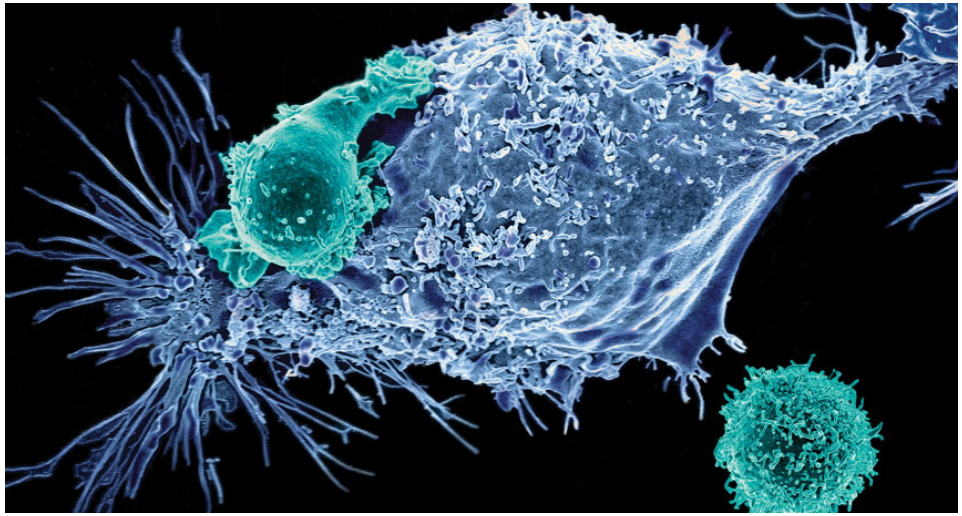
CAR-T THERAPY



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CAR-T cell therapy

Preface

Belinda and Fiona quickly agreed on choosing a genetic engineering technique that is or will be used in the future for cancer treatment. Following an internet search and advice from Fiona's father, who works at Roche in a cancer treatment group, we decided to investigate the area of CAR-T cancer therapy and to request an interview with Chris Vann, Chief Operating Officer for Autolus Therapeutics. Autolus is company in the UK working specifically on CAR-T cell therapy. We agreed on this topic for our paper because the recent use of chimeric antigen receptor T cell (CAR-T) therapy suggests that it could be a very effective method for cancer treatment. Also it appears interesting as it uses very new genetic engineering technology, and the interest and number of clinical trials testing CAR-T therapy has expanded dramatically from just a handful 5 years ago to more than 180 currently ongoing trials. We also found it to be interesting because it's a new approach to treating cancer and has some controversial topics because, like most therapies, there are many advantages but also some side-effects.
Our main questions for this paper are:

- How does the CAR-T therapy work and how effective is this method?
- What are the advantages and disadvantages of this therapy?
- How advanced is this technology and what further research is being made?
- Which patients can currently use it?

Introduction

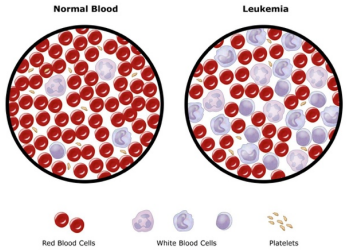
According to the American cancer society approximately 40% of American men and 38% of American women develop cancer at least once in their lifetime. Depending on what type of cancer the patient has and how advanced it is, different treatments are used. Common treatments used to fight cancer are surgery, chemotherapy, radiation therapy, targeted therapy, hormone therapy, stem cell transplant and immunotherapy.

What is cancer even?

Cancer is a disease, or more specifically a group of diseases that develop in the body. It begins when cells start to grow uncontrollably, rather than the old cells dying after dividing. There are different types of cancer. Doctors divide cancer into types based on where it begins. There are four main types of cancer, carcinomas, sarcomas, leukemia and lymphomas. The two main types which concern CAR-T cells are leukemia and lymphomas.

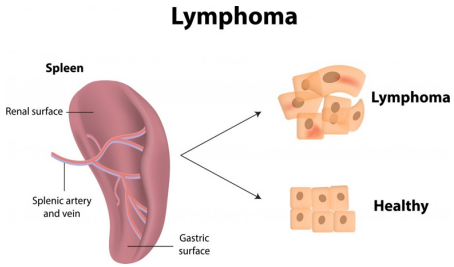
Leukemia

- Leukemia is a blood cancer. It begins when healthy blood cells change and grow uncontrollably. The four main types are acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia and chronic myeloid leukemia.



