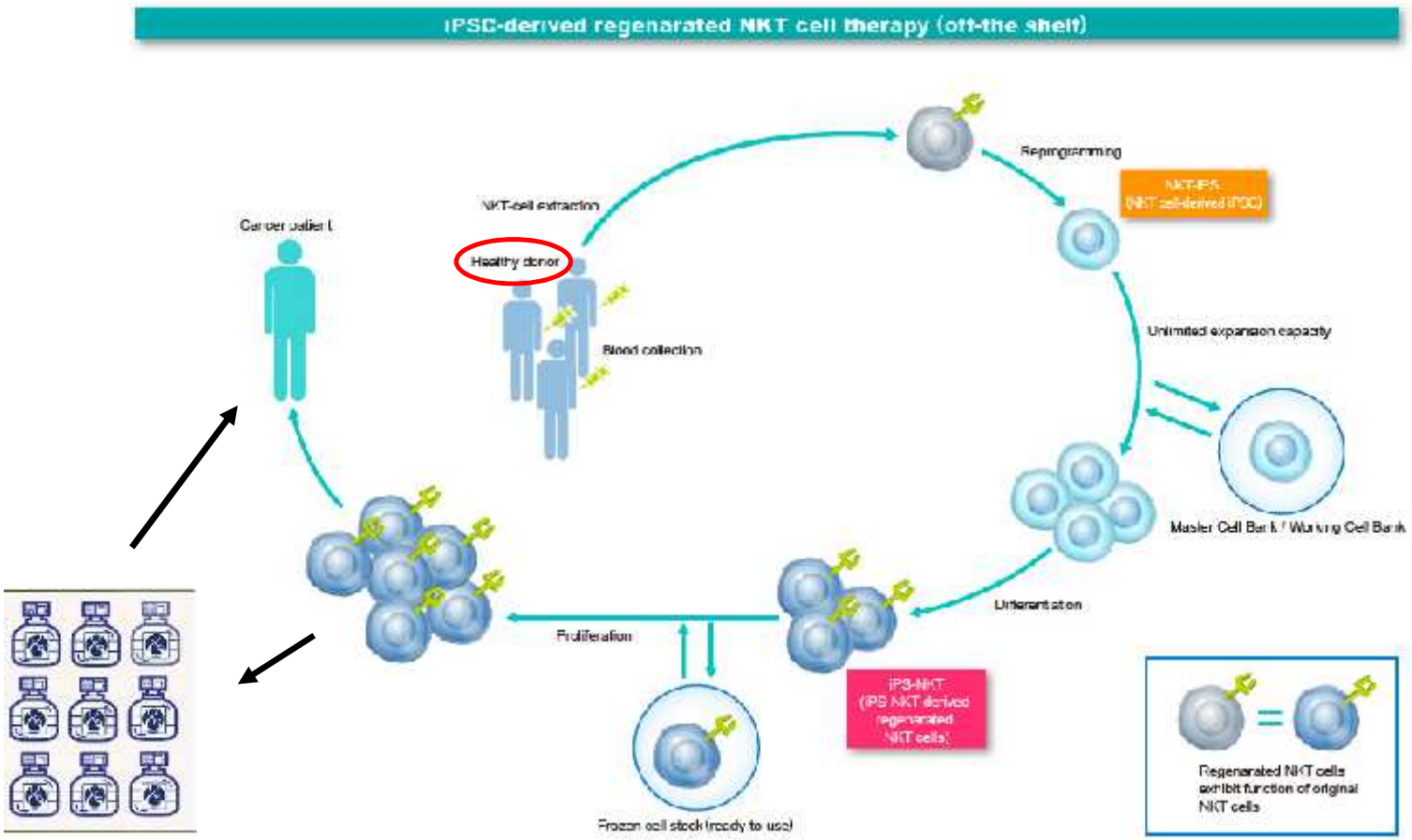


Why NKT cells?

