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In-Hospital Manufacturing of Cellular Therapies Using Automated Systems.
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Guest: Dr. Stephan Kadauke from the University of Pennsylvania and the Children's Hospital of Philadelphia.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Cellular therapies hold tremendous promise for the treatment of a wide variety of diseases but the current approach to producing these cellular products is time consuming and expensive, often requiring dedicated production facilities, specialized reagents, and highly trained staff. This production bottleneck limits the number of units that can be produced, and by extension, the number of patients who can benefit. Automated cell processing systems placed in the hospital allow for on-site production, which eliminates the cost associated with maintaining a separate facility and may increase access to those cell-based products for a larger number of patients.

As these automated systems become more widely available, clinical laboratorians will need to evaluate whether they fit the needs of their health care facility and if so, play a leading role in validation, quality monitoring, and regulatory compliance. A new Q&A session appearing in the September 2023 issue of *Clinical Chemistry* summarizes the current state of cellular therapy production and provides a glimpse of where the field may be headed in the near future.

In this podcast we are pleased to welcome the moderator of that Q&A session. Dr. Stephan Kadauke is an expert at cellular therapies with a focus on the development and manufacturing of these transformative treatments. He is an Associate Professor of Clinical Pathology and Laboratory Medicine at the University of Pennsylvania and serves as the Associate Director of the Cell and Gene Therapy Lab at the Children's Hospital of Philadelphia. So Dr. Kadauke, let's start with this. Briefly explain cellular therapies--how they can be used to treat disease.

Stephan Kadauke: Yeah, that's a great question, thanks Bob. So cell therapies, they're treatments. They are just like, in a way like medications. The thing is that they are living drugs. So the most well-known example of a cell therapy is a bone marrow, bone marrow transplant. So you take the marrow from a donor, you give it to a recipient, and then those cells, the

stem cells in the bone marrow that you've received as the recipient, they start to grow inside and start picking up the function of those stem cells, which is to make blood cells. So, they are a real living drug and CAR-T cells are similar to this. For CAR-T cells, androgen receptor T cells, you take T cells, you genetically modify them to express a protein that helps the T cell recognize and kill a target cell, for example a leukemia cell. These cells are alive. They may proliferate in your body and they're actively killing tumor cells potentially many years later.

So, cell therapies have lots of potential to treat different kinds of diseases, both malignant and non-malignant. So for example, you can use a bone marrow transplant to treat leukemia and solid tumors but also diseases like sickle cell disease or inborn immune deficiency syndromes, and similarly you can use CAR-T cells to treat leukemia since this is what the FDA approved therapies that treat cancers of the blood system, but there is now also trials in which we are trying to use the same therapies to target autoimmune diseases.

Bob Barrett: Now, you recently co-authored a paper about GMP-in-a-Box systems. Can you explain what they are and how they can transform cellular therapy manufacturing?

Stephan Kadauke: So let's unpack this GMP-in-a-Box thing a bit. So GMP stands for good manufacturing practice and that's a set of quality standards that you have to abide by when you manufacture drugs, including cell therapies, which are living drugs as we just talked about. The GMP covers all aspects of production of such a product including the materials that go into it or the facilities, their standards about what kinds of training staff needs to receive to create a high-quality product, so that's GMP.

Now GMP-in-a-Box, it's kind of a marketing term but it really what it means is that we want to apply all of these ideas, all of these principles and build it into a compact automated system, into a machine. So GMP-in-a-Box systems, they are typically benchtop devices. You can put it on a desk and they include the functionality to separate and genetically modify and culture cells that are meant to be turned into cell therapy products. For example, you can take a T cell product from a patient from apheresis as your starting material for making CAR-T cells and a bunch of reagents like buffer, culture medium, and other things, and then connect them to a single-use tubing set, plastic tubing set, or a cassette depending on the system. Then there's a computer that you tell what you wanted to do and a couple of days later you have a CAR-T cell product that's ready for infusion.

And so of course, it's a little bit more complicated than that but because it's automated, there's significantly less hands-on time than the traditional manufacturing process. So a traditional manufacturing process involves a lot of manual handling of cells and moving them into different bags and to different kinds of machines, and because of this just less hands-on time, there's less chance for error, and also less of a chance of contamination.

Bob Barrett: Can you elaborate on the challenges encountered during cellular therapy manufacturing and how GMP-in-a-Box addresses them?

