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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 at Hadassah Hebrew University Medical Centre, Jerusalem, Israel.

What is the journey of patients' cells?

The patient is admitted at the hospital on the day of apheresis collection[1]. The apheresis procedure is performed at the Apheresis Unit at the Bone Marrow Transplantation and Cancer Immunotherapy Department. Collection may last several hours (generally between 2-4 hours, equivalent to two blood volumes or less). At the end of the apheresis process, freshly collected cells are transferred to the Hadassah Medical Organization (HMO)-GMP[2] facility located within the hospital, where the manufacturing process happens. The patient's apheresis-derived cells are either immediately processed into a CAR-T drug product or cryopreserved for later manufacturing. Currently, HBI0101 CAR T-cells are delivered as fresh cells, however, we are now working on the stability of the cryopreserved drug product and within a few months we will move toward infusing patients with a cryopreserved product. At the end of manufacturing and after all the regulatory requirements have been met, the final product is released and transferred back to the Bone Marrow Transplantation Department (BMT), where the patient receives the cells under the close supervision of a physician and a nurse, all in accordance with written Standard Operating Procedures (SOPs[3]).

What happens to the cells?

HBI0101 production is a 10-day process, consisting of the stimulation of the T-lymphocytes; genetic engineering of the proliferative T-cells to make them endow the CAR molecule targeted to the BCMA antigen at the cancer cell surface; expansion of engineered CAR T-cells to achieve the therapeutic dose and, finally, formulation of these cells into a fresh final drug product suitable for infusion into the patient. Briefly, T-lymphocyte cell subset (i.e., T-cells) from the patient's blood are the cells of interest for CAR-T manufacturing. These cells are collected from patients by apheresis and further isolated at the GMP facility. Each patient's T-cells are then stimulated with a specific mitogen[4] in the presence of growth factors, allowing them to proliferate. This is an essential prerequisite step to retroviral transduction, a process by which T-cells acquire the genetic information encoding for CAR expression. CAR-engineered T-cells are then expanded for several days to hundreds of millions, to reach the therapeutic dose. At the end of the process, the cells are harvested, intensively washed to remove any residual material deriving from the culture media and, finally, formulated as a single-cell suspension. Upon completion of the Quality Compliance and sterility testing, the final drug product is released and then infused into the patient.

Who is involved in the process?

The HBI0101 CAR-T drug is manufactured and administered to myeloma and amyloidosis patients within the scope of the registered clinical trial NCT04720313. Point-of-care CAR T-cell production is very advantageous, since both clinical and manufacturing operations are performed at the same location, by experienced teams of the BMT Department, working in close coordination. The patient's journey begins with an initial clinical evaluation by a senior physician from the BMT Department, in the presence of a clinical trial coordinator. Once the patient is found eligible for the study, the treatment schedule is established. The apheresis procedure for a patient's cells collection is performed by highly experienced technicians from the BMT Department.

Dr. Stepensky's team conducts research and CAR-T therapy at the Bone Marrow Transplantation and Cancer Immunotherapy Department, Hadassah Hebrew University Medical Centre, Jerusalem, Israel. At the Facility for Advanced Cellular Therapy, Hadassah Medical Organization (HMO) in Jerusalem, they manufacture HBI0101, an anti-BCMA CAR-T-based drug product for the treatment of multiple myeloma and light chain amyloidosis. This product is currently manufactured and administered to patients within the scope of a registered clinical trial. Several members of the team contributed to answering these interview questions:

- Prof. Polina Stepensky, MD, Director
- Miri Assayag, PhD, Quality Assurance and Cell Collection Manager
- Shlomit Kfir Erenfeld, PhD, Research Laboratory Manager
- Nathalie Asherie, PhD, Research and Development, and Process Manager

"HBI0101 CAR T-cells are delivered as fresh cells, however, we are now working on the stability of the cryopreserved drug product"

1. *A procedure in which blood is collected and then returned to the donor after T-cells are taken out.*
2. *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 ensure processes comply with these standards. The same authorities control the authorisation and licensing of the manufacture and sales of such products.*
3. *Standard Operating Procedures (SOPs) are a set of step-by-step instructions that workers need to follow during routine operations. It aims to achieve efficiency, quality output and uniformity of performance and ensure compliance with industry regulations.*
4. *An agent that induces or enhances cell division.*

The manufacturing process is performed by a highly qualified team of experts (most of them with MSc/PhD degrees). The manufacturing process is tightly supervised by our Quality Assurance Manager who, at the end-of-process is responsible for releasing the final product. Throughout the entire procedure, the patient's health is closely monitored by a team of dedicated physicians and nurses and, if needed, more tests are performed or intensive care (in the case of high-grade CRS) is provided. Lymphodepletion[5] treatment is given five days prior to CAR-T infusion according to the clinical protocol approved by the Israeli Ministry of Health, in order to allow the CAR T-cells better engraftment and activity. CAR-T infusion is performed by a physician and a nurse. For now, our process is not automated, however, we have plans to move towards automation and a shortened manufacturing process. Shorter production time will not only be beneficial to the CAR T-cells, which would display a less exhausted profile, and thus perform longer once in the patient's body, but will also enable the manufacture of cells for more patients and reduce production costs.

