CELL & GENE THERAPY INSIGHTS

EXPERT ROUNDTABLE

Overcoming challenges in analytical assays for cellular therapies

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Jara Joedicke is an immunologist with a passion for the development of novel cancer therapies. She has over 10 years molecular biology experience in both academia and industry. Throughout her career she has managed a variety of employees from students and postdoctoral researchers to technical staff. Her aim is to bring novel cell and gene therapies rapidly from the bench to the clinic, especially those utilizing CAR T cells.

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What are they key trends and challenges facing the development of analytical tools for cell therapy manufacture, and what are the next steps for the space? In this expert roundtable, three experts discuss strategies for overcoming obstacles, best-practice approaches, and the importance of collaboration and standardization.

Q It would be great to begin by framing the key trends and advances with analytical tools for cell therapy manufacture. What do you regard as the most significant current challenges and directions for new innovation?

EA: This is a very timely topic – cell therapies are extremely complicated to make and require a lot of testing. It is a change in mindset; these are living drugs. At the top of my mind are tools to measure potency, for example. As far as analytical testing goes, these drugs have multiple mechanisms of action, so how do you capture that in an analytical testing lab prior to releasing the products for patients?

A lot of these tests are complicated to implement, and not all of the assays are completely worked out. The challenges around potency and how to measure that accurately are some of the most important issues. New innovations and cell-based assays that can do that, in a timely manner, are probably where a lot of focus needs to be.

MM: Another very important point to consider is the time to market. There is a lot of pressure on all of us who are making CAR T cell products, so the race is on. Everyone wants to be first, and of course, that doesn't always leave us lots of time for the corresponding assay development.

What we also see here is that people are using assays that are already established for other purposes, and trying to repurpose them. But they are not tailor-made to what you want to measure. I see that as a challenge. I think sometimes it looks easy to do, but in the end that might backfire and leave you with complicated and slow assays.

As Eric said, we are dealing with living drugs, and we have donor variability in the autologous setting, which of course doesn't make things easier.

I would vote for anything that makes the assays more robust and standardized. This will help you in the end because you always have to deal with donor variability, so you want to try and minimize any other variables as much as possible.

That brings us to reagents and tools. Miltenyi has a dual role here – on the one hand running clinical trials, but also providing tools that we developed for our own CAR T cells, which we also want to spin out for the market.

In the future, there might be tools that will be specifically for CAR T cells, such as manufacturing and quality control (QC) assays. I would also predict more and more movement towards automation of QC assays.

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JJ: I would add that cost reduction is a big topic. Also, the timely translation of these assays is extremely important, especially from a research or academic perspective. We already have to prepare assays in such a way that we can translate them to a contract manufacturing organization (CMO), because we cannot produce these products ourselves.

For us that it is a very important topic, because this is expensive. If we already have assays established in the research labs that are easy to translate and validate at a CMO, that is very important for the field.

Q How would you define the cutting-edge toolkit and best practices for potency assay development?

MM: Probably the biggest challenge with a potency assay is developing assays that correlate with clinical efficacy. We are hoping we will get there one day, but to my knowledge, there is nothing out there yet.

You are highly dependent on reliable reagents, because there is already variability from the donors. It is also important to choose assays and machines that fit best to what you want to analyze. There are many approaches out there regarding how you can define potency and how you can measure it, and many people have different potency assays in their pipelines. But whatever you are doing, I would certainly suggest that you use the assay that fits best to your questions, and also the machinery that goes with it. That goes again to reducing variability.

Looking at best practices, I think that is also something people are struggling with, and we also ran into that. You need to know your regulatory framework. You have to familiarize yourself with that to know what you are doing and where you want to get to. Keep it as simple as possible, then focus on the development and try to standardize as much as you can.

EA: When it comes to developing cutting-edge toolkits, there are a lot of new advanced technologies coming. These drugs are complicated, and the current tools that are being borrowed from monoclonal antibodies and small molecules are insufficient.

