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# EMPOWERING THE IMMUNE SYSTEM

Harnessing the power of iNKT cells and CAR immunotherapies in cancer treatment

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## Objectives

- Describe the biology, classification and anti-tumor function of **iNKT cells**
- Take a deep look into **CAR-iNKT immunotherapy**
- Analyze relevant **clinical trials** and **future perspectives** of this immunotherapy

## Methodology

Bibliographic review searching, reading and contrasting information from scientific publications. Search in official websites of World Health Organization, Clinical Trials, PubMed and Google Scholar.



## Introduction

9.9 million deaths in 2022!

- Cancer is a global health challenge** → there's an urgent need of effective treatment.
- Immunotherapy**, which uses the body's immune system, is a promising approach.
- Invariant natural killer T cells (iNKT cells)** have unique properties that make them attractive for cancer immunotherapy, they are **amazing fighters!**
- The **genetic engineering** of iNKT cells, through **chimeric antigen receptor (CAR)** technology, enhances their targeting of cancer cells, but there are challenges to overcome for a successful clinical translation...

Do you want to know more?

## NKT cells

0.05%  
of T cells

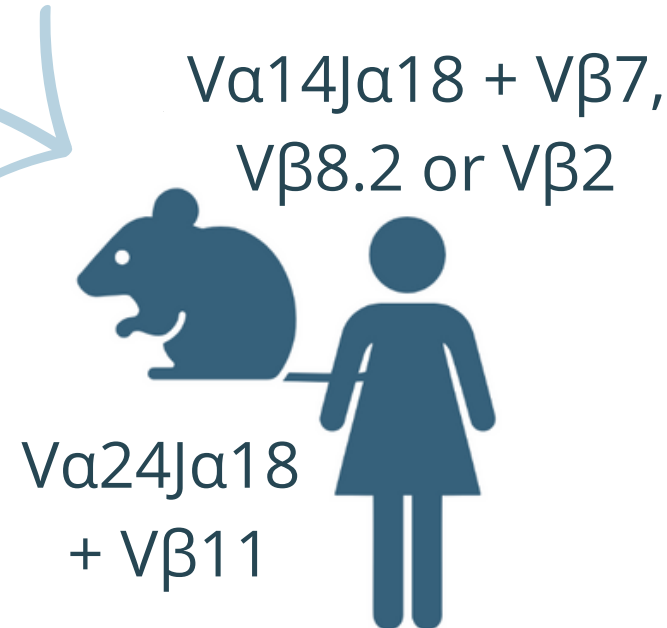
- Natural Killer T cells (NKT cells)** are a **subset of T cells** share characteristics with both T and Natural Killer cells (NK cells).
- Although NKT cells are relatively **low in abundance** in human peripheral blood, they play a **crucial role in anti-tumor immunity**.
- They can **bridge the innate and adaptive immune systems** and can expand and differentiate into **memory cells**.

## Cell comparison

	T	NK	NKT
T + NK killing mechanisms	✗	✗	✓
Naturally suppresses GvHD	✗	✗	✓
TCR dependent	✓	✗	✓
Not MHC dependent	✗	✓	✓
Maturation in the thymus	✓	✗	✓
Tumor infiltration	✗	✗	✓

## NKT types

	iNKT	vNKT	NKT-like
CD1d dependent	YES	YES	NO
Antigens	α-GalCer	Sulfatides	
TCR	Semi-invariant	Variant	
Main role in cancer	Enhance immune response	Suppress immune response	



CD1d is presented by APCs, thymocytes and some cancer cells

TCR recognizes glycolipids presented by CD1d

We're going to focus on iNKT cells

Best cells to fight against cancer!

