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Cell Therapy Analytics: Overcoming CMC Challenges

A Conversation with Akshata Ijantkar, Senior Principal Scientist, CMC Team Lead, Cell and Gene Therapies, Bristol Myers Squibb

ABOUT

Akshata Ijantkar is senior principal scientist, CMC team lead, cell and gene therapies at Bristol Myers Squibb. Here, she shares her experiences and provides advice on how cell therapy companies can develop better flow cytometry assay strategies for cell therapies including: (1) What is/isn't phase appropriate; (2) Understanding regulatory implications; (3) FMO controls and why you need them; (4) Qualification attributes—How many experiments is enough.

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Dominic Clarke: Thank you for joining us today, Akshata. Before we jump into our discussion about flow cytometry can you give some background on your expertise in the field?

Akshata Ijantkar: I started with vaccines and started as a cell-based person in the beginning, then came to flow. Over the years, I've learned so much from the people I work with and give credit to my co-workers, my managers—so many smart people. I started with Juno back in 2016. I worked on the entire Breyanzi journey and it became like a family—I call it the Breyanzi family. It was a commercial product and I don't work on Breyanzi anymore. I then switched from flow cytometry assay development to becoming the analytical lead for one of the gene editing programs by BMS. And, right now, I just became the CMC lead for some of the other assets are in the pipeline.

Clarke: When it comes to your experience and thinking about flow cytometer assay development and cell and gene therapies, what is and/or what isn't phase appropriate? What does the term really mean? And how have you tackled that in your time?

Ijantkar: Focusing on flow cytometry, which is a widely used technique in cell therapy and definitely one of the platforms that are used to report identity and purity, what is it that is phase appropriate? Are we going to look at all of the qualification attributes? How many experiments we need to do and to get to that stage? Specifically, with things like flow cytometry, the one thing I always keep in mind is what do we need to do for Phase One. This is like a trial when we are ensuring that our product is safe. When you're starting with the flow assay, the rule of thumb is to only look for things that are your report events that you need on this assay, report identity and purity. Things which you don't need

those not to be on your QC assay, all of those can go on characterization, and you can design multi attribute assays and use things such tiFC and all of those fancy flow techniques that we use to look at process changes to look at biology and things like that.

So the QC flow assay with a GMP assay, only needs to be minimalistic. What is and isn't phase appropriate, I guess, when we start with Phase One, there is a term now, which is phase appropriate validation. Because it's a release for infusion assay in most cases, we need to look at linearity, for sure; we need to look for specificity, for sure; and we need to look for intermediate precision, definitely. When you're doing the intermediate precision approach, I always like to include QC within our intermediate precision design, just so that you have the site variability also taken into consideration. Add as many variables as you can in your intermediate precision to really know what's the variability of your assay, try and add new operators to your intermediate precision, because usually people who develop the assay may be good at it.

Clarke: When you talk about phase appropriate, what are perhaps the key flow assays for initially getting into Phase One? What are the ones that have to be there versus all the others that you might utilize for characterizing your process and your product?

Ijantkar: Usually, there are two platforms when you think about it. One is the molecular platform and one is the flow platform. But I have noticed one thing, the agency likes to see expression, many times, and that is usually three things that get reported from the flow assay, which is the GMP grade flow assay. One thing is identity of your product, which might be CAR or PCR, whatever is the gene of interest if it's the center of the product. The second thing is the purity that can support it. So whether it's an NK cell, a T cell, any other stuff that you're working on, that needs to be there. If it's a T cell, of course, you need to have CD3 definitely on your on your assay. The third thing is most probably its strength. I know there might be some companies out there that might be using molecular assays for strength, but again, mostly, in my experience at least, the agency likes to see at least at Phase One a protein-based expression assay on release to look at all of these attributes.

