Shahid Beheshti
University of Medical Sciences

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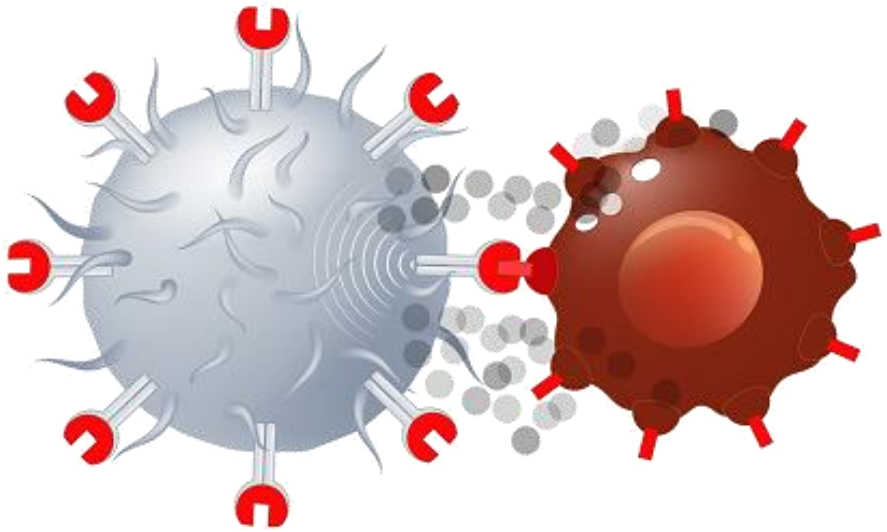

Adoptive T cell Therapy

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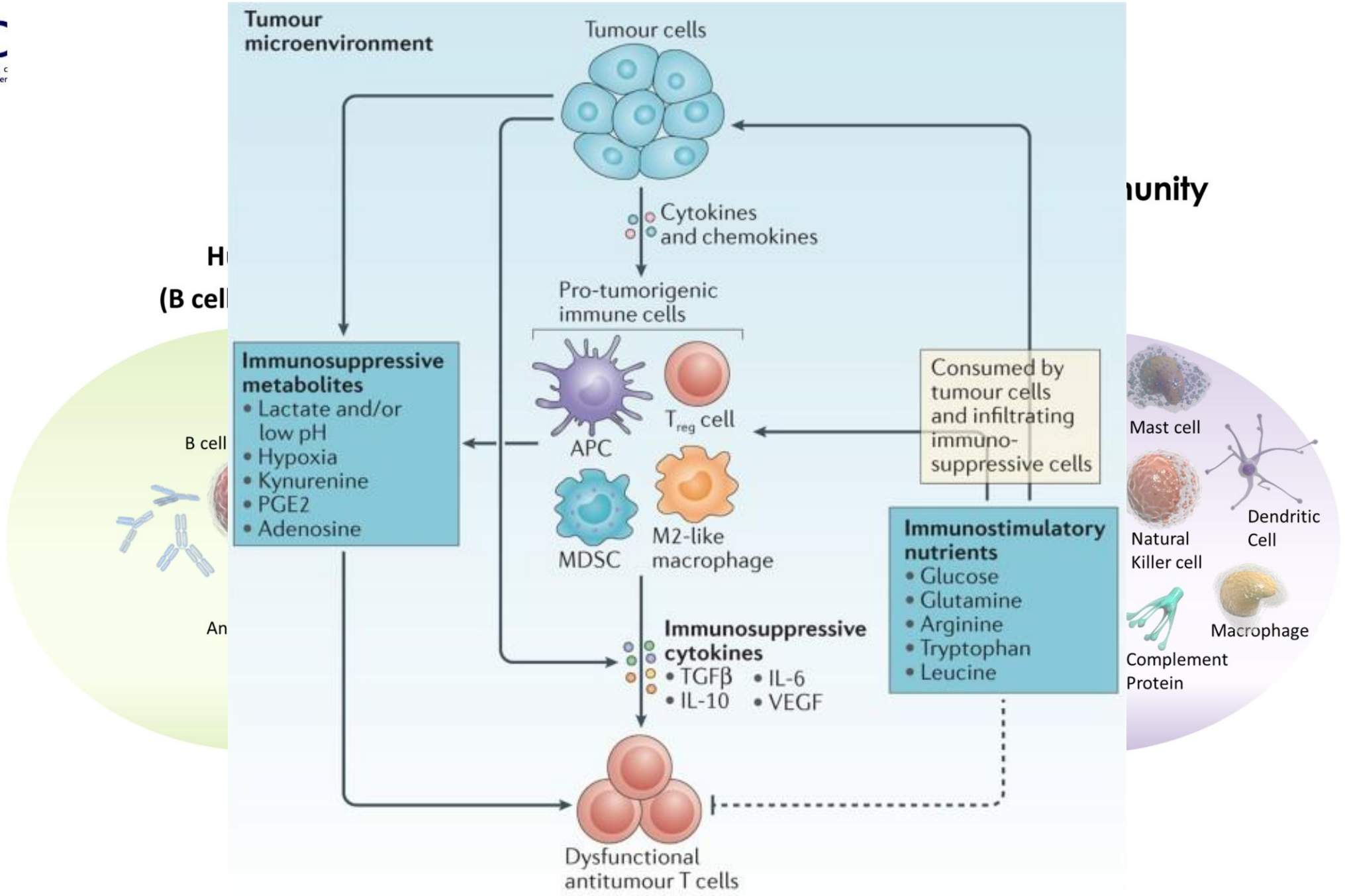
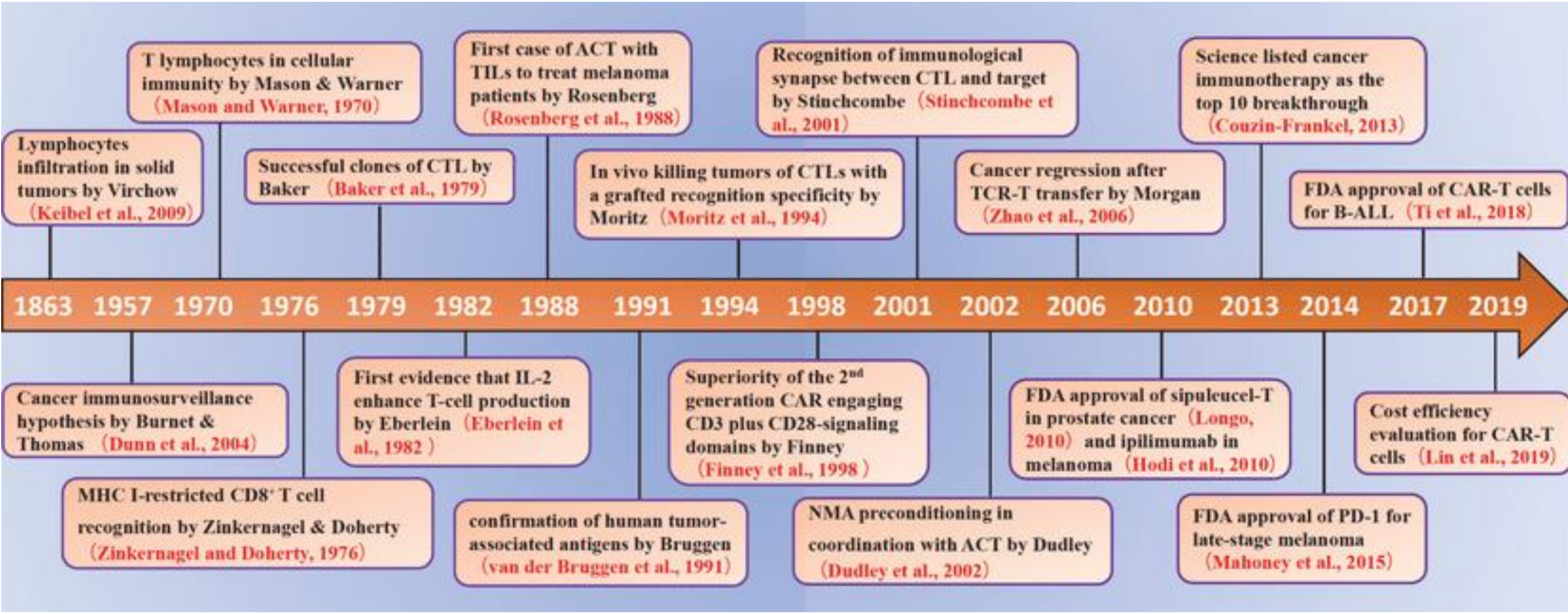
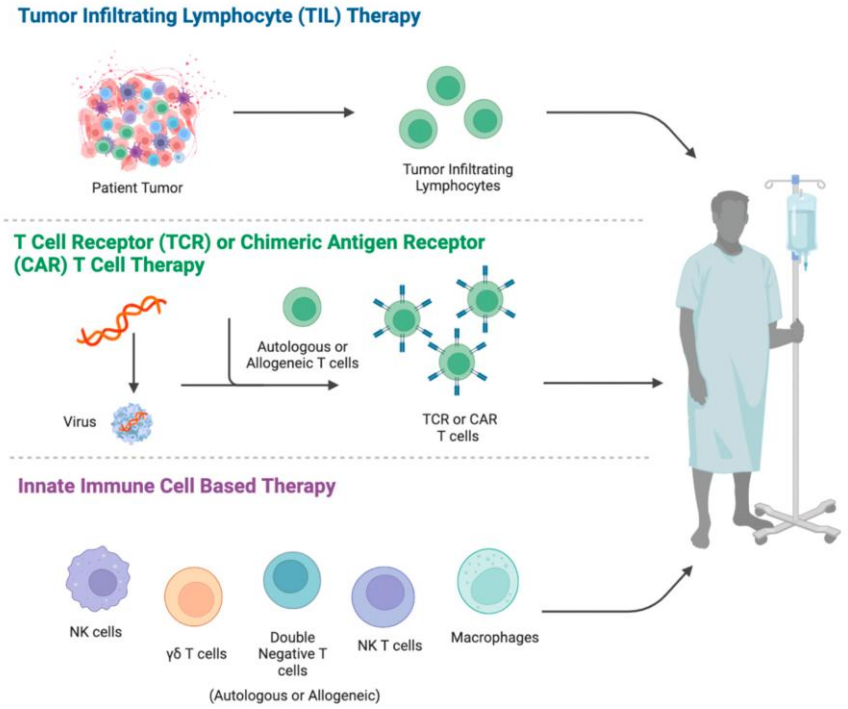