On the other hand, we often have to transfer these methods to a QC lab to perform routinely. And so, cost and complexity are common challenges when implementing cutting-edge tools or sophisticated approaches.

Trying to thread that needle to do your best on capturing potency in a way that is suitable for regulatory interaction, but could also be implemented seamlessly without issues around deviations, or operating systems, or any other technical quality challenges that might happen in running a complex assay, is a challenge.

Trying to think about these complicated things in a simplistic manner is the hardest thing to do, but it should be at the top of our minds. Although you can use a complex approach to understand things, you have to remember that some things need to be done routinely. Striking that balance is a big challenge, and I think it is going to take both innovative thinking from wet and dry lab scientists, and some new tools.

Q In your experience, is the guidance that comes from the regulatory bodies quite clear-cut? We have heard mixed reports that while agencies are very engaged in trying to help

that clarity, the field is moving so fast that it is sometimes challenging for them to keep up.

EA: You have hit the nail on the head. We are moving at a breakneck pace, and it is hard to keep up. Every day there is something new – for example, new editing technologies coming out almost every other month.

In any case, the guidances are there to guide. They are not there to give you the answers. It really takes an eyes wide open approach early in product development with that in mind.

Sometimes that doesn't happen, and that is why it is really important for a quality or analytical development/CMC team to be interfacing with the research teams early on; to understand what the product is trying to achieve and how best to measure that. Hopefully, that allows you to work with your regulatory teams to make sure you are operating within a boundary – whatever that might be, sometimes it is ambiguous – to be successful in delivering your package and to be able to accurately quantify safety and efficacy.

Again, we are in a new space. Cell therapy is often thought of as something that has been around for a while, and everyone is doing it so it should be all figured out. But the truth is we are really in the early stages, still. As technologies and product concepts evolve, it requires everyone to stay even more up to speed.

It is a challenge, but early planning and taking a sober view of what you are trying to do is the best approach.

JJ: As Eric said, you really have to think about these regulatory aspects early on. Even in the academic field, you have to already have an idea about the regulatory guidance in order to structure your assays in a way that they might expect.

Furthermore, I believe we have to come away from these long and tedious assays that delay product release in the end, towards assays that are easy to establish, easy to run, and fast.

There is no assay so far that gives a complete clinical translation or response; we have to adjust our assays to a CAR T cell product. What I see in the academic field is that we are able to do a lot of assays. We are doing chromium release assays, for example. But is that really feasible for product release? We have to come up with new ideas for these assays, and perhaps come away from cellular targets for potency assays.

Q Could you share your thoughts on the need to adopt a less isolated approach to cell therapy potency assay development?

JJ: We have to go more towards assays where target cells are replaced by, for example, artificial beads coated with a peptide. That is an interesting development and needs to be adapted as it's not necessarily available for every product on the market so far. Furthermore, we need to take care of lot-to-lot consistency in this aspect.

MM: As Jara said, you probably need to have an assay tailored to your potency testing. It would also be great if it was possible to somehow harmonize that across different manufacturers. I am aware of one consortium, T2Evolve, that is trying to harmonize regarding cellular therapy, at least across Europe. This is a good start, as

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academia and industry are coming together to develop standardized assays as much as possible. It would be great if there was more going on in that direction.

Q Eric, do you think this is going to be achievable in such a propriety environment, and do you think harmonization is the goal we should be looking for around assays?

EA: We have already touched upon avoiding a siloed approach within an organization. For example, having regulatory input early on. Cross-functional interactions between different teams in cell therapy are required to be successful. That is one way to break down the siloed approach.

But we have also just discussed consortiums and organizations that collect information, or have people come together to talk about the challenges to try and standardize things. There are challenges there too, especially in the competitive world and space we are in. Sometimes it is difficult to do.

One doesn't necessarily want to teach the world how to do everything. That is one of the realities we all have to operate under in this competitive space. There is a middle ground there, and I am certainly trying to work towards that in some of these organizations as well, by providing input and thoughts.