How is the quality of the product ensured?
The quality of our HBI0101 CAR-T product is ensured via a series of Quality Compliance (QC) and sterility testing performed throughout CAR-T manufacture (start-of-process, mid-process and end-of-process). In-house QC tests are performed by our QC team, whereas sterility tests are outsourced to a GMP-certified external laboratory. The drug product is infused into the patient only when all the tests are completed and the results meet the final product specifications.

What could go wrong?
One of the major issues that could compromise successful CAR-T manufacture is the failure to expand the CAR T-cells to the desired dosage, either due to poor cell growth or low transduction[6] efficiency. As both parameters, cell growth and the transduction rate are tightly monitored during CAR-T manufacturing, we have - at a certain level - the ability to interfere with cell numbers (e.g., increase the number of cells at the beginning of the process, at transduction and at expansion). It is noteworthy to mention that, in the case of impaired T-cell proliferation related to a patient's intrinsic characteristics, it is possible that CAR-T production would fail. From our experience, all batches of HBI0101 CAR T-cells produced in our facility (close to 80[7]) were successfully delivered to the patients. Although, for a very few batches we initially were not able to get the targeted dose, we did not really experience a 'production failure'. In those few cases, owing to very close monitoring, we were able to react very fast to the low CAR T-cell expansion rate by initiating another batch in parallel to the one running, and in doing so, we were able to deliver the targeted dose in sequential portions, one week apart.

"The patient's journey begins with an initial clinical evaluation by a senior physician from the BMT Department, in the presence of a clinical trial coordinator. Once the patient is found eligible for the study, the treatment schedule is established"

Another risk associated with CAR-T production, is the detection of contamination[8] during the manufacturing process. In order to minimise the risk of contamination, all the operations of the manufacturing process are carried out in an approved GMP facility by highly trained personnel under constant monitoring, to ensure an aseptic environment and the sterility of the final product infused to the patient. Finally, end-of-process sterility testing provides us with the assurance that no potentially contaminated drug product will be infused into the patient. At patient-level, a rapid deterioration of the patient's clinical status could occur, and in that case, CAR-T infusion should be postponed until his/her status is stabilised. Since we deliver only fresh final products for the moment, this would mean that another batch will have to be initiated.

How many samples can you process and how will this evolve in the future?
For the moment, we are able to process four CAR-T products a month (one patient per week). However, Hadassah Medical Centre's GMP facility consists of three production suites. While today only one production room is dedicated to HBI0101 production, we are considering using additional rooms in the future to considerably increase our capacity. Delivery of our drug product as cryopreserved cells, rather than fresh cells as done today (awaiting approval from the Ministry of Health), will lead to another increase in our production capacity.

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?
For the time being, our product is manufactured as a clinical trial authorised by the Israeli Ministry of Health's strict regulations. As long as we operate in our current location regardless of the origin of the patients enrolled in the trial, we comply with local regulations that are not extremely different from those of the EMA or the FDA. If delivery of HBI0101 to the European market becomes an option, we will be able to comply with EMA regulations as well under the supervision of the Israeli Ministry of Health.

"We plan to shorten the manufacturing time, and to modify the manufacturing process in order to produce more naïve-like memory cells that have a longer survival rate in a patient's blood stream"

What do you think needs to be improved in the near future, with regards to how manufacturing is done now?
Our major goal is to extend access to HBI0101 CAR T-cells to as many patients as possible. To this end, we plan to increase our manufacturing capacity, and we are actively working in this direction. Another way to reach this objective is to use cryopreserved apheresis material as a source for

5. Treatment which usually involves receiving a short course of chemotherapy to kill T-cells, creating a favourable immune environment for CAR T-cells, which will prolong their persistence and increases the effectiveness of the therapy.
6. Process during which genetic material encoding CAR molecules is transferred to the T-cells using viral vectors.
7. Numbers updated on 30/03/2023.
8. e.g., bacteria, fungi.

CAR T-cell manufacture, rather than processed apheresis material as performed today. This will enable us to manufacture the CAR-T product in case a patient's status does not allow immediate cell production and infusion, or in case the collection is performed in a different country.In addition, we are working on a cryopreserved version of HBI0101 to make the final CAR-T product transportable to other countries. Finally, we plan to shorten the manufacturing time, and to modify the manufacturing process in order to produce more naïve-like memory cells that have a longer survival rate in a patient's blood stream.

What could be changed in the manufacturing process to improve a patient's experience?

HBI0101 CAR T-cells are delivered within the scope of the registered safety and efficacy phase I clinical trial NCT04720313. As such, and in order to ensure maximum safety and close monitoring of our patients, the hospitalisation time is pretty long, since it starts on the day of apheresis collection, throughout production, until CAR-T infusion and up to at least 11 days post-CAR-T infusion. We are aiming at shortening the time of hospitalisation, so the patient won't be confined to the hospital for such a long time, however, the cell infusion will remain an 'in-patient' step. When we are able to provide a cryopreserved version of the product, the need to admit the patient for an extensive time period will no longer be required, especially patients from other countries.

"To ensure maximum safety and close monitoring of our patients, the hospitalisation time is pretty long, since it starts on the day of apheresis collection, throughout production, until CAR T-cell infusion and up to at least 11 days post-CAR-T infusion"

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CAR-T Interview Series

The CAR-T Interview Series is composed of four different interviews. Read in the links below the following interviews:

- Janssen Pharmaceuticals
- Bristol Myers Squibb (BMS)
- Dr. Halvard Bönig, Translational Development of Cellular Therapeutics, Goethe University

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