- Better outcomes in patients with high NKT tumor infiltrates
- Ameliorate GvHD
- Restricted by non-polymorphic CD1d molecules
- Easily expandable

] off-the-shelf therapy

NKT cells are easily expandable *in vitro*



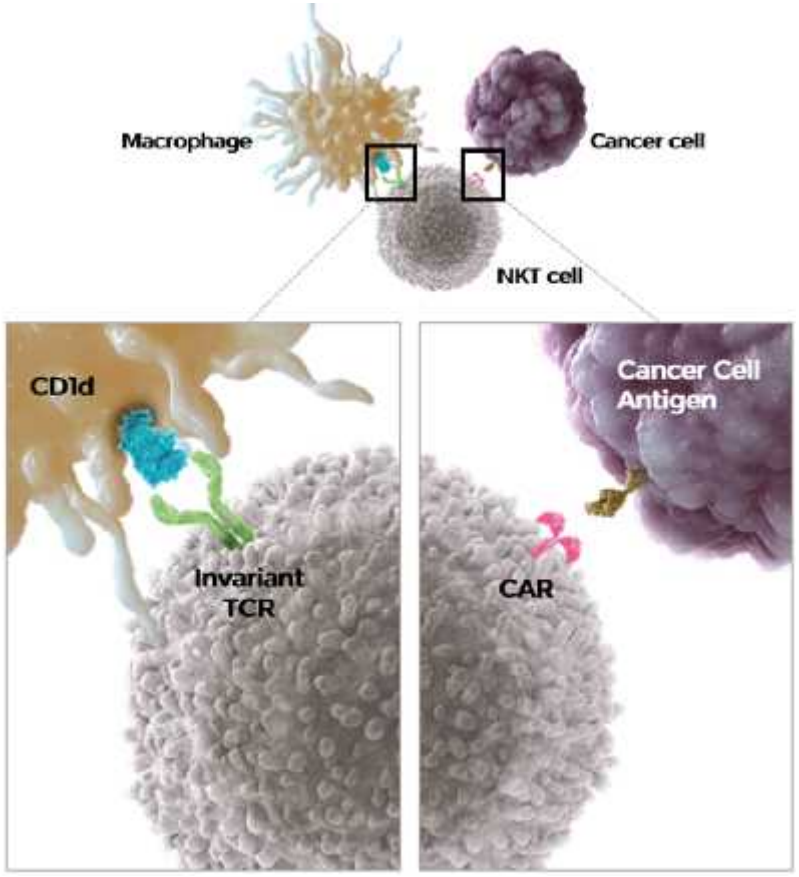
Why NKT cells?

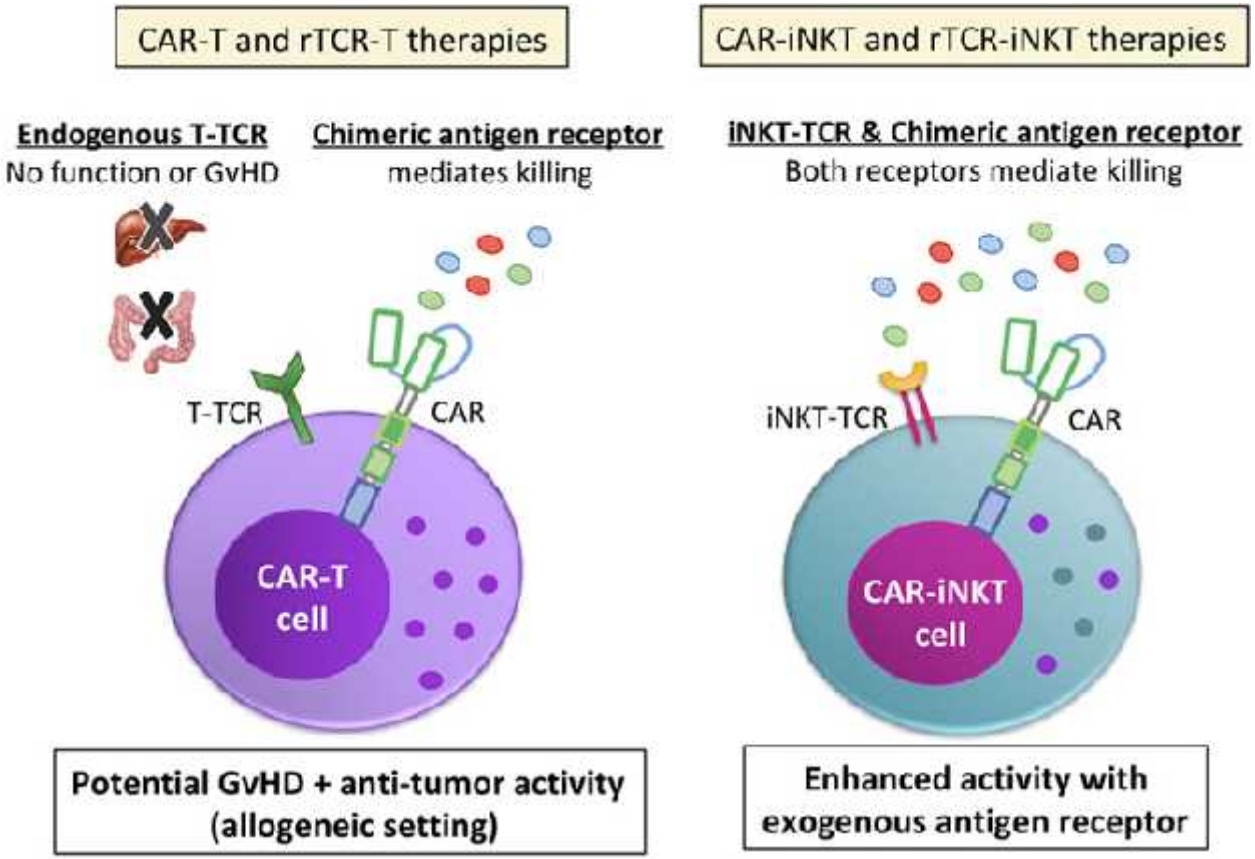
- Better outcomes in patients with high NKT tumor infiltrates
- Ameliorate GvHD
- Restricted by non-polymorphic CD1d molecules
- Easily expandable