Lymphomas

- Lymphoma begins in the lymphatic system, which is a network of vessels and glands that help fight infection. There are two main types: Hodgkin lymphoma and non-Hodgkin lymphoma.



Blood cancers account for almost 10% of new cancer cases in the U.S. each year and are the most common cancers in children. Until recently, the use of CAR-T cell therapy has been restricted to small clinical trials, largely in patients with advanced blood cancers. However, from the results so far, these CAR-T therapies have shown a lot of success in both children and adults for whom all other treatments had stopped working.

All cell-based immunotherapies inspire hope for a more and more cancer-free future. Out of all the engineered T cells that have been developed, the most efficient one, the CAR-T cell

approach has secured regulatory approvals in the United States, Europe, Canada, Australia and Japan.

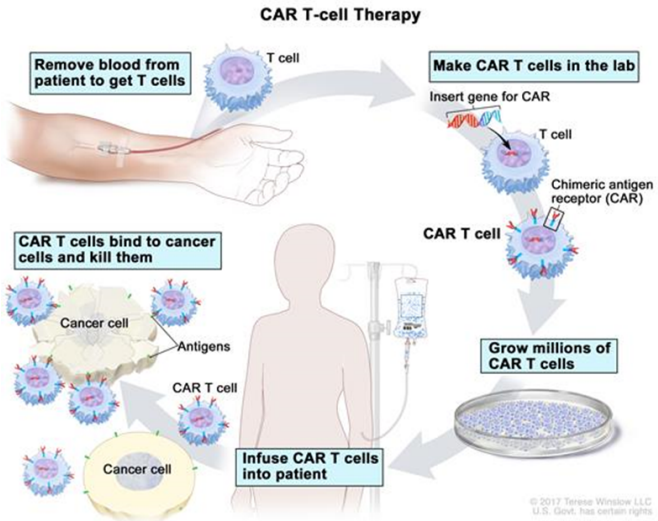
Technique

For most of us, our immune system, in particular T cells, identify individual tumor cells in our bodies early and destroy them before the cancer has the chance to grow, spread, and escape our body's natural defenses. People get sick when those cancer cells are not recognized by the immune system or grow too fast for our body's defenses to bear. For years, the foundations of cancer treatment were surgery, chemotherapy, and radiation therapy.

The immunotherapy approach called adoptive cell transfer (ACT) collects and uses patient's own immune cells to treat their cancer. There are different types of ACTs (for example TILs, TCRs, and CARs), but the CAR-T cell therapy is the most advanced and the furthest in clinical development. T cells are a type of white blood cell and an important part of our immune system. Michel Sadelain told The Scientist: "CAR-T therapy is at the same time cell therapy, gene therapy, and immunotherapy. It represents a radical departure from all forms of medicine in existence until now."

One of the very unique aspects of CAR-T therapy, is that the therapy has to be produced specifically for each individual patient. This is very different from how medicines are usually developed. Instead of just producing a medicine that gets used in all patients with a disease, CAR-T therapy has to be genetically engineered to attack the patient's specific cancer using the patient's own immune system. To prepare and engineer the CAR-T therapy takes a number of steps. These steps usually take place in different labs or facilities and for safety reasons it is usually not possible to visit such facilities. However, the general steps are shown in Figure 1 and described below.

Figure 1: The Process for Making CAR-T Therapies



1. **Blood extraction and T cell separation**

For CAR-T therapy blood is taken from the patients called autologous treatment, or in some cases from a healthy donor called allogenic treatment. The T cells or leukocytes are then separated out from the blood using a blood cell separator in a process known as leukocyte apheresis. The manufacturing process is the same if it`s from the patient itself or from a donor; only the choice of initial blood donor is different.

2. **Antigen introduction**

The next step is introducing the chimeric antigen using genetic engineering. This is done by inserting a gene using a viral vector so that afterwards cells, which have been basically "infected" with the engineered antigen receptor gene, will be able to mass produce the CAR-T proteins.

3. **Production of CARs**

When cells that have been "infected" with the engineered antigen receptor gene produce the proteins for the genes, these proteins are receptors on the surface called chimeric antigen receptors, or CARs. These receptors don't exist naturally but the genetically engineered DNA produces the chimeric CAR proteins that allow the T cells to recognize an antigen on targeted tumor cells. Many different antigens exist on cells, but most CARs have been designed to recognize a marker called CD19, which is found on the surface of all B cells (which are the white blood cells responsible for producing antibodies), including the destructive B cells that cause certain leukemias and lymphomas.

4. **Multiplication**

Once the collected T cells have been engineered to express the antigen-specific CAR, the cells are multiplied in the laboratory to produce a lot of CAR-T cells.

