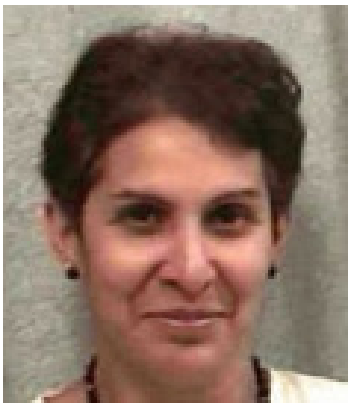
INTERVIEW

Perspectives on the use of ancillary materials for cell and gene therapies



SHIRLEY BARTIDO: Dr. Bartido obtained a PhD in Immunology from New York University and an MBA in Pharmaceutical Management. Her postdoctoral work at Memorial Sloan Kettering Cancer Center involved the development of DNA vaccines using the melanocytic antigen tyrosinase for the treatment of melanoma. Following her postdoctoral work, she joined the Carl Icahn Institute of Gene Therapy and Molecular Medicine as an Assistant Professor to serve as the Assistant Director of the Gene Therapy Immunology Core Laboratory. In this role, she developed several immunomonitoring tools for assaying efficacy of adenoviral directed immunotherapies targeting metastatic liver cancer. This was followed by an 11-year role as the Senior Quality Manager of the MSKCC Cell Therapy and Cell Engineering Facility. In this role, she developed the QA program for the development and GMP manufacturing of autologous CD19 Chimeric Antigen Receptor T cell therapies targeting several indications in leukemia and prostate cancer as well as gene therapy for the treatment of B-Thalassemia using lentiviral transduced CD34+ HPSCs. She was integral to the design and construction of a state of the art GMP facility at MSKCC. Presently, she is the Director of Regulatory Affairs at Cellectis Inc. The company is presently involved in the development of allogeneic CAR T cells as a universal off the shelf immunotherapy for leukemias.

Q What are ancillary materials in the context of cell and gene therapies?

SB: Ancillary materials (AMs) are known by different terminologies, such as ancillary reagents, ancillary products, process reagents, raw materials. They are usually biological and biochemical substances used in processes for manufacturing cell-based therapies and other therapeutics derived

from cell cultures. Importantly, AMs are not intended to be in the final product. In general, AMs include, but are not limited to, cell separation reagents, cell culture media and cryopreservation agents. Other examples are the disposables, such as plasticware and bioprocessing bags associated with many bioreactors in the market right now.

Cell and gene therapies are what we call 'More Than Manipulated products' in FDA jargon. Because of the manufacturing complexity, there are many AMs specific to these cell-based therapies. For example, the starting material is obtained from an apheresis collection and the patient's/donor's cells often undergo processes to isolate target cell populations. Another example is the AM used in for the introduction of genetic material into these cells either *ex vivo* or *in vivo* by utilizing integrating viral vectors (retroviral vectors), non-integrating viral vectors (adeno-associated vectors) or non-viral vectors that are DNA in nature (transposons). We also have gene editing tools such as CRISPR or TALEN®, that come in the form of messenger RNA. Third is the basic media that has a lot of human-derived materials added to it, such as human AB serum or human-derived serum that makes it more complex. As I mentioned, AMs must ultimately be removed from the final product. To do so, we use beads that are typically coated with antibodies to help sort and purify the different desired populations in the final product.

Those are some examples, but definitely not an exhaustive list, of the different AM components found in the cell manufacturing process.

Q How do AMs introduce inherent variability into the cell and gene therapy products and why is it so critical we make sure they are controlled?

SB: Anything that's biological tends to have a lot of variability. Take for example your donor material. Donor material can either come from a patient in an autologous setting, or from a healthy donor. That in itself is a variation. You will have the impact of intra- and inter-individual variations that are associated with how the product was isolated, who performed the procedure, what was the disease state of the individual, for example. Right now, it's becoming increasingly important to identify critical donor characteristics such as gender, age, weight, and in the case of CAR-T cell therapy for example, the ratio of the CD4:CD8 T cells is now becoming an important parameter to gauge. That in itself introduces a great deal of variability within just one component of your therapy.

