ADOPTIVE CELL THERAPY

Lecture – 7

Second Semester

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Course Description

This course introduces the fundamental principles of pharmacology, focusing on:

- Drug classification systems
- Mechanisms of drug action
- Pharmacokinetics (ADME)
- Pharmacodynamics
- Drug–drug interactions
- Toxicology and drug safety

Week	Topic
1	Introduction to Pharmacology
2	Pharmacokinetics (ADME)
3	Pharmacodynamics
4	Steroid & Non-Steroid Drugs
5	Nervous System Pharmacology
6	Cardiovascular Pharmacology
7	Antimicrobial Agents
8	Endocrine & Metabolic Drugs
9	Hematology & Chemotherapy
10	General Toxicology
11	Clinical Toxicology & Drug Safety
12	Student Presentations & Review

COURSE SYLLABUS

Learning Objectives

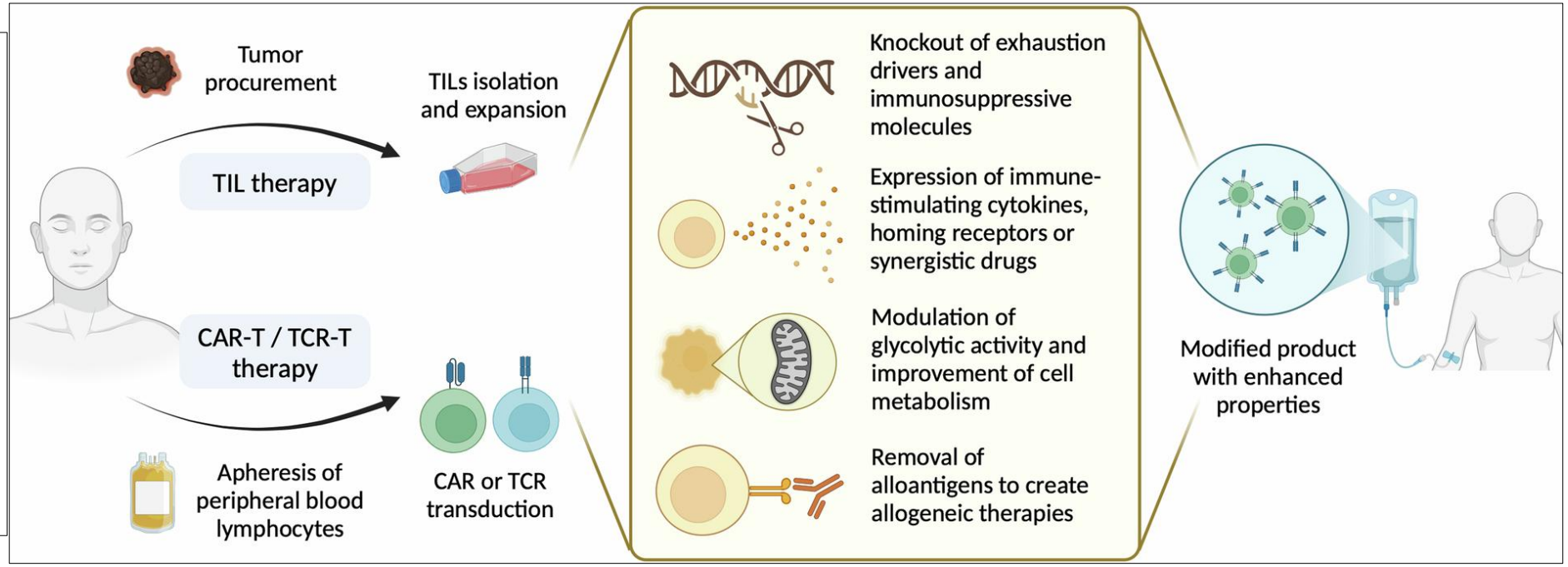
- Adoptive cell therapy
- Tumor-Infiltrating Lymphocyte Therapy
- CAR-T Cell Therapy
- NK Cell Therapy

Adoptive cell therapy

Adoptive cell therapy (ACT) is a personalized immunotherapy in which immune cells are collected, selected, expanded, genetically engineered, or functionally enhanced outside the body, then infused into the patient to mediate anti-tumor immunity.

Basic principle:

Patient/donor immune cells → ex vivo activation or engineering → expansion → lymphodepletion → reinfusion → tumor recognition and killing.



