CELL & GENE THERAPY INSIGHTS

EXPERT ROUNDTABLE

Smooth cell therapy analytical assay translation from analytical development to QC

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In this Expert Roundtable, four experienced industry professionals discuss challenges and strategies for effective assay translation in the cell therapy space, with a particular focus on flow cytometry assays. The panelists share insights on effective communication between analytical development and QC teams, risk mitigation, and regulatory considerations.

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Q What are the unique challenges of developing quality assays, in particular flow cytometry assays, for a cellular therapy?

IS: Flow cytometry assays are integral to cell therapy, in the same way mass spectrometry is to biologics or nuclear magnetic resonance is to small molecules. Over the last decade in

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the space, I have seen many of the historic challenges in developing flow cytometry assays being resolved. We previously struggled with the variability of reagents, difficult-to-use instruments that lacked standardization, and software compliance, but these are no longer challenges today.

There are still three primary areas in which challenges exist. One area is strategic and relates to how assays are designed. For example, many companies design panels that aim to measure identity, purity, and viability in a single flow cytometry panel, using four to five colors. This is fantastic for working towards an IND submission and is highly efficient, but it may cause lifecycle management issues later. If you want to switch to a different viability modality, and your validated panel has viability markers, it might not be so useful. If one of the reagents used to validate your panel becomes unavailable, the whole assay goes offline. For product release, the decision of whether to use one multicolor flow panel or multiple smaller panels must be made early on. Smaller panels will lead to fewer compensation issues and easier lifecycle management. This can be hard to align strategically within companies.

The second area is positive assay controls. Often, a positive control is needed to know if instruments are performing properly. This could take the form of a mimic or something with equivalent fluorescence to the population being measured. There are great reagents out there for some cell types, but not all.

The third area that must be considered is assay transfer during manufacturing site transfer. In many cell therapies, flow cytometry is performed with fresh samples during manufacturing at the QC lab within the manufacturing site. Later, you may want to open another manufacturing site or switch sites. Once products have been manufactured at those new sites, at the stage of applying for Biologics License Application (BLA) or Market Authorization Application (MAA), you must demonstrate comparability of the products across manufacturing sites. To demonstrate comparability, you first must demonstrate assay equivalence. The gold standard is testing the same sample at both the old and new QC labs. If the old lab is already closed, this will be impossible. If you are working with fresh material, it may become impossible if you miss the opportunity to do that during site transfer. Comparability studies can become difficult if you no longer have access to the old QC site to perform site-to-site equivalence.

KY: One key challenge in assay transfer is the controls. As a CDMO business, we have many clients sending us their R&D-stage assays. When an assay comes in, they often do not have sample-accepting criteria. In some unique cases, they do not even have system suitability set up. Other cases may have a positive control, but it may lack robustness, and a negative control may not exist. Having robust controls and the relevant data to back them up is critical.

We also encounter issues wherein the client may have no analytical target profile (ATP) in place, meaning that they have not identified critical reagents or alternatives. This means that if a reagent ran out, they would have no bridging study.

TT: One of the challenges especially in the CDMO business is balancing instrument availability. Currently, there are many kinds of available instruments from various companies. It is critical to ensure the release assay is as simple as possible. Companies offer fluorochromes

of eight or more which can become difficult to manage. Minimizing the number of fluorochromes and focusing on the target is critical.

Currently, for cell and gene therapies specifically, there are no standard commercial positive controls available. You must establish a standard from the beginning, make a cell bank that works well, and characterize it to demonstrate comparability. You do not need to go through the entire manufacturing process to show comparability. You can use a well-characterized positive control. Having a well-established method from the beginning is key.

HA: At the beginning of development, one of the challenges can be material. Usually, the process development and analytical development (AD) are happening side by side and it can be hard to get representative samples. The process is continuously changing, so the samples are changing, which can be a challenge for assay development. Working closely with the process development team to understand the changes and how impactful they are on the final sample, and risk analysis for the samples, is important.