## Activation of iNKT

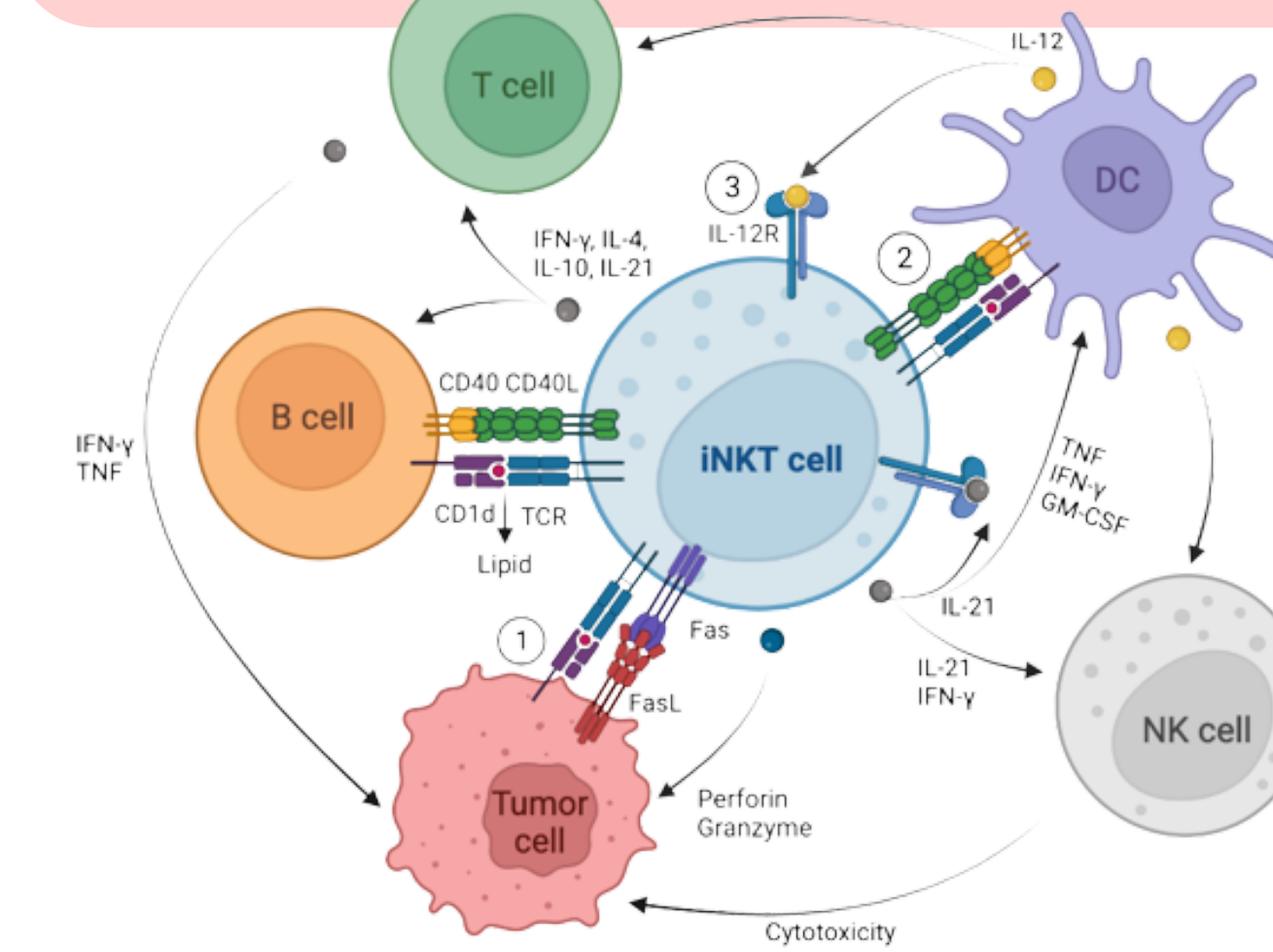


Figure 1: Interactions between iNKT cells and the impact of activated iNKT cells on anti-tumor immunity.

- Antigenic activation by TCR signal**
  - Direct:** direct recognition of cancer cells (1)
  - Indirect:** APCs are activated through their TLRs that trigger the loading of CD1d molecules with endogenous antigens presented to NKT cells (2)
- Activation mediated by cytokines (3)**