Stephan Kadauke: Yeah, so some of the challenges that you encounter with traditional manufacturing are cost, like these things are really, really expensive. The price for commercial CAR-T cell product is about a half a million dollars and the price of a commercial hematopoietic stem cell gene therapy product is closer to two million dollars. So, these things are really, really expensive and part of the reason why they're so expensive is because you need very highly skilled technicians to make these products, and these products need to be made in a facility that has really complex and expensive air handling, and other kinds of processes to prevent the product from getting contaminated during the processing.

Now, the GMP-in-a-Box deals with this in two ways. The first is the automation, reduces the amount of technical skill and the amount of hands-on time that needs to go into making such a product because these systems are designed to really minimize steps that are open and open steps mean that you have some kind of a connection between the outside air and the inside of the products like an open tube or something like that. So, we're using closed systems in the GMP-in-a-Box systems. That means that we can operate at less stringent air handling requirements and that is a lot of the cost.

The last piece is that if you want to make twice as many CAR-T cells, you can put two processors next to each other and they don't take as much space as building a second clean room suite, which would be required if you want to double your capacity for making CAR-T cells or other cell therapies using traditional manufacturing.

Bob Barrett: So how can automated processing systems improve access to cellular therapies?

Stephan Kadauke: That's a wonderful question and I do want to emphasize that I really wanted to drive this point home and that's the reason why I asked discussions from different countries to contribute to this. So we had Martín Bonamino from the National Cancer Institute of Brazil in Rio De Janeiro and Ohad Karnieli, a

manufacturer of next-generation GMP-in-a-Box systems from Israel, on this paper.

Now, it's really related to the same kinds of things, its costs and facilities. If you're in an environment that doesn't have massive GMP facilities, then it becomes next to impossible to manufacture these products on your own. However, if you have a facility like a stem cell cord blood bank lab, and Brazil has many of these throughout the country, you can imagine putting one of these cell processors into a suite like this and manufacture your own CAR-T cells on site. And we have found that if you deploy such a process inside of a hospital system where you can do some of the quality control tests inside of a clinical lab that already exists, and you have an existing stem cell lab where you can put the machine that has some air handling and some clean room environment, you can cut down on the cost of manufacturing a product by more than 90% compared to what the cost of a commercial product is.

So, I think it's a really important topic that we need to solve in the cellular therapy field is how can we, now we have these wonderful therapies, how can we spread them through the world? And I believe that GMP-in-a-Box is going to have to be part of that strategy.

Bob Barrett: Okay. Well, finally Doctor, let's look ahead. What are the future prospects for automated cell processing systems and do you foresee any specific advancements in this area?

Stephan Kadauke: Yeah, so one hot topic in next-gen GMP-in-a-Box system and that is going to be Miltenyi Prodigy but there's all kinds of new devices coming out, and continuous monitoring is a huge trend. So basically, we want to be able to know not only what the temperature is of the culture as we are cultivating the product for, you know, up to 12 days, but we also want to know the CO2 percentage, we want to know metabolites, which is glucose and lactate and ammonia, and the cell density, at all times and newer GMP-in-a-Box device deliver on this.

This obviously can be really useful but there is also a real challenge to this with the question 'what are you going to do with all that data?' Well, one example is that we could potentially improve the timing of culture feeds by analyzing, you know, how much glucose have my cells eaten up already or how much lactate have they produced, so I can provide better timing for when I feed the culture. We could also, if we had a string of slower growing cultures, we can retrospectively look and if there are any characteristics, any things that were different, maybe the temperature was slightly off or something like this. So this is something that's coming. The next thing is the ability to manufacture other types of cell therapies. So for example, you don't want to

only make lentiviral CAR-T cells, which is a very common application for these GMP-in-a-Box Systems.

But you also want to be able to make hematopoietic stem cells such as gene therapies for sickle cell disease, and also cell therapy products that are made not using a virus but with other systems such as CRISPR-Cas-based nucleus and CRISPR-Cas-based base editors, and also direct transfer of messenger RNA or DNA using electroporation or other kinds of methods. So, basically, we want these things to be much more versatile for making different kinds of cell therapy products and then I think there's still development in making these machines more flexible in other ways. For example, remote access is something that's getting built into newer devices or you also potentially want to build a cluster of these things for parallel manufacturing. These kinds of things are being developed right now and I think they'll all together contribute to making it easier to spread cell therapies more widely and make it more accessible.

Bob Barrett: That was Dr. Stephan Kadauke from the Children's Hospital of Philadelphia. He served as a moderator of a Q&A session describing automated manufacturing of cellular therapies in the September 2023 issue of *Chemical Chemistry* and he has been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.