CAR T Fundamentals

Bristol Myers Squibb™

CAR T Fundamentals

01 Mechanism of Disease

02 CAR T-cell Mechanism of Action

03 CAR T-cell Manufacturing and Processing

04 Overview of the Patient Journey

05 Patient Selection

Section 01

01 Mechanism of Disease

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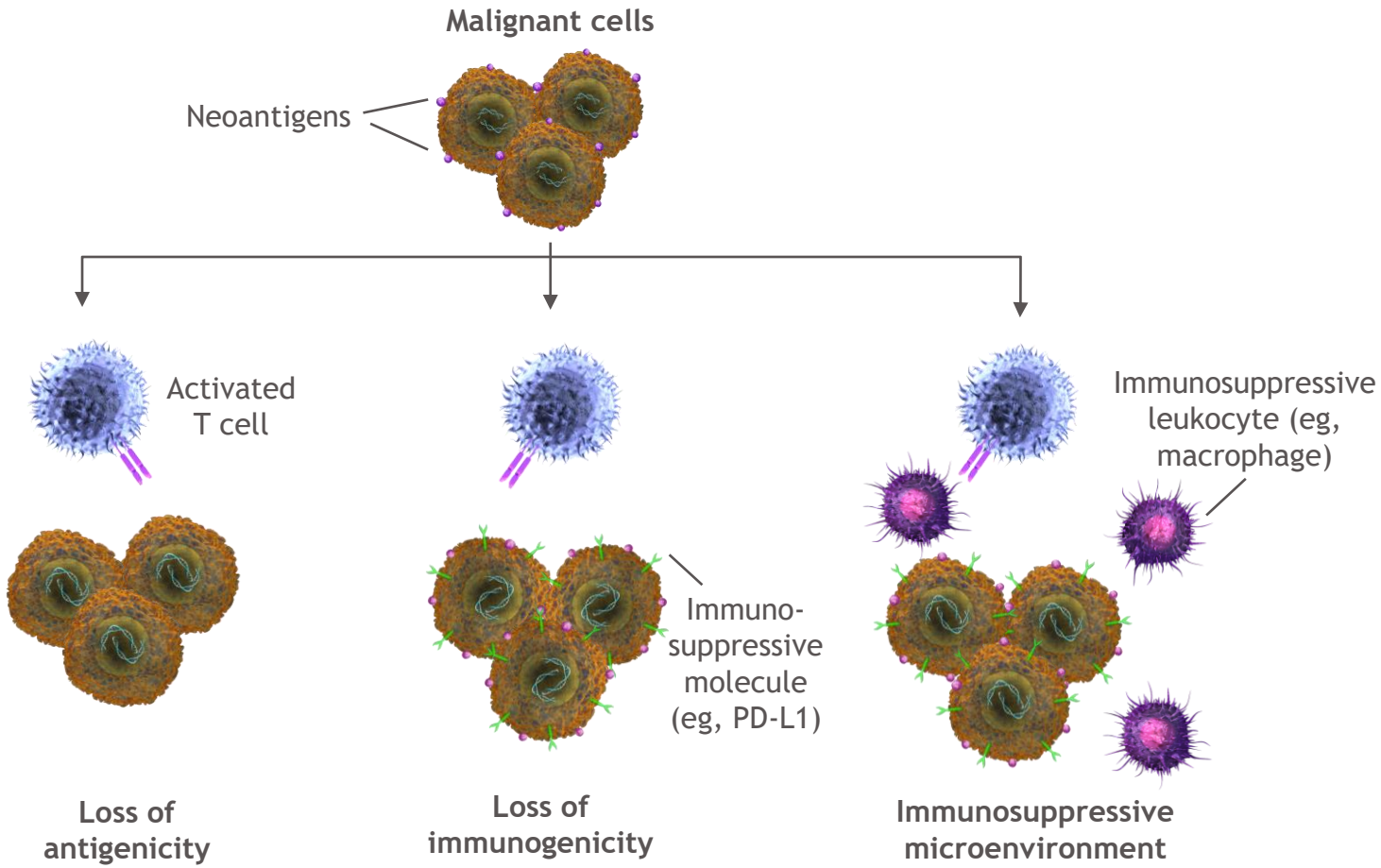
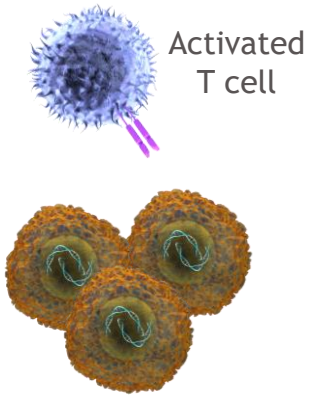
Malignant Cells Can Acquire Mechanisms to Evade Attack By the Immune System



