

# Come sfruttare le cellule della immunità innata nella terapia antitumorale

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## Disclosures of Massimo Massaia

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
AbbVie						x	
Roche						x	
Janssen						x	
Sanofi						x	

# Natural immunity

## PAMPs/DAMPs/PRRs

- ready-to-use
  - rapidity
  - cross-reactivity
- 
- plants
  - invertebrates
  - vertebrates

innate



**1 bacterium (1 hour doubling time) → 20 million progeny in one day**

# Adaptive immunity

## Ags/TCRs/BCRs

- specificity
  - diversity
  - self/non-self discrimination
  - memory
- 
- arisen 500 million years ago
  - confined to vertebrates

1 week to develop

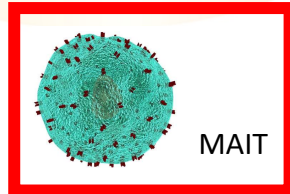
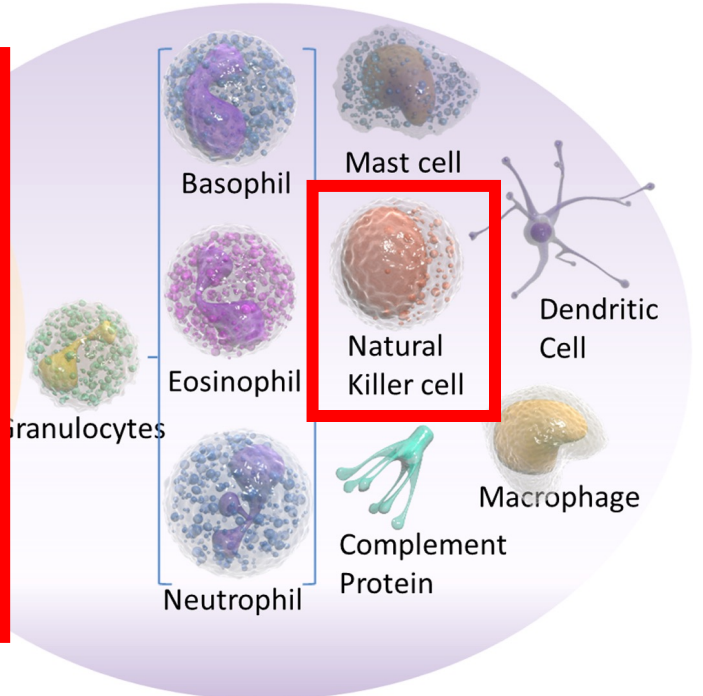
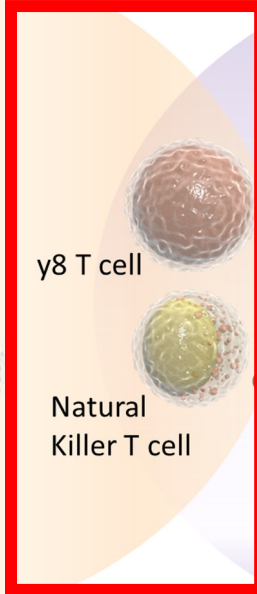
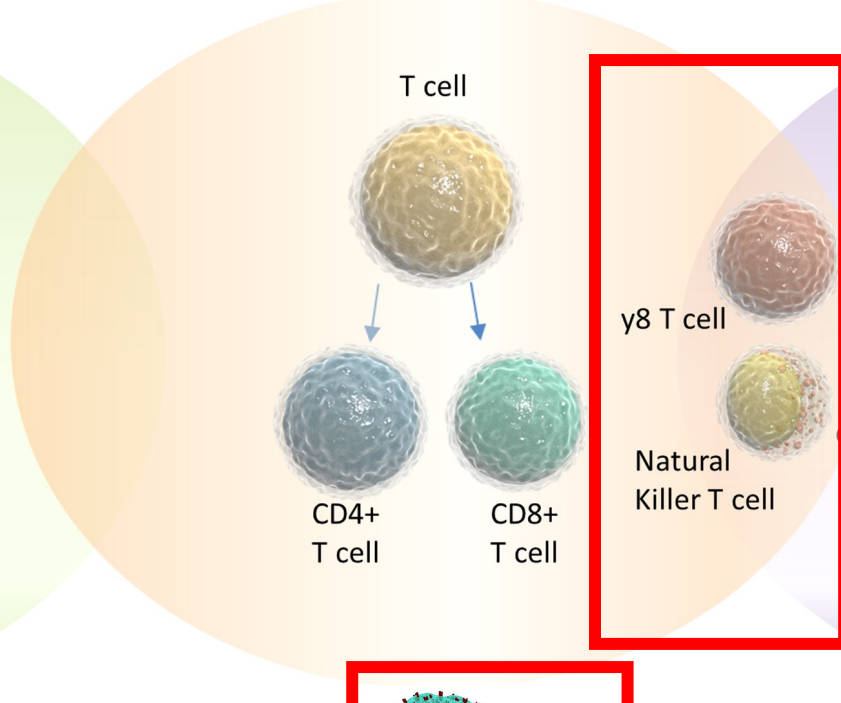
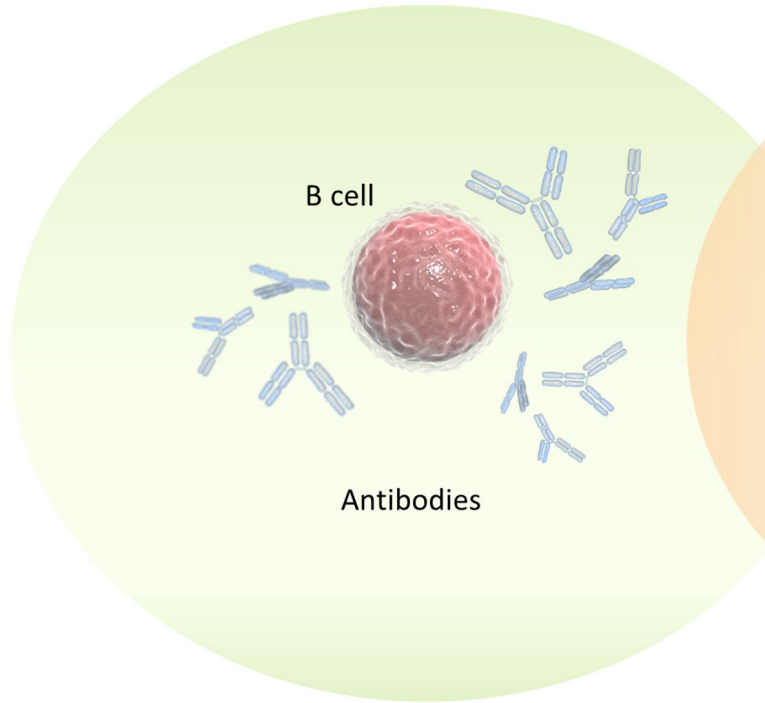


# Adaptive Immunity

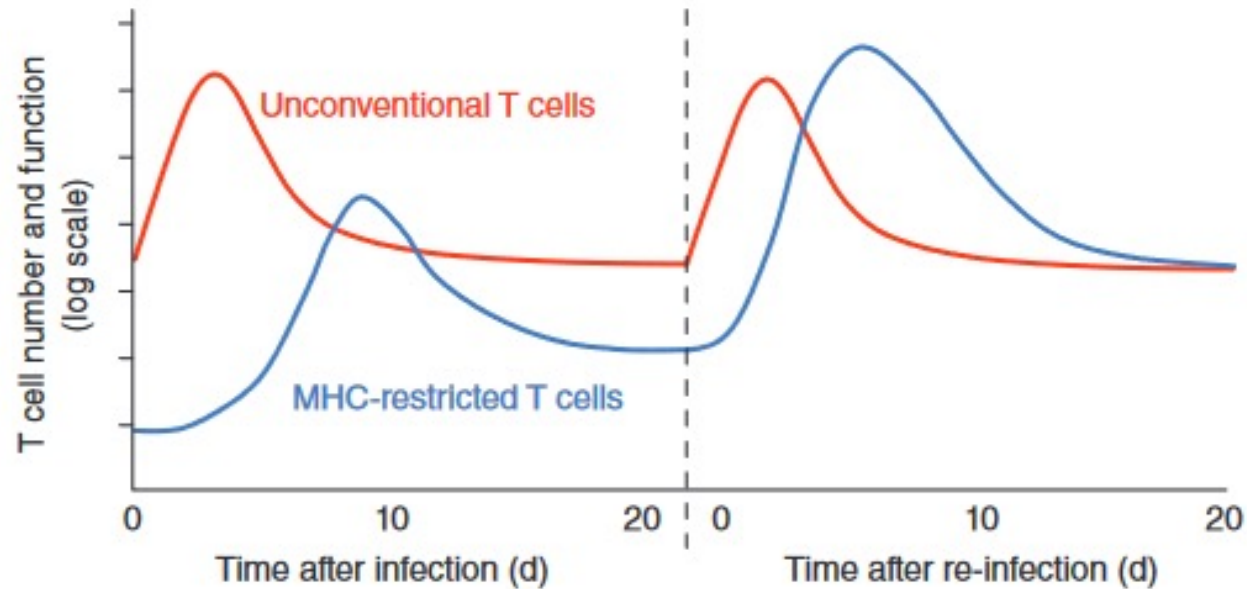
# Innate Immunity

Humoral  
(B cell Immunity)

Cellular  
(T cell Immunity)



# Comparison of unconventional and MHC-restricted T cell responses



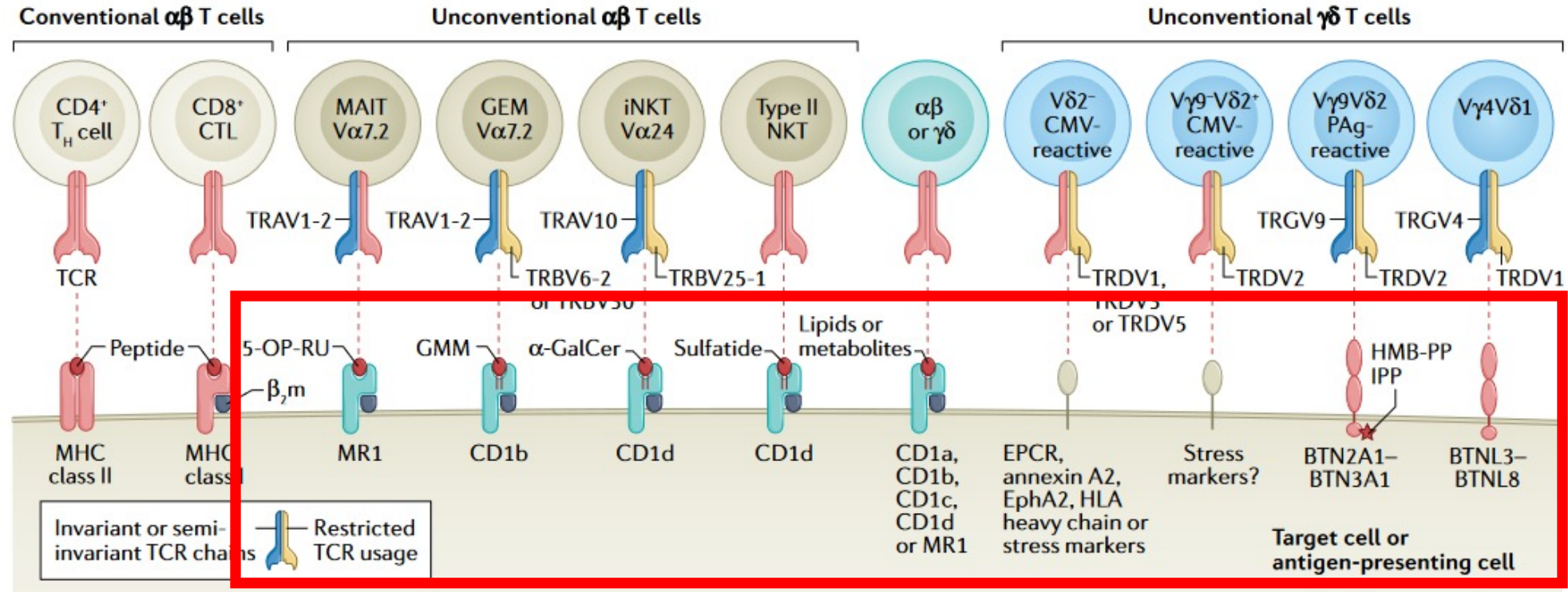
**Table 1 Characteristics of MHC-restricted as compared to unconventional T cell responses**

	<b>MHC-restricted T cells</b>	<b>Unconventional T cells</b>
Precursor frequency	Low (~1–10 per million)	Higher (~ $10^{-2}$ – $2 \times 10^5$ per million)
Time to effector function <i>in vivo</i>	Days to weeks	Hours to days
Memory response	Yes (highly durable)	Controversial
Antigen-presenting molecule polymorphism	Very high	Low (functionally monomorphic)
Inter-individual TCR diversity	Very high	Varies for each subset (Species-wide TCR patterns known)
Population response to antigen	Private (individuals respond differently)	Public (most individuals in a species typically respond similarly)

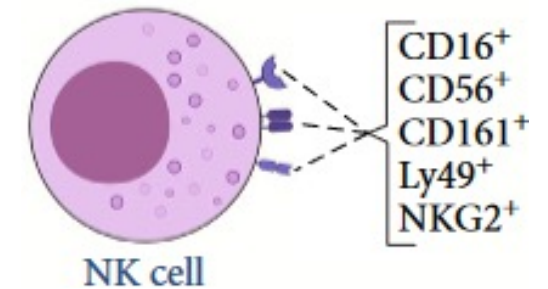
In general, the key differences in the patterns of MHC-restricted and unconventional T cells relate to the initial number of antigen-specific populations, the timing of the response, the role of memory T cells and the extent to which individuals in an outbred population respond to the same kinds of antigens.