You will also need to look at the potency of the materials that are used in media. Not all sera or antibody-coated beads are created equally. For

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example, some lots of serum may have higher transduction efficiencies compared to another batch of serum or to another brand. Similarly, variability in antibody ratio on beads can also have an impact, one lot may work out fine while another lot may not. Therefore, these types of materials need to be controlled in such a way that they are tested for the application prior to being purchased or ordered usually in bulk.

As you can see, that is a lot of variability to consider. One more source of variability that I'd like to point out, which is not getting much attention is the disposable plastic material used in these processes. Most processes use single-use disposables because it doesn't require complex cleaning validation and is therefore much easier. But there is not enough attention being paid to how much material is extractable or leaching out from these plastic materials, and how much is present in the final product. Again, because AMs need to be removed from the final product, as we go through processes we also have to think 'How can we remove this?'.

Q How are AMs currently evaluated and regulated?

SB: AMs are evaluated according to several parameters. One is safety, in particular concerning the use of human- or animal-derived AMs. For example, analyzing the AM for the presence or absence of adventitious agents such as bacteria, human viruses or porcine viruses is critical. Second, AMs are evaluated according to identity. It's easier with a chemically defined AM such as basic medium, for example. But it becomes more complicated with products such as fetal bovine serum (FBS), human serum, human AB serum, because this is not just one ingredient and all the ingredients have to be identified as much as possible. Third, potency of the product has to be evaluated. More often than not with critical AMs, such as cytokines, sera, etc. batches are tested with the process to determine which AM lot works best. Last, but not least, is evaluation of the product purity. Usually this depends on what the product is. If it's a protein you can have protein assays to identify how pure the product is. In terms of DNA- or RNA-based material, one way is by utilizing whole DNA sequencing. Chromatography is another technique that can be used to determine purity of the product. These four parameters: safety, identity, potency, purity are the key parameters on which AMs must be evaluated.

Q Have there been any noticeable changes in efforts to control AMs within the past few years and where do you think the industry is headed?

SB: There has been continued growth in the cell therapy industry. Notably, the commercialization of cell therapies, with the two approved CAR-T products in the market by Novartis and Gilead. This has led to increased awareness of the need for defined, specialized and complex materials utilized in the manufacture of cell and gene therapies that are continuously being developed. For example, growth factors, the DNA and RNA molecules needed for gene editing, and the introduction of chemically defined medium.

There are also many grades of AMs that range from research grade to clinical grade, for example. One of the issues is that many AMs are initially not approved or intended for clinical administration or use. They are often labeled for research-use only. Therefore, it becomes important to start defining the different grades of AMs, ranging from what's typically called laboratory grade, research grade and good manufacturing practices (GMP) grade. But the complexity doesn't end there – there are also many different 'flavors' of GMP-grade AMs. I'm certainly seeing a shift as more clinical trials and processes are being developed, the industry is forging into the area of having these reagents manufactured to GMP standards.

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Q How does the stage of development of a cell and gene therapy influence the quality of AMs that are used?

SB: Because AMs come in contact with cells that are intended for clinical administration, the quality of the AM used will definitely affect the potency and purity of the final cell product. The long-term feasibility of using a given AM in a clinical setting must be considered through a risk-based approach at each stage of the development process of cellular therapies. AMs must be evaluated on the basis of various criteria, including and not limited to suitability in a given application, composition, compliance, cost, availability, packaging and also, ultimately, its risk to patient safety. The more patients involved in a given stage of a trial, the more stringent the evaluation becomes.

Q Do you have any advice on how therapy manufacturers and AM suppliers should navigate this landscape?

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SB: An inherent problem within the industry is the lack of governance and consistency. On the user side, it's very important to use AMs that have been carefully selected and appropriately qualified during cell therapy development. To do this, users must investigate and fully understand the claims made by a supplier. It is very common that suppliers can have different definitions and interpretations of standard terms for quality claims. For example, the collection source for FBS may vary from supplier to supplier. Cytokines are another good example as they can have very different activities depending on the supplier. So, it's very important that there is early and continual communication between users and suppliers. I find this to be very critical so as to make sure expectations are aligned.