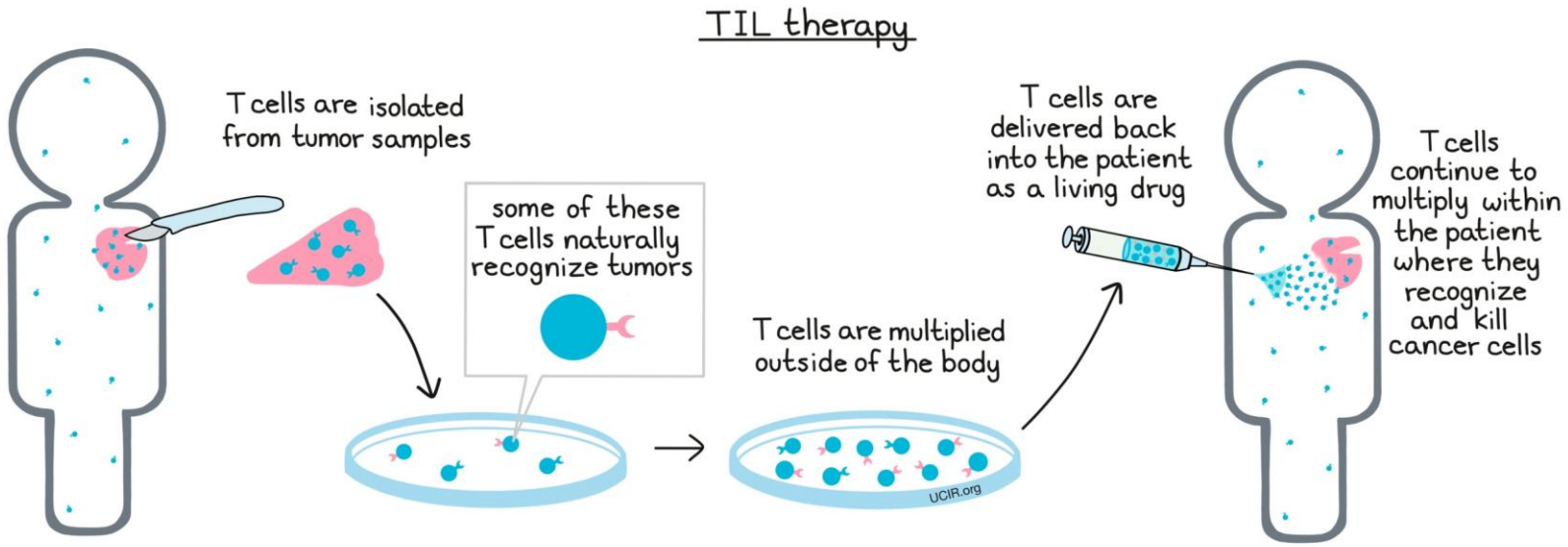


The history of adaptive T cell immunotherapy

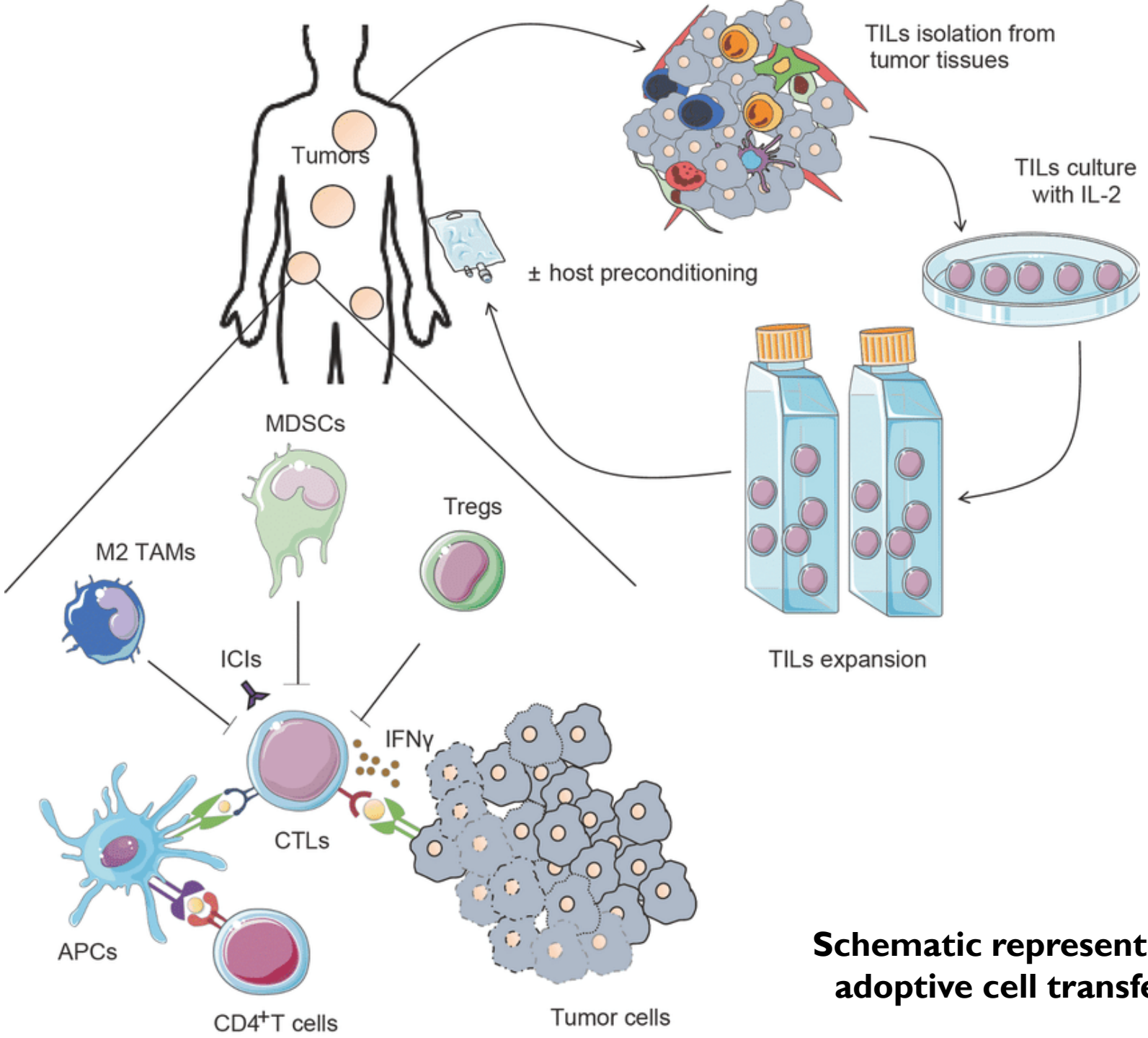
- Immunotherapy has been coined as the “fourth pillar” of cancer treatment.

- ACT has gained interest as an immunotherapy and involves the infusion of ex vivo expanded immune cells into patients for treating cancers, ranging from re-infusing immune cells taken from specific tumor sites to infusing genetically modified donor-derived immune cells.

