Creative Biolabs®
CARepeutics™

WHAT ARE CAR-T THERAPIES ?

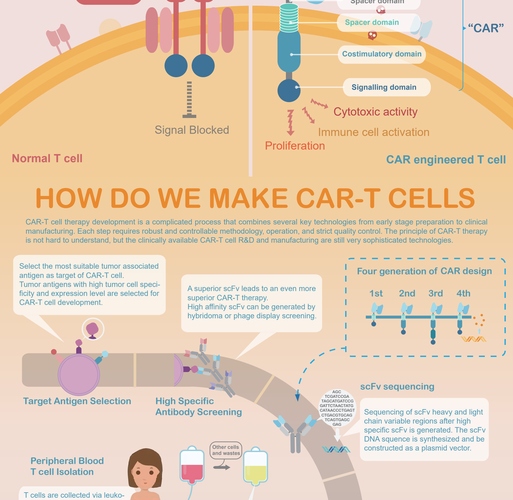
Through actions such as down-regulating the expressions of specific peptide antigens, major histocompatibility complex (MHC) molecules, and costimulatory molecules, tumor cells can avoid T cell recognition and escape immune attacks. This is called the Tumor Cell Immune Escape, explaining why tumor cells can escape from the attack by normal T cells and progresses to cancer. It is well known that the candidate markers recognized by antibodies are much broader than those of T cell receptors (TCRs). Antibody recognition is MHC independent and its binding affinity could be optimized by antibody affinity maturation process. In this case, if we combine an antibody recognition domain with a TCR signaling motif to form a **Chimeric T cell Receptor** - a more robust antigen receptor is formed with superior tumor recognition capability and the ability to trigger T cell cytotoxicity effect. This genetically engineered T cell is called the **Chimeric Antigen Receptor T cells or CAR-Ts.**

Tumor Cell Immune Escape

- ↓ Tumor peptide antigen expression decreased
- ↓ MHC I molecular down regulated
- ↓ Co-stimulation molecule (e.g B7) down regulated

CAR-T Cell Therapy

- ↑ "CAR" engineered T cell, better specificity
- ↑ MHC independent recognition
- ↑ Enhanced immune activity and tumor toxicity



HOW DO WE MAKE CAR-T CELLS

CAR-T cell therapy development is a complicated process that combines several key technologies from early stage preparation to clinical manufacturing. Each step requires robust and controllable methodology, operation, and strict quality control. The principle of CAR-T therapy is not hard to understand, but the clinically available CAR-T cell R&D and manufacturing are still very sophisticated technologies.

Select the most suitable tumor associated antigen as target of CAR-T cell. Tumor antigens with high tumor cell specificity and expression level are selected for CAR-T cell development.

Target Antigen Selection

A superior scFv leads to an even more superior CAR-T therapy. High affinity scFv can be generated by hybridoma or phage display screening.

High Specific Antibody Screening

Four generation of CAR design

scFv sequencing

Sequencing of scFv heavy and light chain variable regions after high specific scFv is generated. The scFv DNA sequence is synthesized and be constructed as a plasmid vector.

Peripheral Blood T cell Isolation

T cells are collected via leukocyte apheresis. Leukocytes are removed using a blood cell separator. Patient's autologous peripheral blood mononuclear cells (PBMC) are then separated and collected from the formed buffy coat. The remaining blood is then returned to the body.

Other cells and wastes

T cells

Specific T cells are activated in a certain environment in which they can actively proliferate.

IL-2
anti-CD3

CAR Vector Packaging

Lentiviral particles containing the coding sequence for the CAR are used for the transfection of activated T cells.

T Cell Engineering

Different approaches can be used to reprogram T cells to express CARs. Current strategies include inactived lentiviruses, transcription activator-like effector nucleases (TALEN) and the famous CRISPR system.

Newly created CAR-T cells undergo expansion and strict quality control.

These CAR T cells are frozen and sent to the hospital or center where the patient is being treated.

CAR-T Cell Expansion

Xenograft animal model for CAR-T *in vivo* validation

Validation of CAR expression and assays for cytokine induction and cytotoxicity *in vitro* and *in vivo*

CAR-T Cell Validation

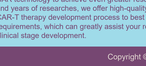
CAR-T Cell Infusion

The re-engineered T cells are infused back into the patient. The patient receives CAR-T cell therapy and is carefully monitored for any signs of adverse reactions.

WHICH CANCER CAN BE TREATED?