Main Classes of Adoptive Cell Therapy

ACT type	Cell source	Engineering?	Recognition mechanism	Main use
TIL therapy	Tumor tissue	Usually no	Native TCR recognizes tumor antigens	Melanoma, solid tumors
CAR-T cells	Blood T cells	Yes	CAR binds surface antigen independent of HLA	B-cell cancers, myeloma
TCR-T cells	Blood T cells	Yes	Engineered TCR recognizes peptide–HLA	Solid tumors, intracellular antigens
NK cell therapy	Donor/patient NK cells	Optional	Natural cytotoxicity or CAR-NK	Hematologic and solid tumors
γδ T-cell therapy	Blood/tissue γδ T cells	Optional	Stress-antigen and phosphoantigen recognition	Experimental solid tumors
Treg therapy	Regulatory T cells	Optional	Immune suppression/restoration	Autoimmunity, transplantation

Tumor-Infiltrating Lymphocyte Therapy

Tumor-infiltrating lymphocytes (TILs) are endogenous T cells extracted from the tumor microenvironment (TME) that already possess tumor-specific TCRs recognizing neoantigens or tumor-associated antigens. However, in vivo they are functionally suppressed (exhaustion, checkpoint signaling, metabolic stress). ACT restores their function through ex vivo expansion and reinvigoration.

Mechanism:

Tumor biopsy
↓
Isolation of tumor-reactive T cells
↓
Expansion with IL-2
↓
Selection of reactive lymphocytes
↓
Patient lymphodepletion
↓
TIL infusion + cytokine support
↓
Recognition of tumor antigen via native TCR
↓
Tumor cell killing

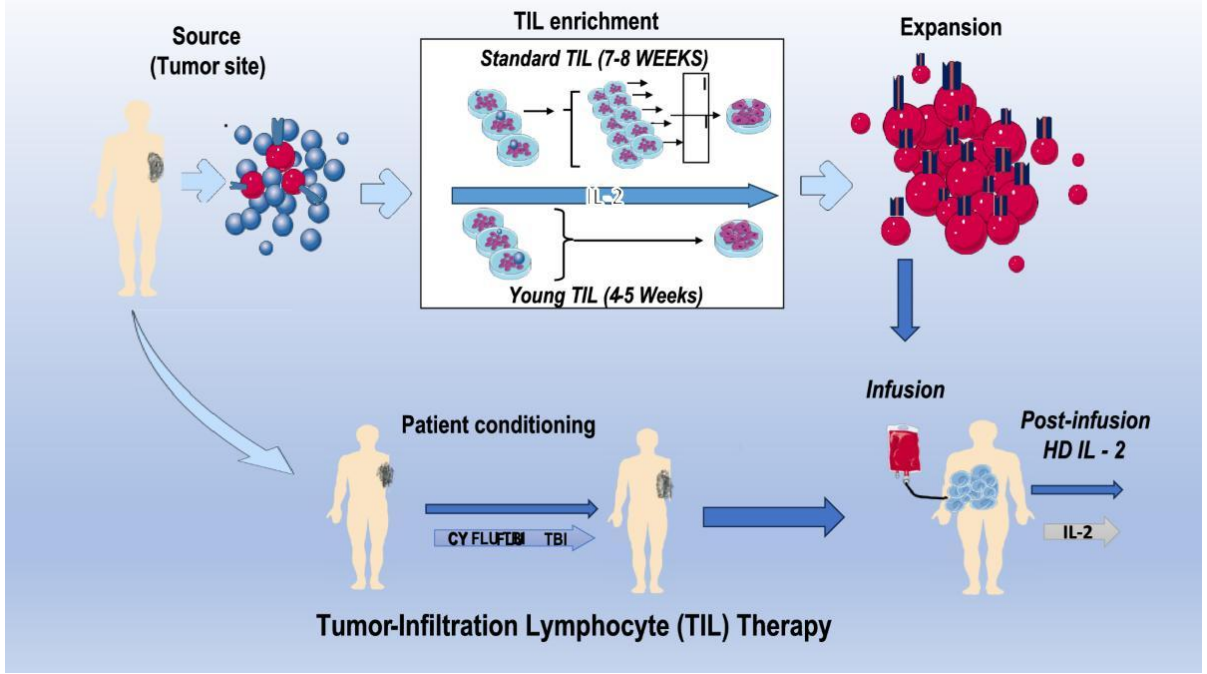
Tumor-Infiltrating Lymphocyte Therapy

Mechanism of TIL Therapy:

Tumor recognition (in vivo origin)

- **TILs** recognize tumor antigens via T-cell receptor (TCR)–peptide–MHC interaction
- **Antigens include:**
  - Neoantigens (mutation-derived)
  - Differentiation antigens (e.g., MART-1)
  - Cancer-testis antigens (e.g., NY-ESO-1

Tumor antigen ⟶ processed ⟶ presented on MHC-I ⟶ TCR recognition



Tumor-Infiltrating Lymphocyte Therapy

Functional impairment in tumor microenvironment

Inside the tumor, TILs become dysfunctional due to:

Mechanism	Effect
PD-1 / PD-L1 signaling	T-cell exhaustion
TGF-β, IL-10	Immunosuppression
Hypoxia	Reduced metabolism
Tregs / MDSCs	Inhibition of effector function
Chronic antigen exposure	Loss of cytotoxicity

Ex vivo activation and expansion

- Tumor is resected → TILs isolated
- Expanded with high-dose IL-2
- Selected for tumor reactivity (sometimes using IFN-γ release assays)

