"The journey starts with the collection of T-cells from the blood. The process is called 'apheresis'"

Myeloma Patients Europe (MPE) has conducted a series of four written interviews on CAR-T manufacturing to better understand manufacturing processes and challenges of various myeloma CAR-T therapies. Four different stakeholders have answered our questions, including two pharmaceutical companies, which each have a myeloma CAR-T product approved in the European Union, and two academic teams which have developed myeloma CAR-T products currently under clinical investigation. In this interview, we will learn about the manufacturing process of the CARAMBA product.

What is the journey of patients' cells?
Today, all CAR T-cells are made from the patient's own cells. The journey starts with collection of T-cells from the blood. The process is called 'apheresis' and most patients will be familiar with it because it's the same technique that is used to collect stem cells from blood, and most patients will have had stem cell collection, high-dose chemo and re-infusion of their stem cells - in short, auto-transplant. Apheresis is mostly uneventful. Blood flows through a tubing set into a machine where the white blood cells, of which the T-cells are a subtype, are separated into a bag. Most patients do not even experience much of a drop in white blood cells in the blood. Typically, the apheresis is done on an outpatient basis, but usually in hospital. The cells are kept at 4 °C when being transported to the manufacturing centre – which may be just next door, in a different country, or even on the other side of the globe. It takes two weeks to make CAR T-cells. During this time, the synthetic CAR gene is inserted into the cells while they are kept in growth-supporting culture media and environmental conditions. To facilitate the logistics, a lot of manufacturers freeze the apheresis product until a manufacturing slot becomes available and freezes again at the end of manufacturing to synchronise quality testing of different patient batches. In this case, it would take longer - a month or more - to deliver the CAR T-cells to the patient. The frozen product is transported in liquid nitrogen vapour at temperatures below -160 °C.

What happens to the cells?
Most CAR-T manufacturing begins with the enrichment of T-cells, or even specific types of T-cells. The CARAMBA process isolates two kinds of T-cells (killers and helpers) and manufactures CAR-killers and CAR-helpers separately. The idea is that a balanced proportion of killers and helpers is associated with the best outcomes. After isolation, we add an ingredient which connects two surface receptors on T-cells so that they think they have met the antigen they are made to react with, and thus become activated and begin to divide. This facilitates uptake of gene ferries, which we use to put the artificial CAR gene into the cell and to get it to insert itself into the genes of the T-cell, so that it will make the gene product (the CAR) for as long as it or its progeny live. CARAMBA uses a very innovative gene ferry, a small naked DNA molecule containing only the CAR and a small RNA from which the cell makes the protein, which splices the CAR gene into the T-cell's genes. Other processes use virus-like particles as gene ferries. We then allow the T-cells to proliferate; in total the process takes two weeks. Specific proteins called cytokines, which instruct T-cells to proliferate, are present in the culture media during that time. At the end of the two weeks, the cells are collected, washed a couple of times to remove any media and cytokines, and then counted and formulated to contain the desired number of CAR-helpers and CAR-killers for the patient.

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Very few cells (one or two million per kg) are a typical dose of CAR-T; the combined volume of such a dose of cells is less than the volume of a pinhead. A lot of tests are performed on the product, to ensure safety and pharmacological activity. Good quality provided, the cells are shipped, either at 4 °C or after freezing at -160 °C or colder, to the hospital. Non-frozen cells are best administered immediately; frozen cells can be held for years. Before CAR-T can be infused, an intermediate-intensity chemotherapy must be given a couple of days prior, to allow the CAR T-cells to continue to expand in the body and to find and attack the tumour.

Dr. Halvard Bönig is the Head of Manufacturing and Qualified Person for the CARAMBA product, a SLAMF7-targeted CAR-T therapy (and some other CAR-effector cell products). He is Professor for Translational Development of Cellular Therapeutics at Goethe University, Frankfurt, Germany, and works in the German Red Cross Blood Service and Institute for Transfusion Medicine and Immune Hematology of the Johann-Wolfgang-Goethe Medical University GMP (cell therapy medicine manufacturing) core unit.
CARAMBA, short name for "SLAMF7-CAR T-cells prepared by Sleeping Beauty gene-transfer for immunotherapy of multiple myeloma – a rare hematologic disease" is a Horizon2020 programme, financially supported for four years by the European commission. A total of 11 partners in six European countries are collaborating to this project to bring CAR-T therapy to patients. The CARAMBA trial is ongoing in Germany, Spain, France and Italy.

Who is involved in the process?
At least three dominant parties are always involved: the patient and their family, the medical team in charge of the cancer treatment and the CAR-T manufacturer with their team. Sometimes, the apheresis team is distinct from the cancer team. A lot more people are involved, especially once the CAR-T product is licensed, such as couriers, hospital administrators seeking confirmation of reimbursement and pharmacists, who order and receive the CAR T-cells. Also involved are the manufacturer's own staff who physically manufacture

the product; quality control staff, who sample the product at defined intervals and measure relevant properties; and many other teams working behind the scenes, such as quality management, vendor supervision and purchasing, the technical infrastructure department, regulatory affairs, pharmacovigilance, legal and contracts, and many more. Cell therapy manufacturing in accordance with GMP[1], the legal framework for drug manufacturing, as CAR-T are drugs, takes a village.