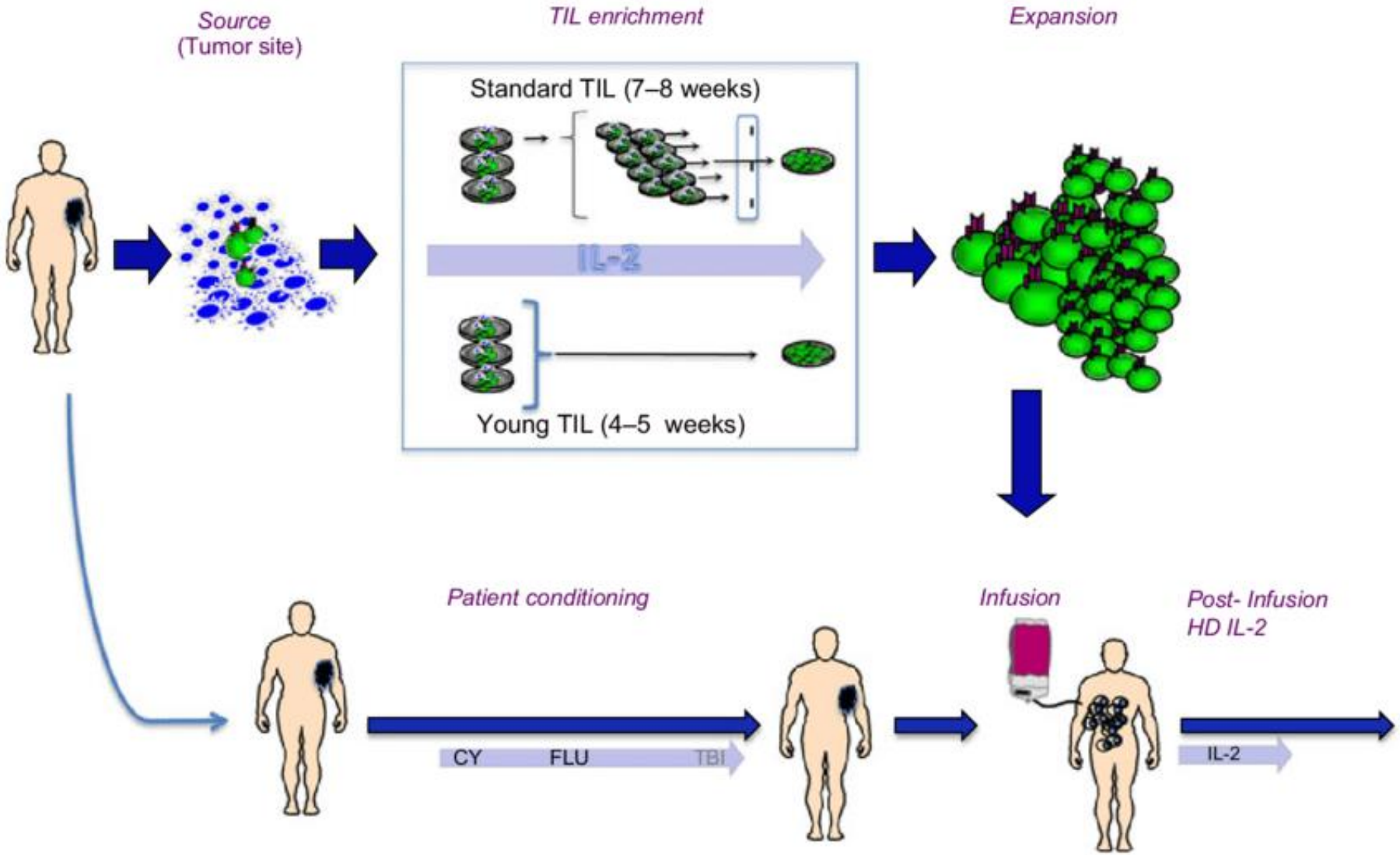




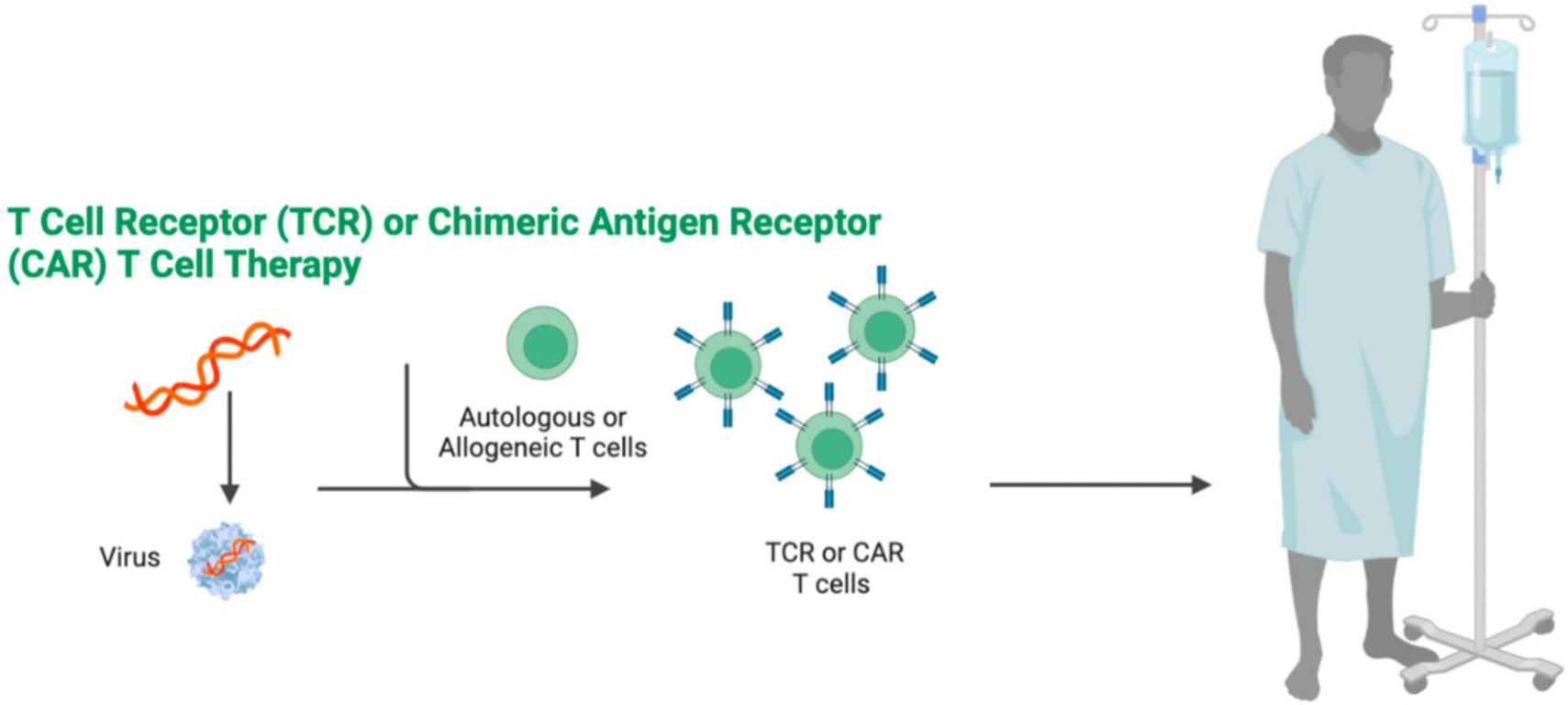
TIL-based adoptive cell transfer



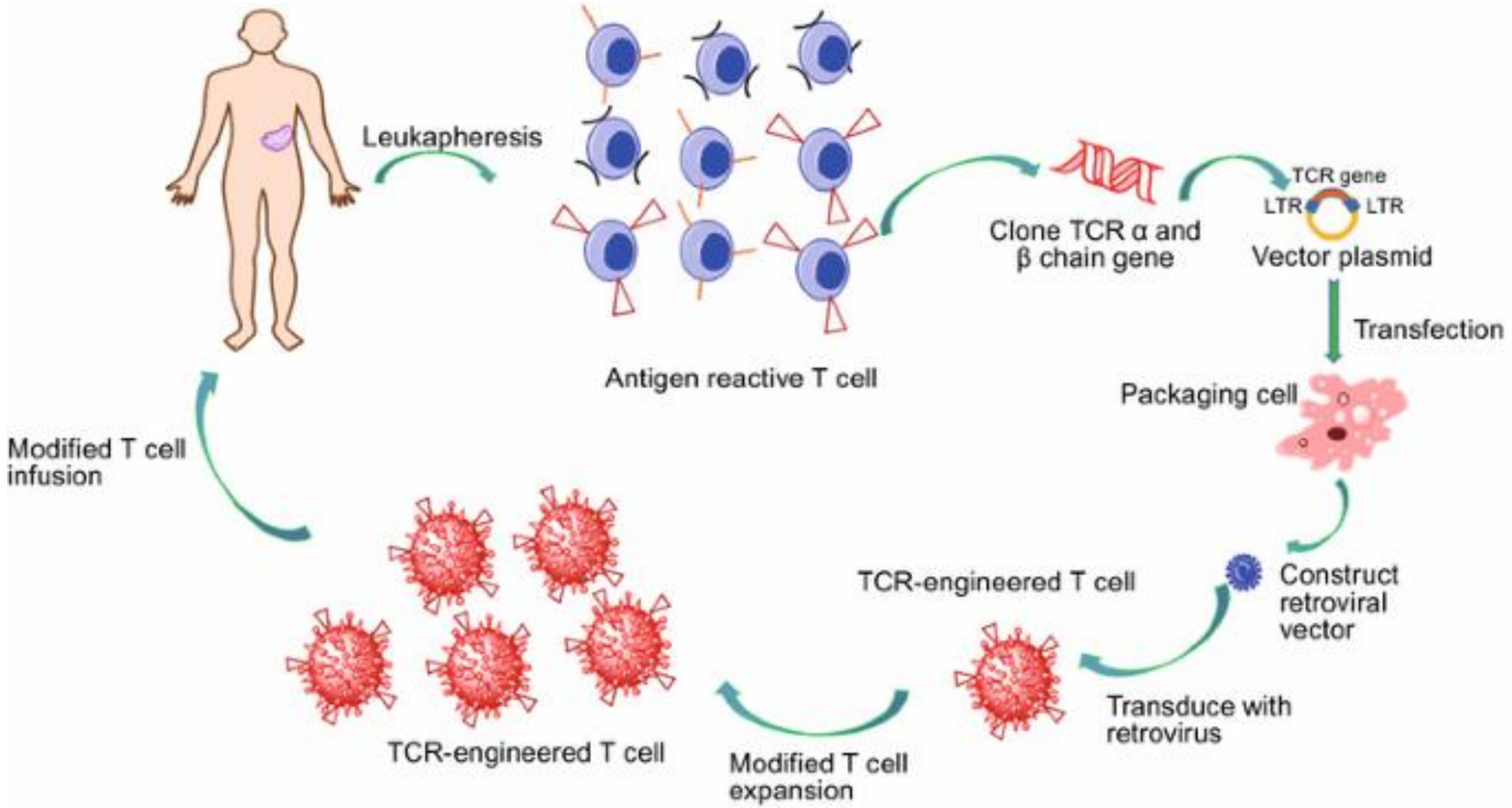
Schematic representation of TIL-based adoptive cell transfer against cancer



A 'young' TIL protocol developed by Tran et al. to address the concern raised by critics of TIL therapy which has been the attrition rate of patients enrolling on the study due to disease progression, failure to cultivate TILs meeting release criteria and intervening comorbidities from the time of tumor collection to TIL product.



Adoptive T cell therapy with transgenic T cell receptor-modified T cells


Leukapheresis
Antigen reactive T cell
Clone TCR α and
β chain gene
TCR gene
LTR
LTR
Vector plasmid
Transfection
Packaging cell
Construct
retroviral
vector
TCR-engineered T cell
Transduce with
retrovirus
Modified T cell
expansion
TCR-engineered T cell
Modified T cell
