INTERVIEW

Progress and challenges in the manufacturing of CAR-T cell therapy

Dr Bruce Levine, Barbara and Edward Netter Professor in Cancer Gene Therapy, is the Director of the Clinical Cell and Vaccine Production Facility (CVPF) in the Department of Pathology and Laboratory Medicine and the Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania. He received a BA in Biology from the University of Pennsylvania and a PhD in Immunology and Infectious Diseases from the Johns Hopkins University. The CVPF develops and tests novel cell and gene therapies in clinical trials in patients with hematologic malignancies, solid tumors, HIV infection and genetic disease. First-in-human trials include the first use of a lentiviral vector, the first infusions of zinc finger nuclease genome-modified cells, and the first use of lentivirally-modified cells to treat cancer. Dr Levine has overseen the production, testing and release of 2700 cellular products administered to >1000 patients in clinical trials since 1996. Through these technologies, personalized and enhanced immunity has been engineered. T lymphocytes from HIV+ subjects have been rendered resistant to HIV infection and reinfused. T lymphocytes from cancer patients have been redirected with chimeric antigen receptors to hunt and destroy their malignancies, an investigational therapy that received the first Breakthrough Designation from the FDA for an academic institution and is currently in commercial development. Dr Levine is co-inventor on 23 issued US patents and co-author of >125 publications with a Google Scholar citation h-index of 66. He has been interviewed by the NY Times, Wall Street Journal, Forbes, BBC and other international media outlets.

Q The field of cell-based immuno-oncology has made some incredible advances over the last 5 years. Which ones stand out as the main highlights for you?

The key highlights in the field of immuno-oncology are the approval of a number of checkpoint inhibitor antibodies, and the emergence of engineered T cells, particularly chimeric antigen receptor T (CAR-T) cells and engineered T cell receptor T cells.

The ability to edit cells is another breakthrough in the field and we have conducted the first ever gene editing clinical trial on humans using zinc finger nucleases to knock out *CCR5*. But within the past few years there has also been the development of CRISPR-Cas9, which according to press releases has been used in trials in China, but we have yet to see the data. We will be the first center outside of China using CRISPR-Cas9 in humans.

Q As we move CAR-T therapies towards commercialization, what are some of the key challenges in translating from hospital-based manufacturing to commercial scale manufacturing?

I think the challenges include transitioning from an academic-based process and academic-based systems and regulatory infrastructure to commercial manufacturing and analytics, and the appropriate documentation and regulatory background.

What we did in our tech transfer process to Novartis was engage with the FDA in the design of transfer and comparability studies that would satisfy the FDA. That was successfully concluded and enabled Novartis to proceed with their own clinical trials, manufacturing and conducting analytics using their own processes.

Q In terms of facilities, what are the key considerations when transferring from academic to large-scale facilities?

All our facilities are designed to support a broad array of cell therapies, which include different types of T cell therapies, dendritic and mesenchymal cell therapies.

In the commercial setting, it's likely to be one process or a series of very similar processes. The facilities may be designed differently and some companies such as Novartis purchased an already built facility. Companies like Kite, Juno and AdaptImmune designed those from the ground up.

Cell therapy is a newly emerged therapeutic and it's not clear whether there is a favored facility design, but what we do know is that the FDA accepts a number of different designs. In Europe, the situation is more complicated due the step down between classifications requiring more corridor space with interlocking doors. On a gross area basis the area available for cell processing relative to the overall area is less in Europe than it is in the states and also in other regions.

Q What efforts are ongoing to optimize the speed of CAR-T development, such as using electroporation to produce a test CAR-T before switching to integrating vectors once efficacy is demonstrated?

