CELL & GENE THERAPY INSIGHTS

IMMUNO-ONCOLOGY: MANUFACTURING & COMMERCIAL BUSINESS MODELS FOR THE NEW DECADE

SPOTLIGHT

INNOVATOR INSIGHT

Simplifying GMP CART and CAR NK cell therapy manufacturing processes

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There are currently around 550 active clinical trials utilizing CAR T cells. The industry is growing by as much as 37.5%, according to recent reports, and in terms of investment, almost $975 million has been spent. Two therapies are now approved, with more set to follow. Almost half of all clinical trials that were initiated in 2019 have a sponsor or involve collaborations, illustrating the importance of collaboration to this field: industry, academia and small biotechnology companies all have a role to play. But even as this dynamic field sees such promising growth and investment, questions remain over what the future of cell therapy manufacture will look like. There are emerging trends which provide clues – for example, the increasing use of allogeneic cell sources as off-the-shelf drugs are developed. This is being seen not only in CAR T cells but also in natural killer (NK) cells and even in macrophages. Switch receptors and control receptors are also areas seeing further development, and CARs are being developed that secrete a range of cytokines and enzymes, enabling them to migrate to different locations within tissues and tumors. Combination therapies may also prove to be key to the further success of the field. However, cell therapies differ greatly from small molecules and other drugs, and the way they are manufactured is complex and involves a variety of steps. Especially when using manual manufacturing systems, a lot of risk is introduced. This increases cost, as skilled staff and stringent manufacturing conditions are required. Concerns over manufacturing challenges associated with cell therapies, such as product shortages/delays that could threaten growth and directly impact the length of time to market, are growing within the industry. In this roundtable, six cell manufacture experts discuss the progress towards standardized and fully automated generation of gene modified CART and CAR NK cells – and address the remaining obstacles.

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Q What are some of the main differences and similarities in working with CAR T versus CAR NK cells?

SF: The biological and functional differences between NK cells and T cells ultimately have a significant influence on the production and manufacturing processes. Aside from this, the main differences between CAR NK and CAR T cells are in the selection process; T cells need a CD3 selection, and the NK cells first need a CD3 depletion and then a CD56 selection, in order to remove the NKT cells. The stimulation processes are also different in T and NK cells. T cells need beads for stimulation, while NK cells need cytokines like IL-2, IL-15, and IL-21.

We also have different time points for transduction processes. Where normally CAR T cells are transduced at the beginning of the process, NK cells are normally more efficiently transduced in later cultivation stages; day 8 is a very important point.

Next, there is influence of cryopreservation. When working with CAR NK cells, they normally have to be re-cultivated after they are cryopreserved, whereas when you work with apheresis material in CAR T cells, you can go immediately into the manufacturing process.

When you look at apheresis material and CAR NK cells, you have to keep in mind the role of impurities. That's a very important point when you work with feeder cells for instance, or if you have T cell impurities in your final product. Occurrences of side effects after transfusion into patients, such as graft-vs-host disease, may be higher than when you work with autologous CAR T cells. The role of pre-treatment and cultivation procedures will have a great impact on the fitness of the cells.

The cultivation times between CAR NK and CAR T cells are also different. Normally, CAR T cells are ready for use a little bit earlier than the CAR NK cells, and the in-process controls in the CAR T process are better developed than in the CAR NK process.

Q What are the key considerations and best practices in transitioning from open manual to closed automated bioprocessing in this particular therapeutic technology field?

> "When you look at apheresis material and CAR NK cells, you have to keep in mind the role of impurities."

KF: As a field we certainly need to see a shift in our approach to manufacturing. We need to move away from the manual open processing steps that we often associate with the early academic processes. These often include many open manual steps which can have quite long, complex protocols and expensive clean room requirements. Often many different pieces of equipment are required, along with skilled operators and extensive operator training requirements. A shift towards automated closed systems will reduce manual handling and contamination. Increased reproducibility in simplified tech transfer should be another goal – all of these changes are

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ultimately going to allow us to reduce cost of goods and improve patient access to cell and gene therapies.

In terms of best practices, you need to ensure that you know your product. You must fully understand the critical quality attributes, so that as you are making these changes, you are able to accurately predict and control how they are affecting both your cells and ultimately your product. You also want to make the changes as early as possible in the development process.

Engage with regulators early, and evaluate as many of the pieces of kit that are out there for automation as you are able. Ensure that your chosen process or your chosen equipment is fit for your specific purpose.

> "We need to move away from the manual open processing steps that we often associate with the early academic processes."

XW: This is something we're struggling with almost every day, especially in the academic setting, where we need to consider the upfront costs of a large instrument. There are so many challenges, starting from your supply chain. It's important to talk about whether it's the right decision to incorporate automation into your system.

Understanding the process is key – as is estimating the scalability of the process, and having staff members properly trained. For us, if we transition from an open process to a closed system, we need to first understand whether the supply chain could pose an issue. Not every reagent you use in an open process can be readily transferred into a closed system. For example, Dynabeads®, versus TransAct™ beads: you may have to choose one if you decide to use a different platform, and change of the manufacturing platform may require additional testing.