] off-the-shelf therapy

- Dual targeting (both CAR NKT antigen and CD1d-restricted antigen)

Dual antigen targeting of CAR-NKT cells




CAR-T and rTCR-T therapies
CAR-iNKT and rTCR-iNKT therapies
Endogenous T-TCR
No function or GvHD
Chimeric antigen receptor
mediates killing
iNKT-TCR & Chimeric antigen receptor
Both receptors mediate killing
T-TCR
CAR
CAR-T cell
iNKT-TCR
CAR
CAR-iNKT cell
Potential GvHD + anti-tumor activity (allogeneic setting)
Enhanced activity with exogenous antigen receptor

NKT cell-mediated therapies

NKT cell activation

- Direct aGalCer activation
- Ligand-loaded APCs
- Ligand and cytokine activation

NKT cell adoptive transfer

- NKT cell expansion
- NKT cell differentiation from HSCs, or iPSCs

CAR-NKT cell generation

- CAR-GD2 (neuroblastoma)
- CAR-CD19 (B-cell lymphoma)

Treatment	Injection site, number of injections/cycles	Tumor type	Number of patients	Safety	Clinical outcome	Immunological response	Reference
Direct α-GalCer injection							
α-GalCer	i.v., 50–4,800 μg/m², 3 days 4-weekly cycle	Solid tumors	24	No dose-limiting toxicity	1. 7/24 patients stable disease for 123 days 2. No clinical response	1. Transient decrease in type I NKT and natural killer (NK) cells from circulation 2. Increased serum cytokine levels of IFN-γ and GM-CSF in 6/24 patients 3. Cytotoxicity in 1/24 patients. 4. The effect was dependent on pretreatment type I NKT cell numbers.	[109]
Ex vivo-generated dendritic cell (DC) pulsed with α-GalCer							
α-GalCer pulsed CD1d-expressing immature monocyte-derived DCs (moDCs)	i.v., 3 doses over 2 week-cycle	Metastatic malignancy	12	No severe toxicity	1. 2/12 patients had decreased serum tumor markers 2. 1 subject developed extensive necrosis of tumor-infiltrating tumor regrowth 3. 2 patients with hepatic infiltration had reduction in serum hepatocellular enzyme levels 4. Clinically apparent treatment specific inflammatory responses at [illegible]	1. NKT cell, T cell activation 2. Increase in NK cell numbers, activation and enhanced cytotoxicity 3. Increased IFN-γ (10/10) and IL-10 (8/8) levels in serum	[110]
α-GalCer pulsed IL-2/GM-CSF cultured PBMCs	i.v., 4 doses, 3 x 10^7 cells (level 1) 5 patients, 2.5 x 10^7 cells (level 2) 3 patients, 3 x 10^7 cells (level 3) 3 patients	Non-small cell lung cancer	11	No severe toxicity	Stable disease in 3 patients	1. Expansion of type I NKT cells in 3/11 patients 2. Elevated IFN-γ mRNA levels in 1/11 patients	[119]
α-GalCer pulsed immature moDCs	i.v., 4 injections at 1 x 10^7 cells	Non-small cell Lung cancer	17	No severe toxicity	Stable disease in 5 patients, median survival time 18.8 months	1. Expansion of type I NKT cells in 10/17 patients 2. Elevated IFN-γ-producing cells by ELISPOT in 10/17 patients	[117]
α-GalCer pulsed immature moDCs	4 treatments total with iv., 2 treatments, and intradermal (i.d.) 2 treatments, doses ranging from 6 x 10^5, 3 x 10^6, and 2–5 x 10^7 cells	Metastatic solid tumor	12	Safe and well tolerated	1. Stable disease in 6/10 patients 2. 1 patient had objective objective defined as reduction in tumor mass/lesion 3. 9/12 had transient therapy related tumor inflammation	Dose of 5 x 10^7 iv i.v. route gave the most unprecedented result of NKT activation resulting in increased circulating type I NKT cells levels with NK and T cell activation and increased serum IFN-γ levels	[118]