Such mechanisms enable cancer cell persistence, growth, and proliferation, eventually enabling the cancer cells to impair immune system function with immunosuppressive effects[1,2]

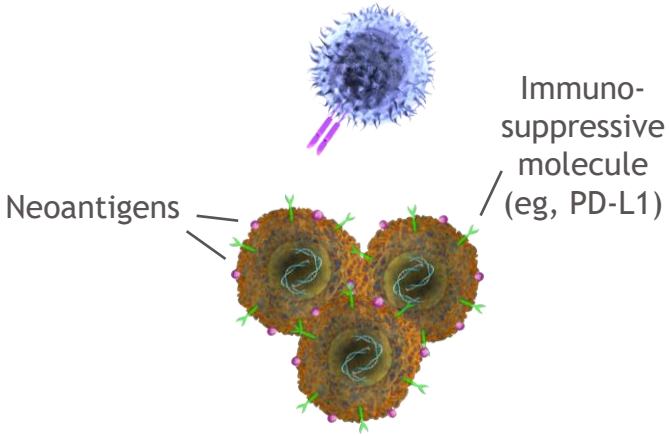
References: 1. Beatty GL, Gladney WL. *Clin Cancer Res*. 2015;21:687-692. **2.** Vinay DS, et al. *Semin Cancer Biol*. 2015;35:S185-S198.

Malignant Cells Can Acquire Mechanisms to Evade Attack By the Immune System



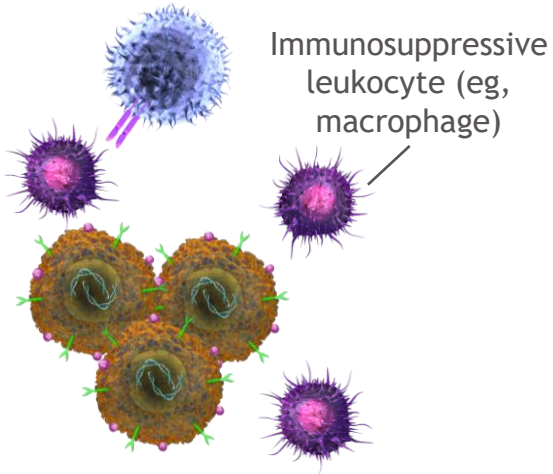
Loss of antigenicity[1]

Cancer cells can acquire defects in antigen processing or presentation, or through the loss of immunogenic tumor antigens



Loss of immunogenicity[1]

Increased expression of immune checkpoint proteins (eg, PD-L1) or increased secretion of suppressive cytokines (eg, IL-10, TGF-beta)



Immunosuppressive microenvironment[1]