infusion

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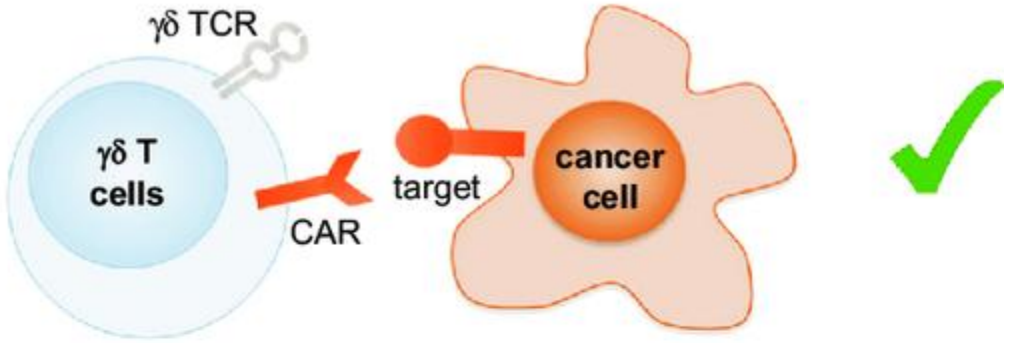
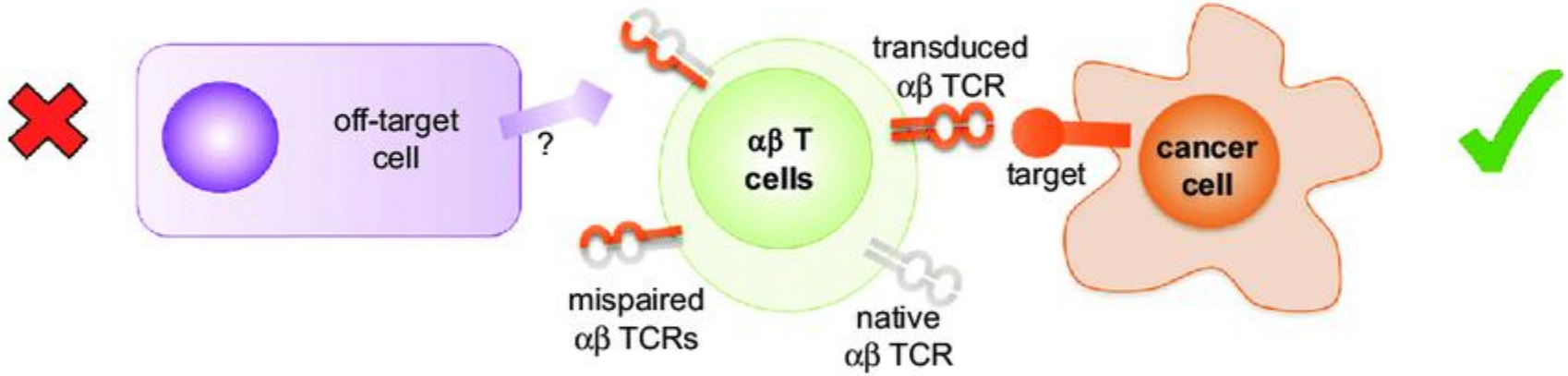
Genetic modification of cd T cells for adoptive therapy approaches to cancer.

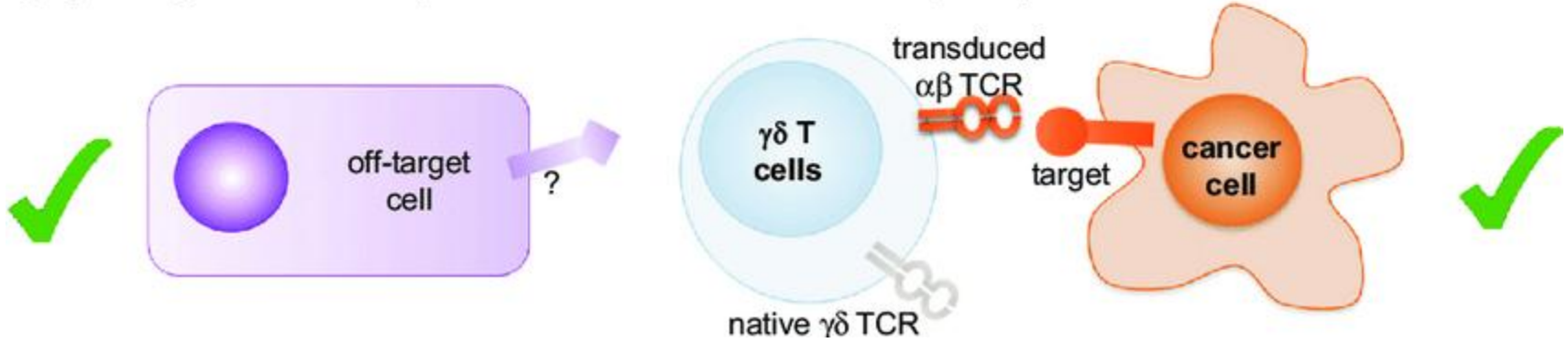
a γδ T cells can be retargeted *via* CAR.



b αβ TCR gene transfer to αβ T cells may result in mispaired TCRs of unknown specificities.



c αβ TCR gene transfer to γδ T cells does not lead to TCR mispairing.



- T cells engineered with TCRs allow for targeting diverse types of TAAs, including proteins overexpressed in malignant cells, those with lineage-restricted expression, cancer-testis antigens, and neoantigens created from abnormal, malignancy-restricted proteins.

- Minor histocompatibility antigens can also serve as TAAs for TCR-T to treat relapsed hematologic malignancies after allogeneic hematopoietic cell transplantation.

