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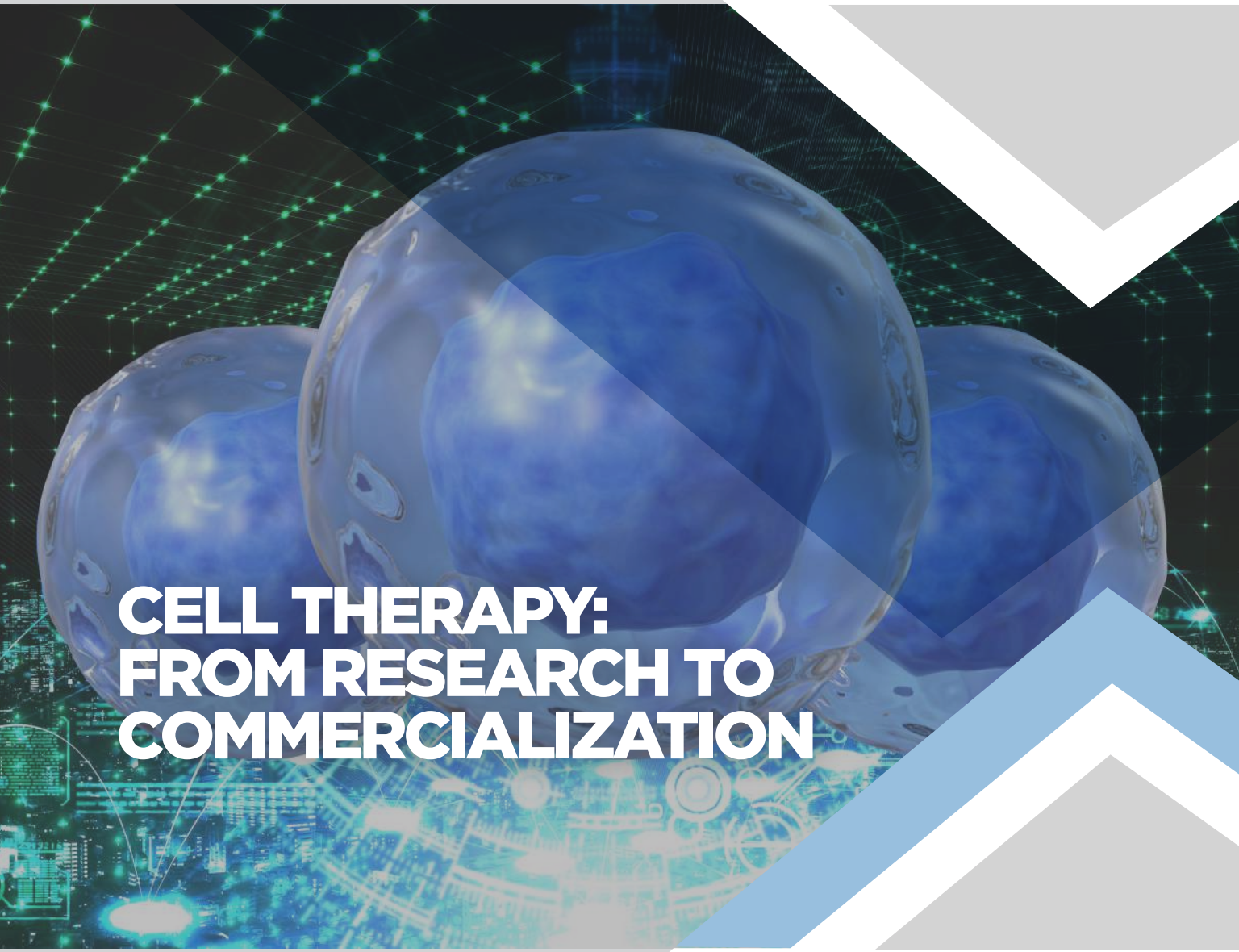