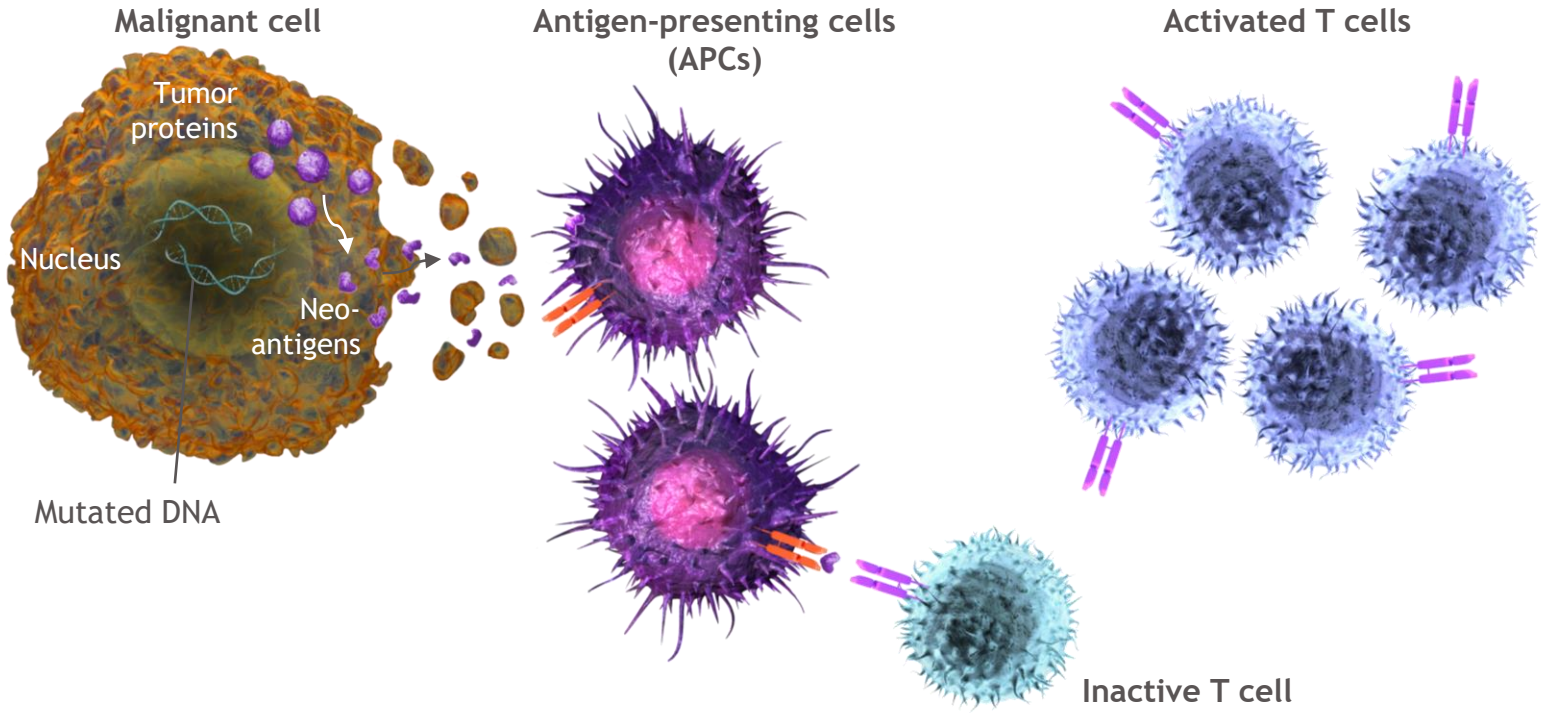
Increased local infiltration of immunosuppressive leukocytes (eg, macrophages) that, in turn, downregulate the activity of cytotoxic T cells

Note that tumors can acquire additional mechanisms of immune evasion beyond those shown here[2]

References: 1. Beatty GL, Gladney WL. *Clin Cancer Res*. 2015;21:687-692. **2.** Vinay DS, et al. *Semin Cancer Biol*. 2015;35:S185-S198.

The Immune System Plays a Fundamental Role in Protecting Against Cancer[1,2]

- Mutations that arise during cancer development are important as they provide the immune system with a means of attacking neoplastic cells[1]
- The adaptive immune system[2]:
  - discerns "self" antigens from "nonself" antigens
  - generates pathogen-specific immunologic effector pathways to eliminate pathogen-infected or cancerous cells



APCs, antigen-presenting cells.