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Applicability

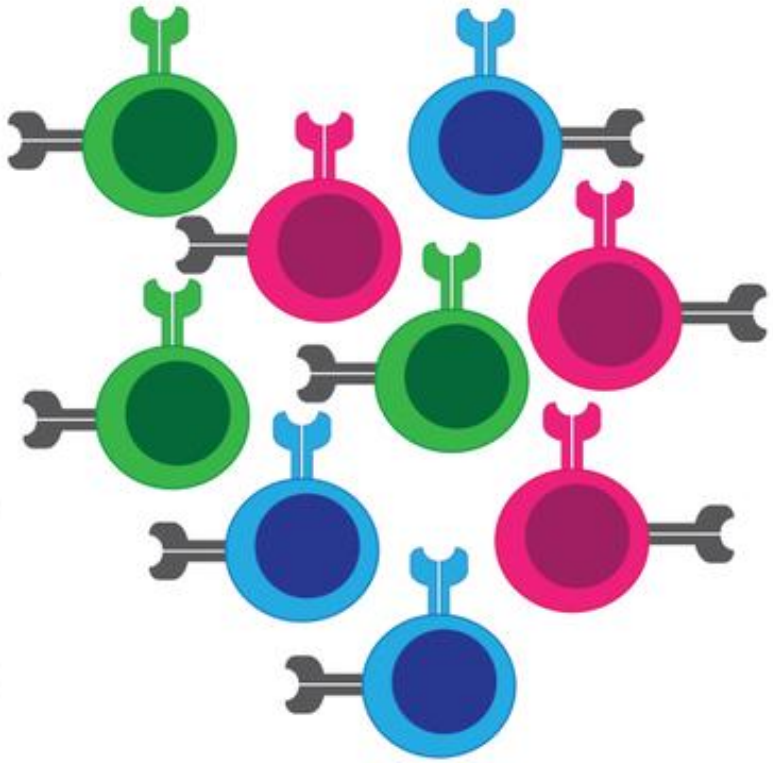
Create "off-the-shelf" universal TCR-T by knocking out HLA and adding NK inhibitory molecules

Safety

Prevent mispairing of transgenic & endogenous TCR chains

- Cysteine modification
- Knock out of endogenous TCR
- Murinization

Enable rapid elimination of TCR-T cells in event of toxicity by including safety switch



Effectiveness

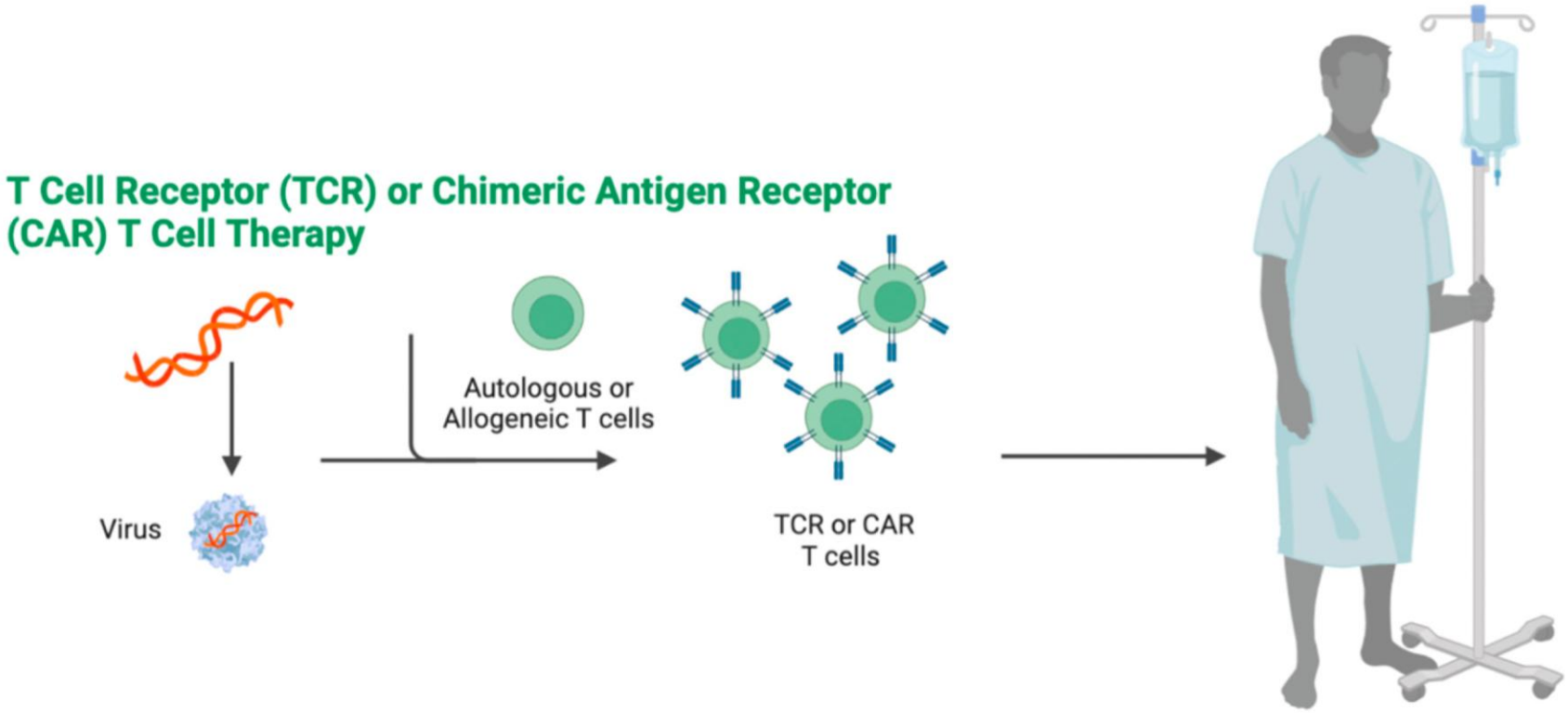
Increase affinity of TCR for antigen with affinity maturation

Allow $CD4^+$ T cell recognition of class I restricted antigens by adding CD8 coreceptor
- $CD4^+$ help
- Improved persistence

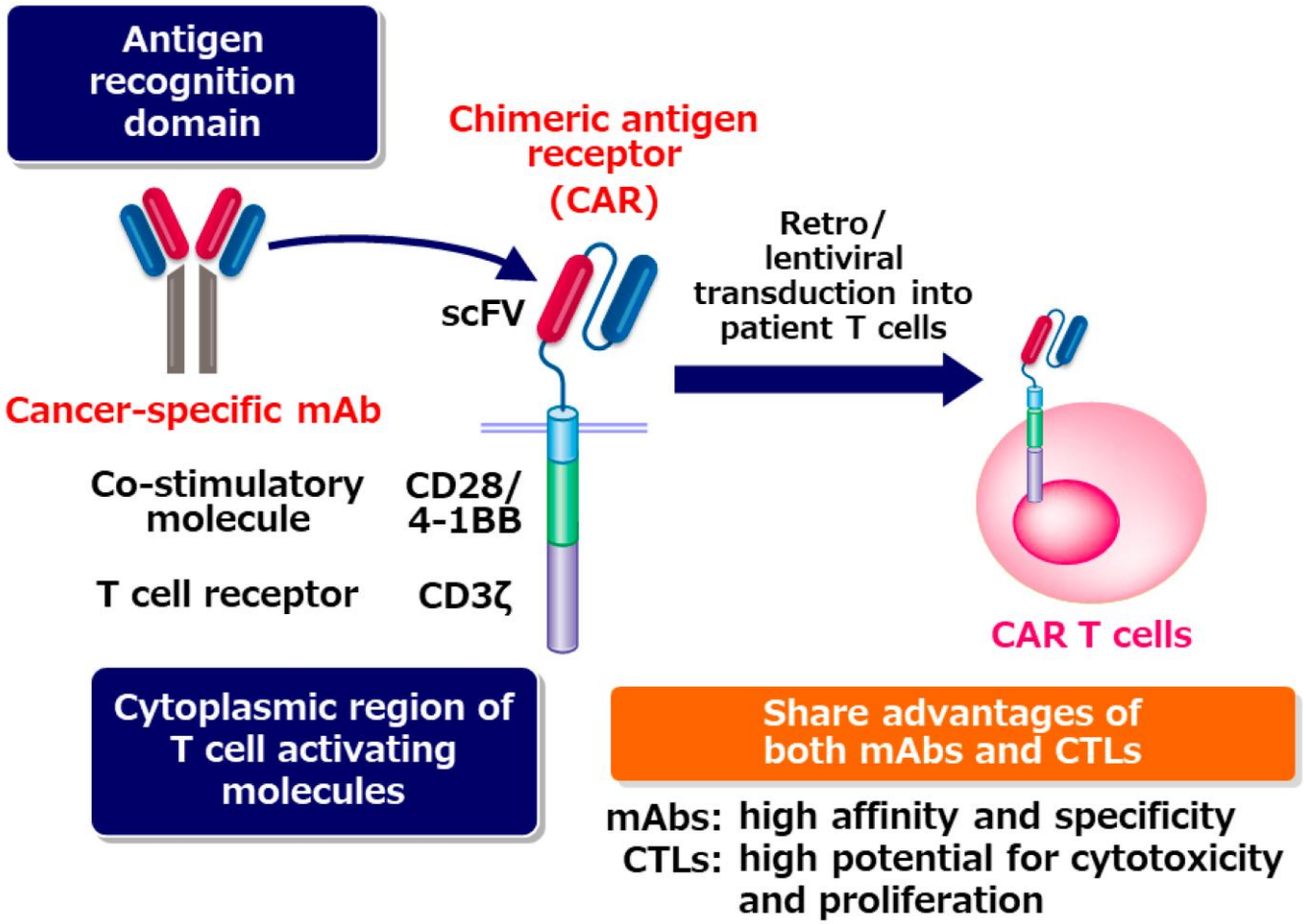
Protect from exhaustion by knocking out exhaustion molecules or converting into stimulatory signal