## Antitumor mechanisms

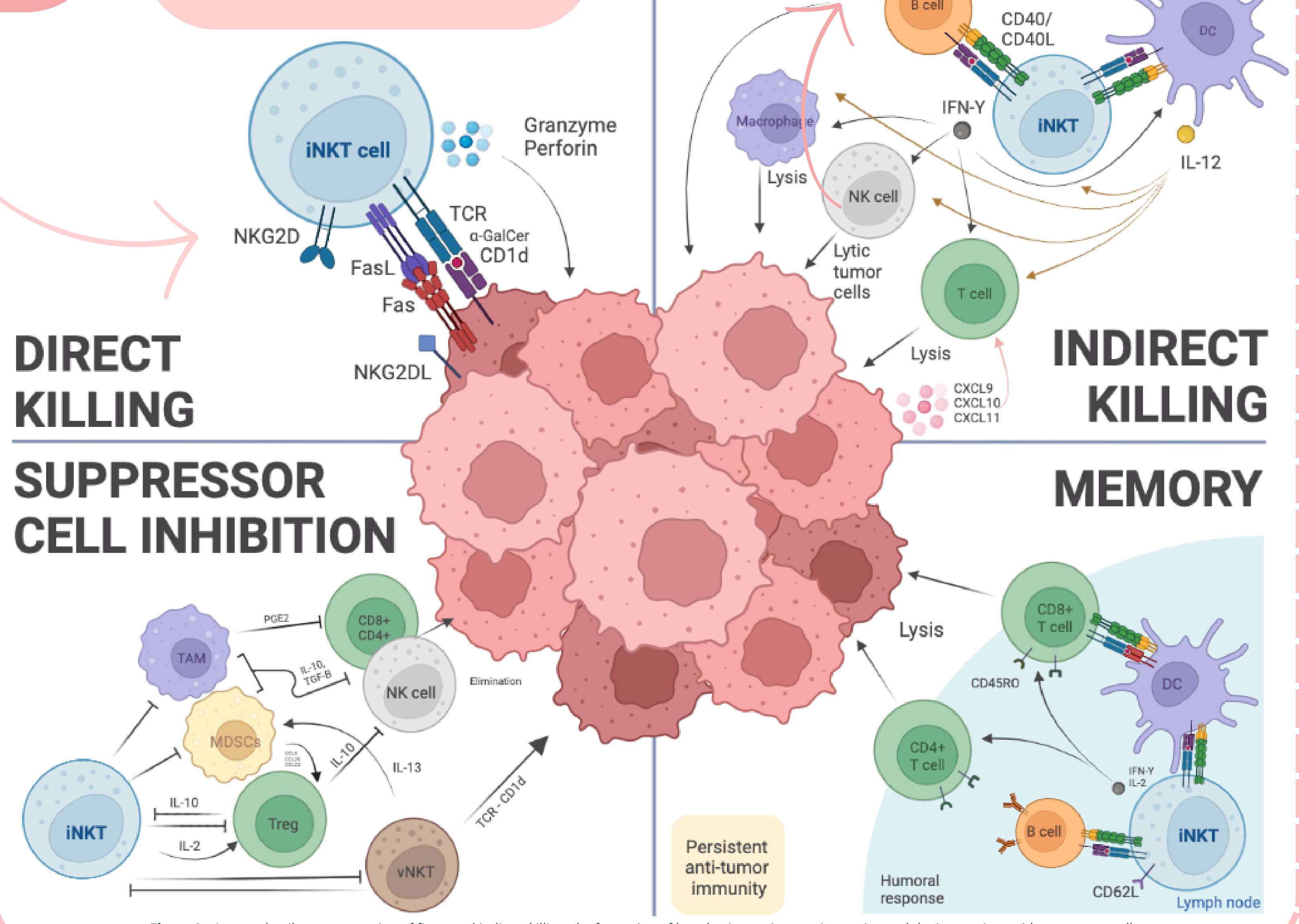


Figure 2: A more detailed representation of direct and indirect killing, the formation of long-lasting anti-tumor immunity, and the interactions with suppressor cells.

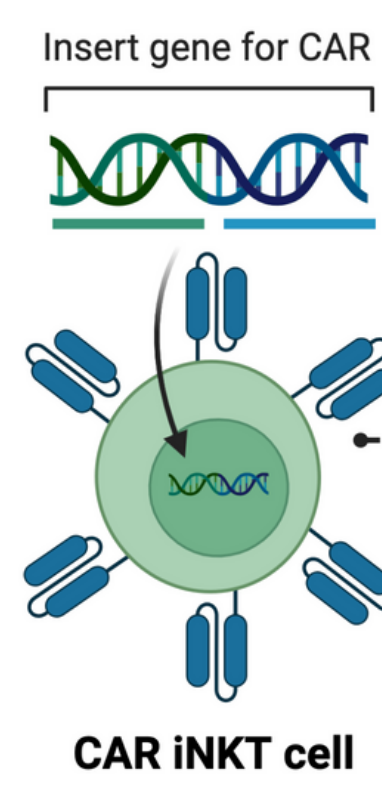


The low frequency of NKT cells, insufficient infiltration into TME, and the majority of the CD1d-tumor cell population make it **insufficient** to maintain their anti-tumor function.

