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The Role of Early Characterization Studies in the Assessment of Advanced Therapeutic Potency

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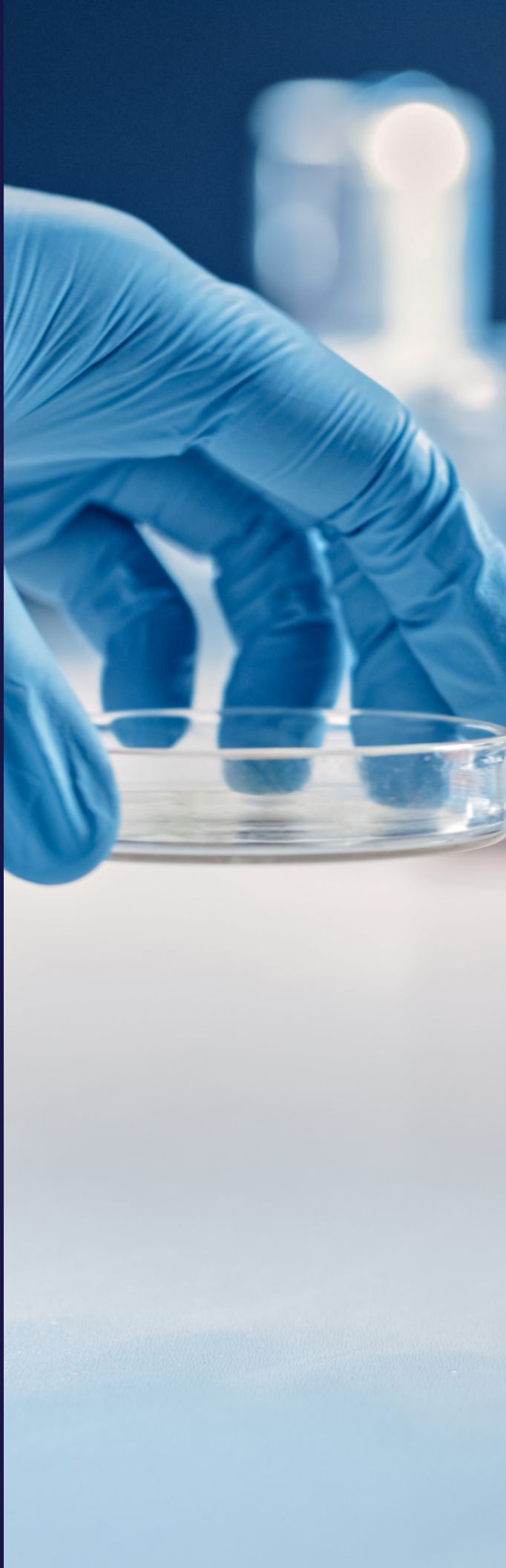

Measuring the potency of an advanced therapeutic is rife with complexity. These drugs, often composed of intricate cellular matrices, require a myriad of decisions for the companies pioneering them, particularly in the early phases of development. Even when the pathway is known, the mechanisms of action of these products are frequently not well understood; as a consequence, many organizations may run into unforeseen challenges when transitioning an asset from in vitro to in vivo testing.

Early product characterization can serve to uncover crucial information about a therapeutic candidate, affording new insight into its critical quality attributes (CQAs) and helping to shape better analytical assays. This applies to more than potency – deep characterization studies can ultimately provide crucial understanding to inform safety, purity, identity, and other core attributes.

Despite the technical advantages afforded by characterization studies, many organizations may be reluctant to spend the time and capital to conduct more detailed evaluation than is deemed necessary in early development. While the imperative to accelerate early development and manage spend is an important driver for industry, deep characterization studies and faster timelines are not mutually exclusive: in the end, the right characterization studies, undertaken early, can help developers establish analytical strategies that better support uninterrupted scaling.

The Importance of Characterization, as Illustrated By CAR-T Therapies

The varying mechanisms of action that typify the advanced therapy space require a broad range of characterization assays to adequately analyze them. Additionally, developers must consider which assays will be most valuable in a regulated environment; identifying tests that can be performed quickly and without a constraining level of operator expertise is an important balance to strike in a landscape where many incumbent release tests can take days to perform and extensive training to execute successfully.

One of the most important advantages deep characterization assays can confer is fomenting an understanding of the interplay between various CQAs. The importance of this interaction can be readily observed in the CAR-T space, where higher potency may go hand in hand with extreme toxicity. The first generation of CAR-T cell therapies demonstrated this challenge, and as a result, the degree of characterization typical for these modalities has ramped up significantly. Characterization for CAR-Ts has likewise evolved to meet technical needs in other ways. For example, nearly all T cells in development were initially cultured using interleukin-2 (IL-2) in the media. However, subsequent characterization studies discovered that other interleukins, such as IL-7 and IL-15, increased the frequency of certain subtypes of T cells that improved the persistence, expansion, and efficiency of CAR-T cells, thereby improving the potency of the product.

Despite the strides made in characterization for CAR-Ts, there remains substantial room for further study. Variables such as metabolic fitness, which encompasses factors such as spare respiratory capacity, stemness, and persistence, are acknowledged as integral to determining the overall performance of a therapy, yet there exists a dearth of clinical data that supports this evaluation. Similarly, the concept of polyfunctionality, wherein CAR-T cells can secrete two or more cytokines, still requires investigation to translate to clinical success.