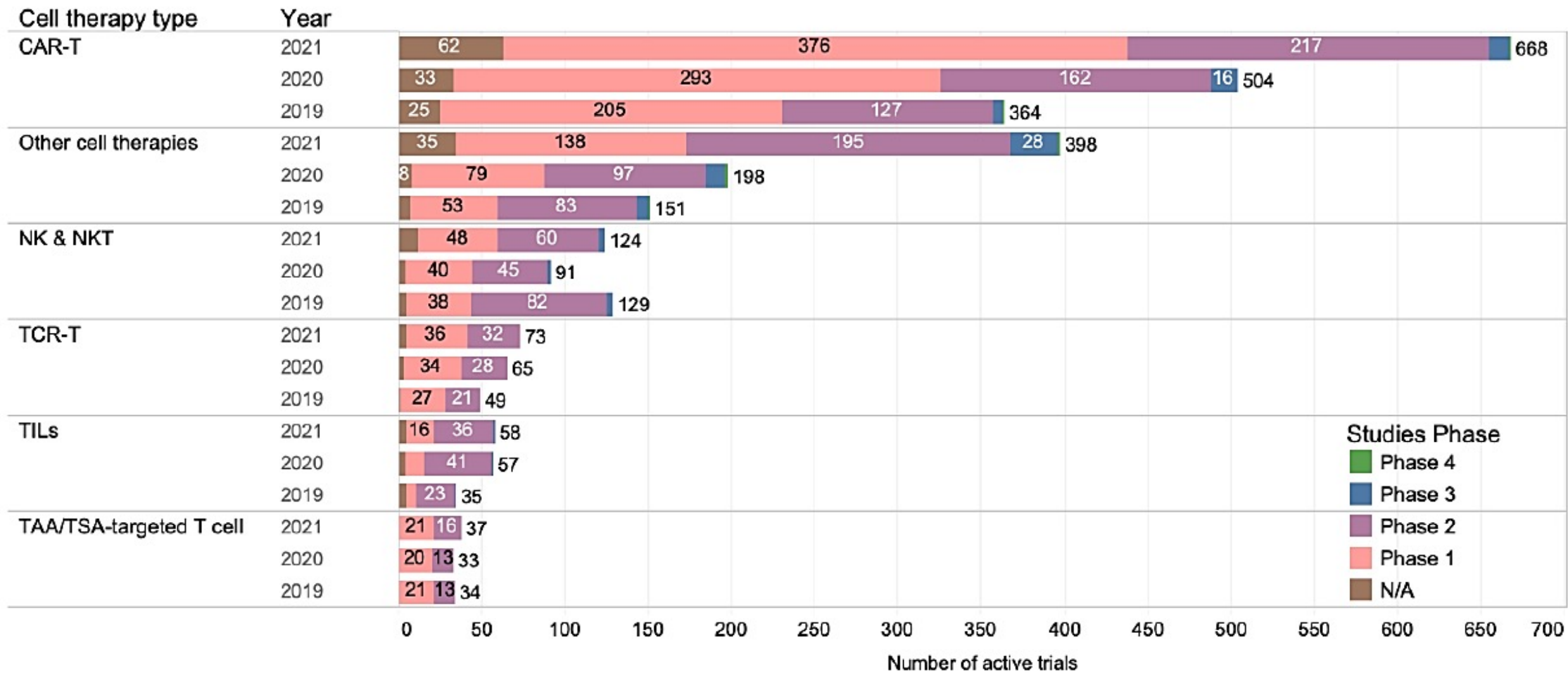
# Recognition of unconventional ligands by unconventional T cells



TCR -dependent	
<b>Non-self molecules</b>	<b>Self-ligands</b>
Pathogens	Stress-induced
Commensals	Constitutive expression in healthy tissues
Environment	



# Comparison of active cell therapy trial landscape in 2019, 2020, and 2021.

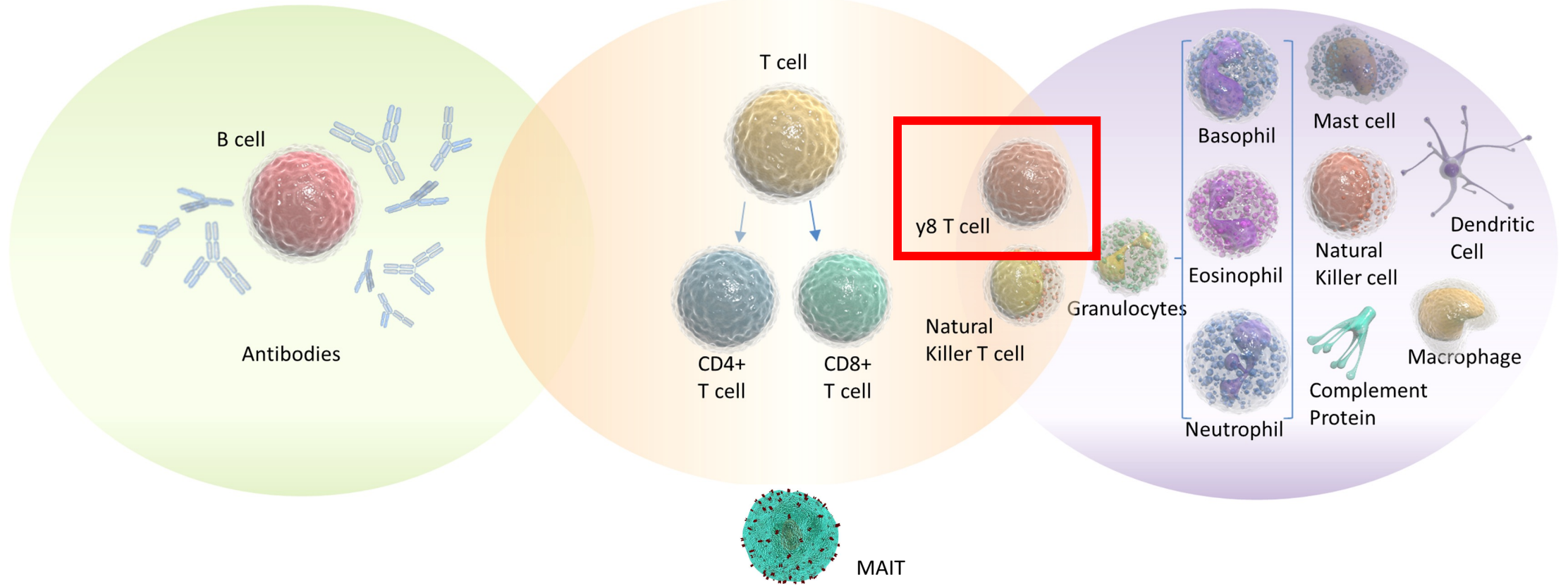


# Adaptive Immunity

# Innate Immunity

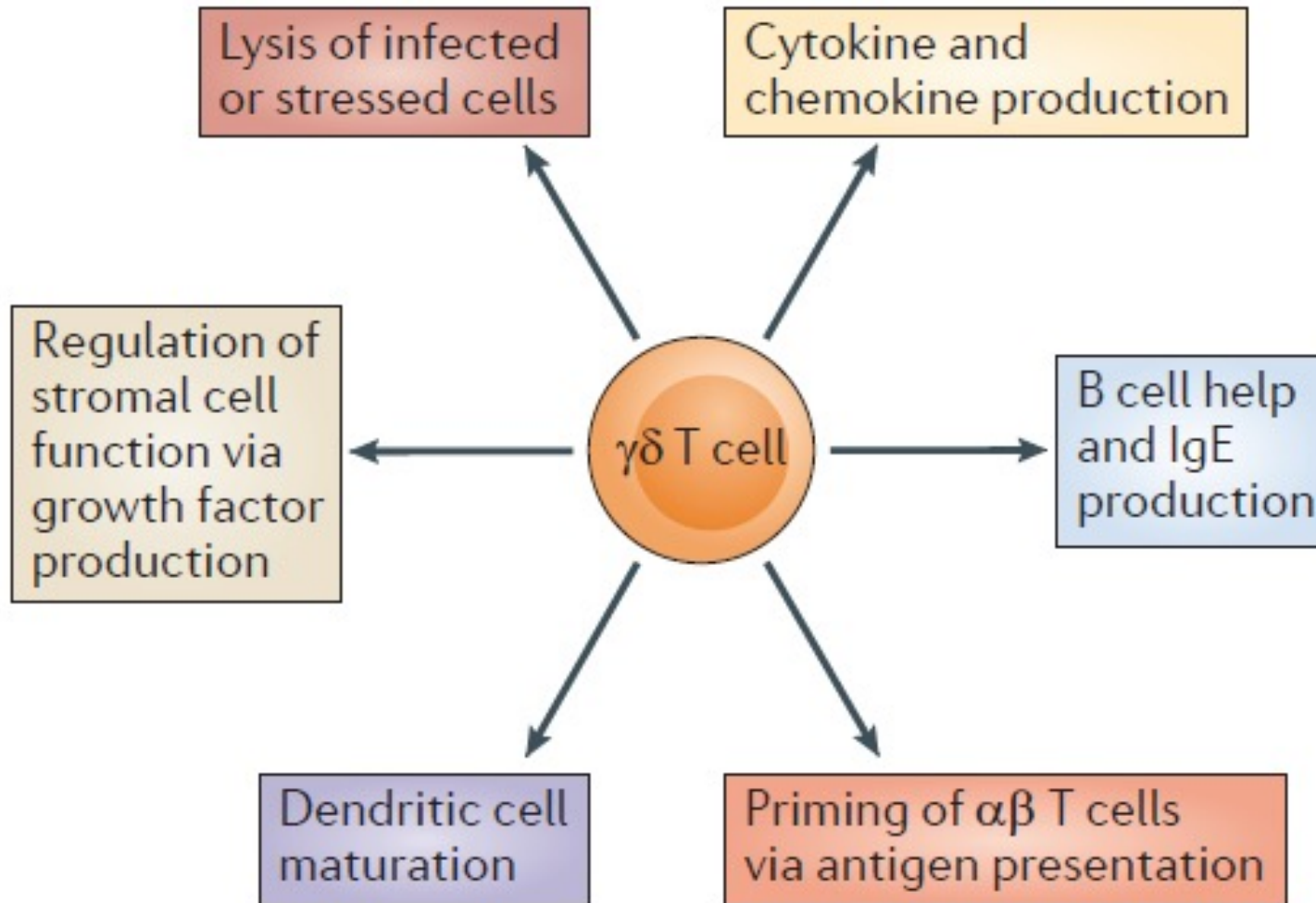
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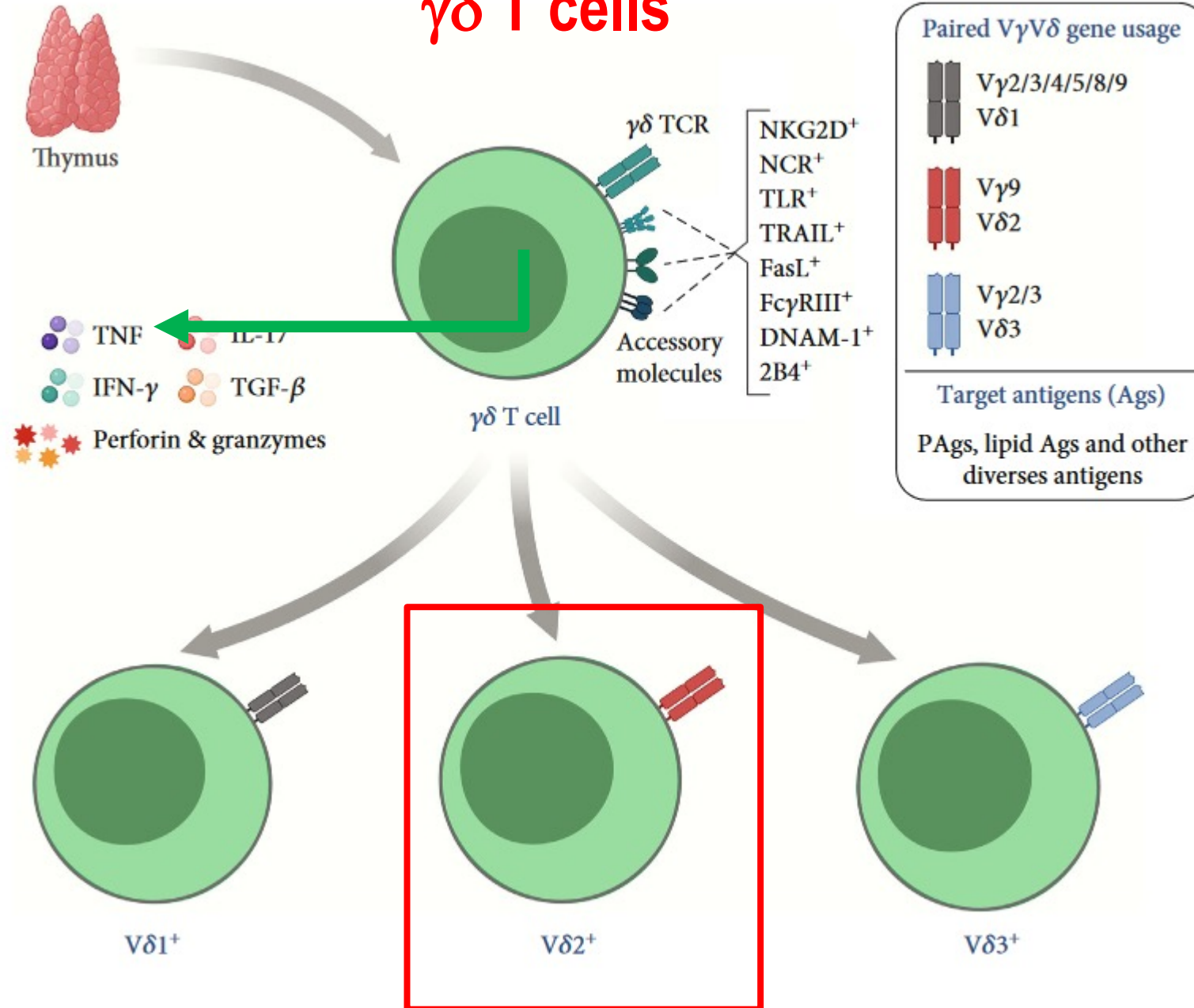