Tumor-Infiltrating Lymphocyte Therapy

Patient conditioning (lymphodepletion)

- Typically cyclophosphamide + fludarabine
- Mechanistic effects:
- Eliminates Tregs and suppressor cells
- Increases IL-7 and IL-15 availability
- Enhances engraftment and persistence

TIL infusion and in vivo function

After reinfusion, TILs:

a) Traffic to tumor: Guided by chemokines (e.g., CXCL9/10 → CXCR3)

b) Recognize tumor cells: TCR binds peptide–MHC complex

c) Form immune synapse: Structured interface between T cell and tumor cell

d) Execute cytotoxicity: Major killing pathways:

Pathway	Mechanism
Perforin–granzyme	Pore formation + apoptosis
Fas–FasL	Death receptor-mediated apoptosis
IFN-γ	Enhances antigen presentation
TNF-α	Pro-inflammatory tumor killing

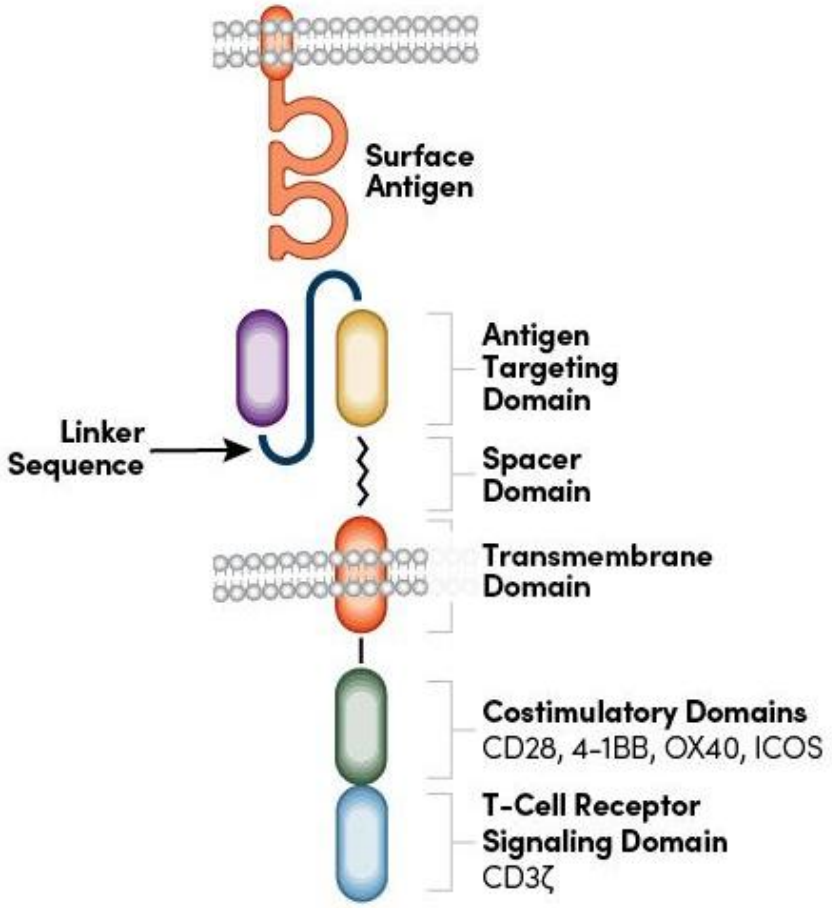
Tumor-Infiltrating Lymphocyte Therapy

Clinical relevance

Lifileucel became the first FDA-approved TIL therapy for adults with unresectable or metastatic melanoma after prior PD-1 therapy, and BRAF-targeted therapy when applicable.

CAR-T Cell Therapy

- **CAR T-cell therapy** is a form of adoptive cell transfer immunotherapy
- Patient T cells are genetically engineered to express Chimeric Antigen Receptors (**CARs**)
- CARs enable HLA-independent tumor recognition