You must also have a plan for quality control – if you change from a manual process to automation, how easy will sampling be? At which point do you want to sample? Maybe your sampling plan will be a lot simpler if it's a closed automated system. The batch record is also a big part of the transition. If it's automated, there is in-line recording, so this may also make documentation easier. How easy you want it to be, and how much control you want to have during this transition period, are other important questions to consider.

UK: At Fraunhofer, we have a lot of experience with manual as well as semi-automated CART cell manufacturing. It is a lot of work, but on the other hand, if you have a very well-trained team, it also saves time and works very well.

I clearly see an advantage in using closed and automated systems like the CliniMACS Prodigy®, but for me it is not the end of the story. Right now, we are only talking about two types of disease treated with licensed CAR T cell products as well as a limited number of patients – either an automated or a manual process is possible here.

What is missing in the development of automated processes is AI-mediated digitalization for triggering the automation that we would need for hundreds of patients in parallel. This is of major importance if we want to address tumors, and not just leukemia and lymphoma.

We need to start with robotics and digitalization right now – and that is not just about the manufacturing process. The same question arises regarding complex quality control. These processes have to be automated and digitized so that everything, including documentation, is contained within an automated system. This is necessary to avoid mistakes.

Q What is the current technological state of the art in in-process controls (IPCs) and quality control (QC)?

MELS: Part of my job is listening to the requests and wishes of the field, and I think one of the main things I have been hearing in the last year concerns these IPC/QCs.

Taking a global perspective, the main issue is it is not harmonized. You can have different requirements when producing CAR T cells in Germany compared to the USA, or in China compared to South Korea. Now that we have multinational companies working in this area this is the beginning of a big challenge.

Regarding technology, I agree with Ulrike that we need to be looking into automated, autonomous robotics. There is a lot of potential to utilize block chain technology to transfer data and make it transparent. Artificial intelligence (AI) is already here, and we are seeing advances in big data analysis and digital platforms. However, in my experience even though some companies may already have the tools available, there is some skepticism and reluctance in the field to make everything connected and available – although I do believe this is where we are going.

We need to embrace it more, and address any concerns people may have about this technology. Especially for IPC/QC the potential is significant – I envision that at some point we could have automated sampling for which you don't even need a person to go into the GMP room. The sample could be taken automatically for you and transferred automatically into MACSQuant® Analyzer, for example. A robot could essentially perform the analysis and send you the results.

UK: It is understandable that people are cautious, because IPC/QC is not only focused on flow cytometric controls, but on a broad range of tests. We need an intelligent system – one that is flexible, modular and automated, that allows different manufacturers worldwide to use their own systems. Martha mentioned the MACSQuant® platform (Miltenyi Biotec) for the flow cytometric side, but there are other similar platforms here as from BD for example.

> "There are so many challenges, starting from your supply chain. It's important to talk about whether it's the right decision to incorporate automation into your system."

For a successful intelligent modular system, there must be interfaces between different devices. In the past, it has been difficult to set international standards for accreditation and validation in QC, and to have such a system work the needs of different manufacturing sites throughout different countries will have to be considered. In my opinion, this will not be easy.

XW: Another question I'd like to raise is the issue of scale out. For example, if we are trying to create allogeneic procedures and we are using a scale out approach, we're going to have many devices. What is the best approach to taking samples – do we sample from each device to show they are comparable, or do we choose one of them as the read out for all?

Further, how do we know instruments and other devices being used are compatible? We often see a lack of standardization in testing. To go a step further, are we

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happy with the surrogate readout of just the transduction efficiency of the CAR T expression or the CAR expression on NK cells as a release criteria?

We are also seeing people within the field report a two-day manufacturing process for CAR T cells – which does not allow enough time for the CAR to express on the surface. This highlights on obvious need for QC to be reconsidered.

> "We need to start with robotics and digitalization right now..."

UK: If you change to a two or three day manufacturing protocol, then you must also change the IPCC/QC procedures, because if you work with the normal lentiviral platform it's currently not possible from the regulatory side to give that product directly to a patient.

If you change the system you're using to a gene editing platform or use transposon sleeping beauty technology, then you can use that sharpened manufacturing protocol, because then it's clear when you have the CAR expression already in place. This is another kind of IPC/QC, in my opinion.

In terms of harmonization, the whole IPC/QC system needs to be modular. There is a variety of manufacturing protocols, both long and short, and differing transduction and even transfection systems, and so on. In a modular system, harmonization is possible because the minimum criteria can be the same for nearly everybody, and then you can add on the specific IPC/QC for your respective system.

Q What specific areas should be prioritized in the quest for standardization?

SF: When we look at the manufacturing process, we can start with leukapheresis, for instance. The time point of leukapheresis is very important, as is pre-treatment of patients. We do not have enough data concerning how the pre-treatment of patients influences the NK cell or T cell fitness, for example, and we should look more closely at when leukapheresis should be done.