Q What are the key challenges (or most common mistakes) in translating assays from analytical development to QC teams?

KY: The most common mistake is that when the assay is translated to QC, the foundations of statistically relevant data are frequently lacking, which compromises robust analytical procedures. In the early development phase, a lot of material used is not representative, and thus it is hard to accumulate statistically relevant data. Later on, that data will be important to setting system suitability and certain criteria as you enter qualification and validation. To solve this, have the ATP in place as early on during development as possible, and carry out a gap assessment.

As a CDMO, we often deal with rapid timelines. We need clients to understand that when dealing with IND filing, less is sometimes more. Many assays are not necessary as release assays. These can instead be classed as categorization assays, for example, potency assays. Adding things such as release assays early on can cause problems when entering Phase 2 or 3, as it can be hard to justify to the US FDA why a release assay is being changed.

HA: Equipment, instrument configuration, and workflow capabilities are all different between AD and QC labs. There are many more things in place in the QC lab and there is a longer checklist to consider. For example, in an AD lab, you may be able to use a research-use-only antibody, but the QC lab may not permit that. These elements must be discussed in advance of the transfer so that the AD lab can ensure that they are developing the right kind of assay that can easily be transferred into the QC lab.

Another element related to that is reagent traceability. We have talked about controls and system suitability, which will come from the AD lab. The documentation and traceability within the two settings can be different. The QC lab might not accept reagents that the AD lab routinely uses if there is not enough traceability, documentation, and characterization of those reagents.

IS: A common situation that can cause issues is AD providing the QC team with only a template for an instrument. This can be a mistake—you must have both a template and a well-described gating strategy in the standard operating procedure (SOP). This should include

descriptions of what you should see at each gate, with representative examples and some flexibility to adjust parts of the gate. It is acceptable to not have an entirely rigid strategy.

TT: Provide a range for critical steps, such as staining times as a range. Robustness, in terms of the hold time, the number of sets, and the instrument, must be shown so that there is no variability. Robustness in terms of time is a critical aspect of translating AD to QC.

Q What practical steps can teams take to minimize risk in assay translation?

HA: The number one step that teams can take is to have well-characterized assays. Robustness in ranges for incubation times and understanding the reagent requirements must be considered. Small elements can become problematic if they are overlooked during transfer. Do you thaw a reagent at room temperature or on ice? How long before you must use it? Each of those small steps can make a big difference when transferring an assay from one lab to another and they are often overlooked.

Identifying the most critical steps is key. In some steps, you can pipette differently. Other steps may require pipetting in a very specific way, such as reverse pipetting. This must be outlined and highlighted both in the SOP and during training.

Another key step is gap analysis. At the beginning of the transfer process, a conversation between the teams should be held, looking at every step of the procedure, including all the instruments, reagents, and capabilities of each lab to identify any key differences. Any differences must be looked at more deeply to understand if these can be worked around and proceed accordingly.

Another key consideration is staffing and training. If you lack a skilled person in the QC lab, then you will need to provide much more information in the SOP about gating. Problems can arise from an incompatibility in training in the different labs. For the QC lab to be aware of all the development and robustness work that the AD lab has done, being able to communicate freely and often is important.

Finally, hands-on training is critical. Training should involve being in the transfer lab and seeing how processes are performed. Knowledge of the workflow and even the orientation of equipment can be important, and seeing how that might be different in the QC lab is critical to identifying any possible challenges. For example, we had a situation where we had two very similar cell counters from the same manufacturer, but one was high-throughput, and one was not. This was fortunately identified during training, as this could have added a significant amount of time if the cell counts had to be performed one at a time.

TT: I agree that training and follow-up communications are critical. One of the reasons for this is that assay development at the AD stage is usually performed using a healthy donor. At QC, this is done using patient samples that sometimes behave differently. The patient sample may not match up to the analysis in the SOP. Having follow-up communication between the two groups is critical to ensure all data is being interpreted in the same way.