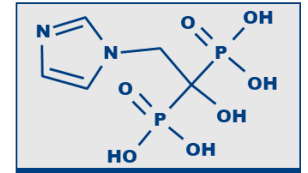
# Immune functions of $\gamma\delta$ T cells



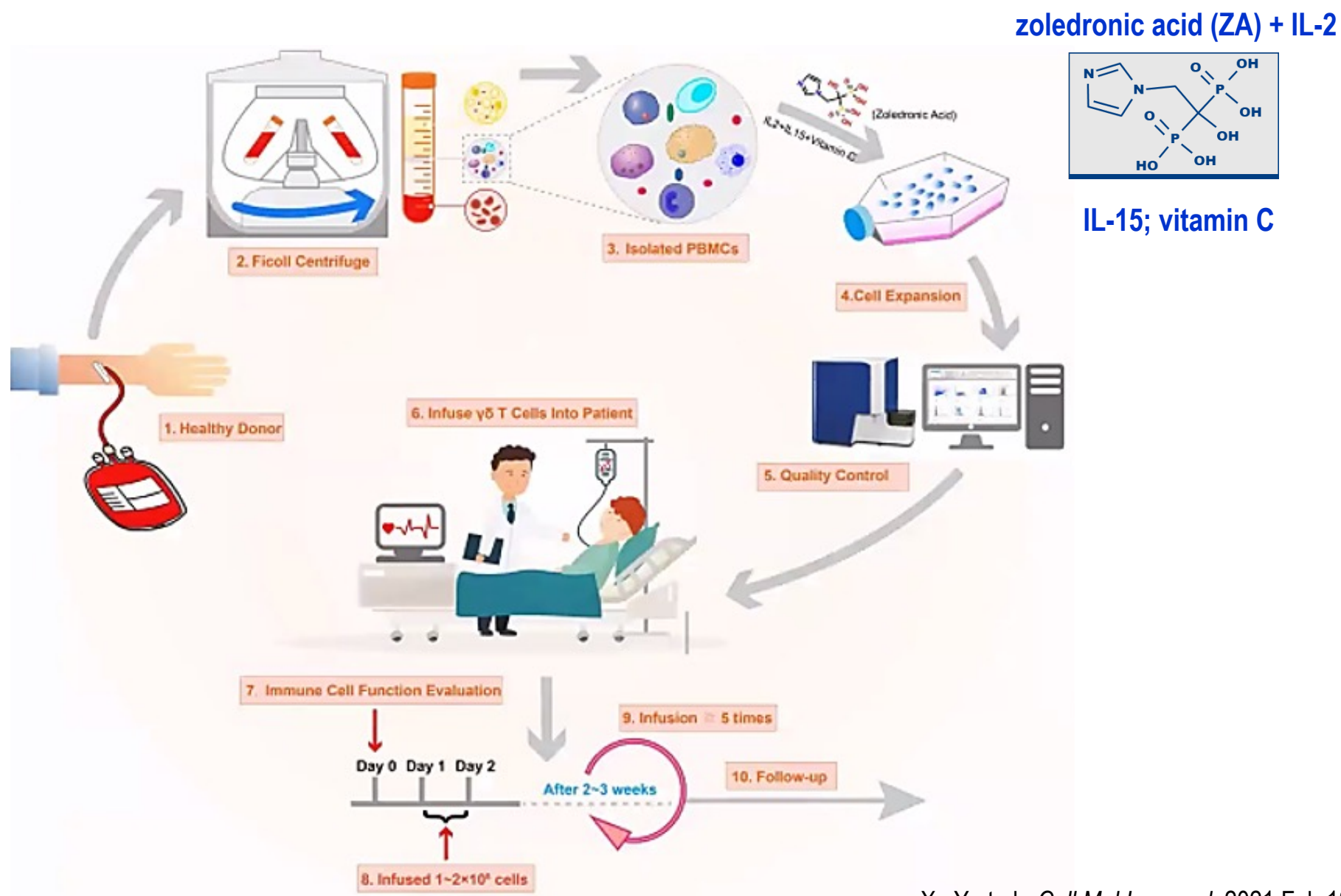
# $\gamma\delta$ T cells



zoledronic acid (ZA) + IL2



# Workflow of allogeneic V $\gamma$ 9V $\delta$ 2 T-cell immunotherapy

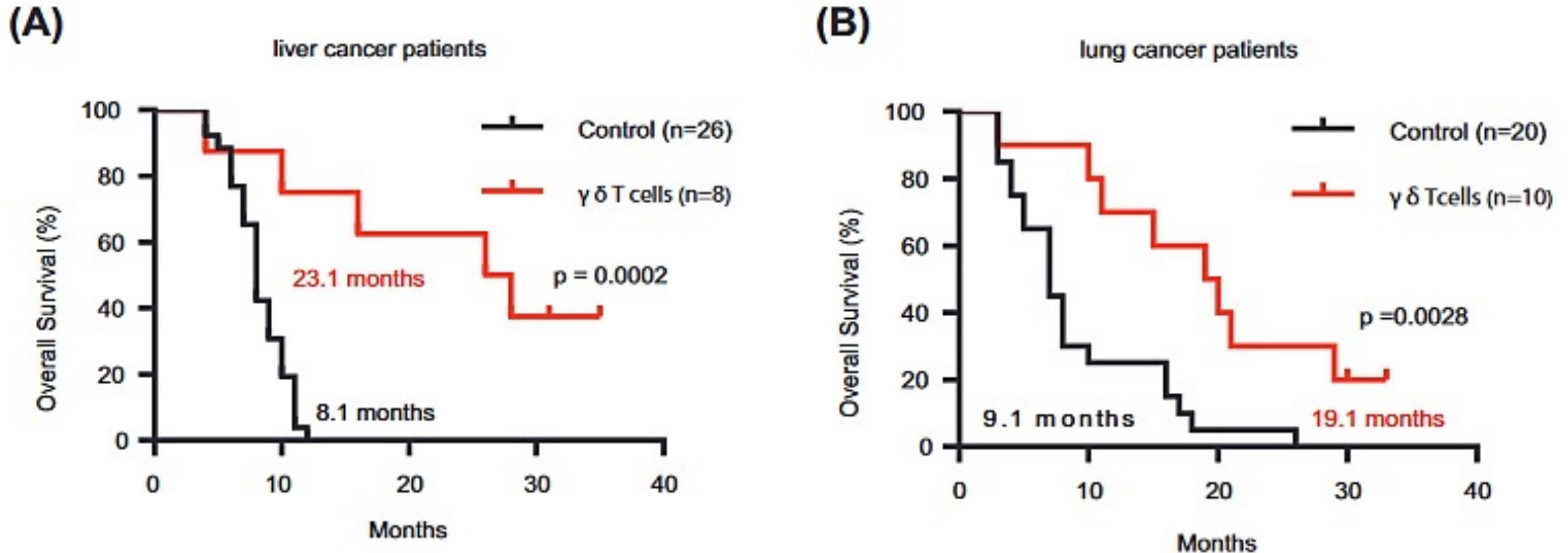


# Investigator-initiated trial with allogeneic V $\gamma$ 9V $\delta$ 2 T cells in late-stage cancer

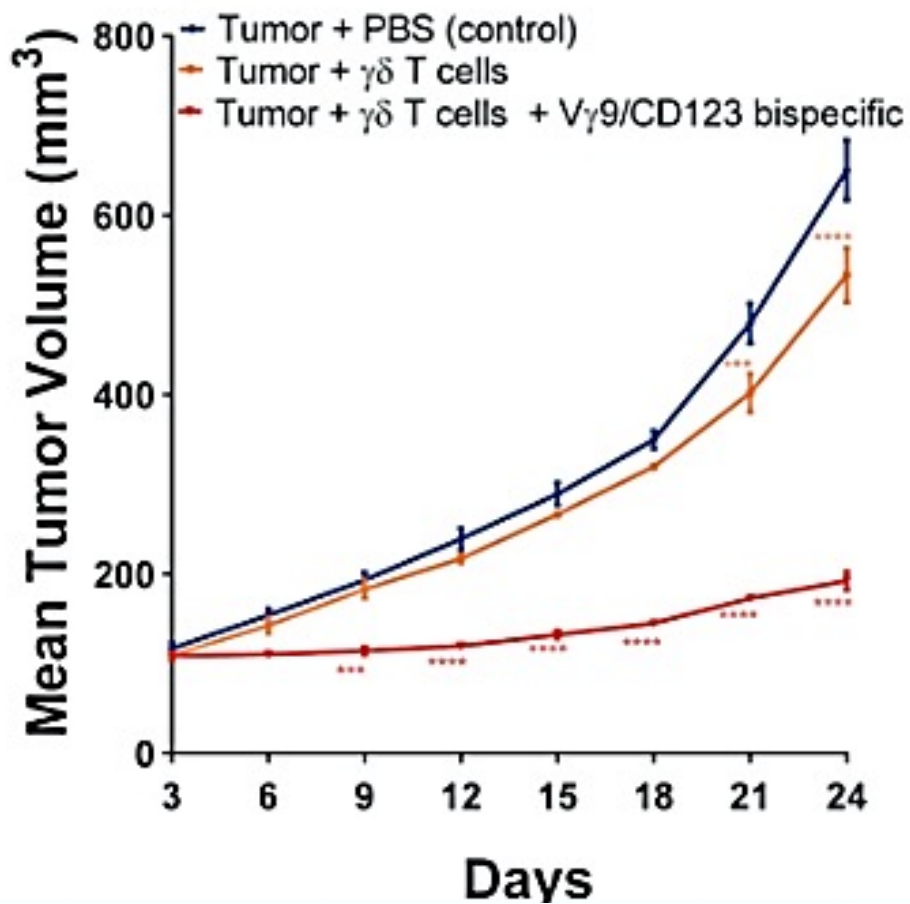
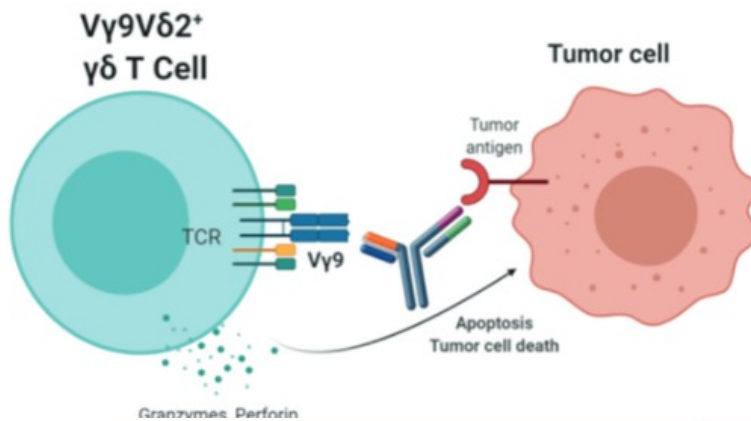
Cancer type	Clinical Registration	Number of Patients	Total Times of Infusion
Lung	NCT03183232	29	107
Liver	NCT03183219	22	87
Breast	NCT03183206	12	42
Pancreas	NCT03180437	16	60
Other	/	53	118
		<b>132</b>	<b>414</b>



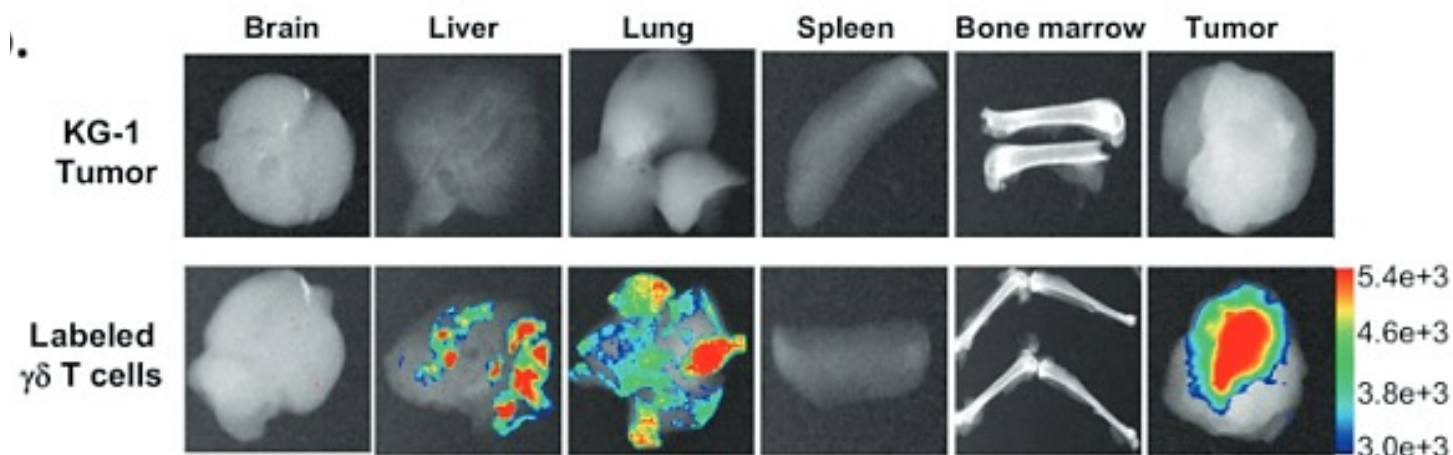
# OS in pts with advanced liver cancer or lung cancer infused with allogeneic V $\gamma$ 9V $\delta$ 2 T cells



# $\gamma\delta$ T-cell recruitment by a bispecific antibody for AML treatment



Samples	Tumor Volume (mm <sup>3</sup> ) Mean $\pm$ SEM	% Tumor growth inhibition
Tumor + PBS (Control)	650 $\pm$ 33	-
Tumor + $\gamma\delta$ T cells	533 $\pm$ 31	21
Tumor + $\gamma\delta$ T cells + V $\gamma$ 9/CD123 bispecific	193 $\pm$ 10	84