What we have used electroporation for is to deliver RNA and enforce transient expression of the CAR and that allows us for

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interrogation of new targets that could be bright on tumor, but expressed on other tissues. It's a built-in self-destruct mechanism. So ordinarily in the design of our electroporated RNA CAR trials, the patients are given multiple doses of RNA CAR-T cells over 2 weeks. And if we were to see toxicity we could just discontinue and not give the subsequent infusion and then the CAR would not be expressed. We, therefore, view electroporation of RNA as a tool to validate new designs and targets, following which one can proceed to lentiviral (or retroviral) vector-mediated delivery to permanently deliver the CAR.

Another approach for gene delivery used by the groups such as MD Anderson and Ziopharm employs electroporation to deliver *Sleeping Beauty* transposon vectors and integrate CAR into the genome. There is much less data with the *Sleeping Beauty* system than there is with integrating vectors such as retrovirus and lentivirus. There are a number of unknowns associated with transitioning transposon-based systems from the academic process to the commercial process. So I think at least in the near term we're looking at improving the efficiency of generating lentiviral vectors and retroviral vectors. But certainly it's simpler to be able to deliver genes without the need for a viral vector, and electroporation is one way to do that.

> In our experience, long-term expression of CAR is better with lentiviral vectors than with retroviral vectors. Also, lentiviral vectors (in model systems) display a lower risk of genotoxicity.

Q What are the major benefits in using lentivirus versus retrovirus vectors for transducing CAR-Ts. And are there any drawbacks especially regarding manufacturing and cost of goods?

Retroviruses, murine leukemic viruses, were first used in humans in 1990. They do require the cells to be dividing, so that there is adequate access to the genes in the nucleus. Lentiviral vectors are able to transduce both diving and non-dividing cells, thus increasing their ability to transduce a wide variety of cells, including quiescent and difficult-to-transduce cells. However, lentiviral vectors require T cell activation to achieve increased transduction efficiency, and therefore these vectors are introduced during cell activation. So there are some differences in the state of the cell cycle that are optimal for the two vectors.

Retroviral vectors are more advanced in terms of the scalability of manufacturing. Lentiviral vectors are more toxic to the vector producing cell lines due to the genes necessary to be included in the vector as well as the VSV-G envelope. So the way to produce these vectors is through transient transfection of three or more often four plasmids. And that leads to some inefficiency when compared to retroviral transduction; however, there is a

lot of research being conducted now on improving the efficiency of lentiviral vector production.

In our experience, the long-term expression is better with lentiviral vectors than it is with retroviral vectors, and there is evidence that lentiviral vectors (in model systems) are less susceptible to gene silencing and possibly also less susceptible to insertional mutagenesis. This is due to the fact that lentiviral integration patterns are relatively random, while retroviral integration more frequently occurs near transcriptional start sites. So when you're thinking about a long-term durable cell product, vectors displaying a lower risk of genotoxicity is a good thing to consider.

Driving down the cost of goods comes not only with more efficient use of materials and more efficiently-producing materials but also with the implementation of automation.

Q How else can we drive down the cost of goods to ensure the commercial viability of CAR-T products? Could a decentralized manufacturing model be a feasible system?

Driving down the cost of goods I think comes not only with more efficient use of materials and more efficiently producing materials such as the viral vectors I just mentioneed, but also with the implementation of automation. The majority of the processes in manufacturing and testing of engineered T cells is very labor intensive. So the more we can automate, the more reduction in cost of goods we can achieve.

On the question of decentralized manufacturing model, I am asked this question a lot. It is inherently attractive for people to envision a box in every hospital around the world where you could generate CAR-T cells.

The main problem associated with decentralized manufacturing. is that you would most certainly need a completely automated process and the addition of the various reagents would need to be automated. That would need to be demonstrated in terms of comparability of the manufacturing. So if you're going to do something in Philadelphia you need to make sure it's the identical process to generate that product in Melbourne, Singapore, Dusseldorf or anywhere around the world. I don't think that is necessary when we can cryopreserve the apheresis product and the final product.

The second major issue is the analytics and testing. It is not just about having a decentralized manufacturing setup, you have to have decentralized testing facility as well. How is all that testing going to be performed and reviewed? For every release test carried out, you need to have an automated way to do it and how can you demonstrate comparability when you have it done at multiple sites around the world?