References: 1. Warrington R, et al. *Allergy Asthma Clin Immunol*. 2011;7:S1. **2.** Lu Y-C, Robbins PF. *Semin Immunol*. 2016;28:22-27.

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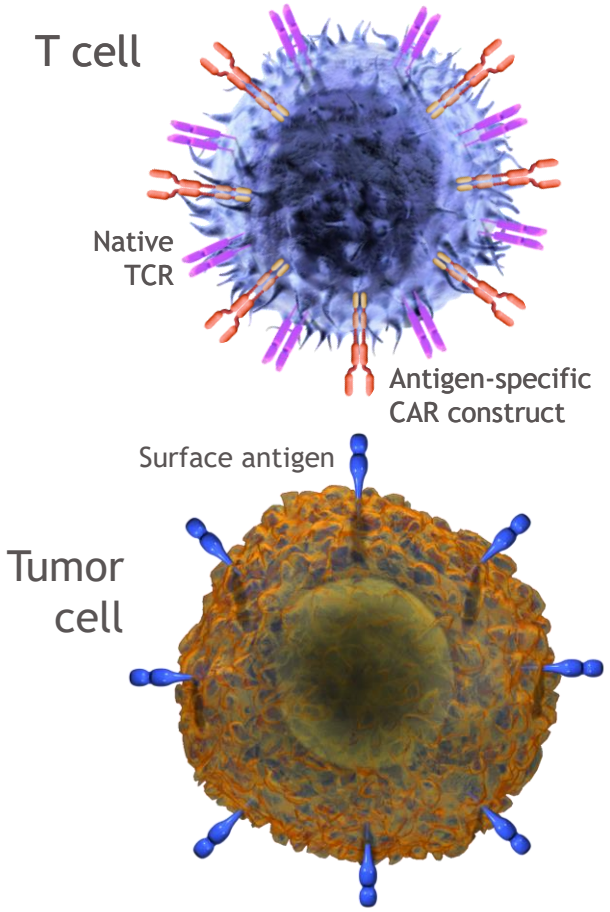
CAR T-cell Therapy Combines Antibody Specificity With T-cell Cytotoxicity and Memory[1]



- Gene transfer technology is used to stably express CARs on T cells, conferring antigen specificity[2,3]
- CAR T-cells can be directed to a specific surface antigen found on target cells[2]
- CAR T-cell therapy takes advantage of the cytotoxic potential of T cells by binding target cells in an antigen-dependent manner[2]
- However, unlike T-cell receptors, CARs can recognize target antigens without the need for MHC presentation[1]
- CAR T-cells may expand and persist, providing T-cell memory for a period of time[2,4]
- Persistent CAR T-cells consist of both effector (cytotoxic) and central memory T cells[3]

MHC, major histocompatibility complex; TCR, T-cell receptor.

References: 1. Maus MV, Levine BL. *Oncologist*. 2016;21:608–617. **2.** Oluwole OO, Davila ML. *J Leukoc Biol*. 2016;100:1265-1272. **3.** Kalos M, et al. *Sci Transl Med*. 2011;3:95ra73. **4.** Boyiadzis MM, et al. *J immunother Cancer*. 2018;6(1):137.