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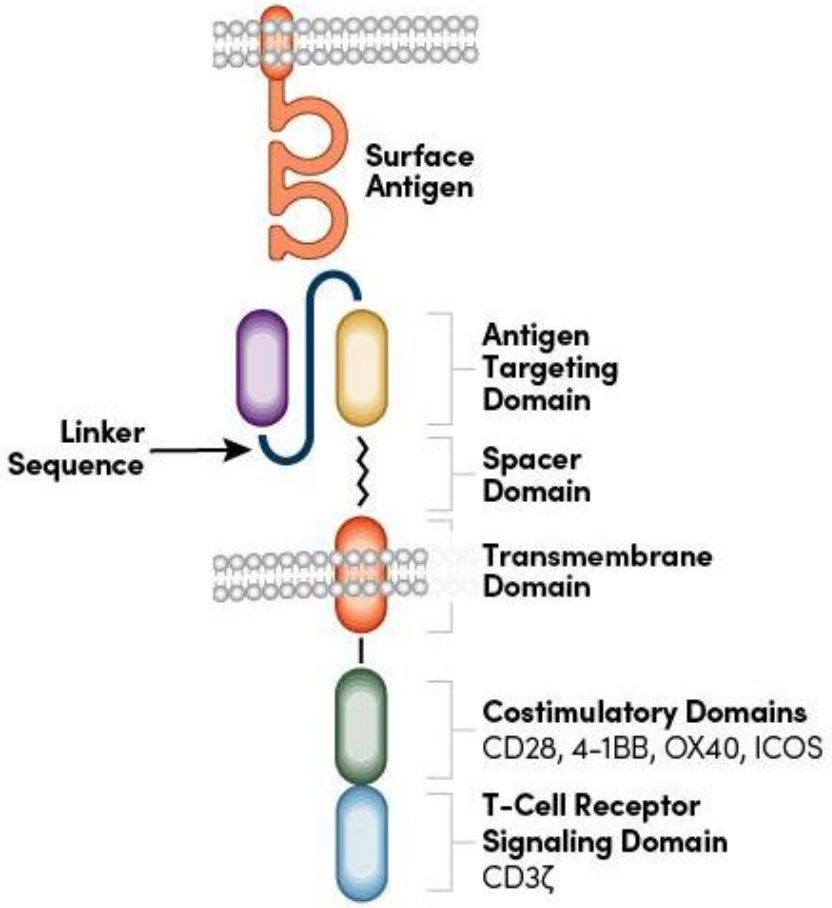
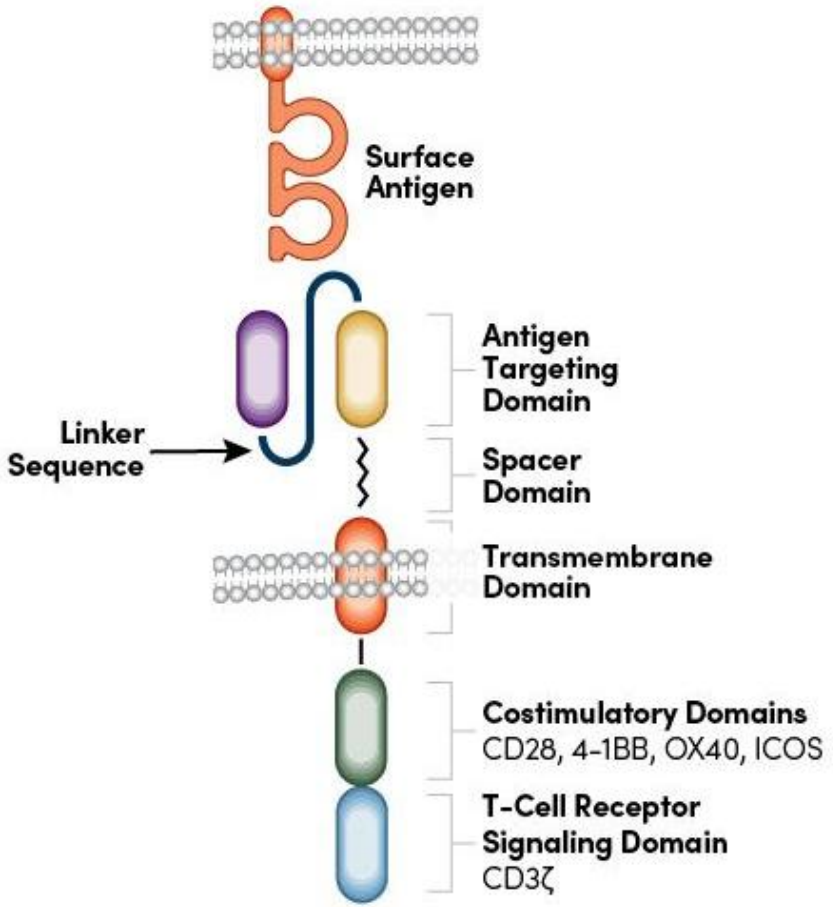


Structure of a Chimeric Antigen Receptor (CAR)