5. **Insertion into patient's body**

Before patients are treated with the newly engineered CAR-T cells, patients are often treated with lymphodepletion chemotherapy in order to reduce the existing immune cells and increase the effects of the introduced CAR-T immune cells. The reduction of the number of circulating leukocytes in the patient upregulates (increases) the number of cytokines that are produced and reduces competition for the CAR-T cells which helps them expand and have a quicker and better impact.

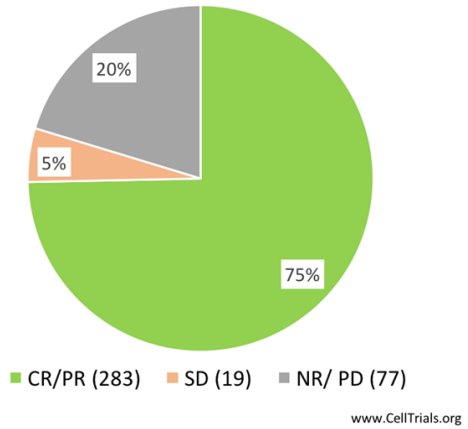
Finally, the CAR-T cells are infused into the patient with just a single infusion. If these steps go as planned, the engineered CAR-T cells further multiply in the patient's body, recognize the antigens on the cancer cells and then kill the cancer cells through the normal T-cell immune response.

Outcome of CAR-T cell trials

Alexey Bersenev summed up the outcomes from all published CAR-T trials and subdivided them into 3 groups: (1) overall response (complete + partial CR+PR), (2) stable disease (SD) and (3) no response/ progression of disease (NR/PD). The total number of publications for CART therapy targeting CD19 (a biomarker for B cell development, lymphoma diagnosis and therapy) was 25 and the total number of evaluable patients were 379 (as of Sep 1, 2017). The

results for CD19+ blood based cancers are shown in Figure 2 below. Only evaluable patients were included in the following diagram.

Figure 2 Clinical Outcomes of CAR-T Cell Therapy Trials targeting CD19



This graphic shows the significant outcome of CAR-T therapy trials. 75% of the patients in the mentioned trials treated with CAR-T therapy showed complete or partial response to therapy meaning that the tumor was either completely eliminated or at least reduced by 50%. These numbers are from trials before 2017 so, as there have been additional new trials since 2017, the success with CAR-T therapy may even increase in the future.

Interview with Chris Vann

(Chief Operating Officer for Autolus Therapeutics UK.)

1. What problem occurred in the past that had/have to be overcome before using this therapy on patience?

There have been many great advances in medicines to treat patients with cancers, but sadly few cures. The reason for this is that the cancer has the ability to find ways to overcome the single-target approaches we use in modern medicines to control them. So the cancer may get smaller or not get bigger, but when they find a way around the specific

thing the cancer treatment is doing, then they can continue to grow again, which is called progression.

In nature, our white blood cells control cancer and infection. They are very effective because they kill the cancer cells very efficiently but also in a more general way (inject granules which kill the cell completely using many paths to do so). So once they interact with a cancer cell if it is dead, clearly it cant find a way around treatment. That's why many researchers are looking to use this approach to make the body's own immune system more effective.

2. Why is the cost of CAR-T therapy so high?

It is an individual medicine, with each product being made seperately for one patient.

As with all medicines, over time the relative cost will come down as we improve the manufacturing and as we share the costs of manufacturing across more patients.

One thing we do at Autolus already is to use automation (a machine called a Miltenyi Prodigy), so we reduce the number of people who are needed to make a single product.

Please bear in mind, the price appears high, but you only pay it once. And if you cure a patient, they don't need the same hospital care and can live a normal life. The total treatment for a patient is not so different from the cost of what that patient would need to help them until they die if they were not treated, the main difference is that it comes all on one time.

3. Some sources say that this therapy can affect the patience brain negatively. Are these side effect common and is further research done to minimize these side effects?

CAR-T cells are powerful medicines, so we are very careful how we give them, and this is usually in a specialist hospital.

The most common things that can happen are cytokine release syndrome (CRS) and neurotoxicity.

The CRS is caused by chemicals released by the cells when they kill cancer, which in small amounts are good because they are signals to encourage the T cells to divide and make more of themselves. This gives like a flu (because it's a similar type reaction) but if unchecked can lead to something more severe like a coma.

In the newer products, like ours, we see less of this because there are ways of designing them to avoid this. The centers are also getting better at managing it and now give a different product when they see an increase in temperature which blunts the effect of the cytokines and so makes it less bad.