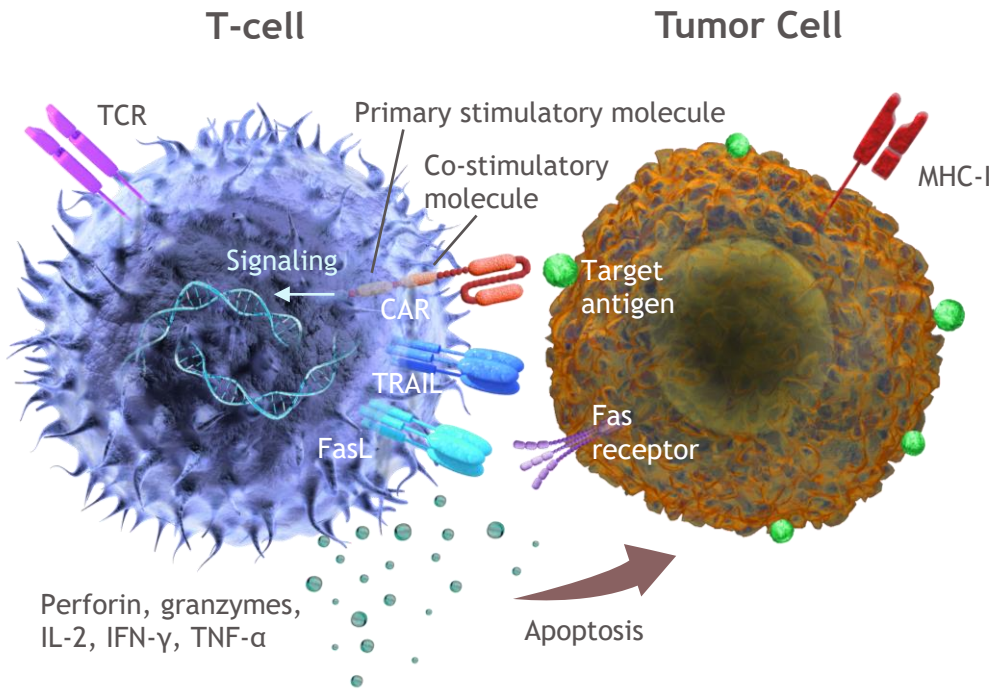
CAR T-cell Mechanism of Action

CAR T-cell therapies stimulate MHC-independent T-cell responses against target cells[1,2]

Current Understanding of the Mechanism[2]

1. The CAR T-cell recognizes and binds to a specific antigen on target cells; CAR T-cells can also attack normal antigen presenting cells
2. Binding induces a conformational change that transmits a signaling cascade inside the T-cell to promote its activation
3. Once activated, the T-cell:
   - Releases lytic granules containing perforin and granzyme, inducing cytotoxic activities
   - Expresses FasL and TRAIL to induce apoptosis of the target cell upon binding to death domain–containing receptors
   - Secretes pro-inflammatory cytokines like IL-2, IFN-γ, and TNF-α to activate other tumor-infiltrating immune cells

CAR T-cell and Target Cell Interaction[1-3]



FasL, Fas ligand; FasR, Fas receptor; IFN, interferon; IL-2, interleukin-2; MHC, major histocompatibility complex; TAA, tumor-associated antigen; TCR, T cell receptor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

References: 1. Maus MV, Levine BL. *Oncologist*. 2016;21:608-617. **2.** Cartellieri M, et al. *J Biomed Biotechnol*. 2010;2010:956304. **3.** Benmebarek MR, et al. *Int J Mol Sci*. 2019;20(6).