How is the quality of the product ensured?
Before the regulatory agency can allow a pharmaceutical manufacturer to manufacture a CAR-T for a clinical trial, the manufacturer must define critical properties of such products and demonstrate that they have built a manufacturing process that can reproducibly generate products meeting these properties. The starting material is tested, samples are taken as the cells grow in the incubator and the end product is also sampled and tested.
What is tested on each of the samples is precisely defined, as is the method which is used to perform the test. Briefly, safety is ascertained by demonstrating that the product contains neither viruses nor bacteria, and identity and quantity of the active substance is measured, as well as other inactive components. These will mostly be white blood cells that are not CAR-T, because no pharmaceutical manufacturer purifies the end product such that it only contains CAR-T – in fact, often the CAR-T are not even the most frequent cell type in the final product. Careful analysis is conducted to ensure that neither tumour cells nor blood stem cells are contained in the final product. The responsibility for those tests is the pharmaceutical manufacturer's, although they can outsource some assays to third parties.

What could go wrong?
The most common problem is that, predominantly because of the years of chemotherapy that the patients have received, the T-cells are not healthy, are not long-lived, have an impaired ability to proliferate, or have poor killer or helper capacity. Because of this, the ideal dose of CAR-T may not be met, the cells may not persist in the patient's body, or their therapeutic function may be sub-par. Sometimes, a product might contain bacteria or other contaminants precluding infusion. If the understanding that a product is not suitable for infusion is reached very late and the plan had been for infusion of a fresh product, the patient may already have received their chemotherapy, which may cause some transient toxicity such as suppression of blood counts, impaired immune function or blood in the urine. Overall, the pharmaceutical manufacturer must have established very robust manufacturing processes before they are allowed to treat patients, to ensure the risk of not receiving a product is quite low. Treatment with CAR-T that are effective obviously also has its risks, although we have learned that toxicity from CAR-T is, to a certain degree, predictive of efficacy.

How many samples can you process and how will this evolve in the future?
The way the process was built, we can currently only start on Tuesdays with cells that were collected on Mondays. If we wanted to accommodate more samples, we would have to work on Sundays and holidays, which we don't currently do. Also, we have limited space in our manufacturing facility and are manufacturing many different cell-based medicines. Thus, at this point in time, we are limited to one product per week minus a couple of weeks for facility maintenance, so we could process maybe 40 CARAMBA samples per year.

What changes will be required in the manufacturing process, if you want to put your product on the market, and what would be the timeline for this procedure?
CAR-T are medicines, and the class of medicine they belong to (the ATMPs[2]) requires a European license a.k.a. 'approval' or 'marketing authorisation'. Before the European Commission can approve such an authorisation, large international trials unequivocally demonstrating effectiveness must be performed, and the entity seeking a European license must be large enough to satisfy the entire European market. Academic manufacturers are inherently unable to perform such studies and serve such large markets. Invariably, therefore, a 'real' pharma company must be solicited as a partner for commercial development.

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What do you think needs to be improved in the near future with regards to how CAR-T manufacturing is done now?
In principle, this very manual manufacturing process is suitable for broader patient access, since this is a patient-individual medicine. Nevertheless, automation would be attractive as qualified labour is increasingly hard to come by and much progress along this avenue has been achieved. Specifically with the CARAMBA process, the targeted antigen is also expressed by normal T-cells, not only by tumour cells. For that reason, as soon as the first cells express the CAR, they start killing their not (yet) CAR-armed brethren. A future manufacturing process may want to put the CAR in 'neutral' during manufacturing – by adding a drug compound that stops the CAR from triggering the killing machinery. We could, alternatively, put a cap on the targeted antigen to make it invisible to CAR-T. The most progress is actually expected not from improvements of manufacturing itself, but from insight into how to recognise that patients have 'good' T-cells for CAR-T manufacturing.

What could be changed in the manufacturing process to improve a patient's experience?
In an ideal world, patients will not have to wait for their apheresis slot and will receive their cells back 3-4 weeks later. Local manufacturing may seem like the obvious solution, but the transportation of cells to and from central manufacturing sites is not actually the bottleneck – redundant manufacturing capacity is! In the interest of cost control, manufacturing capacity is used to its fullest; currently patients have to wait for a "production slot". The strongest real improvement would be a better understanding of how to recognise the ideal time in a patient journey for treatment – when the patients are certain to need CAR-T, when their T-cells are certain to support in vitro and in vivo expansion, killing and persistence, so that the CAR-T are most likely to eradicate cancer.

1. *Good manufacturing practice (GMP) are guidelines describing the minimum standard that a medicines manufacturer must meet during production processes. Inspections are coordinated by relevant authorities to verify processes comply with these standards. The same authorities control the authorisation and licensing of the manufacture and sales of such products.*
2. *Advanced therapy medicinal products (ATMPs) are medicines that are based on genes, tissues or cells.*

CAR-T Interview Series

The CAR-T Interview Series is composed of four different interviews about CAR-T manufacturing, from cell collection to cell infusion. Click on the links below to read the interviews responded by:

- Hadassah Hebrew University Medical Centre
- Bristol Myers Squibb (BMS)
- Janssen Pharmaceuticals

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