The dialogue between users and regulatory officials is going to be important in defining the user qualification requirements. Eventually users will need to hold suppliers accountable for all their labeling and marketing claims.

Q Are there key differences between global territories and how will the industry bridge the gaps to achieve standardization?

SB: There is guidance that describes both quality and regulatory requirements for the manufacture of cell therapies. But the present regulations do not specifically describe the quality requirements for many of the AMs. They do, however, provide a framework for strategies to control these starting materials through guidance on their eligibility in producing functionally specific cellular therapy products.

There are a number of inconsistencies between regional and international guidances, but in general many of the agencies do consistently recommend that the highest grade of AM available that performs as required should be used in a given application.

It's important that cell therapy manufacturers use a risk-based approach to qualify AMs. For example, in addition to testing for functionality of the AM, appropriate testing to demonstrate that AM removal from the final cell product can be carried out efficiently and that the resulting final cell product is safe and effective should be carried out. In an industry where no standard regulations exist, it becomes vital to prioritize patient safety above all else, and work with individual stakeholders to define applicable compliance requirements for applications on a case-by-case basis.

Ultimately, it's patient safety that is the responsibility of regulators that work with AM users or sponsors of an investigation or commercialized

product. Regulators will generally hold the users responsible for working with their suppliers of reagents and materials to ensure that the compliance requirements as defined by the regulatory authority have been met.

Q What challenges in raw and starting materials management need to be overcome for decentralized or point-of-care manufacturing of CAR-T therapies?

SB: There are two types of CAR-T therapies – autologous and allogeneic. For autologous CAR-T therapies you obtain the starting lymphocyte population from the patient, introduce a genetic modification by using vectors so as to get the T cells recognize the cancer and then putting this back to the patient. This is certainly in the realm of point of care. For allogeneic therapies, starting cellular material comes from a healthy donor and is manufactured similarly to that described for autologous CAR-T cell therapies. But with allogeneic therapies there is more flexibility because patients are not really waiting, counting the days to get the product and this approach is considered decentralized manufacturing.

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One issue that becomes very glaring is the supply chain because of the materials that are needed, especially in terms of vectors. Vector manufacturing is currently a lengthy process and the sector is experiencing a supply and demand discord. There are queues as long as several years to make vectors that are needed to transfer genetic modifications into these cells. So, the supply chain becomes a major limiting problem in getting these critical AMs to the manufacturing station or facility.

However, people are starting to come up with a variety of solutions. In the area of vectors, investigators are looking beyond the usual lentiviral vectors used in the early clinical trials for CAR-T cell therapy. Now they are looking at adeno-associated viruses and non-viral methods of delivery such as transposons, *Sleeping Beauty*, for example.

Another area where considerable work is being done with AMs is using components that are purely chemically defined. For example, developing serum-free products to replace materials such as bovine serum or human AV serum, with something that is chemically defined. If chemically defined materials are used, their components will be easier to track and remove at the end of the process.

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Q What advice would you give to companies entering the cell and gene therapy space?

SB: It's evident that the early cell therapy research innovations and initial clinical trials have really opened up a very big industry. You used to count on one hand the number of manufacturers you could source AMs from. Now there are so many companies offering their products and it can be a little confusing – for example, it may be the same product, but everyone has a different nuance or naming convention. However, the growth in AM providers is helping to create a much more competitive environment for media, bioreactors, plastics, single-use disposables etc.

It can be overwhelming, so it's important to establish the relationships with your suppliers to really get to know the products and how they're made. It will save you a lot of grief later on when you start working with the regulator.

I also advise that when in doubt, reach out to the regulator as early as possible. In Europe, you can initiate a scientific advice, in the USA you can have what we call pre-IND meetings. The regulators are there to help.

This is a relatively new field, only 10–15 years old with a lot of potential, but there's still a lot of work to be done especially in the area of comparability, uniformity, potency and characterization of AMs.

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