Clarke: How do you understand and work with the regulatory implications that come with the flow assays and which ones are required versus which ones are for information use only? How does that impact as you get to an IND and look to work with the regulatory advisors to get your baseline started? Are there key implications there that can impact your assay development for your flow that are going to perhaps cause challenges?

Ijantkar: I think the answer to that question would be just have things that are required on the assay. Like for inflammation only, that should be on characterization. And coming to the next point, why is that? There are two things. The biggest thing is that the gating controls—each thing that you have in your assay needs to have a gating control. Let's say if you're using FMOs, each of them need to be gated using an FMO, so that increases the time and the amount of reagents that you're using on the assay and increases, obviously, chances of failures and things like that. The second thing is data management. If we add more and more biomarkers, all of those numbers that we get through the GMP assay need to be cross-checked by somebody. All of those points, let's say something like 90% needs to be verified so many times, 90% before it lands into spec or before it lands into a data set. So data verification, the more markers we add, the more data we add, the more data verification required.

Clarke: Is there a risk when you are interacting with the regulatory bodies of having too much FIO, too much information? You're trying to gather as much as you can to understand your process and your product. But where is that balance?

Ijantkar: Yes, some assays which were on characterization, luckily didn't happen with any flow assays, or some assays which were on characterization, were asked to be put on release. So that's maybe going to happen. The best suggestion that I learned not in my years within flow, but my years being the analytical lead, is engage with the agency as soon as you can. With the flu assays, luckily, now that they have seen so many identities, they know what to expect

from a flu assay. But there might be some gene editing assays where they might not know. So they might ask if it is on characterization. Why is this on characterization? And then, if you believe that it should be on characterization.

Clarke: As we transition from the assays, you talked about FMO and controls, so why do we need them? To take that a little bit further, not only why do we need them, but which controls are needed?

Ijantkar: Yeah, Fms (1) is the one which is popularly used in flow cytometry, but they don't specify that we need to use FMO controls, they just want any kind of objective gating to be used. That could be anything—that could be even threshold gating. As long as the analyst is not getting something by guesswork: this is my CP3 population, or this is my CAR population. I think it needs to be objective for consistency, just from operator to operator, because where I would draw the gate is not where somebody else could draw the gate. So for consistency purposes, any kind of gating control should work, but it needs to be objectively gated.

arke: You talked about the gating. Is that still problematic today with having different operators and different individuals understanding that gating and interpretating it?

Ijantkar: Absolutely, yes. Because the thing with cell therapy is, specifically autologous therapies—allergenic is a different piece of its own— but in a way, we control the starting material. So mainly, it's good. For the different patients with different processes any process changes, that's the other thing I usually recommend is always be on the same page with your process colleagues. Any time they make process changes, their assays might get impacted. That's the very, very important. Things might change, and FMO might work today, but because of any process changes, or differences with the starting material, it might not work. So the assay needs to be assessed every time a major process change happens.

Clarke: Qualifying and validating—what does that mean to you from a flow assay? A lot of times that qualifying is okay, but then validating a flow assay probably goes back to making sure that you don't have too many of those assays—you need to find the right ones. How does that factor into your timeframe? What's the requirement going back to phase appropriate thinking about this? When does something need to be moved from FIO, to qualified, to validated?

Ijantkar: Great question. Again, anything in Phase One needs to be phase appropriately validated—the old term was qualification. So, qualification needs to happen before Phase One. And validation needs to happen. Those are the two different stories. Let's take linearity as an example. If you take a guarantee for Phase One, linearity with one operator, three times should be more than enough. For a validation on the same linearity, you need to add multiple operators and maybe multiple instruments. That's when validation becomes like a larger piece to capture, I feel. Now you're adding too many things to the experiment. So those are the bigger differences between a validation and a qualification.

Clarke: Predominantly, in everything we do in cell and gene therapies, maintaining that balance between the FIO and the number of domains and the ones that you're going to move forward—especially if you've got multiple sites—trying to maintain that from one site to another, there's got to be a large challenge. Having too many would make that tough to keep up with.