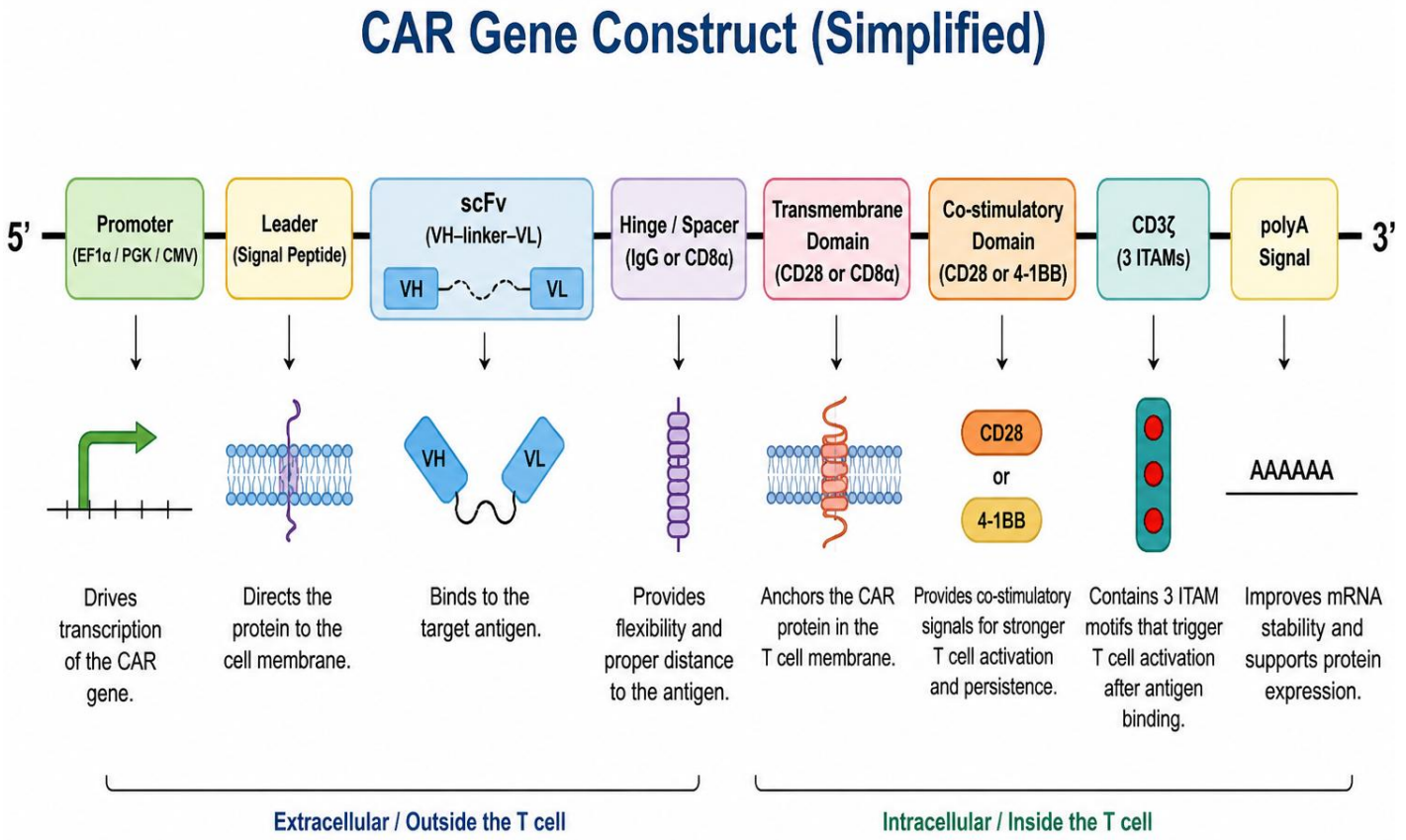


Region	Component	Structure / Example	Function
Target (outside cell)	Surface Antigen	Tumor antigen	Target recognized by CAR
Extracellular	Antigen-targeting domain (scFv)	VH + VL + linker	Binds specifically to antigen
Extracellular	Linker sequence	Flexible peptide	Connects VH and VL, maintains structure
Extracellular	Spacer (Hinge) domain	IgG or CD8α	Provides flexibility and proper distance
Membrane	Transmembrane domain	CD28 or CD8α	Anchors CAR in T-cell membrane
Intracellular	Co-stimulatory domains	CD28, 4-1BB, OX40, ICOS	Enhances activation, proliferation, persistence
Intracellular	TCR signaling domain	CD3ζ (ITAMs)	Initiates T-cell activation signaling