Enhance anti-tumor function with cytokine expression (e.g., IL-12)

Construct modifications to enhance TCR-T cells



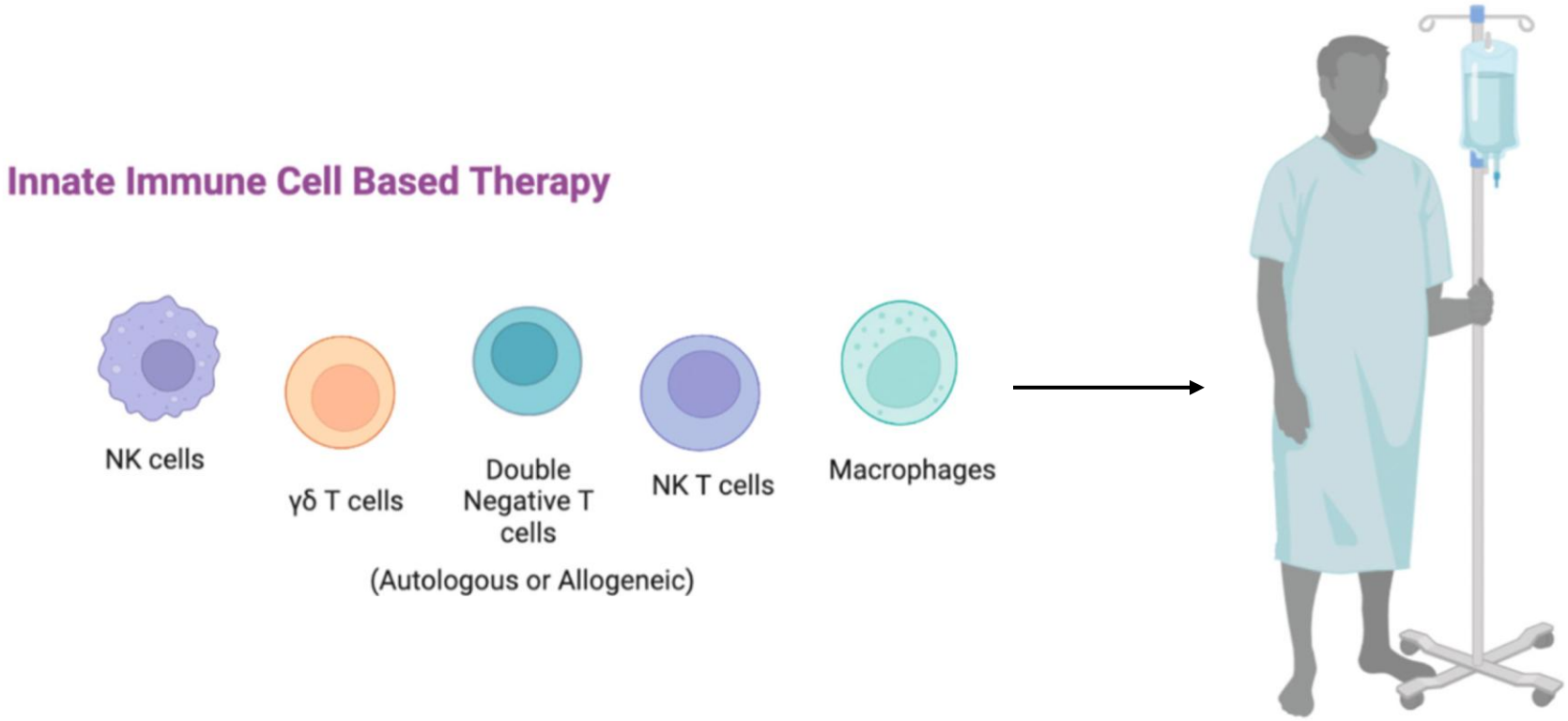
Adoptive T cell therapy with Chimeric Antigen Receptor T cells (CAR-T cells)



- The fratricide or self-killing of CAR-T cells is a major obstacle encountered for the development of CAR-T cell therapy
- The second challenge lies in the potential contamination of autologous CAR-T cell products.
- T-cell aplasia is the third obstacle.

New disease settings for CAR T therapies

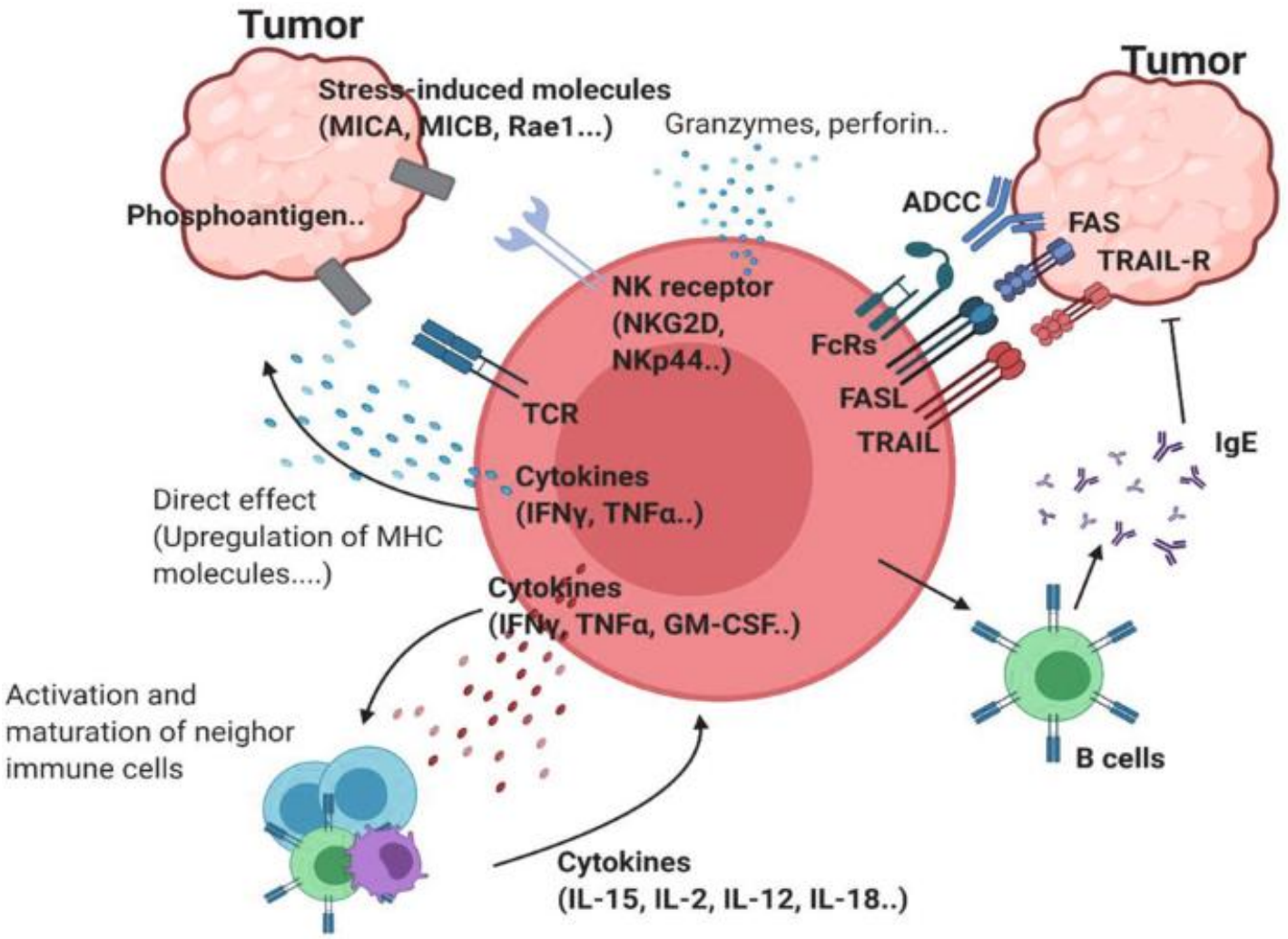
- Multiple myeloma
  - bb2121, LCAR-B38M
- Chronic lymphocytic leukemia
- Acute myeloid leukemia
  - As CD19+ AML is considered rare, alternative antigens – including CD33, CD38, CD56, CD117, CD123, Lewis-Y, Muc-1, and NKGDL – are being considered as targets for developmental CAR T strategies)
- Solid tumors
  - Many challenges concerning optimal cellular targets, tumor immune resistance, and toxicities will need to be resolved.