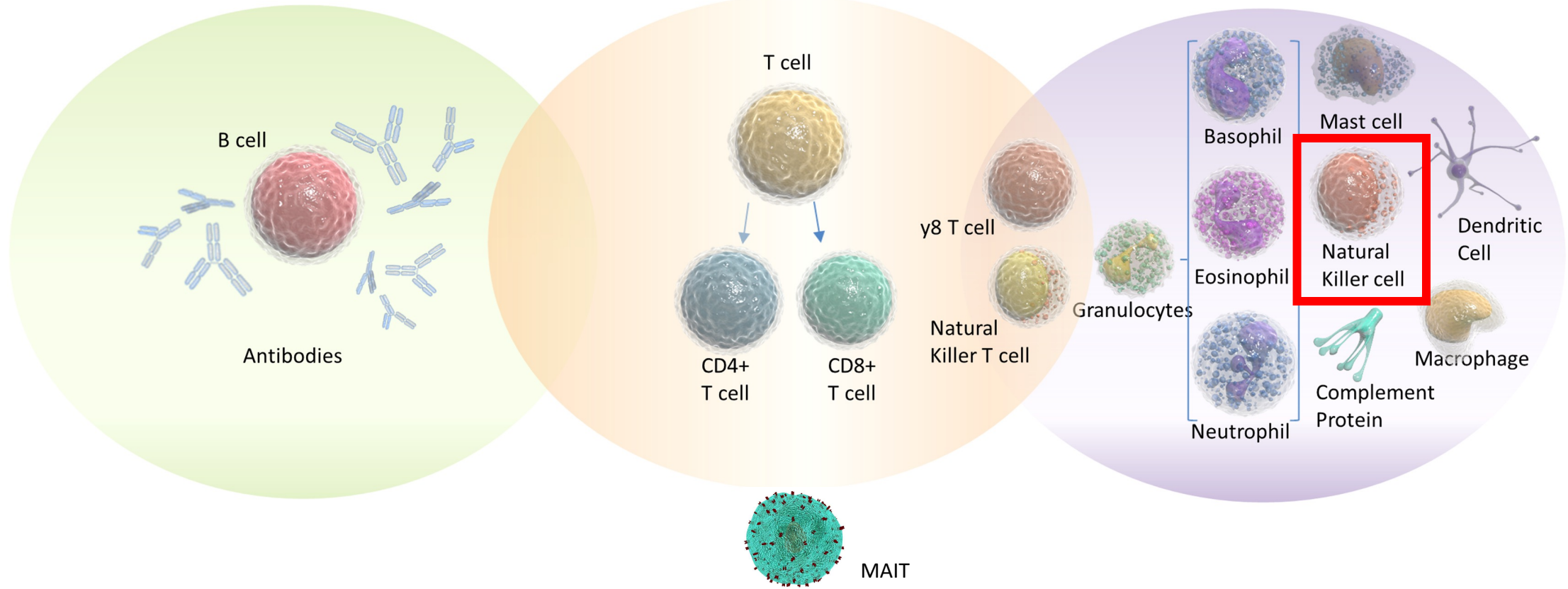


# Adaptive Immunity

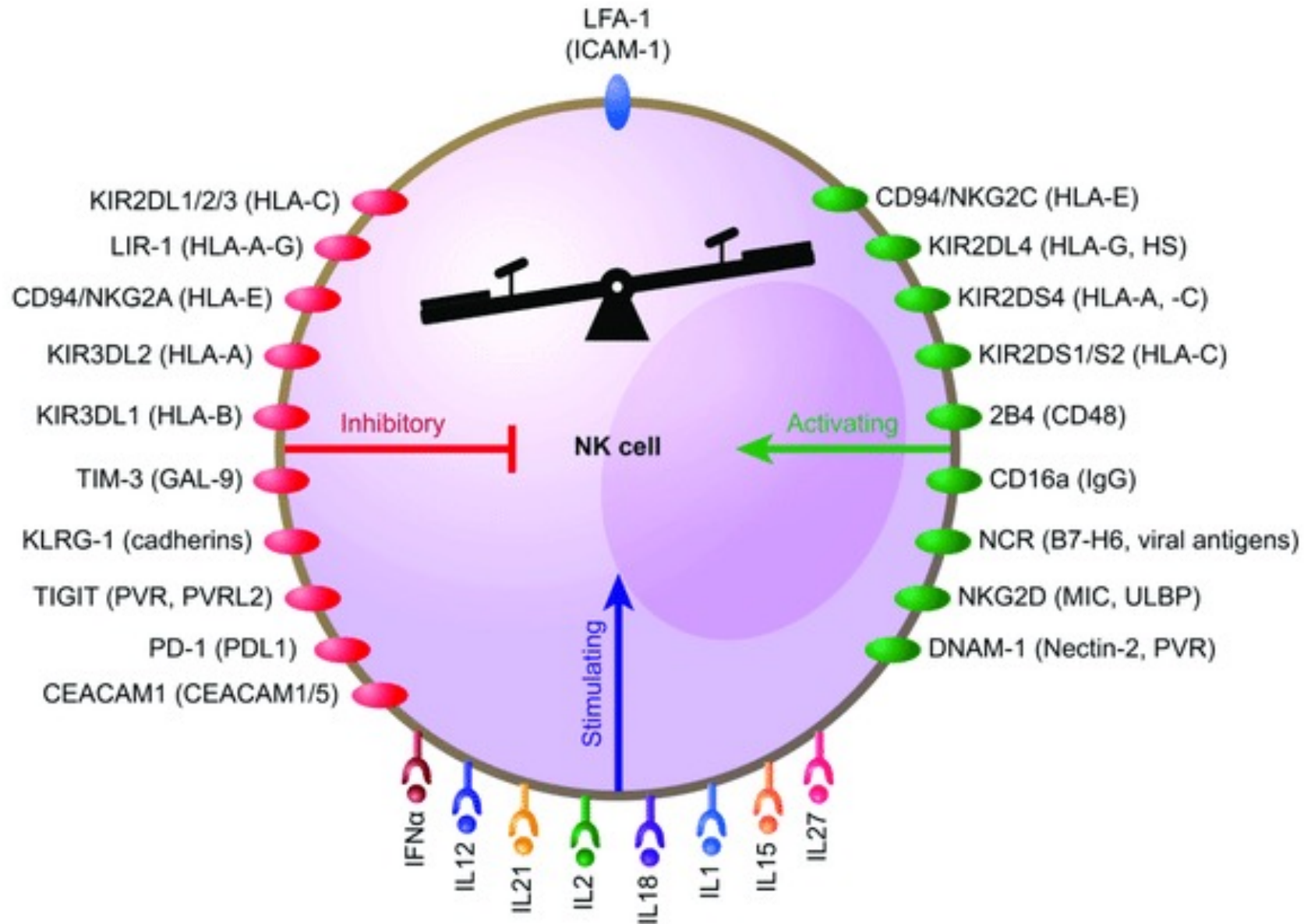
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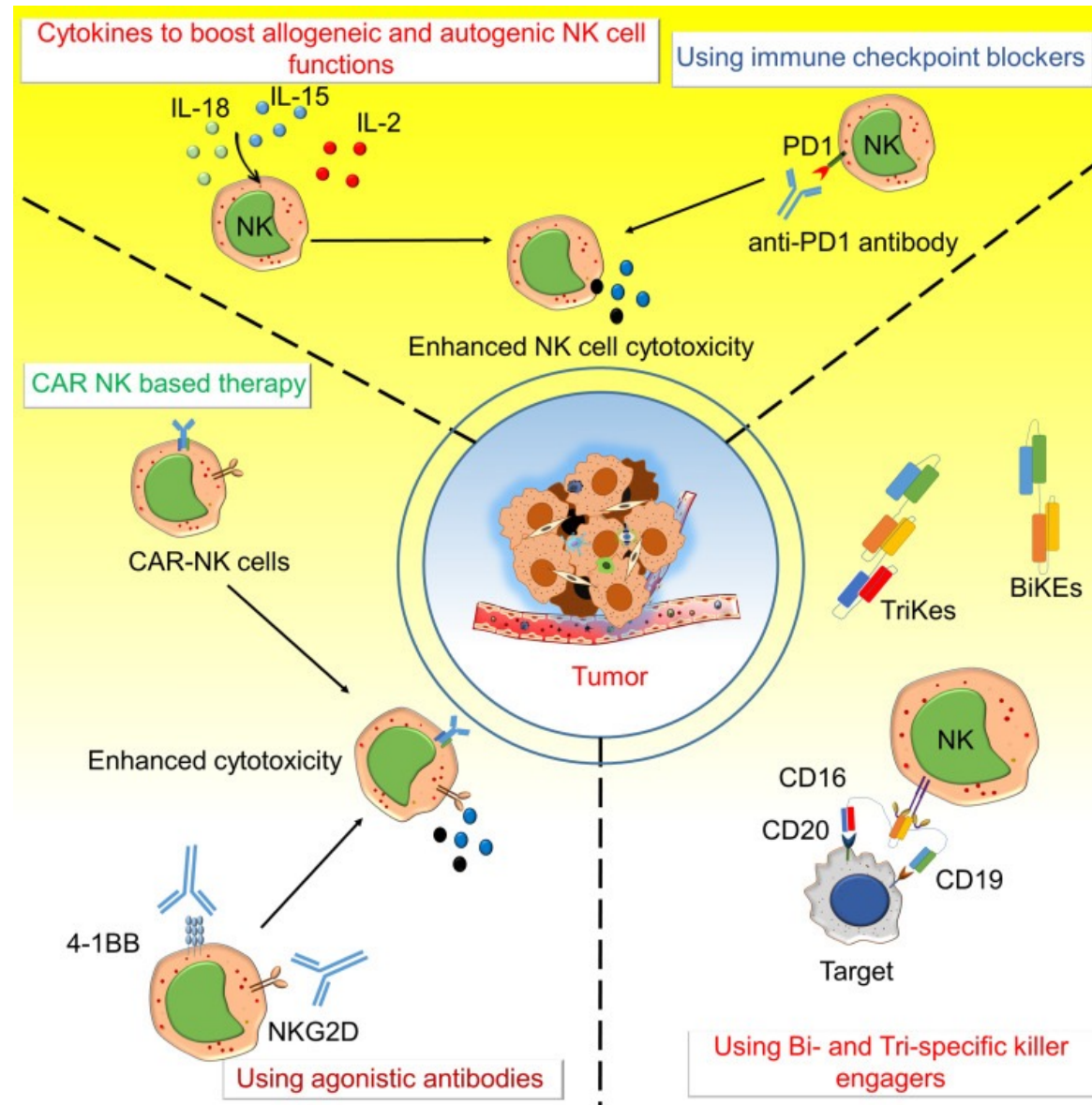