We are not entirely sure of all of the reasons behind the CNS toxicity. It is probably due to the fact that when the T cells find cancer they expand, making many more copies of themselves, and if this happens too fast, things get crowded and so they have to go somewhere. When they cross into the brain, if too many are making the passage in a short time, they can disturb a membrane called the blood brain barrier, causing swelling and things like confusion and other brain symptoms which in some cases can be severe. Again, some of the newer products seem to be better for this and have less of these types of events.

4. What is the next step in CAR-T research?

In addition to giving cells a new ability to see cancer, we will add other properties. We are also improving the manufacturing and looking to come up with cell banks that will allow one source of cells for all patients ("off-the-shelf" autologous products) so individual products are not necessary.

So, for example, in Autolus we have a way of temporarily switching off CAR-T cells using an antibiotic so the patient can rest a little if they get side effects. Once the peak for the side effects is over, we the patient stops taking the antibiotic and the CAR-T cells switch themselves back on.

We also can give cells a protein which makes them immune to the chemicals a cancer secretes to protect itself. So, if you like, the CAR-T cells still receive the signal from the cancer cells not to attack the cancer but ignore it and attack anyway.

5. Who is entitled the treatment?

Currently, CAR-T cell therapy is a standard care for some forms of aggressive, refractory non-Hodgkin lymphoma and for adults and children suffering from relapsed acute lymphoblastic leukemia who haven't responded to other forms of treatment.

In addition, there are many ongoing trails of CAR-T cell therapy for other forms of blood cancer. We are also starting to explore CAR-T cell therapy for solid tumors, but we don't have a treatment

6. Which patients are considered good candidates?

Patients enrolled in CAR-T cell trials are those with advanced relapsed disease. While protocols vary for each trial, in general, patients eligible for CAR-T cell therapy must:

- *Have tumors that are positive for the CAR target*
- *Have an adequate number of T cells for collection*
- *Not have an active, uncontrolled infection, including hepatitis B or HIV*
- *Have adequate performance status and organ function*
- *Not have certain relevant comorbidities*

Comorbidities are often long-term or chronic conditions. It refers to one or more diseases or conditions that occur with another condition in the same person at the same time.

7. What does the recovery process require?

Patients who receive CAR-T cell therapy have a risk period of about 2-3 months, during which they will be evaluated for side effects and treatment response. The acute recovery period is in most cases 30 days after the CAR-T cell infusion. During that time, they patients are advised to stay within close proximity of the hospital, for their regular follow-up care and must have a caregiver with them at all times to monitor their reaction to the treatment. It is not uncommon for patients to be re-admitted to the hospital during this period to manage complications

8. Will the insurance pay for the treatment?

That depends on the country and the insurance system in the patient's country. In countries where CAR-T therapies are approved, and where insurance covers such therapies, they would generally be paid for similarly to other cancer treatments. Nevertheless, the institution running the treatment normally obtains any prior information that may be needed and a patient would be notified of any potential financial responsibility before the treatment would be started.

Discussion

The area of CAR-T therapy is still relatively new and research is continuing in how best to treat patients. Researchers estimate that the overwhelming majority of tumor antigens reside inside tumor cells, out of the reach of CARs, which can only bind to antigens on the cell surface. CAR-T cells have garnered the lion's share of the attention when it comes to the cellular therapies that fall under the ACT umbrella. But other forms of ACT have also shown promise in small clinical trials, including in patients with solid tumors.

There have also been many advances over time in the development of CAR-T therapies. Over time, advances in the intracellular engineering of CAR-T cells have improved the ability of the engineered T cells to produce more T cells after infusion into the patient (expansion) and survive longer in the circulation (persistence). Advances have also been made in how long it takes to produce a batch of CAR-T cells. Although it initially took several weeks, many labs have now reduced the production time to less than 7 days.

Other refinements or reconfigurations of CAR-T cells are being tested. One approach is the development of CAR-T cell therapies that use immune cells collected not from patients, but from healthy donors. The idea is to create so-called off-the-shelf CAR-T cell therapies that are immediately available for use and don't have to be manufactured for each patient. Nevertheless, researchers caution that, in many respects, it's still early days for CAR-T cells and other forms of ACT, including questions about whether they will ever be effective against solid tumors like breast and colorectal cancer. In this scenario, if early indicators suggested that these high-risk patients weren't having an optimal response to chemotherapy or other cancer treatments, they could be stopped, and the patients could be treated with CAR-T therapy. For patients who respond well, "they could be spared 2 more years of chemotherapy," Dr. Fry said. "That's amazing to think about."