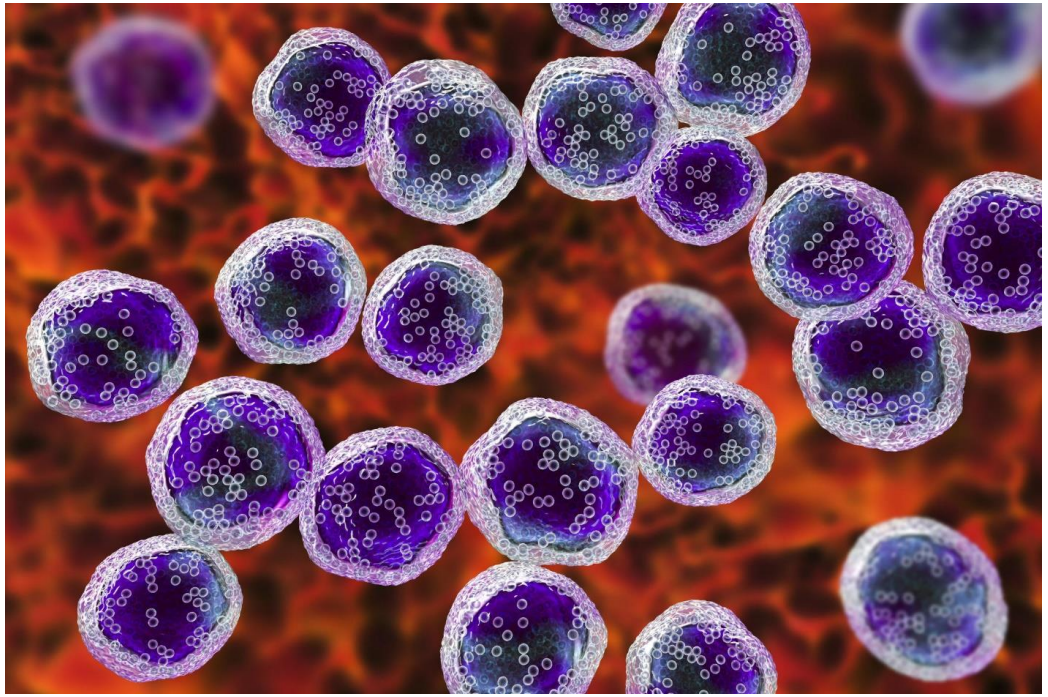
CAR T-cell Therapy in Hematologic Malignancies[1,2]

Features of Hematologic Malignancies Utilized for CAR T-cell Therapy

Antigen expression is specific to target cells[3,4]

Cancer cells are disseminated without physical barriers or immunosuppressive microenvironment of solid tumors[4,5]

Cancer cells typically reside in the same locations as migrating T-cells (eg, peripheral blood, lymph nodes, bone marrow)[4]



References: 1. Miliotou AN, Papadopoulou LC. *Curr Pharm Biotechnol*. 2018;19:5-18. **2.** Ogba N et al. *J Natl Compr Canc Netw*. 2018;16:1092-1106. **3.** June CH et al. *Science*. 2018;359:1361-1365. **4.** Filley AC, Henriquez M, Dey M. *Front Oncol*. 2018;8(OCT):1-19. **5.** Kakarla S, Gottschalk S. *Cancer J*. 2014;20(2):151-155.

Target Antigens in B-cell Malignancies

B-cell malignancies express several potential antigen targets, many of which are being explored for CAR T-cell therapy approaches[1]

Malignancy	Potential CAR Targets in Hematologic Malignancies[2-4]								
	CD19	CD20	CD22	CD30	CD38	BCMA	Igκ light chain	NKG2D ligand	ROR1
B-ALL		✓	✓						✓
CLL	✓	✓					✓		✓
DLBCL	✓	✓	✓						
FL	✓	✓	✓						
HL				✓					
MCL	✓	✓							
MM	✓				✓	✓	✓	✓	
NHL	✓	✓	✓	✓			✓		
SLL	✓								✓

AML, acute myeloid leukemia; B-ALL, B-cell acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HL, Hodgkin lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; SLL, small lymphocytic leukemia.

References: 1. Hartmann J, Schüßler-Lenz M, et al. *EMBO Mol Med*. 2017;9:1183-1197. **2.** Leukemia & Lymphoma Society. Facts about chimeric antigen receptor (CAR) T-cell therapy. 2018. **3.** Morandi F et al. *Front Immunol*. 2018;9:2722. **4.** ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT01747486. Accessed April 29, 2021.