CAR-T cell therapies have brought hopes and expectations for cancer treatment. This can be reflected from the number of registered clinical trials: more than 250 since 2004 while 116 for 2016 alone. After years of clinical trials, in 2017, the US Food and Drug Administration approved the use of two CAR T-cell therapies. Both approved therapies use genetically modified cells recognizing a protein (CD-19) on the surface of cancerous B cells.

The number of clinical trials registered has rapidly increased since 2013. As of 2017, this number is over 250 in total.



Most of these trials are therapies for hematologic tumor, accounting for 69.7% of the total. Solid tumor trial only accounts for only 30.3%.

Leukemia, Lymphoma and multiple myeloma are the most frequently studied indications. Acute lymphoblastic leukemia is the most common cancer being studied.

CD19 is regarded as the star biomarker in CAR-T therapy. 50.6% of the world's CAR-T programs are targeting the CD19 molecule. The second place is mesothelin, accounting for just 4.5%, then followed by BCMA and GD2, each accounts for 4%.

Only **2** FDA approved CAR-T Therapy

Novartis is currently leading the race for the commercialisation of CAR-T therapies, followed by Kite pharmaceutics which developed the second approved CAR-T therapy YESCARTA. These CAR-T is directed against cancer cells expressing CD19 on their surface.

83% of 63 patients who received treatment with Kymriah achieved complete remission.

Of 101 patients with non-Hodgkin lymphoma who had failed other treatments, YESCARTA was shown to help 51% achieve complete remission.

PROBLEMS AND CHALLENGES

Although CAR-T therapy, as an immunotherapeutic approach, may show less toxicity and risks than conventional radiotherapy or chemotherapy, it can still cause several worrisome, and sometimes fatal side effects.

Possible side effects

Cytokine Release Syndrome (CRS)

Large quantity of cytokines are released. Elevated amount of cytokines. High fevers, low blood pressure or poor lung oxygenation. The symptoms are reversible.

B Cell Aplasia

CAR-T cells can destroy cancerous B and normal cells expressing the target antigen. This results in a ***low number of normal B cell***. B cell aplasia limits the production of antibodies and thus the normal immune response.

Neurologic Toxicities

Common symptoms include language impairment (aphasia), confusion, delirium, involuntary muscle twitching, hallucinations, or unresponsiveness.

Tumor Lysis Syndrome (TLS)

TLS results in complications during the treatment as dead cancer cells release their toxic contents in the bloodstream. It is a life threatening complication common to all cancer treatments and requiring close monitoring of the patient

Anaphylaxis (Life-threatening Allergic)

Potential for patients receiving CAR-T cell therapy to have an overwhelming immune response against the CAR itself is called "anaphylaxis". Symptoms include hives, facial swelling, low blood pressure and respiratory distress.

Challenges to overcome

Manufacturing Challenges

The production of CAR-T cells is difficult
Time: autologous approach takes 14-21 days
Scaling up: allogenic approach could be difficult

Handling and Storage

The handling of CAR-T cells is difficult. Risk of cross-contamination between patients.
T cells are extremely sensitive cells.

Transportation Challenge

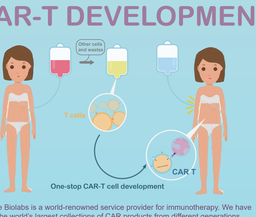
The cold-chain transportation of CAR-T cell is difficult.

Cost Challenge

Combining all other challenges, the cost of this technology becomes very expensive. Kymriah costs $475,000 for one therapy session.

The technology has a huge potential to treat cancers which have few alternatives when the standard treatment fails. The success of CAR-T will require the identification of tumor-specific antigens and the development of "safety switches" in order to turn on and off the engineered T cells. Such advances will minimize the toxicity risks of the CAR-T. Overall, the first CAR-T therapy on the market opens the way for exciting new and better ways to fight cancer.

CAR-T DEVELOPMENT PLATFORM



- Antibody screening
- Hybridoma sequencing
- CAR vector design
- Lentivirus production
- CAR-T cell generation
- CAR-T cell expansion
- CAR-T cell optimization
- *In vitro* cytokine assay
- Xenograft animal studies

Creative Biolabs is a world-renowned service provider for immunotherapy. We have one of the world's largest collections of CAR products from different generations targeting various biomarkers, and we are continuing to innovate the next generation CAR technology to achieve even greater results. Based on advanced technologies and years of researches, we offer high-quality custom services covering the entire CAR-T therapy development process to best suit your technical program and budget requirements, which can greatly assist your research, preclinical investigation and clinical stage development.

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