α-GalCer pulsed IL-2/GM-CSF cultured PBMCs	i.v., 1 injection	Non-small cell lung cancer	4	No serious toxicity	NA	1. Increased mobilization of type I NKT cells into primary site of the lung cancer 2. Augmented IFN-γ-producing ability of tumor-infiltrating type I NKT cells	[116]
α-GalCer pulsed antigen-presenting cell (APCs)	Nasal sub-mucosal injections, 2 treatments with 1 week interval	Head and neck squamous cell carcinoma	9	Safe and well tolerated	1 patient showed partial response, 3 patients showed stable disease	Increase in circulating type I NKT numbers (4/9) Expansion of α-GalCer reactive IFN-γ producing cells in PBMCs (5/9)	[120]
α-GalCer pulsed mature moDCs	i.v., 2 injections	Advanced cancer	5	Safe and well tolerated	[illegible] had stable disease, 3 patients had decreased M spike [illegible]	1. ~100-fold expansion of type I NKT cell subsets sustained up to 5 months after vaccination 2. Type I NKT cell activation was associated with increased serum levels of IL-12p40, IP-10, and MIP-1β	[121]
Adoptive transfer of autologous ex vivo-expanded NKT cells							
Ex vivo-expanded NKT cells with autologous α-GalCer pulsed PBMCs	i.v., 2 doses, 1 x 10^7 cells (level 1) 6 patients, 2.5 x 10^7 cells (level 2) 3 patients	Non-small cell lung cancer	9	No adverse effects	No tumor regression Stable disease in 3/9 patients	1. Absolute number of circulating type I NKT cells increased in 2/3 cases receiving level 2 dose 2. IFN-γ production augmented in all 3 cases receiving level 2 dose	[122]
Ex vivo-expanded NKT cells	i.v., 3 infusions of 25 x 10^7 cells/infusion spaced 2 weeks apart with pretreatment of GM-CSF before cycle 2 and 3 to enhance DC functions	Advanced melanoma	9	No adverse effects	1. Patients deceased (3/9) 2. Patients progressed (3/9). Median follow-up for 43 months	1. Type I NKT infusion appeared to cause transient peak of circulating type I NKT cells that were enhanced by GM-CSF pretreatment 2. Increased number of activated monocytes 3. Elevated IFN-γ production (6/9)	[123]
Combination therapies							
Ex vivo-expanded NKT cells (intra-arterial) and autologous α-GalCer-pulsed PBMCs (s/c nasal submucosal)	1 x 10^9 α-GalCer loaded APCs submucosal injections (2 injections) followed by in vitro activated type I NKT cells (i.a) into tumor feeding artery (1 injection)	Head and neck squamous cell carcinoma	9	Serious adverse event (1). Mild adverse events (7)	1. Partial response (3/9) 2. Stable disease (4/9) 3. Progressive disease (1/9)	1. Increase in circulating type I NKT numbers (8/9) 2. Expansion of α-GalCer reactive IFN-γ producing cells in PBMCs (7/9)	[112]
Ex vivo-expanded NKT cells (intra-arterial) and autologous α-GalCer pulsed PBMCs (s/c nasal submucosal)	1 x 10^9 α-GalCer loaded APCs submucosal injections (1 injection) followed by in vitro activated type I NKT cells (i.a) into tumor feeding artery (1 injection)	Head and neck squamous cell carcinoma	10	No adverse effects	1. [illegible] regression (5/10) 2. Stable disease (5/10) 3. Antitumor effects (8/10)	1. Expansion of type I NKT in PBMC (7/10) and 3L circulating with partial response (6/6) 2. Elevated expansion of IFN-γ spot forming cells in PBMCs (9/10) and in tumor tissue	[113]
α-GalCer pulsed mature moDCs + LEN	i.v., LEN (oral 10 mg/day), 28 day x3 cycles	Multiple myeloma	9	Safe and well tolerated	2 patients show reduction in serum associated M spike and [illegible]	Activation of NKT, NK, monocytes, and eosinophils	[114]