CAR Gene Construct & Molecular Architecture

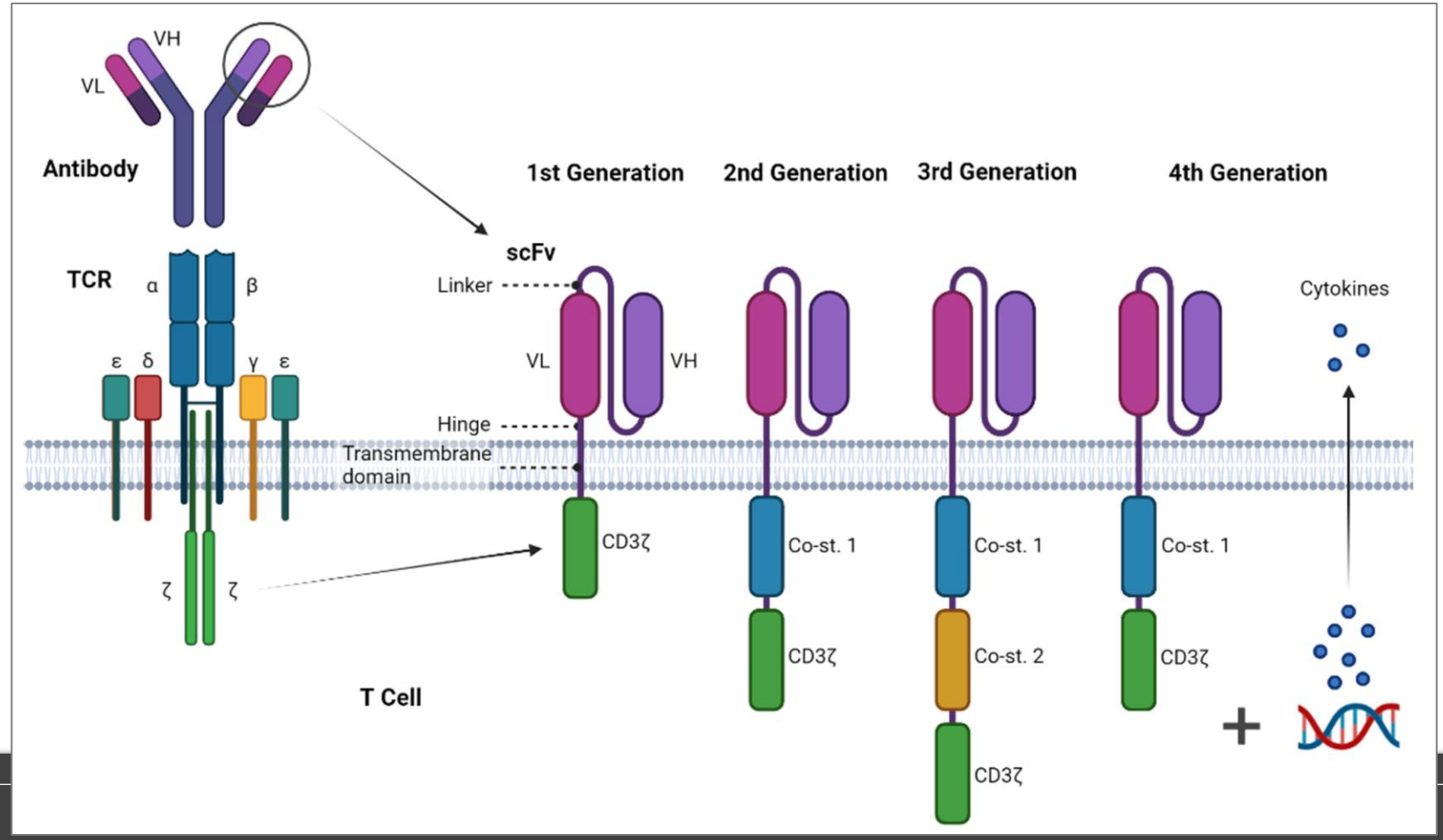
Molecular Components:

- **Promoter:** EF1α / PGK / CMV ⟶ drives transcription
- **Leader (signal peptide):** directs protein to membrane
- **scFv coding sequence:** VH + VL linked via flexible linker
- **Hinge/spacer region:** IgG-derived or CD8α
- **Transmembrane domain:** CD28 or CD8α
- **Intracellular domains:**
  - CD3ζ (3 ITAM motifs ⟶ phosphorylation sites)
  - Co-stimulatory domains (CD28 / 4-1BB)
- **Polyadenylation signal (polyA):** mRNA stability



Generation	Structure	Functional significance
First generation	scFv + CD3ζ	Weak persistence
Second generation	scFv + CD3ζ + CD28 or 4-1BB	Strong activation and persistence
Third generation	Two co-stimulatory domains	Enhanced signaling
Fourth generation / TRUCKs	CAR + inducible cytokine gene	Designed to remodel tumor microenvironment
Armored CAR-T	CAR plus resistance or cytokine modules	Improved solid tumor activity

CAR generations



CAR generations

Mechanism of CAR-T activation

- CAR binds tumor surface antigen.
- CD3ζ ITAMs become phosphorylated.
- ZAP70 is recruited.
- Downstream signaling activates NFAT, NF-κB, and AP-1.
- T cells proliferate, secrete cytokines, and kill target cells.

Major CAR-T targets

Target	Disease
CD19	B-ALL, DLBCL, mantle cell lymphoma
BCMA	Multiple myeloma
CD22	B-cell malignancies
CD20	B-cell lymphoma, investigational
GPC3	Hepatocellular carcinoma, investigational
HER2	Solid tumors, investigational
Mesothelin	Mesothelioma, pancreatic, ovarian cancer, investigational
Claudin 18.2	Gastric/pancreatic cancer, investigational

TCR-Engineered T Cell Therapy

- TCR-engineered T cell therapy is an adoptive cell therapy in which patient T cells are genetically modified to express a tumor-specific T cell receptor (TCR).
- Unlike CAR-T cells, which recognize surface antigens directly, TCR-T cells recognize intracellular tumor antigens presented as peptide fragments on MHC/HLA molecules.