Ijantkar: Yes, what you need, needs to be on this GMP assay for the very reason that you said: multiple sites. Because if there are 20, 30 markers in a GMP assay, it becomes very difficult to control that assay now, because it needs to have given controls, we need to have reportables, the trading strategy becomes very complex. So that's the reason on a GMP assay, we need to have minimum reportables—just what we need. Then a transition assay could be a bigger assay because it's used to see process changes. It's there to know more about the product and usually characterization teams might not be across different sites. They might be across one or two sites looking at product performance over time, or process changes over time. If there are one or two sites working on that, I think it's relatively easy to manage. Again, categorization is a challenge of its own. I agree with that.

Clarke: So the distinction between characterization assays and safety assays for the product quality attributes, how do you distinguish between the two of those as you're developing this out and then moving it through? How important is one versus the other for the phase appropriateness?

Ijantkar: Release for Phase One needs to be safety, for sure. All of these things—identity, purity, strength—need to be done based on specifications, and usually works in simpler but at least you know what to look for. As we progress more, it note in your correlative analysis what is important. Just knowing something is important for the performance, then yes, let's move on to this next version of the flu vaccine. That you'll come to know through correlative analysis.

Now what comes under characterization? What do I need to measure? I need T cell biology or NK cell biology. There might be so many receptors on the surface of the cell and everything that is going on within the pathways. It's really important to know the product biology and process through early build. Step two, once you know that, think about the critical quality attributes, what are those attributes I need to measure in terms of phenotype or potency? What are the things that will be important? Then your CQA in a Phase One will have minimal data—you might have some literature search, things like that, that will be your CQA. As you move forward, you will know from your qualitative analysis what are those critical quality attributes, which need to be there on future potency assays.

Clarke: when you when you're running these experiments you want to make sure that you're thinking FIO versus which ones are actually going to be qualified, validated, and necessary. But how much is enough when you are working through each of these?

Ijantkar: I think, because it's going to go into infusion most of the time, you might have to do all of the qualification attributes, because the assay availability should be known. But there is a way to be creative. You can clump intermediate precision and repeatability together. You can talk to your statistics team, they are very useful. So, you can combine those two things, intermediate precision and repeatability. That cuts down on the plates and the number of experiments. Can we do it with two operators or three operators instead of six operators? Knowing the performance of the assay, and whether adding two or three operators, versus six operators is going to change my intermediate precision could be another thing to keep in mind.

Things like linearity—how much linearity should I perform? I usually like to do linearity all the way. For flow assays, the upper limit of quantitation is always 100%, but it might be possible to find something which is 100%. I do like to see the entire range of what my analyte is showing within this assay. Those experiments, for sure, need to be done for Phase One.

Clarke: We've talked about the flow cytometry space and assays within cell and gene therapies. Where do you see is the biggest challenge today versus the biggest opportunity? Are we limited on technology? Are we limited on just understanding? What would you like to see happen in the near term that can improve this for the field?

Ijantkar: In terms of flow, guidance from the agency would be good. For the most part, for at least GMP assays, we know what we are doing. I guess we can start engaging something like pressure gating, just so that we don't have to use FMOs very often, that could be great. Better data analysis platforms could be good as we move forward. Specifically for flow, the field is also moving towards spectral analyzers. That's something new: we can explore a number of different fluorophores and different marker combinations. The place, I think in my perspective we are struggling, or where it might be good to know more things would be all of the [unintelligible] assays—what is expected versus what we need to absolutely have.

As we move forward, the biggest thing that we will need to keep up with as an industry is we are generating so much data and how to get the best out of it is the biggest challenge. By using sophisticated techniques, such as ML or AI, that's going to be the next biggest thing.

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Details of future events can be found here.

You can watch Thompson's presentation in full and on-demand here.

Cell Therapy Analytics: Overcoming CMC Challenges

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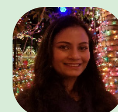

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