KY: To solve the problem of instrument transfer, at Miltenyi Bioindustry, we use the MACSQuant® System with Smart Gain technology to align instrument settings when transferring assays from site to site. Using the Smart Gain function can minimize those problems.

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Teams like Analytical Science and Technology (ASAT) are still required to draft all the details on the gating, but this technology can help a lot.

Q Can you suggest strategies for effective communication between teams during assay translation?

KY: We all need a collaborative mindset, including the client and the CDMO. Early on, we perform a gap assessment. We do a paperwork exercise including a detailed and comprehensive Program Scope Development in place to work through all the details to identify the must-haves and the good-to-haves.

I also recommend leaning heavily on the project manager (PM). A PM should have a satisfactory technical background, because sometimes when we speak about these technologies and platforms in meetings it can sound like a foreign language to people without a cell and gene therapy background or even a science background. A PM should orchestrate all meetings, understanding who needs to be in each meeting and involved in each decision. For example, if a decision impacts the QC team, a QC representative needs to be involved in the decision-making.

IS: AD must have a say in the transfer. As the originating lab, once the assay is within QC, the AD team should still be involved in solving problems. AD probably has more experience with the assay, so when things go wrong, AD should have access to the raw data (FCS files) and trending. In an ideal situation, this should go as far as allowing AD to have a part in training new analysts in QC. Another approach is that AD can provide training videos. An SOP only goes so far; a recording of someone doing a procedure can be highly effective for assay transfer to QC.

TT: The AD team's communication across all stakeholders is critical. Once the assay is transferred to the QC team, there may be unforeseen outcomes such as an invalid assay. The subject matter expert from the AD team will have the expertise to help. From the technical perspective, it makes sense to maintain communication with the originator for backup information. Assays sometimes need improvement in the latter phases so help from the originator is critical during clinical testing.

HA: Face-to-face communication is critical. Things get lost or misinterpreted in emails so I would not rely heavily on these. Having frequent face-to-face check-ins for the QC and AD labs to get questions answered and check on minor things is essential. If things are slightly misinterpreted, it can make a big difference.

Before initiating the transfer, have all the documentation handed over from AD to QC so that they can review the development work and the procedure thoroughly. This ensures they

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are as informed as possible and ready to ask questions at the transfer stage. It is important to have the analysts who are actually in the lab as part of this conversation, not just managers and supervisors.

Q What are the impacts on communication when running QC internally versus externally?

IS: I have seen both models, and they can both work well and poorly.

Often, even with internal QC, the QC teams tend to silo themselves away to manage the risk of GMP non-compliance. They do not want AD walking in and watching over their shoulders. This is valid, but we want the QC to be inclusive to allow effective communication. If QC becomes too isolated, you cannot effectively have face-to-face meetings or proper training.

One of the differentiating factors that makes QC work, whether internal or external, is its mission. If the QC team is invested in the success of your product, they will behave differently in terms of how they communicate with you. You must work hard to establish that mission-driven approach, whether your QC is external or internal.

KY: Being inclusive, even very early on, is key. At Miltenyi Bioindustry, we include our QC team in the Program Scope Development and ATP planning. The QC team is on the front line holding the pipette, so they should know what assays are going to come into their lab.

HA: It is a common misconception that internal QC is better and external is harder. It is important to understand the differences between the AD and QC settings It may be easier to get information from an internal QC in terms of processes, workflows, and systems, but this is not always true. One of the things that can be more challenging with an external QC is coordinating the onsite training. This must be considered early in the agreements between the two companies. Factors such as how many onsite visits are permitted should be pre-decided. It is important to include the AD and QC personnel in those early conversations because hands-on training is critical.