## iNKT-CAR immunotherapy

immune system + genetics

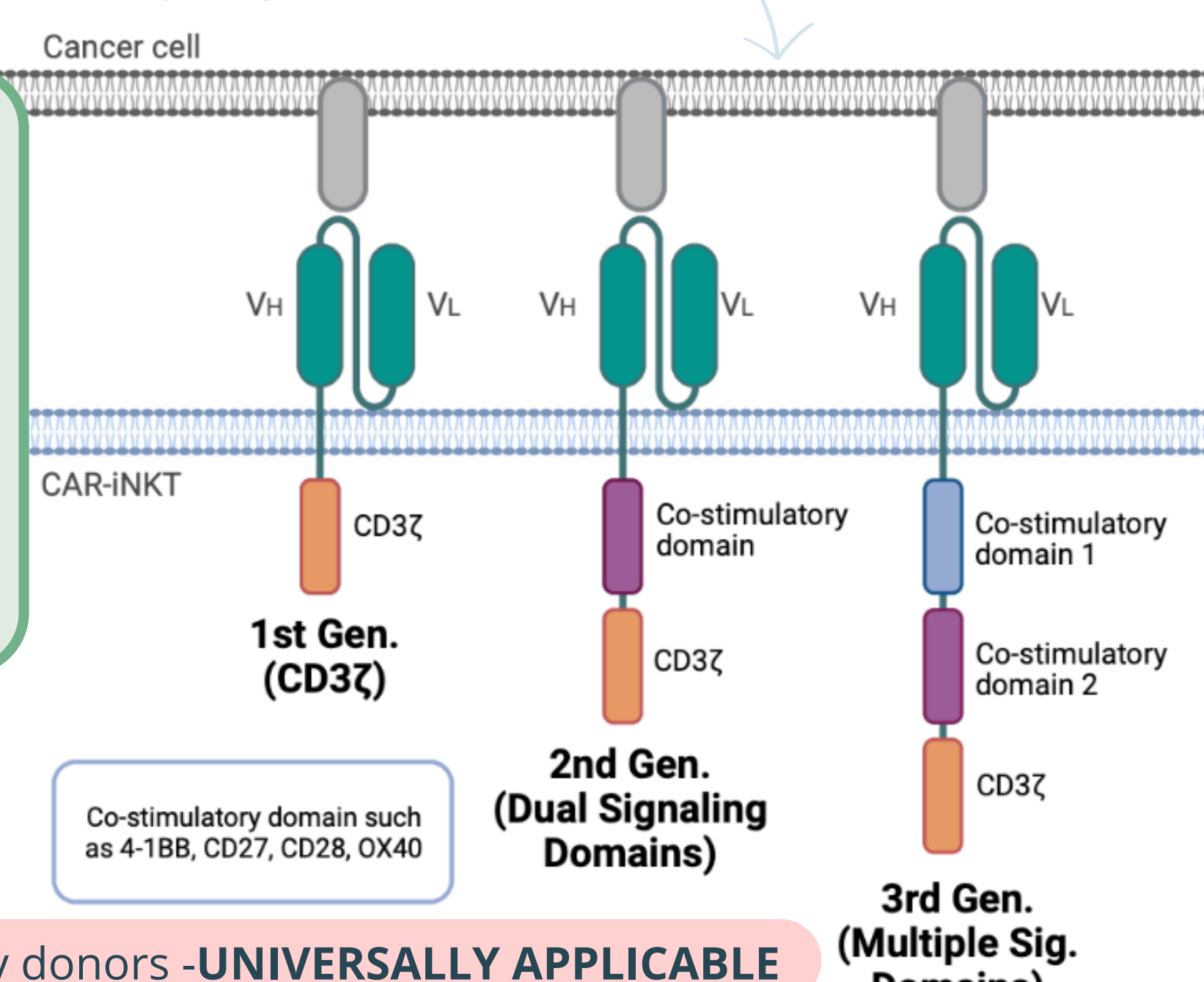
Genetically engineer immune cells to express CARs that specifically target cancer cells



- Design of CAR: Selection of a **target antigen** that is specific to the cancer
- Isolate and expand** iNKT cells
  - Allogenic** - healthy individuals
  - Autologous** - cancer patients
- CAR construct is **introduced** to iNKT cells by viral vectors or electroporation
- Tested** - preclinical and clinical trials

CAR helps iNKT cells by providing them with an **artificial receptor** that can recognize specific antigens on cancer cells, enabling enhanced tumor targeting and immune response.