Research and tests have shown that these treatments can extend life expectancy or even eliminate highly advanced cancers in patients with only a few months to live. One of the most promising aspects is that it targets a single antigen, that is present at high levels in a multitude of different cancer types. Not only does the life span of the patient increase but the treatment time is rather short due to the fact that only a single infusion is required that may require at most two weeks of inpatient care. Because no aggressive chemotherapy is used, most patients have a much faster recovery. Clinical trials have shown that in patients whose cancer came

back after treatment, CAR-T cell therapy has helped achieve suspension of the cancer that lasted for years.

However, CAR-T cells have their issues. One of them being that their target antigen must be on the surface of the cancer cell, for the chimeric antigen receptor to be able to bind to it and do its' job. Another problem is that there are only a few antigens on the tumor cell surface. If the target antigen is also present on healthy cells the CAR-T cells could attack those and cause serious damage. Adding to that, the CAR-T cells can expand in the body causing immune responses that can kill many healthy cells in addition to the targeted cancer cells.

Manufacturing challenges of producing large quantities of CAR-T cells is also a big disadvantage concerning this therapy and leads to its high price. As for now researchers have not been able to come up with a solution for this problem, even though they are on the verge of developing a new therapy that aims to reduce the manufacturing challenges of producing large quantities of CAR-T cells and create a synthetic CAR molecule to be genetically engineered into a patient's own T cells.

Like any other treatment there is a chance for side effects to occur. CAR-T cell therapy can trigger a range of side effects including neurotoxicity and cytokine release syndrome (CRS), mentioned by Chris Vann in our interview. Neurotoxicity has been observed in trials with CD19-directed CAR-T cell therapy. Symptoms include confusion, delirium and seizures. They may not occur at the same time as CRS and may be able to be treated by injecting steroids.

However, CRS can be a very severe side effect coming from CAR-T therapy. Once they enter the body, CAR-T cells initiate a massive release of immune proteins called cytokines, which summon other elements to join and attack the tumor cells. The results of CRS include high fevers, extreme fatigue, difficulty breathing, and a sharp drop in blood pressure. In many cases patients having CRS often suffer from a second side effect that involves the nervous system referred to as treatment-resistant large B-cell lymphomas, which can cause headaches, tremors, confusion, loss of balance, seizures, trouble speaking and sometimes even hallucinations. Even though the causes of the symptoms are not well understood, the symptoms last only for a few days, although sometimes they can last up to a few weeks.

However, they are generally clinically manageable, according to Marcela Maus, M.D., Ph. D., director of Cellular Immunotherapy at the Massachusetts General Hospital Cancer Center.

Future research steps include strategies to maximize outcome of CAR-T cell therapies, minimize toxicities, widen targets beyond CD19 and to develop CAR-T therapy to target solid tumors.

Summary

CAR-T (chimeric antigen receptor T cell) therapy is an immunotherapy approach to treating cancer. Instead of just producing a medicine that gets used in all patients with a disease, this approach makes use of targeting the patient's own immune cells to attack their cancer. The patient's T cells are genetically engineered in the laboratory so they will recognize, attack and kill cancer cells. Because the CAR-T therapy approach is specific for individuals, and is based on genetic engineering, the production of CAR-T cells involves steps to collect, genetically modify, and reintroduce T cells from the individual being treated.

At this moment CAR-T cell therapy can only be used on patients with a certain type of blood cancer but so far there have not been any successful trials on solid tumors. Even though this fairly new therapy has not been researched to its fullest, it is already considered as an effective alternative to chemotherapy for some cancers. For now, the treatment is only available to patients under 25 years old and like all cancer treatments CAR-T cell therapy comes with its side effects including cytokine release syndrome (CRS) and neurotoxicity. Research is continuing in CAR-T therapy and researchers are constantly figuring out new and different ways to prevent or weaken such side effects.

Even though it may seem to be a more expensive treatment than other cancer treatments, overall costs for CAR-T therapy are actually not higher than other cancer therapies such as chemotherapy due to the fact that it only needs to be injected once and has a high probability of success. Currently it is approved in Europe, United States, Canada, Japan and Australia. In the future, CAR-T cell therapy may even replace aggressive chemotherapies used to treat cancer due to the lower side effects, the quicker recovery and higher rates of success with CAR-T therapy. In any case, CAR-T therapy is very interesting as a new approach to make use of genetic engineering to develop individual treatments for cancer.

Further information and an easy visual explanation

CAR-T cartoon: https://www.youtube.com/watch?v=Mt5C5fhuU_0

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Pictures

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