TT: For Mitenyi Biotec's San Jose site, currently, most of the analytical methods are in-house QC testing, but we do have some safety tests performed in an external lab. We have biweekly meetings to follow up on progress with this external lab. For instance, if we send samples for lot release, we have a follow-up meeting so they can be prepared to test the sample on time and ensure they have the resources to do so. This meeting is critical, whether it is done with internal or external QC.

IS: It is helpful to have a common SharePoint® set up with external QC labs, so any preliminary results and raw data can be placed there. This cuts down on the number of emails

needed to check how samples are going. If an assay has been run, it will appear in SharePoint® way before a certificate of testing is received.

Q Which technological advances can smooth the transition from assay development to QC—now and in the future?

HA: Regarding flow cytometry assays, the biggest challenge is the gating. Flow cytometry is highly automated, but the gating can be very manual. There is a lot of work being done on this, which is fantastic. Having simple assays can also help here, as this enables simpler gating. If you have many different colors and populations, things can get more complicated as automated gating is not an option. To minimize the variability seen in flow assays, automation is key.

Another consideration is having good controls. Reference standards are not always available, but having good positive controls will assist in troubleshooting. If there is an issue in the QC lab, good controls will help pinpoint the cause of the issue.

TT: Miltenyi Biotec's Express Mode automated flow cytometry software is beneficial here. However, when clients come from AD, they may not see the benefit of this as they are used to manually adjusting gating. In addition to making the assays simpler for analysis, the software is also good for regulatory impact. It removes any subjectivity within flow cytometry. Although the result might not vary much between analysts, in the case of getting results quickly for manufacturing processes to continue, automation is critical. The MACSQuant Analyzer's automation system, specifically Express Mode, can be adapted for different kinds of assays.

IS: Automated gating has a lot of potential. The danger is that you may validate an assay, for example using Express Mode version 3, but if the vendor releases Express Mode version 4 a year later, you may have to revalidate that assay. If you are planning to use an automated approach, you need to have an understanding with the provider that you will be able to continue using that automated module without any updates in perpetuity. If a new version comes out that automates slightly differently, it could pose issues. This is a common challenge when talking about technology that is constantly evolving.

The other technological piece that I find intriguing is microfluidic-based flow cytometry systems, in which staining is automated and all reagents are preloaded. This parallels how cell counting has evolved, where, 5 years ago, almost everyone was doing manual cell counting with trypan blue. Now, the industry standard has become single-use fluorescent chips. I believe flow cytometry will move that way once the technology matures to accommodate slightly more complex gating. With any evolving technology, you need to have a lockdown version that exists in perpetuity, to prevent issues arising in lifecycle management.

KY: Standardization is one of the ways that we can transfer assays from AD to QC more smoothly. At Miltenyi Bioindustry, we have the standard Express Mode and all-in-one reagents in a dried form. During AD, we use the StainExpress™ Cocktail to collect data for QC. We have already collected a lot of data using Express Mode and a standardization gating strategy. We are also developing a robotic system to help standardize the workflow.

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Q What are some of the key regulatory considerations (e.g., validation, qualification, regional differences)?

TT: The terms assay validation and qualification are sometimes used interchangeably. In general, both assay qualification and assay validation are performed to check how fit for purpose a method will be down the line. Typically, ICH Q2(R1) is the guideline used for both assay qualification and validation.

However, there are some differences between assay qualification and validation, particularly in terms of the study depth, as well as the phase in which they are completed. In assay qualification, the attributes that are being evaluated are similar to those in assay validation. However, assay qualification generally happens at an earlier stage in development, during Phase 1. Typically, during assay qualification, a single representative lot can be evaluated to demonstrate that any assay is fit for purpose, particularly a release assay. There are no clear written regulatory requirements to qualify an assay from region to region. However, it is in the best interest of the sponsor to qualify an assay to demonstrate that the method can be validated in the future. In the simplest terms, qualification is an early stage of demonstrating the method can perform as intended in the future. It also helps to establish acceptance criteria in the future.