So in my view, decentralized manufacturing where people say this will be happening in every community hospital at the site is decades away. I think we will start first with centralized manufacturing per regulatory region, and as we get more efficient we'll be able to produce more patients

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from those centralized sites, and then there will be more sites built in various subsets of the regulatory regions.

Q What are some of the additional areas of active investigation in the development and manufacture of CAR-T cell therapy?

One big area of investigation is the discovery and validation of new targets for CAR-T therapy. These are targets that we know, but also neoantigen and other targets we don't know. There are groups that are investigating neoantigens and deriving T cell receptors targeting these neoantigens.

A second area is related to the element of control. We want these engineered T cells to go in and do their job to kill cancer cells and persist there, but we don't want the initial response to be so severe that there are life-threatening adverse events. That type of a control mechanism, whether you call it a switch, suicide switch or inducible expression, is required to modulate the expression of CAR. There are several systems currently being developed in academia and companies to achieve that. I think in the next few years that's going to be an exciting area to see new trials testing these control mechanisms in patients.

Another line of research is testing how the quality of the patient cells used for CAR transduction affects the efficacy of the final CAR-T cell product. Reduction of an exhausted T cell phenotype has been shown to be associated with improved CAR-T cell efficacy. Therefore, additional research needs to be conducted to devise the best strategies to generate the highest quality CAR-T cell product.

Q What is the potential impact of using gene editing processes on the manufacture of CAR-T?

Gene editing is an area of great promise for CAR-T therapy. To be able to edit CAR and engineered T cell receptors to knock in or remove genes or even add in accessory genes including control mechanisms I just talked about would make an impact to the field.

We have come to the possibility of transitioning from using viral vectors to deliver genes to using non-viral mechanisms to deliver the Cas9 and guide RNA, or other systems including zing finger nucleases, TALENs and mega-nucleases. The CRISPR intellectual property landscape is complicated, we've yet to see how that will finally play out when it comes to clinical trials and pivotal trials and commercialization of a product that would include gene editing.

Q As CAR-T products enter Phase 3 trials, how do you see the field emerging in the next 5–10 years in terms of CAR-T manufacturing?

We have already seen in 2017 submissions to the FDA for CAR-T cells from Novartis and from Kite. I think we're going to see additional submissions from those companies and others in the space in the next couple of years. And we're looking also to the inclusion of engineered T cell receptors as they advance into clinical trials, currently they are a bit behind in development compared to CAR-T cells.

And in terms of the manufacturing I think it gets to envisioning not only the integration of automation into manufacturing that many groups and companies are working on, but also thinking of this as a new type of drug that includes all of the logistics from vein-to-vein. So there are parameters involved in the collection. How do we demonstrate the comparability of the collection protocols, the logistics of the transport to the manufacturing facility and the transport back?

And then the area not to be overlooked is the reimbursement landscape. This requires some very deep thinking and the models for drug pricing and reimbursement where we have for the most part now, drugs that are given either continuously or in a series of treatments. There are very different considerations adapted for the reimbursement policies for gene therapies like Glybera and Strimvelis, that are one time or very few time treatment that has durable long lasting effect. That's beyond my area of expertise. But it should not be overlooked in its importance, and has political, economic and health policy considerations.

One last thing I would like to add is recognizing the efforts of investigators, not only at the University of Pennsylvania and Children's Hospital of Philadelphia, but around the world. In 2010 when we treated our first patients, there were just a handful of clinical trials of CARs around the world and now there are several hundreds. There are now, depending on how you count them, around three dozen companies developing engineered T cells or engineered natural killer cells in the field of cancer and infectious disease, and what we have witnessed is an explosion of interest based on solid clinical evidence and leading to significant investments. We owe it to the patients that enrol in our clinical trials to ensure that we are proceeding in the most effective way forward.

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