Mechanism Diagram

Tumor protein
↓ proteasome degradation
Tumor peptide
↓ loaded onto HLA-I
Peptide–HLA complex on tumor cell
↓ recognized by engineered TCR
T cell activation
↓
Cytokine release + cytotoxic killing
↓
Tumor cell death

Structural Biology of the TCR Complex

The **T cell receptor (TCR)** is a multi-subunit signaling complex composed of:

A. Antigen Recognition Module

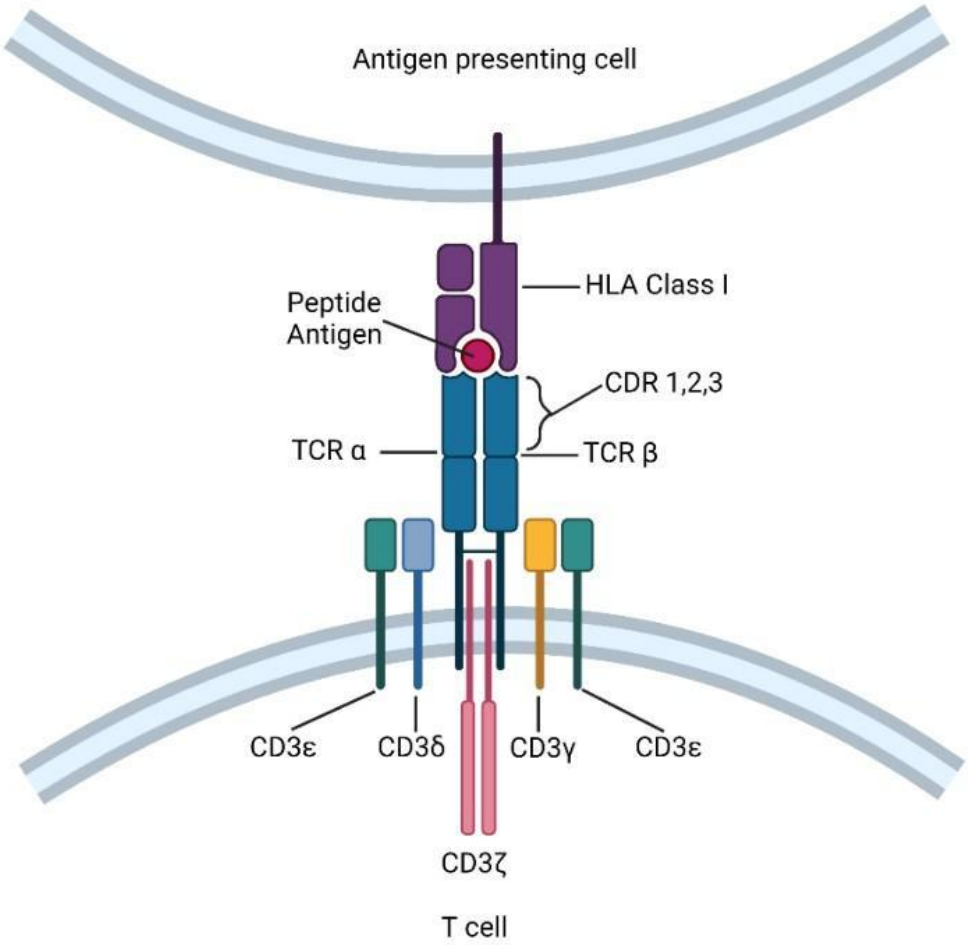
- **TCR α-chain + β-chain (heterodimer)**
- Recognizes:
  Peptide–HLA (MHC) complex, not free antigen

B. Signaling Module (CD3 Complex)

C. Co-receptors

- **CD8** → binds MHC-I (cytotoxic T cells)
- **CD4** → binds MHC-II (helper T cells)