However, for validation, there are clear requirements from the regulatory agencies, particularly in the late phase of IND application or BLA submission. Qualifying an assay at the beginning will help to validate a method. The acceptance criteria are typically similar to those in qualification, but they can be stricter. During validation, you must validate not only your final product but also the intermediate controls.

In general, from a sponsor point of view, qualifying a method early in development, even in Phase 1 before IND submission, is critical. In general, the requirements for validation are clear, but for qualification there are none. Most small companies do qualify their assays, particularly release assays. There is some confusion from companies who believe every assay needs to be qualified, but that is not the case. There will be some characterization assays down the line that are critical in decision-making, but that do not need to be qualified. When data is available, particularly patient data, this can be critical to evaluate those kinds of qualification methods.

IS: In the US, at the BLA stage, there is greater scrutiny of robustness than is needed earlier on in development. After you validate your assay, during your pivotal trial, make sure you invest in robustness studies, or this can become a post-approval commitment.

With these assays, we are typically aiming to capture some precision during qualification. That is the acceptance criteria for validation. The precision might be good, but it must be considered that there is a certain temporal variation in flow cytometry that is not captured by precision. Over a few years, you might see that the precision of your assay changes dramatically. This should be factored into how specifications are set.

HA: One thing that needs to be discussed early on with a QC partner is where the qualification needs to happen. Everybody understands validation needs to happen in the QC lab, but in the early phases, oftentimes AD does the qualification, and then they transfer the method. Some QC labs want qualification performed in their lab. This needs to be understood and agreed upon in advance. I have even seen co-qualifications shared between the two labs.

The idea that characterization assays do not necessarily need to be qualified was mentioned. New potency guidelines came out recently describing how critical potency assays are. From my reading, it seemed that the recommendation was that potency assays should be qualified. It did cover characterization versus release, but my interpretation was that because potency assays are so critical, having the assurance that the assay is performing robustly and well is important.

KY: Statistics are also key here. I think there should be standardized 'magic numbers' in terms of the number of replicates needed for qualification.

BIOGRAPHIES

HADAR H ADAMS is the Director of Analytical Development at Atara Biotherapeutics, an allogeneic T cell immunotherapy company. She has over 10 years of experience in development, optimization, troubleshooting, qualification/validation, and transfer of analytical methods for diagnostic testing, release testing, stability, and characterization of biological and cell therapy drug products. In her current role, Hadar leads a team focused on developing and qualifying a wide range of assays to support an IND for a CAR-T drug product.

ILYA SHESTOPALOV is currently the Vice President of Analytical Development and Analytical Product Lead at bluebird bio. His research focuses on development of cell-based assays for hematopoietic stem/progenitor products, CAR-T products, and lentiviral vectors. Prior to bluebird bio, Shestopalov was a postdoctoral fellow in stem cell biology at Boston Children's Hospital and Harvard University working with zebrafish hematopoietic stem cells.

KITMAN YEUNG is a biopharmaceutical specialist at Miltenyi Bioindustry, a division of Miltenyi Biotec, with 15 years of experience in cell and gene therapies. Her expertise in technology transfer, analytical development, and quality control testing supports the production of Phase 1/2 GMP cell and gene therapies. Yeung is currently an MSAT Analytical Manager at Miltenyi Bioindustry, where she leads technical and operational teams in developing, characterizing, and qualifying analytical tools for cell and gene therapies across multiple modalities.

TAKELE TEKLEMARIAM is a Quality Control Professional at Miltenyi Biotec with 15 years of experience in cell and gene therapy. He has broad experience in QC assay design and development, assay transfer, and qualification/validation. Currently, Takele is Associate Director of Analytical Development and QC Assay Transfer at Miltenyi Biotec, where he leads QC assay development and transfer, assay qualification and validation, stability studies, and QC in-process and release testing in cell and gene therapy for multiple clients.

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