# Expression of activatory and inhibitory receptors in NK cells

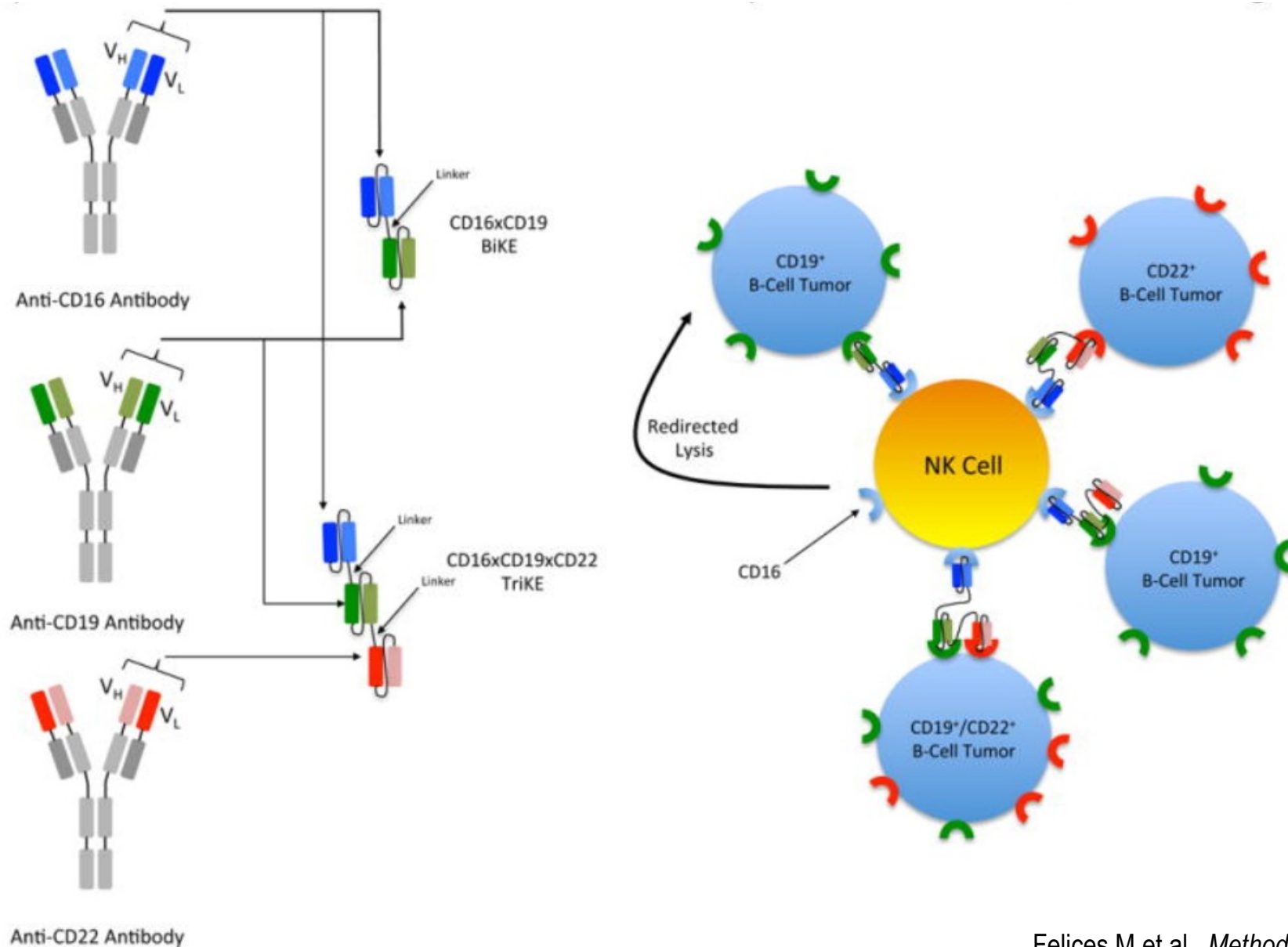




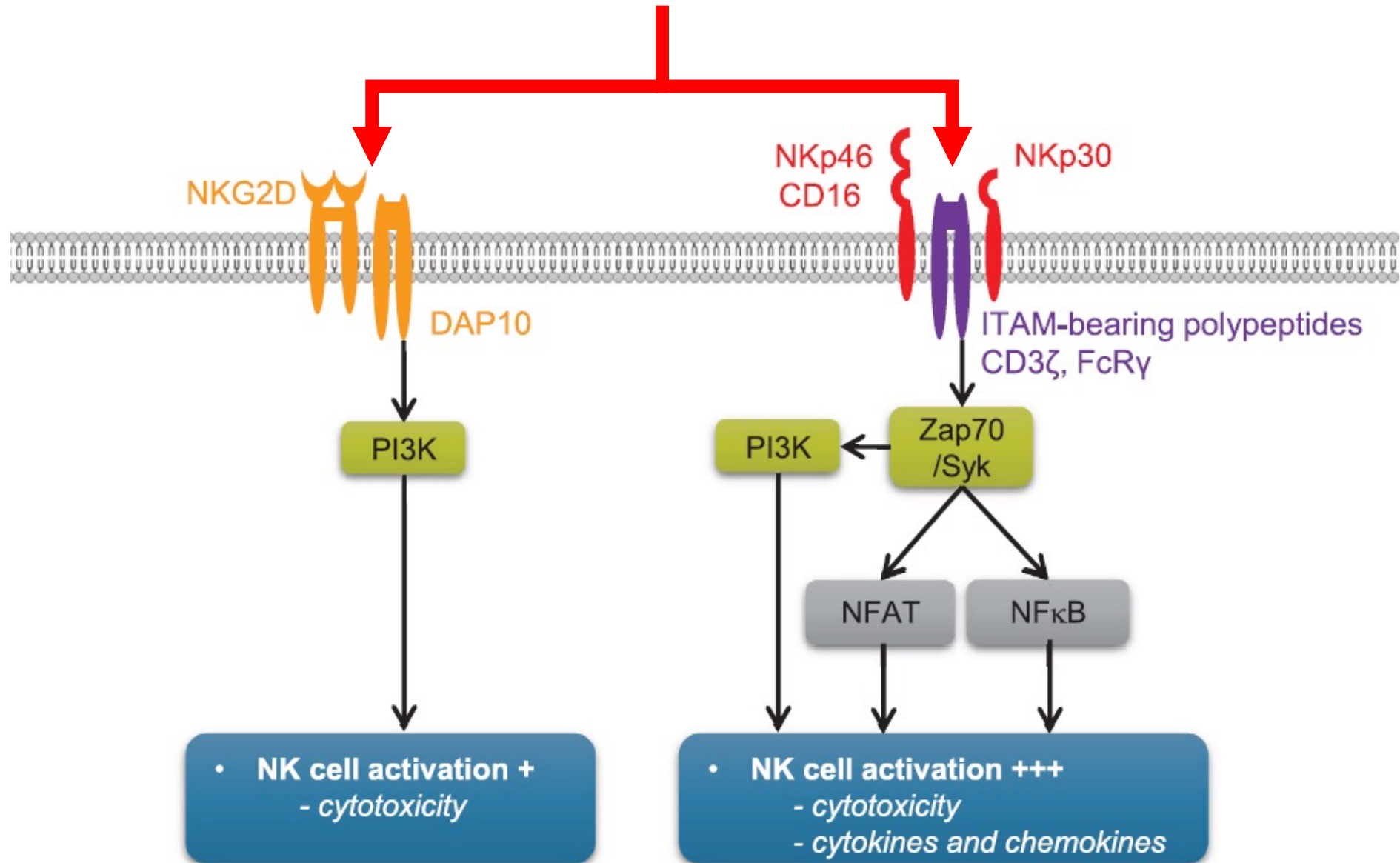
# Strategies to reinforce NK-cell anti-tumor immunity



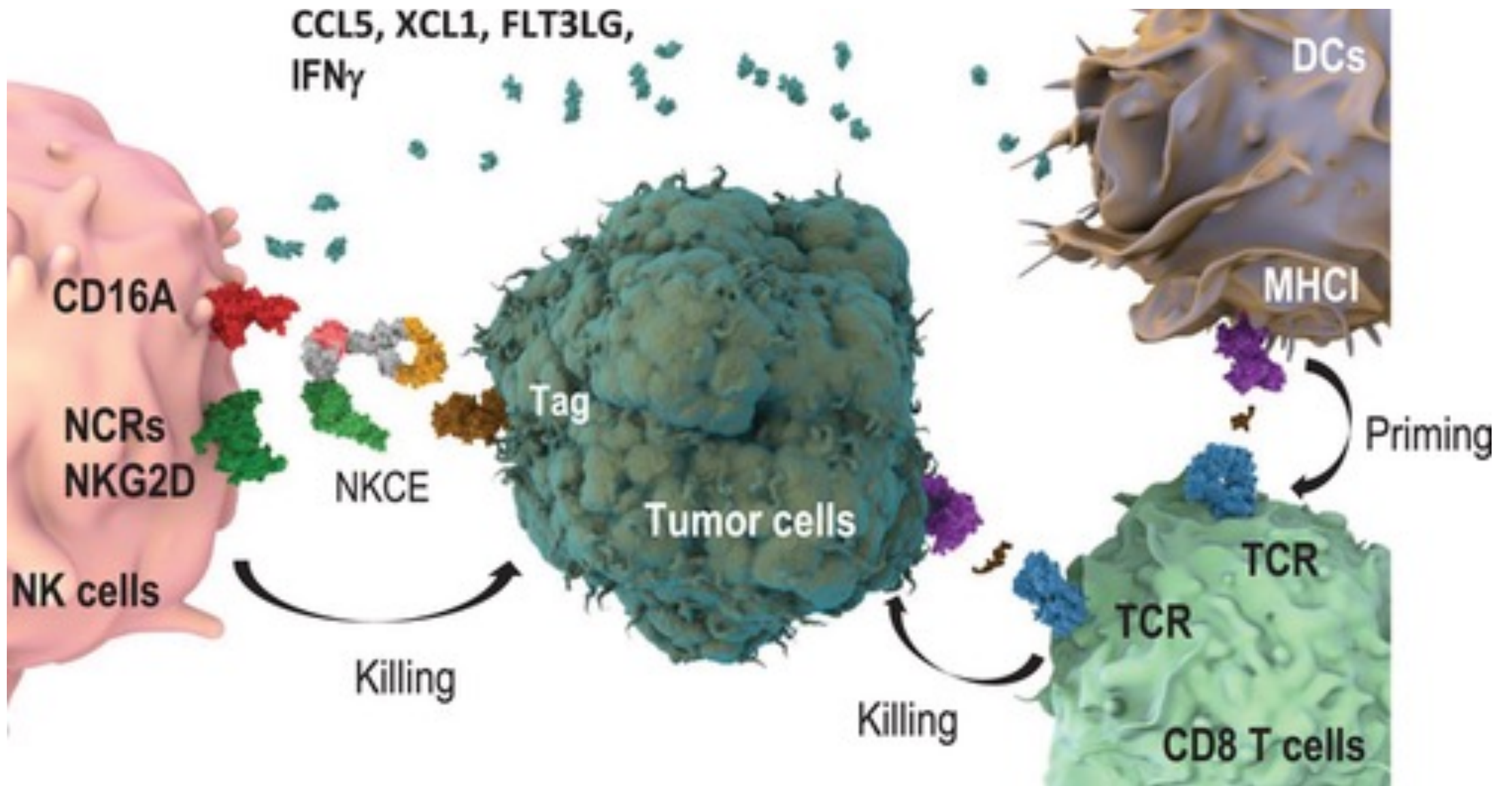
# BiKEs and TriKEs redirected NK-cell mediated anti-tumor activity