# CELL THERAPY: FROM RESEARCH TO COMMERCIALIZATION



MULTI-4899CP02.19

# Accurately Measure Cell Counts and Volume with the Coulter Principle


## The Multisizer 4e Coulter Counter

- Cell volume is a critical parameter of cell health and state
- Cell volume is a useful way to discriminate cell types following elutration
- Volume changes, down to femtoliters, are often too small to be detected by imaging or flow Cytometry methods, but can be captured by the Coulter principle
- CAR-T cells have been shown to swell upon activation and their persistence is often marked by sustained increases in cell volume

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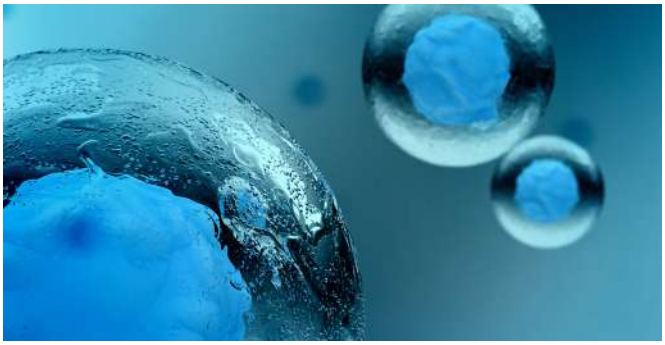

# One Step at a Time: Obstacles to Cell Therapy Success

In cell therapy, cells are introduced into the human body to either replace endogenous dead/non-functioning cells or to modulate dysfunctional cellular mechanisms. Since its establishment, cell therapy has been closely linked to regenerative medicine, under the premise that stem cell transfer could potentially restore tissue/organ damage previously considered irreparable.[1] More recently, the therapeutic potential of non-progenitor cell therapy has been more extensively investigated, particularly the anti-cancer potential of immune cell transfer.[2]

## To Be or Not To Be: Creating the Correct Phenotype

In general, cells are highly dynamic in vivo, responding constantly to direct interactions and indirect environmental cues. This plasticity allows cell therapy candidate cells to respond to experimental manipulations, adapt to pathological environments, and integrate properly into host systems. It's also a double-edged sword, and researchers must take care that candidate cells do not assume an undesired phenotype or differentiate into an unintended cell type either pre- or post-injection.[3] The inability to maintain the desired phenotype has posed a challenge in both stem cell and immuno-oncology cell therapy research, with the loss of targeting selectivity, loss of efficacy, and unpredicted side effects being the consequence.[3,4]

In many cases, unforeseen changes in candidate cell properties are caused by environmental conditions. Many pathologies (especially cancer) involve the creation of local microenvironments promoting continued disease development. Insufficient cellular efficacy and/or persistence upon transfer inevitably leads to the injected cells being overwhelmed by the inertia of the pathological system already present.[5] Scientists can help their own causes by ensuring that this phenomenon does not occur pre-injection by keeping their culture conditions as consistent as possible.

## Curbing Cancer: CAR-T Cell Therapy

Despite these potential pitfalls, real progress has been made in cell therapy research over the last few decades,[2] and one of the most prominent examples of this progress is the advent and continued development of chimeric antigen receptor-T (CAR-T) cells. CAR-T cells are T cells that have been extracted and transduced with custom engineered chimeric antigen receptors (CARs), allowing them to selectively target, in theory, any ligand. In practice, CAR-T cells are generated most commonly against cancer cell-expressed antigens.[4] Decades of laboratory and clinical work, resulting in multiple generations of CAR designs, have yielded promising therapeutic results against B-cell malignancies, resulting in subsequent FDA approvals. The development of CAR T-cell therapy continues to face challenges, including improving efficacy against solid tumors, limiting adverse effects, and scaling up production to meet increasing demand,[4,6] but CAR-T cells have the potential to overcome these obstacles and expand their utility beyond just cancer treatment towards a potential therapeutic purpose for other disorders such as autoimmune diseases.[4]

Cell therapy research has made great strides and yet still faces great obstacles. At the same time, the potential of cell therapy has never been greater. Developments in cell biology, cell characterization technology, and gene editing have made the old issues of cellular plasticity, persistence, and off-target/adverse effects more manageable than ever before. Now, as cell therapy moves more and more away from the bench and towards the clinic, the growing necessity of mass production adds a new wrinkle to an old field.

