THE CAR T CELL JOURNEY

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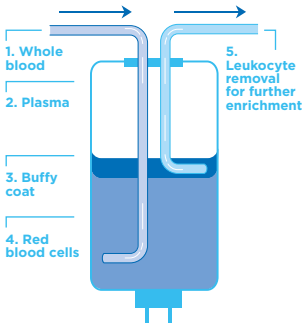
A GILEAD Company

1. APHERESIS

Mononuclear white blood cells make up the body's immune response. These are collected from the patient's peripheral blood.[1]

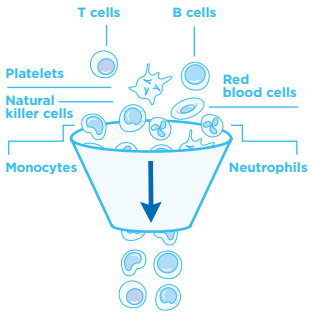
Collected apheresis material may be frozen or otherwise cryopreserved before transportation to the manufacturing facility.



2. T-CELL ENRICHMENT

At the manufacturing facility, patient cells undergo an enrichment step that can be accomplished by a variety of methods, such as centrifugation, fractionation and the use of immunomagnetic beads.[2,3] Specific cell populations, such as CD4+ and CD8+ T cells, can be further selected.[3] The method used may also depend on the need to remove circulating tumour cells (blasts) from the sample.[4]

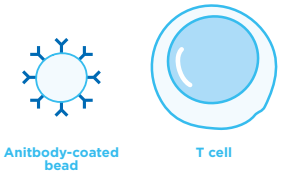
The T cells can be used directly or cryopreserved for future use.[2]



3. T-CELL ACTIVATION

The enriched T cells must next be activated to increase the efficiency of the transduction process that introduces the CAR into the isolated T cells. This requires sustained and sufficient activation, which can be achieved via different processes, including:[2]

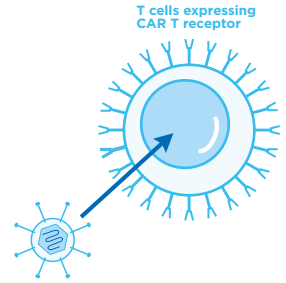
- Cell-based activation using the patient's dendritic cells, which process the cancer's antigen and present to T-cells for a strong immune response
- Antibody-coated beads that stimulate T cells
- Anti-CD3/CD28 monoclonal antibodies that support T-cell activation in the presence of interleukin-2 (IL-2)



4. GENE TRANSDUCTION

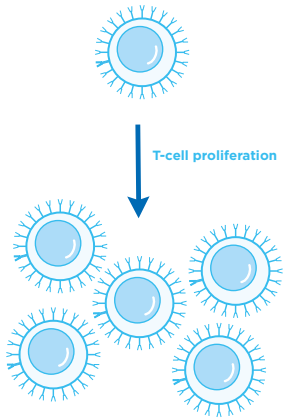
The CAR gene targeting the patient's cancer cells can be introduced into the activated T cells through different viral approaches:[2]

- Retroviral vectors, which are stable and result in high gene expression
- Lentiviral vectors, which are stable with high gene expression and may be able to support transduction of non-dividing cells



5. T-CELL EXPANSION

The newly made CAR T cells undergo their first expansion *ex vivo*, typically in the presence of growth factors such as IL-2, until sufficient numbers of CAR T cells are obtained.[2]



6. FORMULATION AND TESTING

The CAR T cells are washed, concentrated, formulated to dose and cryopreserved.[2,3] Quality tests of the CAR T cell product assess for:[2]

- Appearance and product identity
- Dose and potency
- Purity

The product is released upon successful completion of all testing and quality assurance checks. The product is then ready for transportation back to the medical centre.

Patients receive lymphodepleting chemotherapy to prepare them for CAR T cell infusion. This allows for expansion and anti-tumour activity of the cells after infusion.[5,6] After a few days, the CAR T cells are thawed and infused into the patient.

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1. McGuirk J, *et al. Cytotherapy* 2017; 19:1015–1024. 2. Wang X & Rivière I. *Mol Ther Oncolytics* 2016; 3:16015. 3. Levine BL, *et al. Mol Ther Methods Clin Dev* 2017; 4:92–101. 4. Stroncek DF, *et al. J Transl Med* 2017; 15:59. 5. Gattinoni L, *et al. J Exp Med* 2005; 202:907–912. 6. Klebanoff CA, *et al. Trends Immunol* 2005; 26:111–117. CAR: chimeric antigen receptor