Section 03

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CAR T-cell Manufacturing Methods

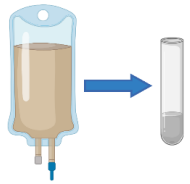
Overview of the CAR T-cell Manufacturing Process

1. Leukapheresis[1,2]

- Patients undergo leukapheresis to collect PBMCs; the PBMCs are then shipped to a central manufacturing facility
- Collected apheresis products may be processed differently depending on the downstream procedures using one of several commercially available devices

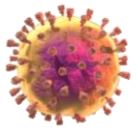
2. Selection & Activation[1,2]

- Lymphocytes are isolated from the PMBCs and T-cells are activated



3. Gene Transfer[1,2]

- Isolated patient T-cells are transduced with a viral vector to insert the CAR sequence



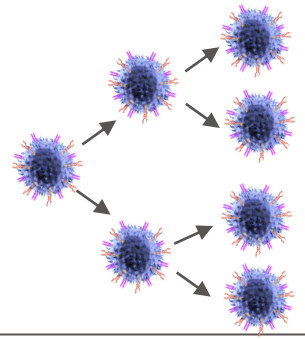
References: 1. Wang X, Rivière I. *Mol Ther Oncolytics*. 2016;3:16015. 2. Levine BL et al. *Mol Ther Methods Clin Dev*. 2016;4:92-101. 3. Better M et al. *Cell Gene Ther Insights*. 2018;4:173-186.

CAR T-cell Manufacturing Methods (cont'd)

Overview of the CAR T-cell Manufacturing Process

4. Cell Expansion[1,2]

- Engineered T-cells are expanded to a therapeutic dose
- Drug product is concentrated and cryopreserved in a low-volume container before being shipped to the infusion site



References: 1. Wang X, Rivière I. *Mol Ther Oncolytics*. 2016;3:16015. **2.** Levine BL et al. *Mol Ther Methods Clin Dev*. 2016;4:92-101.

Section 04

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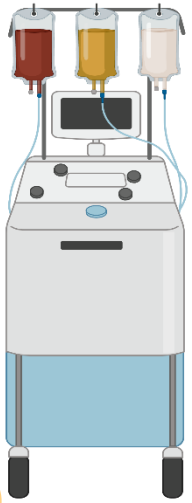
05 Patient Selection

Patient Journey Through the CAR T-cell Therapy Process

Patient selection → Patient consent & education → Patient screening → Leukapheresis and cell transport → CAR T manufacturing → Bridging and lymphodepleting chemotherapy → CAR T infusion & patient monitoring → Post-infusion monitoring → Long-term monitoring

1 Patient Identification

- Appropriate patients will be identified for treatment at qualified treatment sites.
- Patients will be enrolled and leukapheresis and treatment dates will subsequently be scheduled.

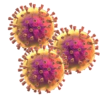


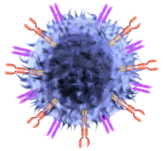
2 Leukapheresis

- Patient will undergo apheresis, which entails white blood cell collection through a catheter.
- Collected apheresis product will be sent to the manufacturer for production.

3 Manufacturing

The CAR T-cell product is created at a manufacturing facility.





4 Prep

- Patients may require bridging chemotherapy to maintain disease control while the CAR T-cell product is being manufactured.
- Shortly prior to CAR T-cell administration, the patient is prepared for treatment with lymphodepletion.

5 Treat

- The CAR T-cell product is delivered to the treatment site.
- Product is administered.