# Signaling pathways NK cell engagers

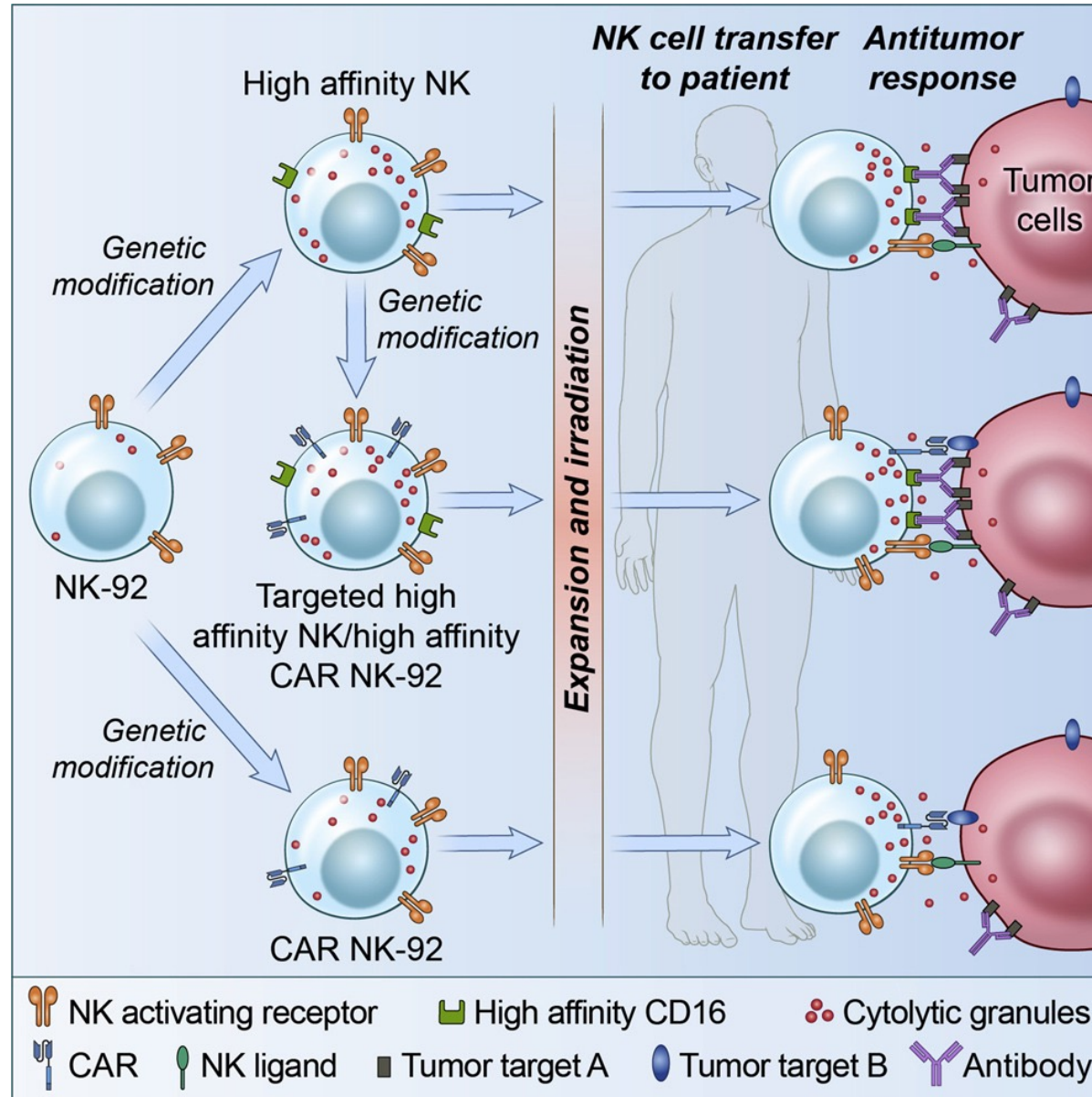


# Second generation TriKEs





# Off-the-shelf engineered NK cells for cancer immunotherapy

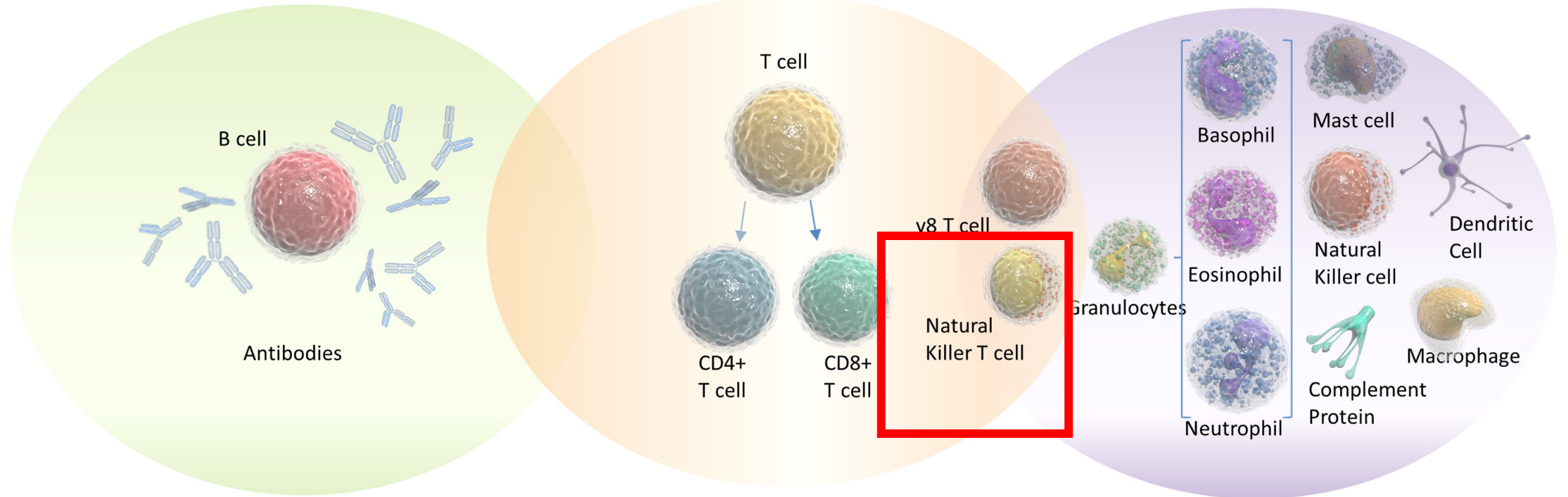


# Adaptive Immunity

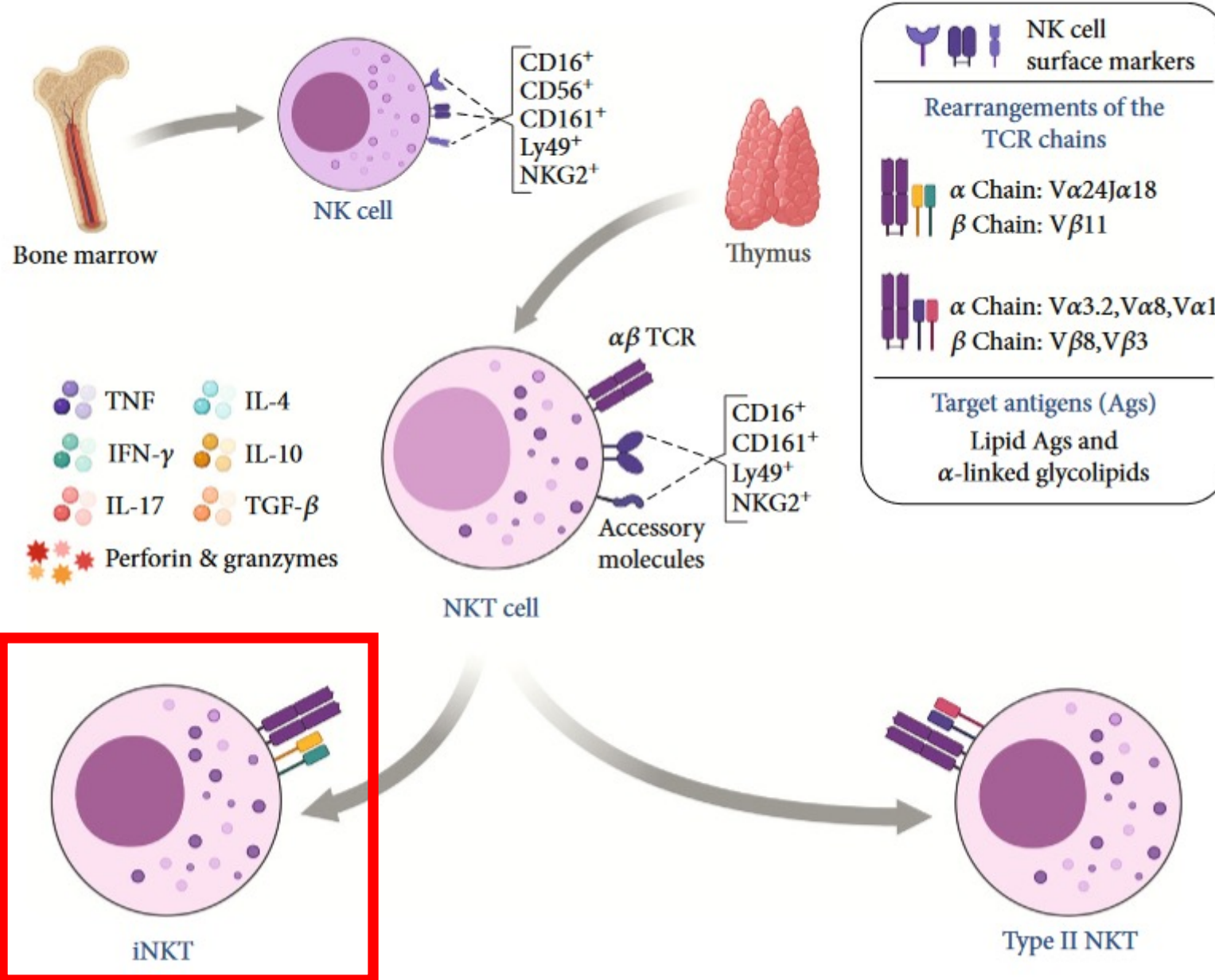
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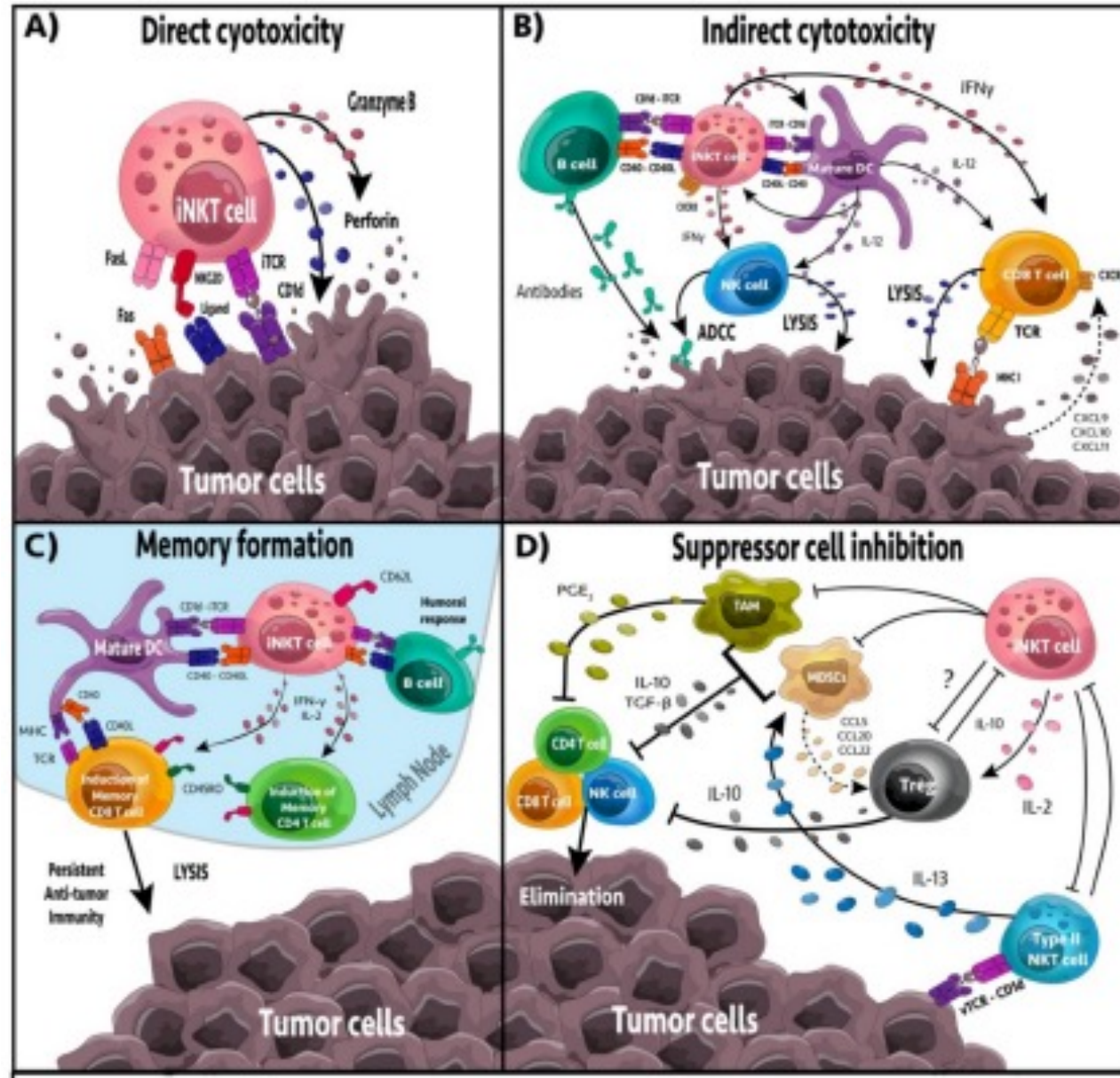