Next, the selection processes could be standardized. What kind of cytokines and beads we should use, and so on. There are different protocols in the USA and in Europe – we also talked about harmonization, so is what we do in these different areas truly comparable?

Ulrike also mentioned transduction methods. We have the retroviral methods and lentiviral transduction methods, and the sleeping beauty, but what about CRISPR CAS technology, for instance? We do not talk about it, but it is probably more able to be used in a standardized way.

The expansion is very important and there are a lot of different protocols: cytokines, combinations, IL-2, IL-15 and IL-21, but nobody knows the exact time points. We also need to standardize formulation and cryopreservation. There are protocols with 5% dimethyl sulfoxide, or 10% or 7.5%. Nobody knows the "right" way to formulate the final product. When we look at the clinical side, there are a lot of chemotherapy protocols before infusion of our final product, which also have a great impact on the functionality.

The last point I would mention is that we have no standardized functional test assays to compare how effective our product is. Maybe we have the wrong functional tests. When we say the produced cells are functional, they may in fact be less functional if we use another test system – or possibly not functional at all.

There are a lot of points to be addressed where we could all work together to get the best results.

Q Turning to in-line analytical tools, what is the current state of the art, and where do you hope to see further innovation in this regard?

SD: In the field of analytical tools, there are standard tools such as automated cell counting, pH or dissolved oxygen. They directly measure one distinct parameter, but not all of these tools are firmly established and possible for use in in-line probes.

On the other hand, there are several powerful non-destructive in-line tools on the market, or under investigation, that use surrogate measurements. These include for example Raman, infra-red or fluorescent spectroscopy, and the procedure is the same for all of them. You collect data, and use it in a preliminary study together with manually measured data to create a multivariate model.

In this way, you can predict parameters that cannot be measured directly with the respective technology, for example glucose concentration. This means that with in-line probes, and a suitable multivariate model or algorithms within software, you can monitor interesting parameters without sampling.

In my view, in-line measurements would especially benefit critical process steps like thawing, or harvesting, or even cell collection. They would also be useful for the time consuming cultivation steps, and then you can utilize adaptive process strategies such as automated feeding.

Q How do starting materials affect the automation picture – and what are the strategies for measuring or minimizing this impact?

> "...in-line measurements would especially benefit critical process steps like thawing, or harvesting, or even cell collection."

KF: I think this question is affecting nearly everyone in the field at the moment. The problem is that we still aren't sure what qualities in the apheresis are going to make an effective high-quality product. We need to be retrospective and make sure we are compiling historical analysis; looking at which products in the clinic have a good clinical outcome, and performing tracing studies to see what the attributes of the apheresis were.

Validation is another really interesting aspect to consider. There are of course ethical considerations when using

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patient material for validation, and you often need large volumes of cells. If you need to use healthy donor tissue to validate your process, it's important to understand the differences between the patient samples and that healthy tissue. The problem you may encounter is that you are setting quite a high bar for your release criteria. We've heard from Novartis and other companies that a drug product is sometimes not meeting those release criteria, but then goes on to work well when it is infused into the patient. We have to make sure that the release criteria are realistic and take into account these differences between donor and patient material.

> "Taking a global perspective, the main issue is it is not harmonized."

Q What does the cell factory of the future look like, and where do you see the remaining obstacles to its realization?

SD: The cell factory of the future will definitely include automated and modular process plans, which are digitally controlled in some way. The modules should be flexible and connectible to adapt different processes, and AI-based robotics could be used to minimize manual steps. At points where manual steps are still included, virtual or augmented reality could be used as a guide.

As already discussed, an important point is also automated documentation. All process steps should be monitored, and this can be summarized in electronic batch records over the whole lifecycle of the product. This could facilitate and speed up the release in the end.

XW: What Ulrike just discussed is definitely my dream – and I suspect the dream of everyone in cell manufacture. My personal experience is that sometimes when we go towards automation, the instruments bring their own risks. I would like to see the handling/trouble-shooting of an instrument as simple as possible, and real time autonomic data generation/communication. It is a lot of pressure for people working on an expensive and important product. Therefore the simpler the design of the instrument, the better. Of course, we're talking about complicated procedures, but this would be my dream – making these complicated processes as simple as possible.

KF: Short term, I would like to see a better understanding of the properties of our cells and our drug products. This is likely to require better analytical testing, and perhaps a move towards functional systems that allow us to understand our CQAs better. Ultimately we'll be able to control these properties better and keep improving our systems once we understand them more.

Further into the future, it would be interesting to think about treating patients vein-to-vein at the bedside, but this is quite far away I suspect.

MELS: I want to work towards connectivity everywhere, by utilizing artificial intelligence, the cloud, and digital connectivity of all kinds. Ultimately the goal is to minimize the amount of risk as much as we possibly can when manufacturing these precious samples.

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