Perfect for **allogenic** or **"off the shelf"** cells derived from healthy donors -**UNIVERSALLY APPLICABLE**



## CAR-iNKT advantages

Antitumor and anti-inflammatory properties, potential to target a **wide range of antigens**, long **lifespan**, and low final **price!**

## CAR-iNKT disadvantages

Low **frequency**, possible **off-target effects**, need for **further research**, risk of Cytokine Release Storms (CRS).

## Pre-clinical studies & clinical trials

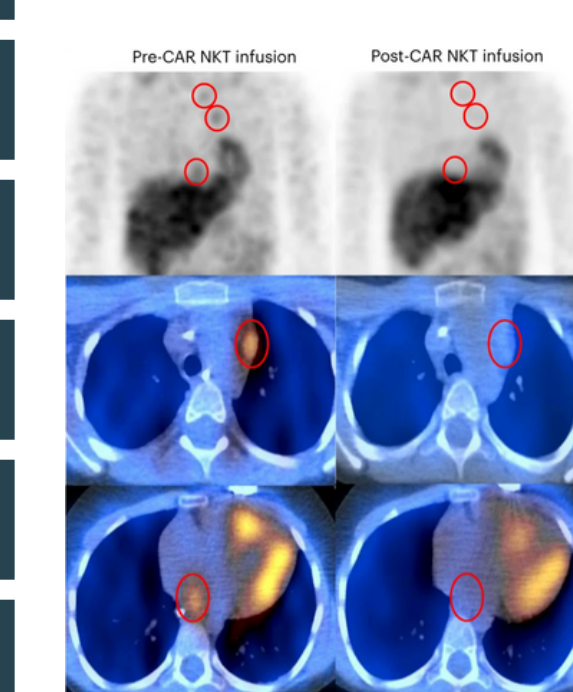


Figure 3: GD2-CAR NKTs expand in peripheral blood, traffic to metastasis sites and induce complete remission.

CAR-iNKT has lead to **tumor regression!** Most studies have focused on **hematological malignancies** targeting **CD19**.

Heczey et al. were the pioneers.

Ongoing clinical trial → **Anti-GD2 CAR-iNKT** cells against neuroblastoma (solid tumor) were very cytotoxic against neuroblastoma expressing GD2 and CD1d+ inhibitory macrophages, no evidence of GVHD.

**Pediatric patients** - cell infusions were well tolerated!

## Improvements

Key strategies to improve CAR-iNKT cell therapy

- Optimize CAR design** - engineering antigen-specific CARs
- Identifying safe and effective target antigens** - ↓ off-target effects
- Combination therapy** - ↑ maximize immune response
- CRISPR/Cas9** - genetic modifications to enhance the tumor response

## Ethics and regulatory considerations

Patient safety and ethical conduct must be prioritized.

Patients must be **informed** of all potential **risks** and **benefits** of the therapy.

## Conclusions and future perspective

- NKTs are a **subtype of lymphocytes** that play an intermediary role between innate and adaptive immunity (memory) and their activation relies on both cytokines and TCR-derived signals responding to antigens presented by CD1d molecule.
- NKTs are divided in iNKT, vNKT and NKT-like cells → **iNKTs** are the best fighters against cancer!
- iNKTs have **direct and indirect tumor killing mechanisms**, they can also modulate the **TME**.
- Perfect cells for CAR immunotherapy** → CAR-iNKT are + effective than CAR-T and CAR-NK.
- The use of CAR-iNKT cells does not induce aGVHD among donors as these cells are highly conserved in the population - great for **allogenic** or **"off the shelf"** therapy! Way **cheaper!**
- Results indicate that CAR-iNKT cell therapy is a promising strategy. However, further research is needed to pave the way for this powerful immunotherapy.

UAB

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