Assays are one of those things that people probably want to keep to themselves a little more, typically because that would give someone a great advantage if a breakthrough is made. But while there is a push and pull, but we are all humans trying to help other humans, and we want to do our best to try to share ideas in order to develop the best therapies possible.

Q Let's discuss some of the key challenges you encounter in the adoption and implementation of state-of-the-art technologies for cell therapy product identification, characterization, and potency testing. What is your advice for addressing them?

EA: My previous role at Bluebird Bio was to do drug product characterizations and build that capability for our oncology programs.

When we think about this living drug concept, we are talking about hundreds of millions of cells that are individual. They are going through their own stuff, they are in distinct cellular states, and this is not a static thing. It is very dynamic, in fact. The drug you start off with in manufacturing is not going to be the same thing 5 hours later. So, how do you measure this? And how do you

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use technologies to understand this in the lab and in the clinic?

The key challenge is that these newer technologies that allow you to disentangle these complexities are relatively new. I don't even think the complexities are necessarily in the wet lab – they are actually in the data analysis world.

There are something like 17,000 different computational pipelines that exist to analyze drugs and cell therapies. How do you know which one is the right one, and how do we standardize that analysis? You can take the same data, use a different analytical pipeline, and get a different answer.

We talked about standardizing wet lab potency assays a minute ago, and I think one of the bigger challenges in these advanced analytics is standardizing the data analytics in a way that doesn't stifle innovation.

MM: There is one thing we always should keep in mind, and that is the distinction between doing research on your CAR T cells or cellular products, and what you need in the end for your QC release testing. Many researchers get carried away by how interesting it is to define what your cells look like, but that might not be what you need.

Of course, we all want to do as much research on the cells as possible and to squeeze out as much information as possible. It is great to do that on the side, and any information we get, and any knowledge we generate, is great. But I would advise not to lose focus on what you need when you want to build up an assay for the release test.

JJ: I totally agree that you have to standardize this and narrow down the actual panels you need for analyzing and release testing. For example, for the product characterization, perhaps we should not let people analyze the data so that the analysis is fast, and not error-prone due to a different technician analyzing the data on a different day.

Q What are the chief considerations when bringing analytical assays into a GMP environment, and could you share any examples of your approaches here?

MM: It is key to consider all the regulatory requirements very, very early on. That requires closely interacting with the QC lab that will be running the assays.

What you are doing in your research R&D lab does not always work in a GMP environment or QC lab, and some people have learned that the hard way. Constant feedback with the QC or GMP QC lab is key.

We may have an advantage here because we are a tool provider as well. If we do need special reagents, in many cases we can develop those ourselves and have them produced for our own purposes. One example that comes to mind is that for flow cytometry we are using dried-down pre-mixed cocktails.

Of course, that makes it easy because we are certain that in our development and in the QC lab they will use exactly the same reagents. That then abolishes any pipetting needs, so we can make sure there is a high degree of standardization during development, and then during routine assay development.

One more thing to mention is automation. This is a tricky one. Automation might not always fit into the GMP and QC environment, and you have to think about how you are doing your validations on automated

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platforms. On the other hand, I am sure that this is the way to go in the future.

EA: Again, I think we are all on the same page here, and these are great examples.

I am a former R&D person myself. You want to do all the fancy stuff and learn a lot about your product, but as you translate it to more of a clinical program, those assays don't always translate well.

When you talk about tech transferring a method to a GMP lab, whether it is internally or externally, my advice is to start that process as early as possible. With a method, in particular, have a clear standard operating procedure (SOP), and procedures that are well thought through and communicated to the QC lab.

I would add that the critical reagent aspect is key. There is sometimes so much variability between batches of antibodies, or whatever you are using, that even if you have a good SOP and technology transfer, if you drop the ball there, you can get very different results and run into all kinds of challenges.

It is incumbent on sponsors to think through these challenges, and it is very valuable to have cross-functional communication, from research to CMC to regulatory, to inform the best way to bridge these assays into a real, routine testing environment.