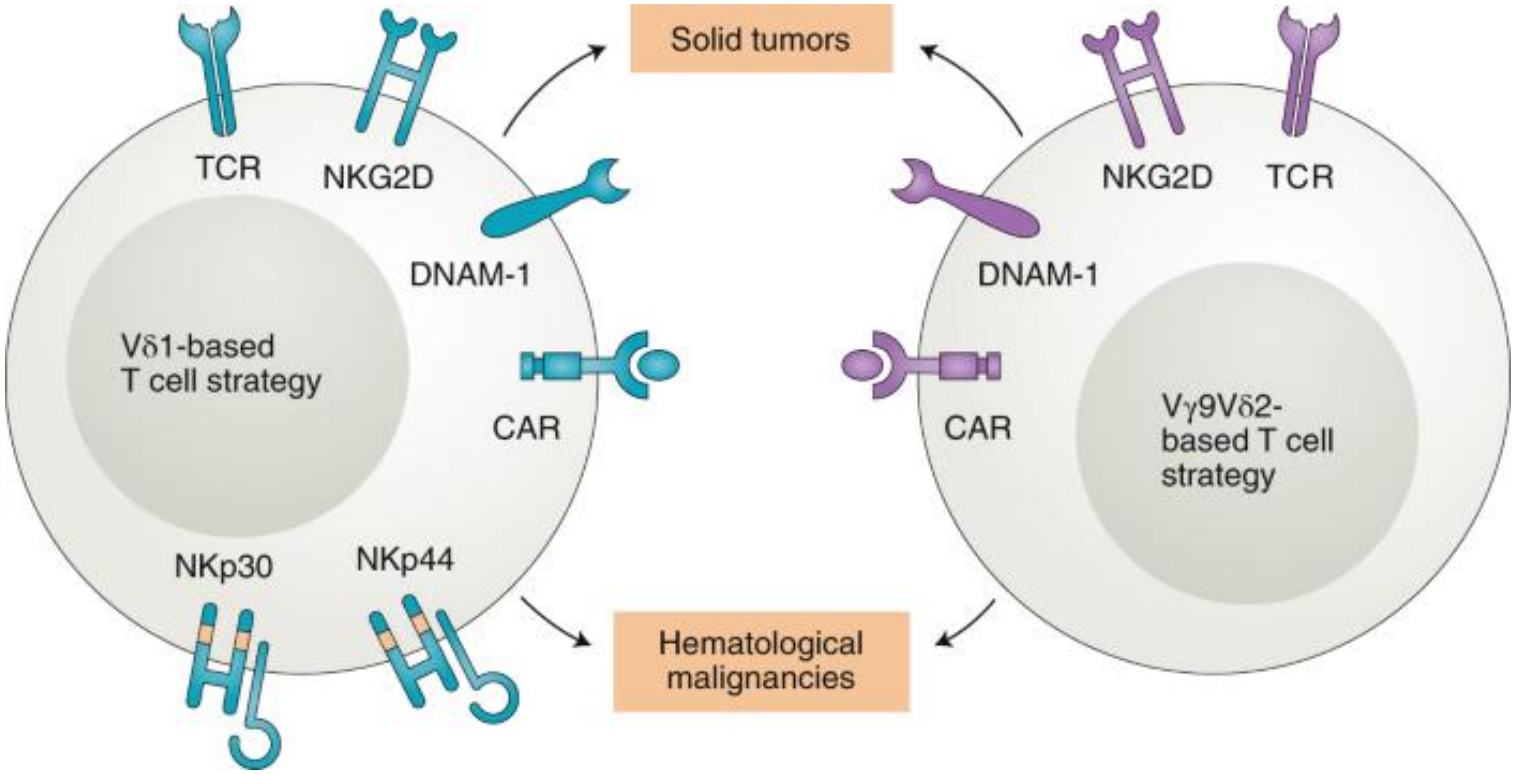


Adoptive T cell therapy with by Innate Immune Cells

γδ T Cells

- γδ T cells account for 1–10% of circulating T cells in the peripheral blood and more than 20% of intraepithelial T cells in the intestine .
- Around 70% of γδ T cells are CD4−CD8−, and 30% are CD4+ or CD8+ .
- γδ T cells typically recognize ligands independent of MHC restriction.
- γδ T cells have multiple roles in the immune system, with both effector and regulatory functions.
- γδ TCRs can be activated by endogenous tumor-derived phosphoantigens presented by butyrophilin molecules.
- γδ T cells can act as antigen-presenting cells (APCs) to present tumour-derived peptides and activate the adaptive immune response





- Studies have particularly focused on developing γδ T cell-based ACT using Vγ9Vδ2 T cells, which comprise roughly 90% of γδ T cells in the peripheral blood.
- Allogeneic Vγ9Vδ2 T cell-based ACT has also been evaluated in post-HSCT to control residual cancer or as a standalone therapy for advanced hematological malignancies
- Recent studies have explored the use of Vδ1 T cells for ACT. Unlike Vγ9Vδ2, Vδ1 T cells are primarily tissue-resident

DNT Cells

- DNTs are mature unconventional T cells defined by the expression of CD3 without CD4 or CD8 expression. (3–5% of peripheral blood T lymphocytes)
- DNTs comprise both TCRαβ+ T cells and TCRγδ+ T cells, with proportions that vary between individuals.
- In the host immune system, both TCRαβ+ DNTs and TCRγδ+ DNTs have been shown to have effector and regulatory functions and are capable of recognizing target antigens without MHC restriction
- DNTs express cytotoxic receptors, including NKG2D, DNAM1, TRAIL, and FasL, for the recognition and lysis of malignant cells
- DNTs show the capacity to expand to therapeutic numbers and to maintain viability and antitumor function upon cryopreservation for at least 600 days. Further, allogeneic DNTs are resistant to immune rejection , and αβ-DNTs can actively suppress GvHD.