# NKT cells

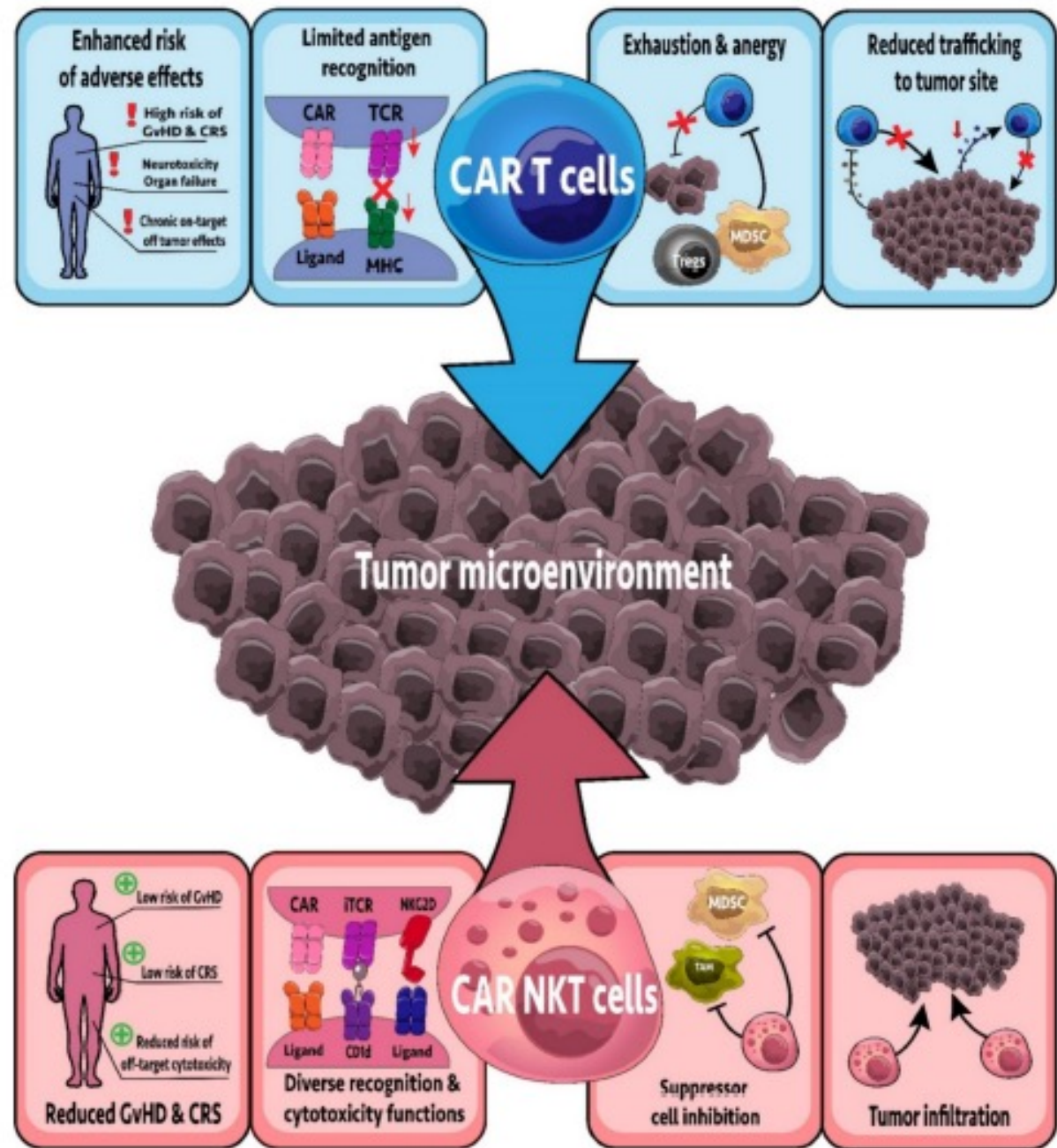




# Immune interactions of iNKT cells in the TME



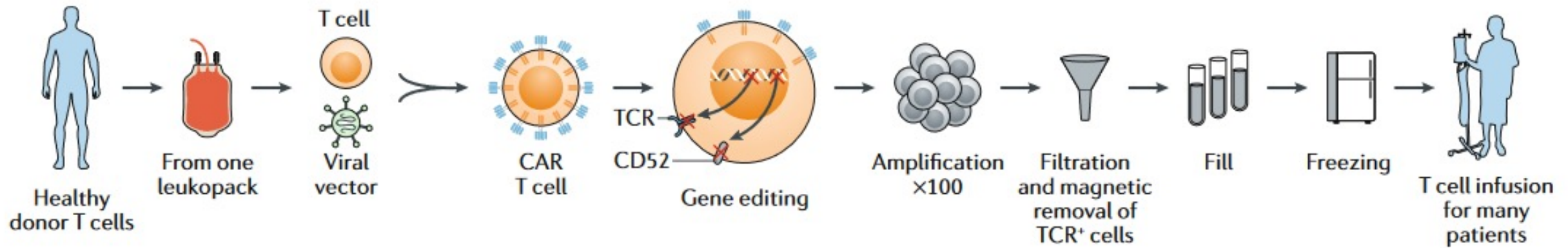




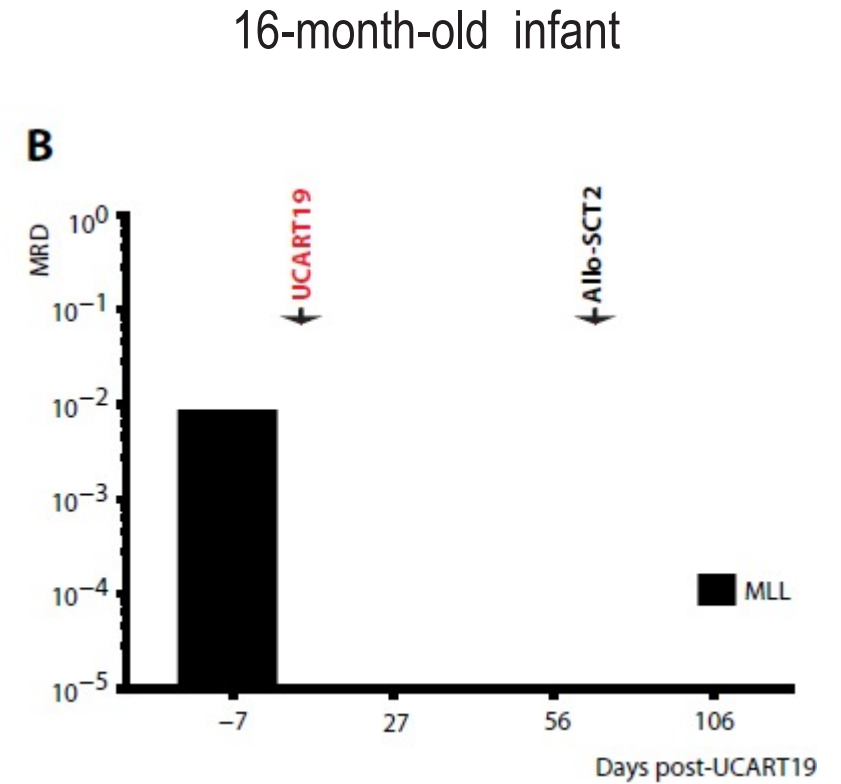
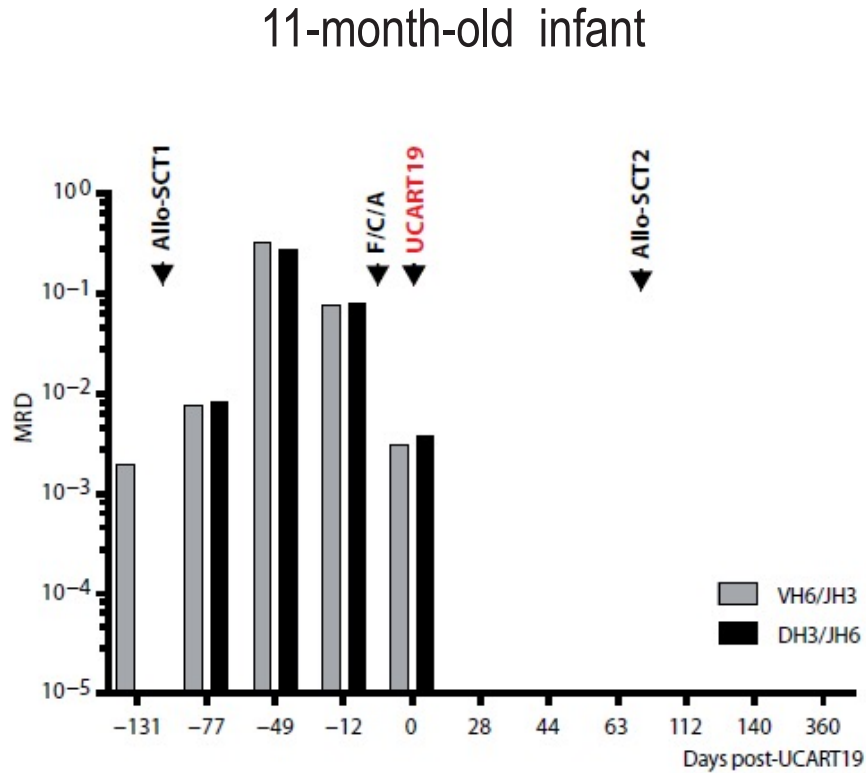
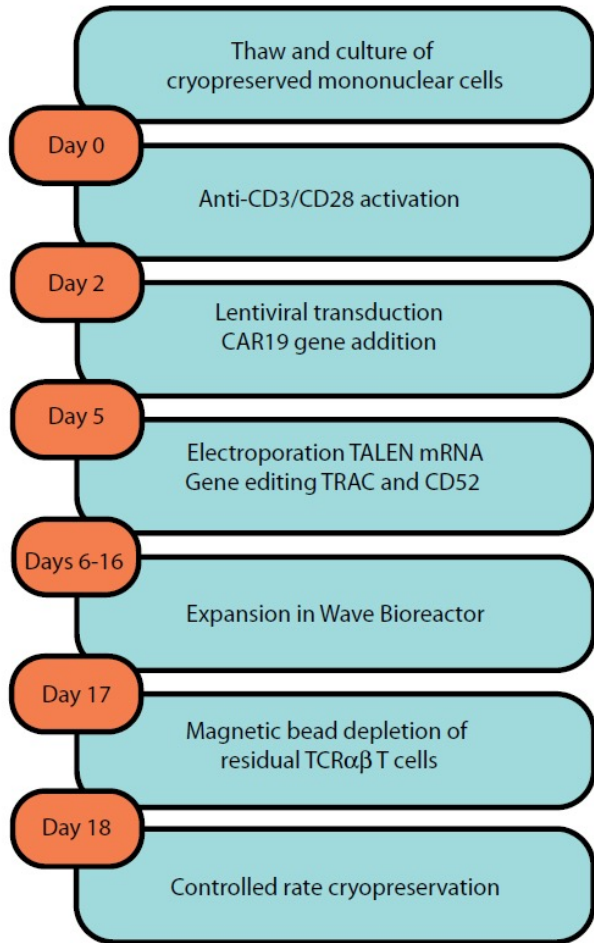
## Comparison of the Various Approaches to Off-the-Shelf CAR-Based Product Development.

	NKT cells (type I)	$\gamma\delta$ T cells	$\alpha\beta$ T cells	NK cells
<b>Gene editing to remove TCR</b>	No	No	Yes	No
<b>Blood frequency</b>	0.1% of T cells	5-10%	45-70%	5-20%
<b>GVHD potential</b>	Low	Low	High	Low
<b>Additional tumor specificity</b>	Yes	Yes	No	Yes
<b>Primary location</b>	Tissues & Blood	Tissues or Blood	Blood	Blood
<b>Proliferation post-activation</b>	Yes	Yes	Yes	No
<b>Memory persistence</b>	Yes	No (?)	Yes	No

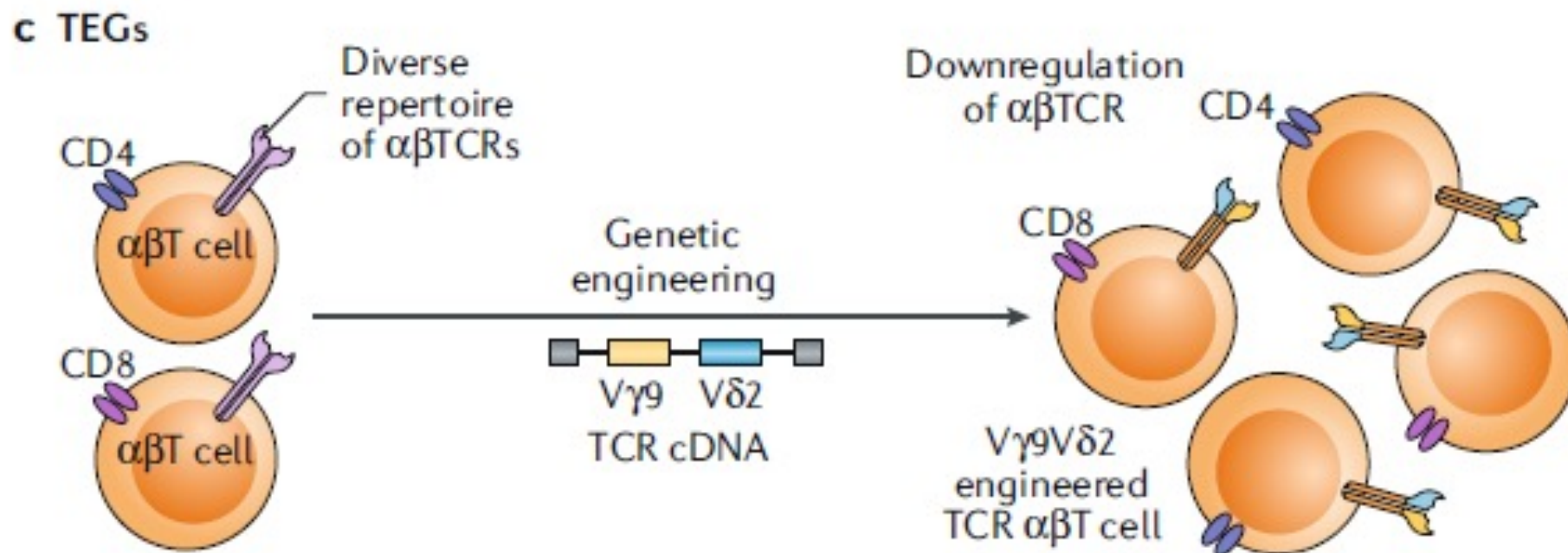
# Manufacturing “off-the-shelf” allogeneic CAR T cells



# Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells



# Genetic engineering to empower $\alpha\beta$ T cells with unconventional T-cell properties

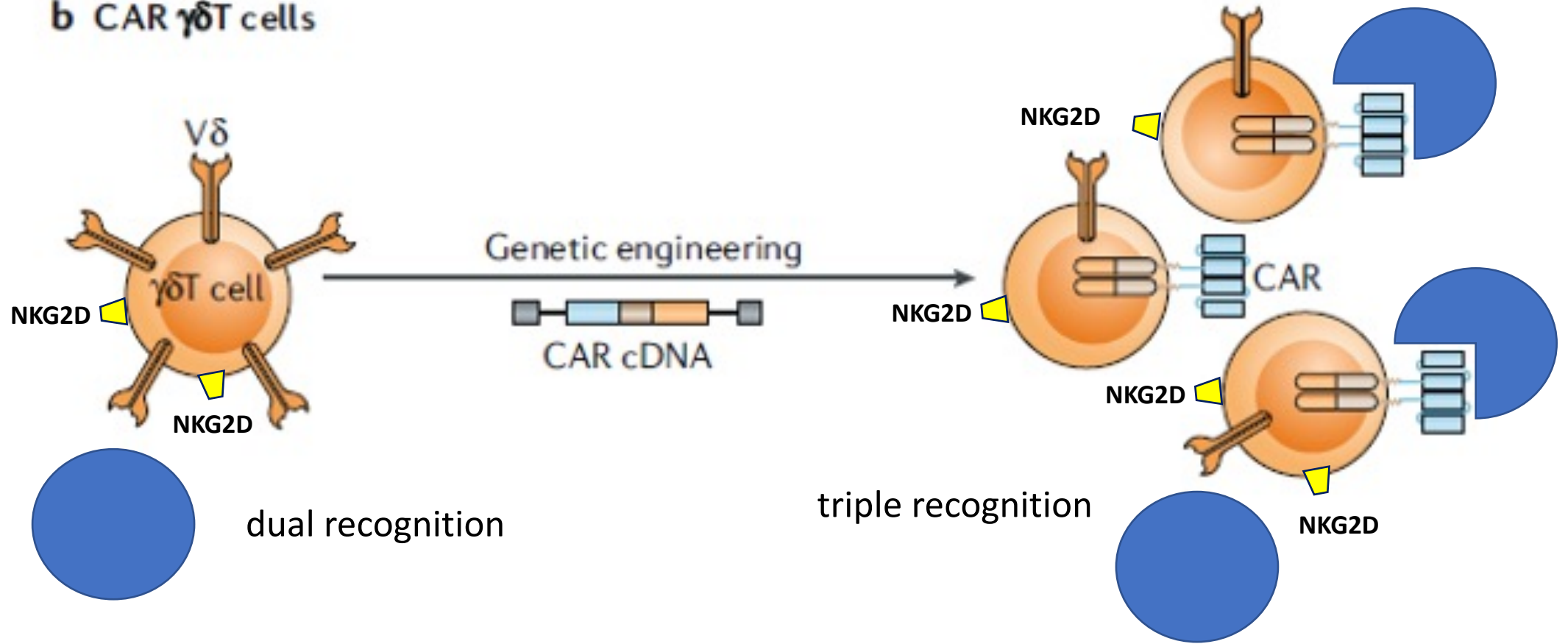




# Comparison of the Different Approaches to Allogeneic CAR-T Development

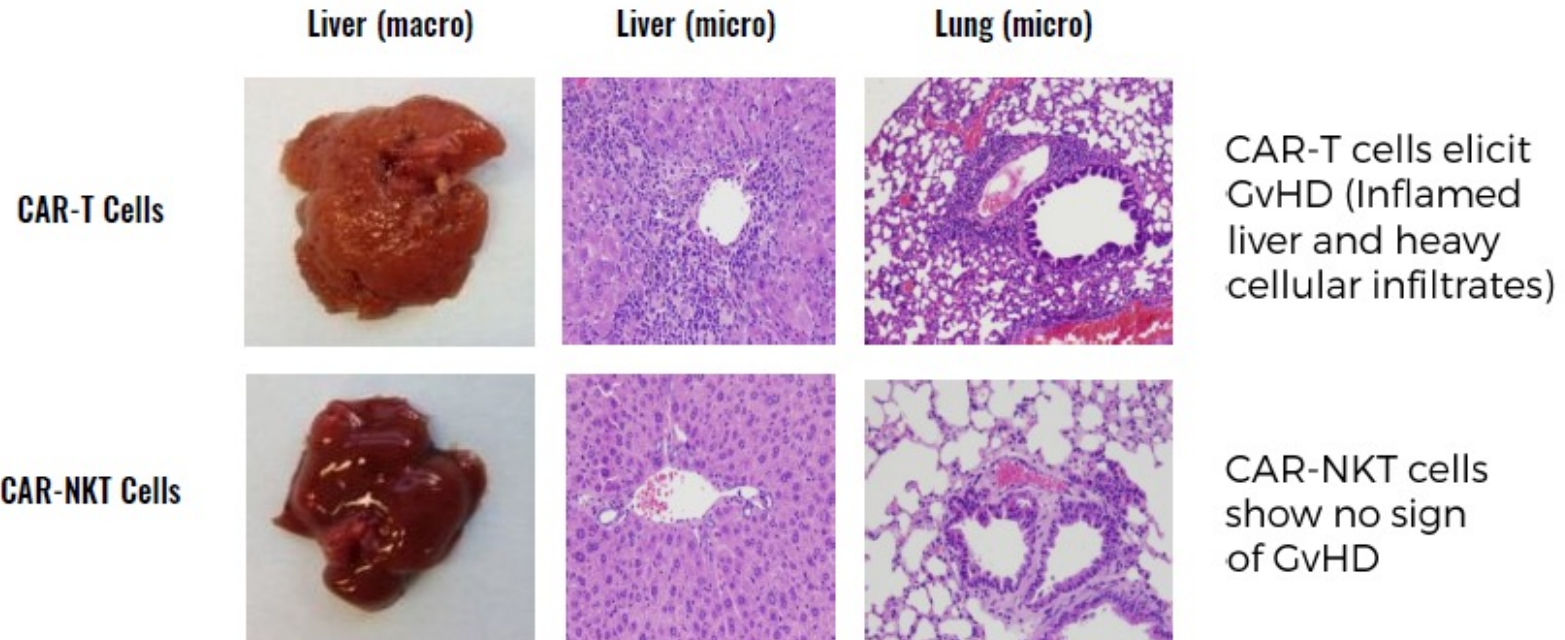
	Pros	Cons
<b>Gene Editing</b>	Continuity vis-à-vis autologous CAR-T technology	Limitations to manufacturing yields
	More than one gene can be deleted	Increased complexity and cost of manufacturing process
		Potential of insertional mutagenesis is less well understood

**b CAR  $\gamma\delta$ T cells**



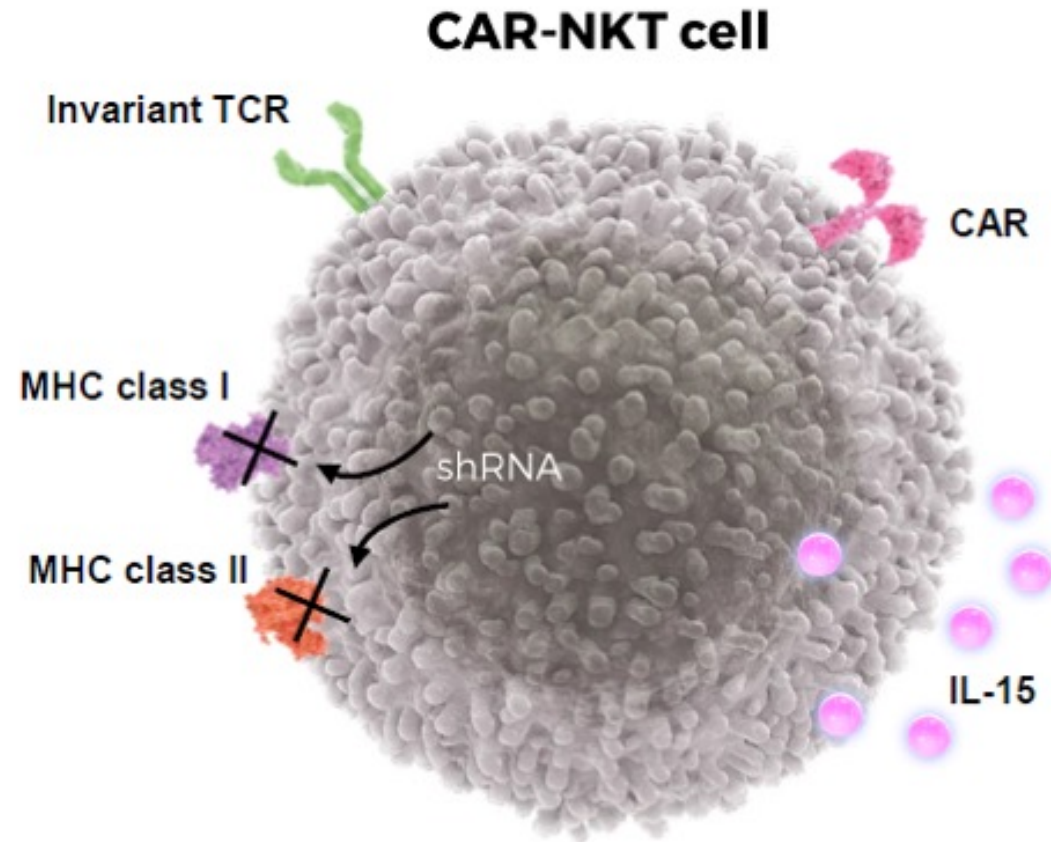
# Preclinical Data Demonstrates NKT Cells Do Not Cause GvHD

Mice receiving CAR-NKT cells showed no sign of GvHD over 11 weeks



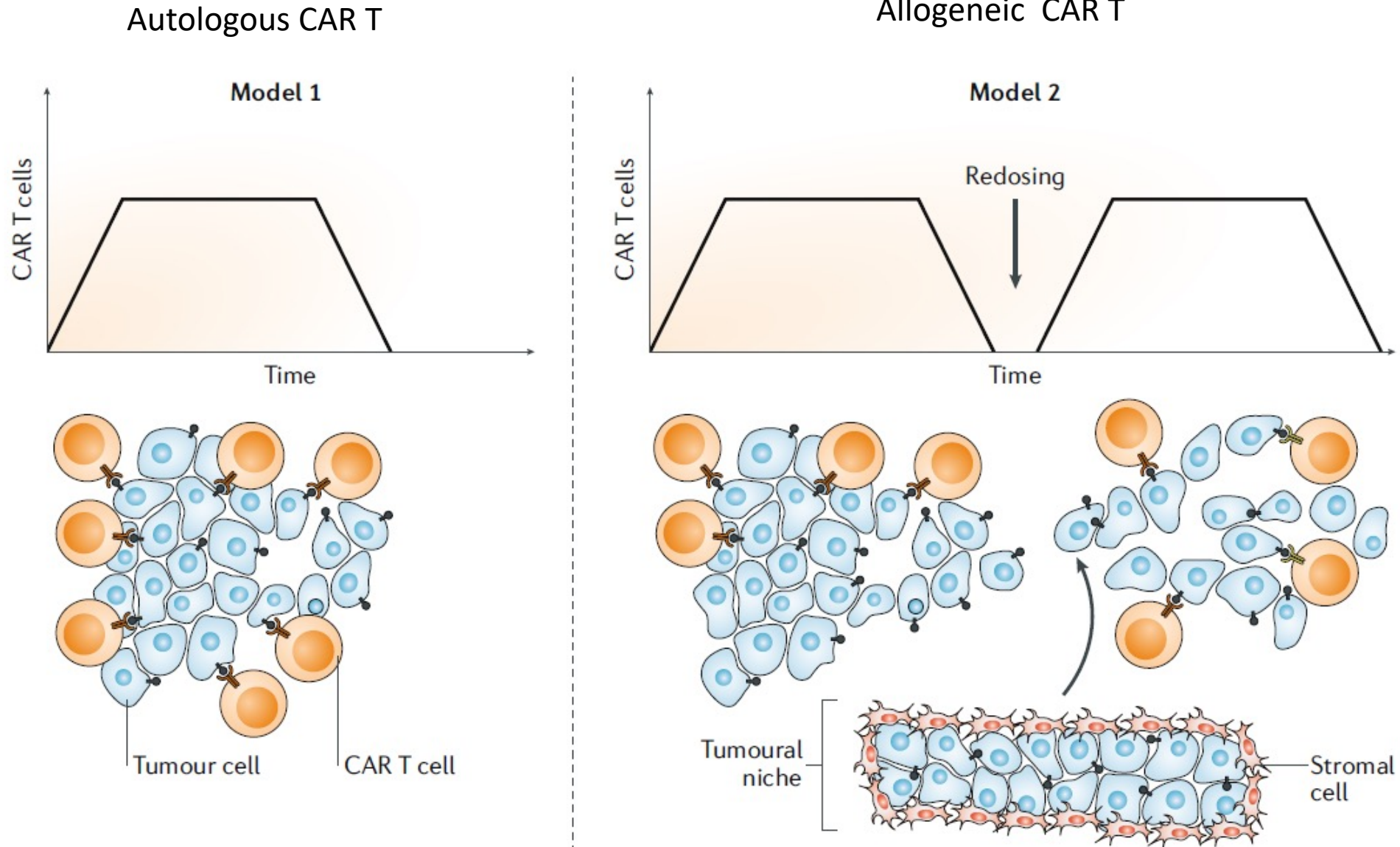
- T cells or NKT cells from a single human donor transfected with the same CAR
- Injected into tumor-bearing hu-NSG mice; analyzed after 4-5 weeks (above) or 8-11 weeks (not shown) for xeno-GvHD

# Engeneering CAR-NKT cells to improve antitumor activity and *in vivo* persistence

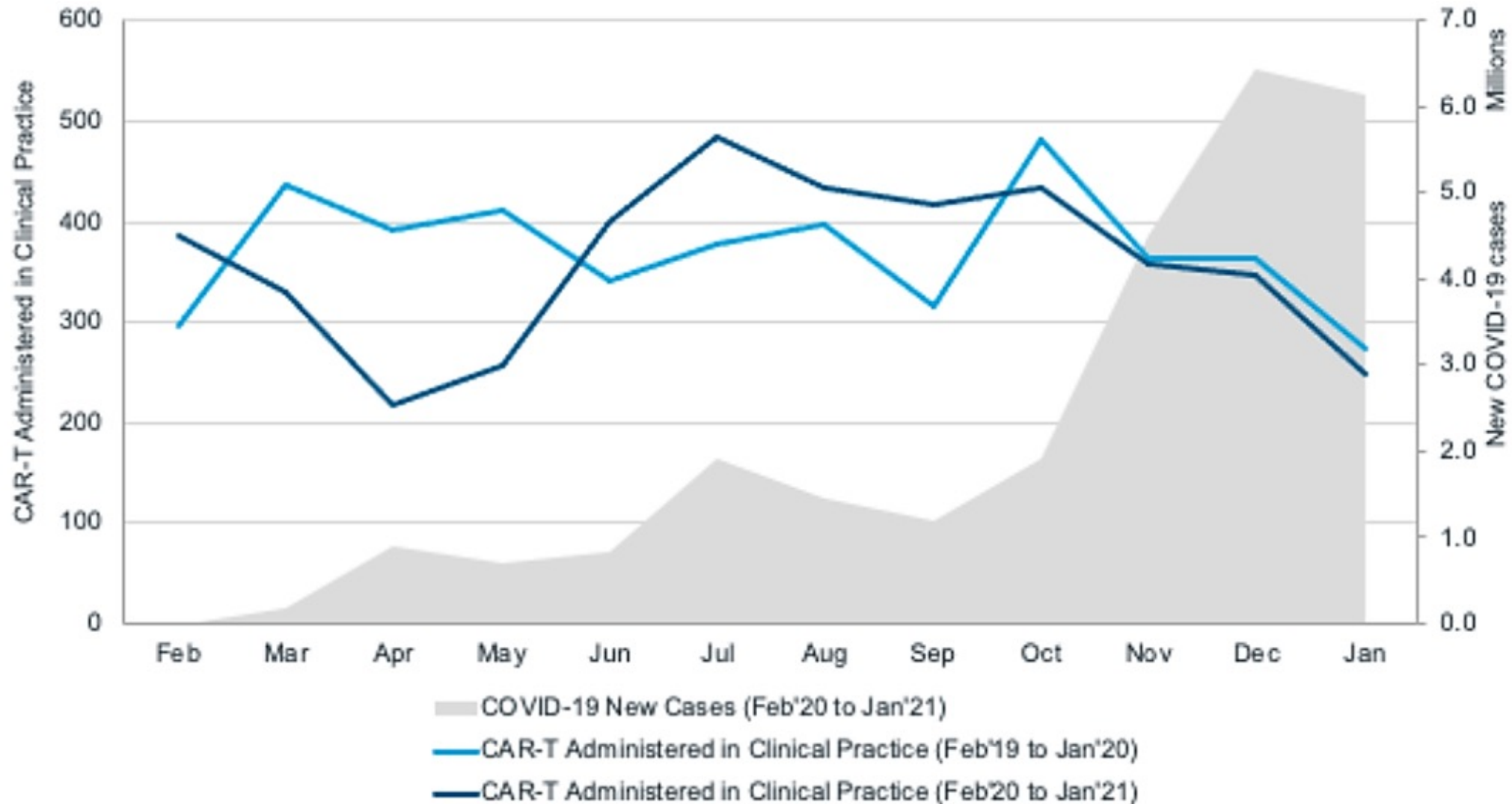




# Persistence of CAR T cells and tumor evolution



# Number of CAR-T cell therapies administered in US clinical practice tracked along with COVID-19 cases.



STUDIO PILOTA IN PAZIENTI ONCOEMATOLOGICI A CATTIVA PROGNOSI:  
SOMMINISTRAZIONE SEQUENZIALE DI T LINFOCITI  
ATTIVATI EX-VIVO CON OKT3  
E INTERLEUCHINA-2 IN INFUSIONE CONTINUA

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