JJ: We have contacted regulatory personal very early on, especially to get advice on our products, and we recruited people into the team who are very experienced with that. It is unusual, and not that easy in an academic setting. But we managed to do it, and it has helped us greatly to standardize and also to make the translation a lot easier later on.

Furthermore, we are also trying to use as close to GMP products as possible. This is sometimes an economic challenge, especially for a research lab, but for us it has worked very well. It comes with some drawbacks in that you cannot analyze everything that you want to. Therefore, we have not used antibody cocktails, for example, because that would limit us too much. However, I can see the benefit of that for later stages.

Q How are strategies evolving to ensure fast batch release of cell therapy products, and where do you see future gains being made in this regard?

EA: Turnaround times for fast batch release products are one of the bigger challenges that the field is facing.

These patients are in desperate need of help, and often don't have a lot of time when you are doing a Phase 1 trial. When you have these patients in your trial, there is not a lot of time to get the cells from them in an autologous setting, manufacture them, then test and release, in the timeframe they have before it is too late. This is a critical thing that we need to holistically work together on.

Another thing that I want to add about the challenges in analytical technology transfer is that assays are becoming more complicated. And as we touched upon earlier, they do require some type of data analytical software pipeline.

That is going to become more prevalent with automation. How do you interface all of the new data software analytics into a manufacturing setting or testing release environment? That is one of the other challenges that could also help solve the fast batch release challenge.

It is a double-edged sword. If you don't do it well, you might actually prolong things. If you do it well, you might be able to use it to your advantage. All in all, this is a general

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- Jara Joedicke

challenge that we have to work through, and having a clear-eyed vision about this *a priori* is going to be helpful.

There are other things that sponsors can do to address this issue. Oftentimes, the people who make the products know them best. One of the strategies we are taking at Bluebird Bio is to try to internalize as much testing as possible in order to reduce back and forth with outside organizations.

This does come at a cost, but it is a good approach to take if one is able to. There is no one solution to this problem, and it is going to be one that we continue to face as these product concepts become more advanced.

MM: Going one step further, it would be great to automate not only data acquisition but the data analysis itself as much as possible. Then at the end it would only require a critical review of the data by a qualified person who can then release the product.

In the future the analytical machines might be able to talk to each other, or even talk to a centralized platform, software, you name it. Because one of the big hurdles eating into time in the QC labs is the documentation. Streamlining as much as possible here will also help with a fast batch release.

JJ: I would add that we also need to take care of other release testing aspects that take a long time. We need to shift to other, faster, methods. For example, instead of 2 weeks of testing for sterility, taking a day or two by switching to PCR testing.

We always analyze in-process controls, just in case we might be able to use these for early batch release. This is just something we are developing on the side. If we are really sure that our process is stable and reliable, so that we can use these in-process controls for an early release, that might be quite critical.

Q Jara, you mentioned earlier that keeping costs as low as possible is an important factor for consideration. What would you define as the next steps forward in trying to drive down those costs?

JJ: One aspect is to cut down on all of the analysis that you do during the process, and concentrate on the critical parameters. In development you can analyze everything that you need, then you specifically cut down everything that is not needed. This will definitely cut down cost, especially if it is developed at an early stage, and then later on these things do not have to be translated to a CMO, for example.

Additionally, staying on the same platform can help. For example, for potency assays, if you stay with a FACS-based potency assay, then you already have the device available, since FACS is needed for the description of the product. It definitely helps to standardize this, and can cut down on the cost for materials and machines.

Furthermore, automatic analyses will definitely cut down staffing costs, which will

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drastically help in cutting the costs for the products.

MM: It is also very important that the assays are robust, because you don't want to have any failures, and then people have to repeat these assays because something went wrong.

You also want to make sure that you have the best reagents possible. You also don't want to start fiddling around if there is a new batch coming in, and then you have problems with your testing.