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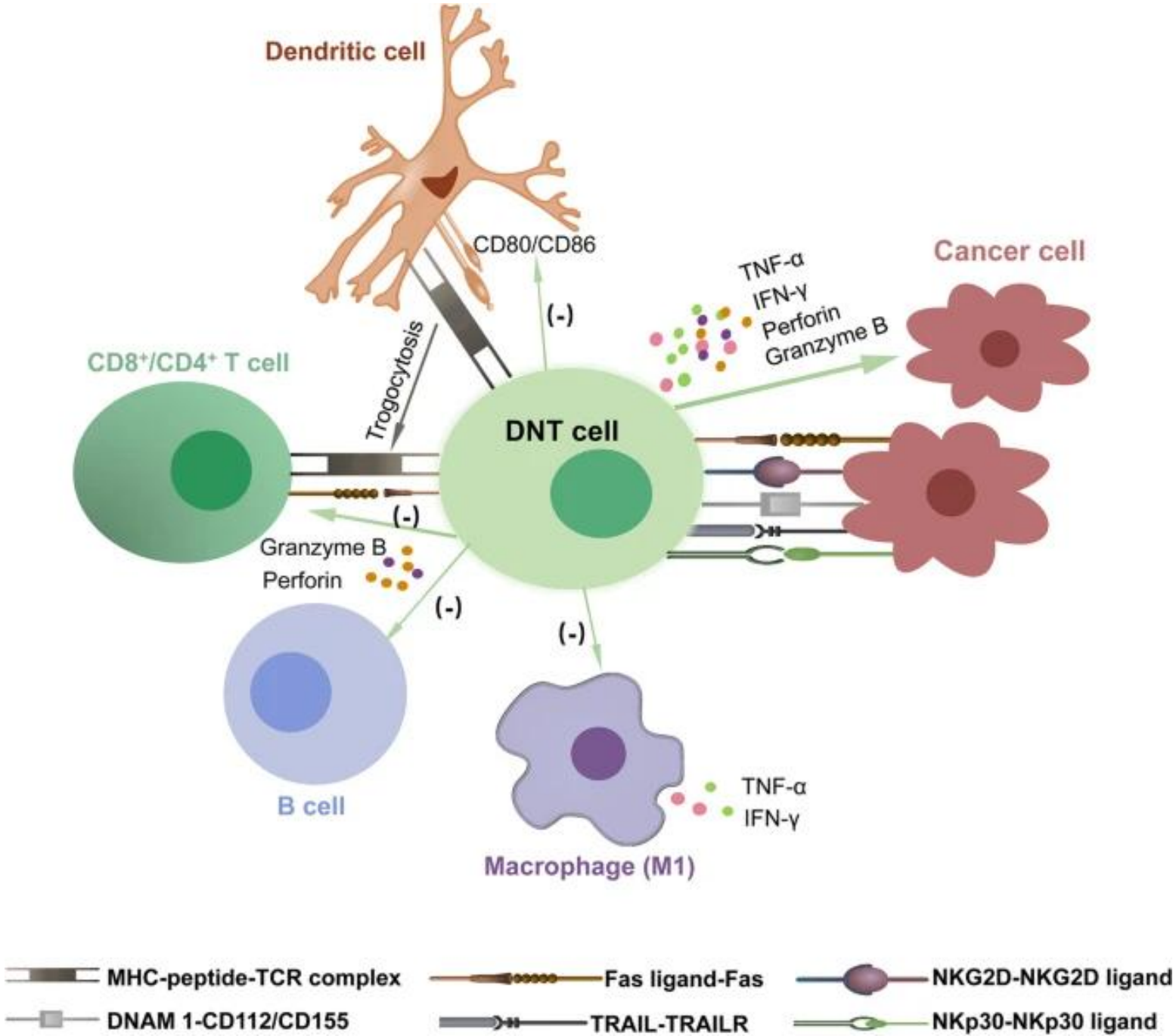
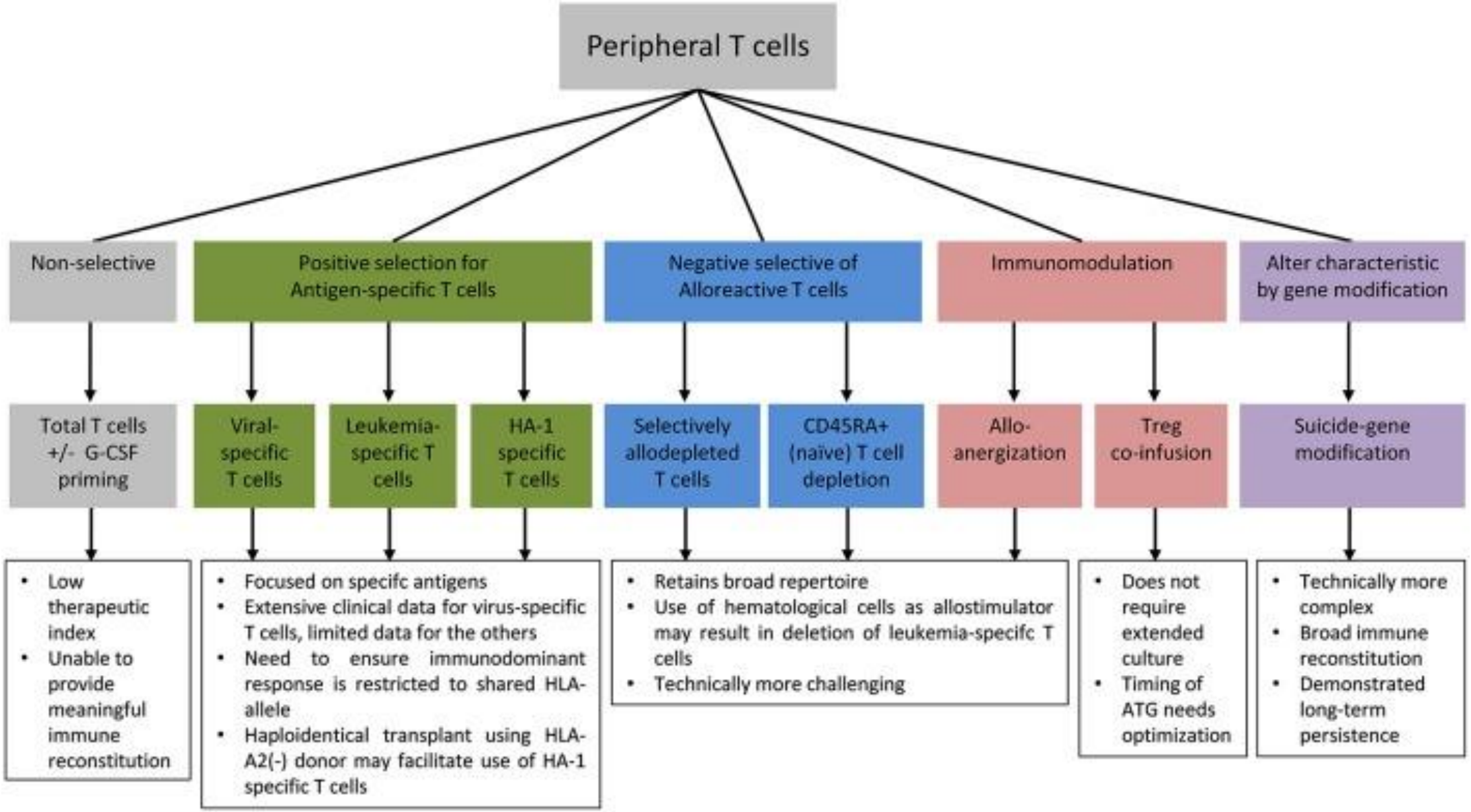
The regulatory and antitumor functions of DNTs.



	CAR- Conventional T/CD8⁺	CAR-NK	CAR-γδ T	CAR-DNT
Risk of GvHD	High due to alloreactive TCRs - Studies investigating genetic modifications (e.g., TCR KO) or non-alloreactive T cells (e.g., virus-specific)	Low - Protective against GvHD activity by targeting recipient APCs	Low	Low - GvHD suppressive activity
Risk of Immune Rejection	High due to MHC mismatch - Studies investigating genetic modifications (e.g., MHC I/II KO) or inhibition of T cell & NK cell cytotoxicity (e.g., certain MHC I alleles)	Present - Require lymphodepletion to suppress T cell activity to minimize NK graft rejection (especially when IL-15 supplementation is used) - Studies investigating genetic modifications (multiple KOs/knock-ins)	Unclear	Low - Resistant to rejection
Risk of Fratricide	Present - Studies investigating surface antigen KOs - Antigens restricted to specific T cell subsets	None for T-lineage specific antigens	Depends on target antigen - No fratricide for TCR αβ	Depends on target antigen - No fratricide for CD4 or CD8
Lifespan/ Persistence	Longest persistence - Detectable for 6 months to years after therapy	Shorter - Detectable for only 3 weeks; lacks long-term antitumor efficacy - Requires multiple doses, increasing risk of rejection - Studies investigating memory-like NK	Shorter	Shorter
Antitumor Cytotoxicity	MHC-dependent - No endogenous killing ability with TCR KO	MHC independent - NK cell receptors - ADCC, potential use in combination with antibody treatment	MHC independent - TCRγδ - NK cell receptors - ADCC	MHC independent - TCRγδ - TCRαβ - NK cell receptors
CAR Construct Suitability	Superior cytotoxicity - CAR originally designed for T cells	Inferior cytotoxicity compared to conventional T cells -NK signaling might affect performance (studies investigating NK-specific CAR constructs)	Comparable with conventional CAR-T	Comparable with conventional CAR-T
Toxicities/ Side Effects	- Cytokine release syndrome (CRS) - Studies investigating use of safety switch to prevent T-cell aplasia or severe adverse events	- Reduced risk of CRS due to limited cytokine secretion profile - Studies suggesting limited persistence can reduce risk of T-cell aplasia	Limited CRS	Unclear
Cost	High due to necessary modifications	Depends on source of cells	Low	Low
Sources	Readily available - Peripheral blood (PB) - Umbilical cord blood	- 10% PB, mature phenotype, harder to expand and standardize product - Umbilical cord blood, immature phenotype - NK-92 cell line commonly used, needs to be irradiated before use, reduced proliferative capacity, derived from lymphoma - iPSC	1–10% of PB T cells	3–5% of PB T cells
Culture and Expansion	Can be expanded to therapeutic numbers	- Poorer expansion (from PB) than conventional T cells - May involve use of feeder cells (risk of contamination, more complicated/costly)	Can be expanded to therapeutic numbers	Can be expanded to therapeutic numbers
Transduction Efficiency	High	Lower - May require multiple transductions or cell sorting	Comparable with conventional CAR-T	Comparable with conventional CAR-T
Cryopreservation	Can be cryopreserved	More sensitive to freezing/thawing than conventional T cells	Sensitive to freezing/thawing	Maintain viability and antitumor functions
Dependence on Cytokine Support		Yes	Yes	Yes

Strategies for adoptive T cell transfer.



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Thanks for your attention!