NKT-CAR clinical trials

ClinicalTrials.gov Search Results 10/24/2019

	Title	Status	Study Results	Conditions	Interventions	Locations
1	GD2 Specific CAR and Interleukin 15 Expressing Autologous NKT Cells to Treat Children With Neuroblastoma	Recruiting	No Results Available	•Neuroblastoma	•Genetic: CINAKIT Cells •Drug: Cyclophosphamide •Drug: Fludarabine	•Texas Children's Hospital, Houston, Texas, United States
2	CD19 CAR Allogeneic NKT for Patients With Relapsed or Refractory B-Cell Malignancies (ANCHOR)	Not yet recruiting	No Results Available	•Refractory B-Cell Non-Hodgkin Lymphoma •Refractory B-Cell Small Lymphocytic Lymphoma •Relapsed Adult ALL •Relapsed CLL •Relapsed Non Hodgkin Lymphoma	•Genetic: CD19 CAR-aNKT cells •Drug: Cyclophosphamide •Drug: Fludarabine	•Houston Methodist Hospital, Houston, Texas, United States •Texas Children's Hospital, Houston, Texas, United States

Advantages of CAR-NKT cell therapies

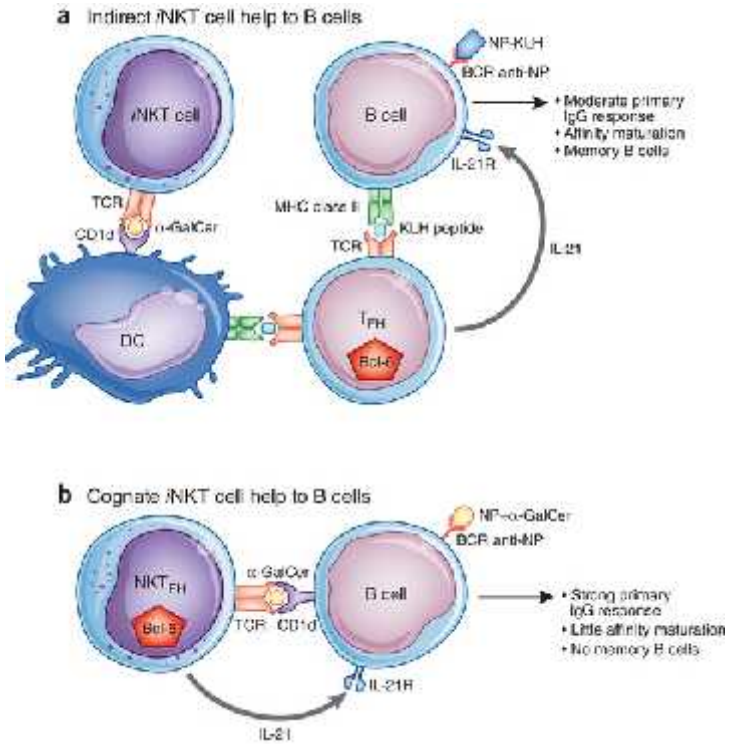
CAR-T cell therapy	CAR-NKT cell therapy
Long time required for manufacturing	Off-the-shelf therapies immediately available
Product-to-product variability due to patient factors	Large number of uniform doses manufactured from a single donor
Limited success in solid tumors	NKT cells naturally home to tissues
CAR-T cells cause GvHD	NKT cells modulate immuno-suppressive cells
Uncertain persistence	NKT cells do not mediate GvHD
	Fully functional TCR and no TCR gene editing required
	IL-15 prolongs persistence