It is also extremely important to monitor your machines. You want to know that the machines are always in their prime condition and that you can rely on them. You don't want to have any downtime or have to look for additional machines, because then you are in big trouble and that is certainly not reducing costs.

Q Eric, how prominent are costs in your mind when you're looking at the analytical processes?

EA: Cost is a huge factor. You can't do anything without money, so we need to be real about it. With that in mind, I think that costs aren't going to go down. They are just going to keep going up, especially with the supply chain issues we have seen during the COVID pandemic. With cell and gene therapy being so promising, everyone is in the game now. There is a lot of competition, and costs are going up everywhere. But as you develop your product, there are a lot of things that sponsors can do to mitigate explosions in costs. It comes back to planning, and having a very sober approach.

We heard about reagents, and we heard about instrumentation. Oftentimes, what I see is that if you don't have a good plan or good communication between groups, things go all over the place. Looking at your bottom line, it can be quite expensive to do a lot of advanced fun stuff, and outsource, and do a lot of the great technology-driven stuff we talked about before. And if it doesn't translate to anything, particularly in a QC lab, what good is it? That money could have been allocated for something else.

Being honest with yourself and planning ahead of time are the best ways at this moment to keep costs under control.

Q What do you anticipate will be the most significant points of further evolution in terms of the regulatory landscape and the analytical requirement for cell therapy products? Do you have any take-home messages in terms of how to best prepare for them?

MM: What I see coming in the future is the regulatory bodies asking for even more in-depth characterization of your cell product, and probably also that you better define what potential impurities could be in your product.

As a take-home message, what we have seen is that in many developments, people jump on the cell manufacturing first, and the assay development is a bit behind. It can't be stressed enough that even if cell manufacturing is the fun part, you need a robust QC

release strategy there, in place, at the time when you need it.

I remember once I heard, and this still rings in my head, that often the assay development is not done when it is needed, but when time is up. Of course, that is totally understandable. We all have timelines and time pressure. But if you are not careful, in the end, it can break your neck. I would stress not to compromise on assay quality.

EA: It goes back to planning. In our own experience at Bluebird Bio, the analytical challenges have often generated the most regulatory interactions, and if you don't prepare and do all the stuff we have been talking about, that could really hurt you.

That said, we are also all learning how to do this. Nobody has a crystal ball, and there is no roadmap. If I were talking to my team, or to young people getting into this space now, I would also say that we need to have new ideas. We need bold innovations and bold plans, and a rethinking of things.

We are all doing killing assays the same way, but we all know that it needs to be improved. One thing we are trying to do in my group is to dump the bag out and see what is good and what is not good, work on the good stuff, and recognize that we don't know it all. Collaboration with academic institutions, and with our research groups internally, will help facilitate better assays in a timelier way during production development. This will also allow for a more seamless technology transfer to a QC environment.

There is a ton of stuff to solve. It is hard to have any one-sentence parting words, other than that we have to stay engaged. Oftentimes we borrow practices from very well-established fields that don't necessarily fit perfectly in cell therapy. It requires people to challenge each other internally on assumptions that X, Y, or Z are necessarily going to work for cell therapy, particularly when you have gene editing and all these new things like combination therapies, very advanced molecular structures, and on/off switches.

It is very complex, and therefore it can be very hard for a CMC group to keep up with the research team. That is also true of our health authorities – it's a ripple effect.

I don't know if there is a greater solution other than to stay engaged, understand the landscape, and be as sober as possible about it. Stay rooted in reality about what those challenges are, so you can direct your resources appropriately.

JJ: We have to focus more on the communication between the different fields, and see where we can take the assays from. What can we develop, what is important, what do we want to find out, which assays are the robust ones, and which are the most feasible ones? This evolution is ongoing. There are not any predictions I can make in that respect, because we haven't found the solution yet. We are still looking for the assays we need. We know we need the potency assay, but which one is the best for our specific product? Or do the authorities want to see specific assays? This is not set in stone yet. Communication is one of the key aspects to keep in mind to establish these cellular products.

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