Mechanism Diagram

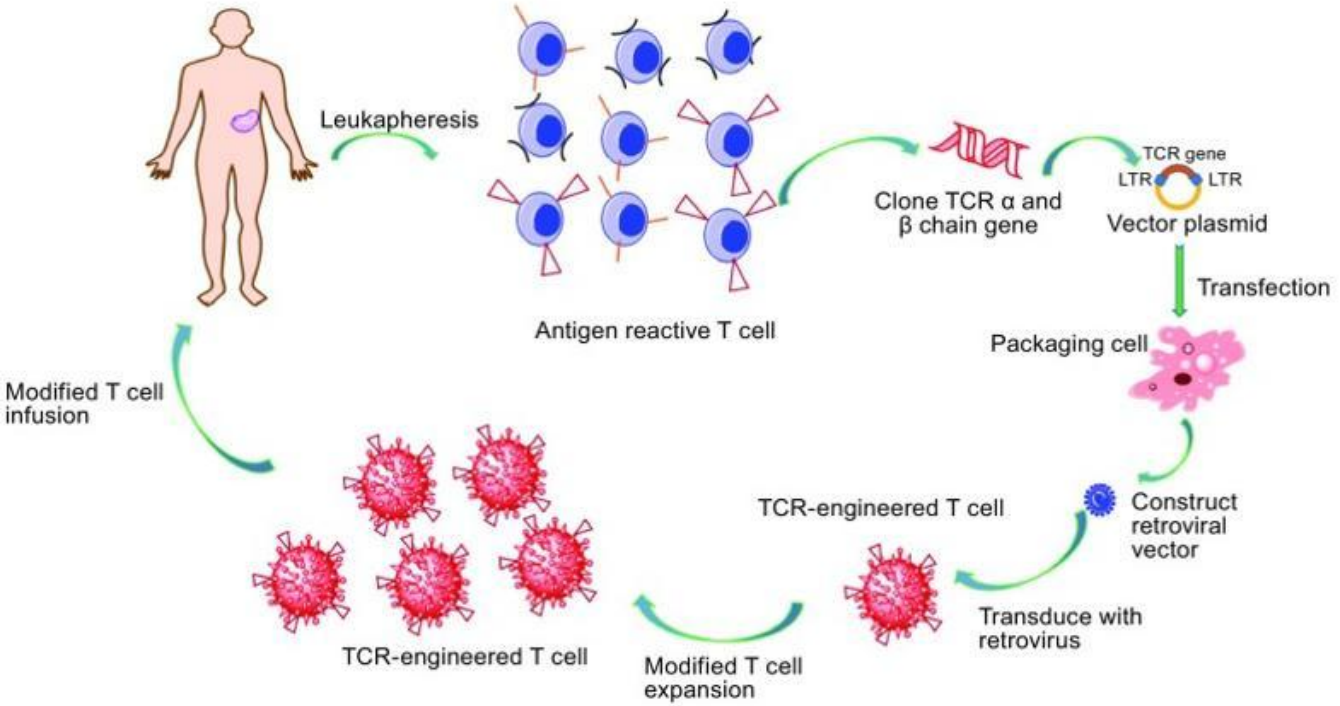


Engineered TCR Design

Engineered TCR design refers to the genetic construction and optimization of T cell receptor (TCR) α and β chains so that patient T cells can specifically recognize tumor-derived peptide–HLA complexes.

- Isolate or design a high-affinity tumor-specific TCR
- Insert its genes into patient T cells
- Generate a population of tumor-targeting cytotoxic T cells

Engineering Process



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Mechanism of Action

- **Tumor antigen processing**
  Intracellular tumor proteins are degraded into peptides.
- **Peptide presentation**
  Peptides are presented on MHC-I/HLA molecules.
- **TCR recognition**
  The engineered TCR binds a specific peptide–HLA complex.
- **T cell activation**
  CD3 signaling triggers T cell activation, proliferation, cytokine release, and cytotoxic function.
- **Tumor cell killing**
  Activated T cells release perforin and granzymes and may also induce apoptosis through Fas–FasL signaling.

Antigen type	Examples
Cancer-testis antigens	NY-ESO-1, MAGE-A4
Viral tumor antigens	HPV E6/E7, EBV antigens
Neoantigens	KRAS, TP53, patient-specific mutations
Differentiation antigens	MART-1, gp100

TCR-T cells can target intracellular proteins

Comparison of CAR-T, TCR-T, and TIL Therapy

Feature	CAR-T	TCR-T	TIL
Antigen type	Surface antigen	Intracellular or surface peptide	Natural tumor antigens
HLA restriction	No	Yes	Yes
Engineering required	Yes	Yes	Usually no
Best current success	Blood cancers	Selected solid tumors	Melanoma
Main limitation	Antigen loss, toxicity	HLA restriction	Manufacturing complexity
Personalization	Moderate	High	High

NK Cell Therapy and CAR-NK Cell

- Natural killer cells are innate lymphocytes that kill abnormal cells without requiring antigen-specific TCR recognition.
- CAR-NK cells combine CAR specificity with natural NK cytotoxicity.

Advantage	Explanation
Lower risk of severe CRS	NK cells often produce less IL-6-driven inflammation
Donor-derived potential	Can be developed as off-the-shelf products
Natural anti-tumor activity	Can kill through CAR-dependent and CAR-independent pathways

CRS: Cytokine Release Syndrome

References

- June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science*. 2018;359(6382):1361–1365.
- Rapoport AP, Stadtmauer EA, Binder-Scholl GK, et al. NY-ESO-1–specific TCR–engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. *Nat Med*. 2015;21(8):914–921.
- Robbins PF, Kassim SH, Tran TLN, et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1–reactive T-cell receptor. *J Clin Oncol*. 2015;33(4):355–361.
- D'Ippolito E, Schober K, Nauerth M, Busch DH. T cell engineering for adoptive T cell therapy: safety and efficacy of TCR- and CAR-based approaches. *J Immunol*. 2019;202(4):1090–1098.
- Yang JC, Rosenberg SA. Adoptive T-cell therapy for cancer. *Adv Immunol*. 2016;130:279–294.