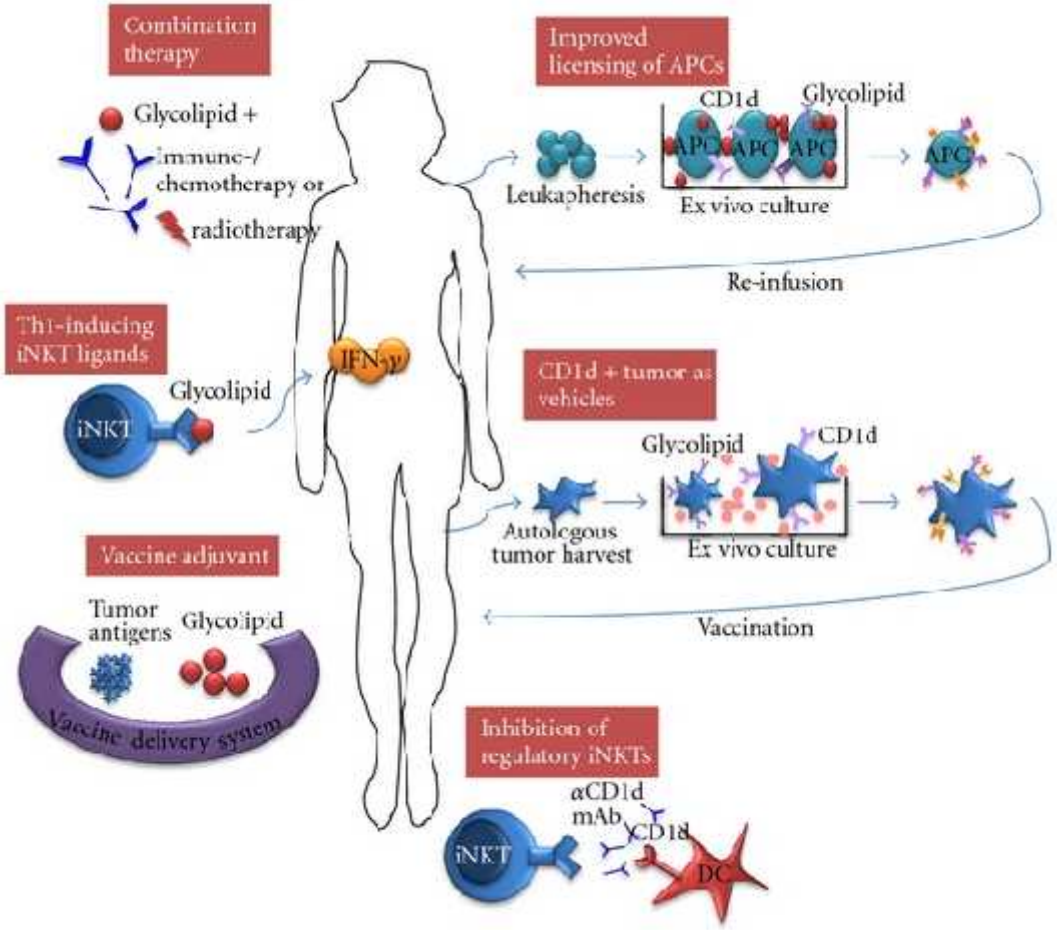
Summary

- Multiple subsets of innate-like T cells
- Both rearranged (although invariant) and germline encoded receptors
- Poised/activated phenotype
- Ample production of various cytokines
- Dependent on non-polymorphic CD1D or MR1 molecules
- Lipid/metabolites recognition
- Distinct developmental pathway
  - DP-DP interactions
  - SLAM-SAP dependency
- PLZF expression
- Potentially better/complementary targets for immunotherapy

THANK YOU!

NKT-B cell interactions: helping humoral immunity




Combination therapy
Glycolipid +
Immuno-/ chemotherapy or radiotherapy
Improved licensing of APCs
CD1d
Glycolipid
APC
APC
APC
APC
Leukapheresis
Ex vivo culture
Re-infusion
Th1-inducing iNKT ligands
Glycolipid
iNKT
IFN-γ
CD1d + tumor as vehicles
Glycolipid
CD1d
Autologous tumor harvest
Ex vivo culture
Vaccine adjuvant
Tumor antigens
Glycolipid
Vaccine delivery system
Vaccination
Inhibition of regulatory iNKTs
αCD1d mAb
CD1d
iNKT
DC

Why NKT cells?

- Better outcomes in patients with high NKT tumor infiltrates
- Ameliorate GvHD
- Restricted by non-polymorphic CD1d molecules (off-the-shelf therapy)
- Easily expandable
- Dual targeting (both CAR NKT antigen and CD1d-restricted antigen)
- Potent anti-tumor cytotoxic activity (aGalCer discovery)
- Very quick and diverse cytokine production: IL4, IFNγ, IL17
- Pre-activation with lipid ligands
- Continuous expression of cytokine receptors

NKT cells: Multiple functions