The Increasing Regulatory Emphasis on Characterization Studies

The varying levels of complexity that can be observed between advanced therapeutic modalities – from gene therapies targeting a single gene to stem cell therapies working to replenish an entire immune system – can make it difficult for developers to get a full picture of their functionality in in vitro studies. Introducing these assets to animal models and first-in-human studies can therefore fundamentally upend prior assumptions, as the complexity of a therapy encounters the complexity of a living organism.

This reality has prompted a closer look from regulators on developing the right science- and risk-based strategies for potency assurance. In December

2023, the FDA released the draft update to its 2011 guidance for industry regarding potency assurance for cell and gene therapies. The updated draft guidance offers approaches to potency assay development founded in quality risk management, noting that "due to the diversity of CGT products and the product-specific nature of potency assays," its recommendations related to selection and design are "necessarily general." The emphasis on characterization studies in the FDA's 2023 draft guidance considerably exceeds that of its 2011 predecessor. Importantly, it recommends that those looking to accelerate development timelines "thoroughly characterize the product and manufacturing process to help… rapidly establish a well-controlled manufacturing process that consistently yields a potent product."

Utilizing a matrixed approach to characterization, one that works to link a product's CQAs to its mechanism of action, often requires looking beyond the existing literature many rely heavily on when selecting potency assays. This is especially important when trying to understand a product's performance for different indications and at various phases of development. Not every assay leveraged during development, particularly early on, will be needed for release testing, yet employing as many different analytics as possible during earlier phases can inform both potency assurance strategies and later assay development.

Finding ways to incorporate artificial intelligence (AI) and machine learning (ML) techniques early can help to streamline the heightened data aggregation and interpretation demands that accompany deeper characterization. For example, trends such as single-cell sequencing, which can provide high-resolution insight into a cell's function within its microenvironment, consequently produce huge data sets that make managing the wealth of information they provide its own challenge. Additionally, integrating process analytical technologies (PAT) that can afford operators real-time or near-real-time in-process monitoring and sampling and deploying novel advanced bioanalytical technologies will enable more precise, faster assessment of a product's consistency and quality.

Addressing Complexity with Characterization

The advantages of engaging in a full genomic or proteomic study early in development are manifold. Though these efforts are not designed to translate into later stage analytical assays, they can offer a great deal of insight to inform those late-stage assays by enabling developers to select the right biomarkers and connect more specified assays to them. This can help mitigate the complexity of later validation efforts, which are then focused on more quantitative biomarkers for a particular cell. The more often developers can make decisions based on characterization data, the less likely a program is to land on assays that require later redevelopment and revalidation.

Characterization can prove especially crucial when evaluating autologous cell therapy samples and products. These drugs, derived from individual patient samples and genetically manipulated to produce a therapeutic effect, are highly bespoke, making their characterization all the more difficult. Moreover, the starting material for an autologous therapy is often highly variable and dependent on a patient's health and previous treatment.

While there are ongoing efforts to find ways to reinvigorate these cells, the ability to adequately assess their potential is key to helping manufacturers avoid lengthy and costly processing for a product that ultimately will not be efficacious. This, again, requires a thorough understanding of the desired characteristics of a starting material and how those characteristics align with treatment outcomes. Similarly, for allogeneic cell therapies, this understanding enables manufacturers to set donor selection criteria to optimize a process. Often, manufacturers can control variability to some degree this way, analyzing starting materials and employing relevant subprocesses and additional manufacturing steps to accommodate for variability.

Choosing a Better Potency Assay Through Characterization

Developers should work to continuously adapt characterization studies to inform study design, derisk scaling, and identify the right assays for potency and other key product characteristics. At the earliest stages, this looks

like wide-ranging exploratory studies, while later strategies will naturally contract and employ more specificity, leveraging foundational characterization. Typically, developers must generate their own internal controls and reference standards and are often required to revisit them as assays are transferred between phases.

Assays for potency are often complex in nature, time consuming, and highly manual; the time and operator handling intrinsic to many of these tests increase the chances of variability. Advanced therapeutics with short half-lives necessitate the development of surrogate assays based on key characteristics, which alongside more time-consuming functional assays, can give operators a more complete picture of a product's potency while enabling more flexible release testing.

Ultimately, the advanced therapy industry is rapidly evolving, and many of these curative therapies are reaching patients for the first time for certain conditions. With this growth and advancement, developers are learning more about the requirements and benefits of product characterization, as well as the technological advancements and process analytical technologies that might support characterization studies. Investment in the right technology or study needs to be weighed against many other equally critical deliverables and prioritized appropriately, which can be difficult in the face of competing priorities, time, and cost constraints. Strategizing on which studies to employ at what scale should be data-driven and may also depend heavily on the phase the product is in, the modality, and technologies that are feasible.

The timelines for early development continue to accelerate as the pressure to generate clinical data to attract investors and beat the competition increases; for many emerging biotech companies, the value of deep characterization may take a backseat to other considerations. But, on the receiving end of every cell therapy is a patient who depends on a product's safety and efficacy. By taking a step back and learning from other delays and regulatory pushback linked to inadequate characterization, companies can position themselves to bridge significant gaps in their own understanding and protect the patients they aim to treat, all while differentiating their assets and maximizing their ultimate potential.

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