6 Monitor

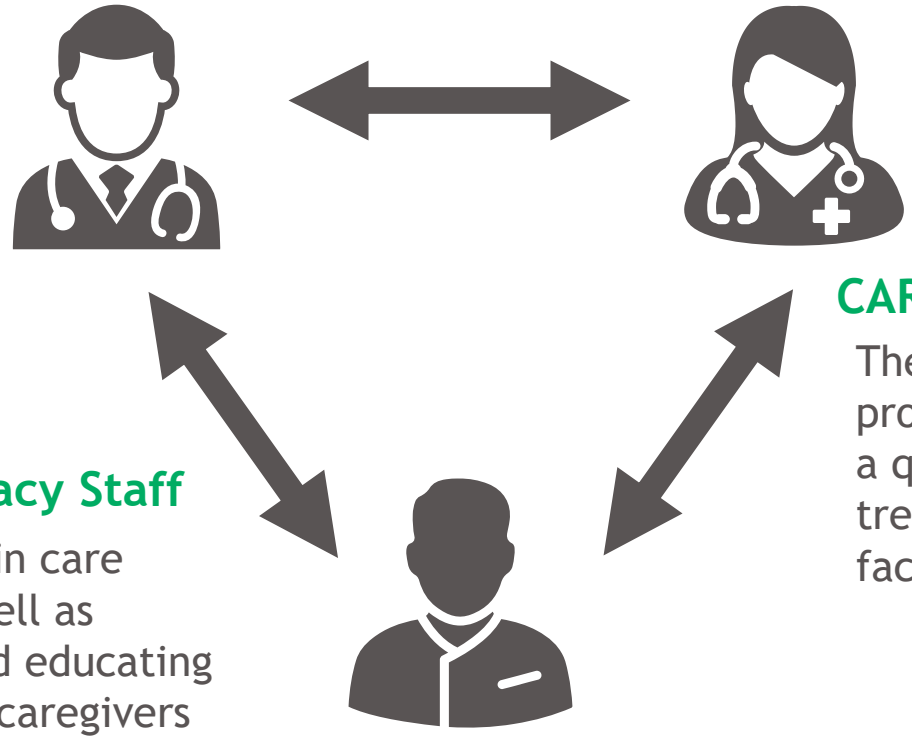
- The patient is monitored closely for at least 4 weeks and adverse events are promptly managed. Caregiver support is critical during this time.
- Thereafter, the patient is periodically monitored long term.

Reference: 1. Beaupierre A et al. *Clin J Oncol Nurs*. 2019;23:27-34.

Treatment and management requires care coordination between referring and providing institutions

- An increasing number of patients are referred for CAR T-cell therapy and acquire treatment outside of their community or state, collaboration between referring and treating providers is required to coordinate appropriate care

Patients will be co-managed by the primary oncologist and CAR T specialist leading up to and immediately following CAR T-cell therapy. Care will eventually be transitioned back to the primary oncologist.



Reference: 1. Beaupierre A et al. *Clin J Oncol Nurs*. 2019;23:27-34.

Section 05

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Eligibility Criteria for CAR T-cell Therapy

General eligibility requirements for CAR T-cell therapy[1,2]

- Tumor positive for the CAR target
- Adequate numbers of T cells for collection
- No active, uncontrolled infections, including hepatitis B, hepatitis C, or HIV
- Adequate performance status and organ function
- Absence of clinically relevant comorbidities (eg, select cardiovascular, neurologic, or immune disorders)

Precise criteria for eligibility vary by:[1]

- Malignancy
- Treatment regimen or protocol
- CAR T-cell product

References: 1. Leukemia & Lymphoma Society. Facts about chimeric antigen receptor (CAR) T-cell therapy. 2018. **2.** Beaupierre A et al. *Clin J Oncol Nurs.* 2019;23:27-34.

Summary

- The immune system plays a fundamental role in protecting against cancer; however, cancer cells develop mechanisms to evade immune system attack
- Immunotherapy (eg, CAR T-cell therapy) leverages the power of the immune system to fight cancer by enhancing the ability of immune cells to recognize and eliminate target cells expressing a specific antigen
- CAR T-cells are also susceptible to tumor immune evasion mechanisms
- CAR T-cells are manufactured from autologous T cells derived from patients through several carefully performed steps
- The patient journey through the CAR T-cell therapy process features 6 key steps: patient identification, leukapheresis, CAR T-cell manufacturing, preparation with bridging and lymphodepleting chemotherapy, CAR T-cell treatment, and monitoring
- Potential candidates for CAR T-cell therapy are carefully evaluated to ensure eligibility