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# Movin' on Up: Scaling Up Cell Production

Transitioning from cell therapy research to the commercialization of a cellular product inevitably requires a scaling up of the workflows used during the research phase, as researchers move from experimenting with small amounts of source material to the mass production of desired agents. Scaling up brings several obstacles for scientists. Besides the increased financial investment required, researchers must contend with the physical requirements of new instrumentation (*e.g.*, space, electricity, temperature). They must ensure that sufficient operational capacity, materials, labor, and time are available for all workflow steps to maintain throughput and prevent bottlenecks. Most critically, they must maintain production workflow consistency in order to limit variability in the final product.

## Onward and Upward: Workflow Expansion

In order to increase throughput, a cell therapy production workflow needs to be expanded, accelerated, or both. The fixed nature of cellular growth cycles generally precludes acceleration (although extra time can be potentially gleaned by performing workflow tasks during non-standard working hours), meaning that researchers focus primarily on workflow expansion. Expansion, at its most basic level, means increasing the number of cells in the workflow at any given time. This can be achieved through elevated source material extraction/purification, denser culture/storage conditions, and/or extension of expansion stages. The exact amount of throughput increase necessary will depend on the therapeutic properties of the agent itself (*e.g.*, dosage, dosing regimen, stability).[1]

Inevitably, having more cells in the workflow will translate into a need for additional workstations, additional staff, additional storage capacity, and additional reagents. Here, while equipment and staff requirements can be potentially alleviated through the use of automation (*please see the Infographic, page 5, for more information*), a proper supply chain is critical to acquiring and replenishing the reagents necessary to keep the workflow running as desired.[2] Cell therapy workflows are particularly susceptible to supply chain logistical deficiencies because they use autologous cells and thereby require a greater range of reagents (*e.g.*, media, growth factors, differentiation factors) than production workflows for small-molecule biologics.[2,3] Whether short-term or long-term, supply chain planning needs to factor in both supply capabilities and demand requirements to be truly effective.[3]

## Conducting a Cell Culture Symphony

Variability is the biggest problem faced by both small- and large-scale biological agent production workflows, and cell therapy is particularly susceptible, given that the source material is often patient-specific. Scaling-up has great potential to exacerbate the impact of potential variability sources. For example, prolonged culture duration designed to expand cell counts can lead to culture environment shifts, cellular phenotypic changes, and senescence.[1] Similarly, the introduction of additional sets of equipment can result in culture condition variations between them. Optimized culture parameters can – and should – be defined prior to scaling up, but researchers need to understand that culture conditions must be scrutinized throughout the scale-up process, adjusted if necessary, and then constantly monitored afterward.

Scaling up is necessary for the scientist to transition from research to commercialization, but it must be done in a carefully planned manner. Moreover, scaling up cell therapy workflows presents additional complexity in that individual workflows must often be customized and optimized based on the properties of the autologous source material. The researcher needs to develop a robust and consistent workflow in order to minimize variability, but also set up a logistical network capable of accommodating small adjustments as needed – something that automation is well-positioned to assist with.

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# THE ADVANTAGES OF AUTOMATION

## MAINTAIN WORKFLOW CONSISTENCY

A small difference in mixing technique, a timing that's slightly off. Cell therapy workflows are multi-step affairs with many opportunities for inconsistencies to creep in.

Automation helps eliminate preventable variation from your workflow by ensuring that every step, every process, every run is conducted consistently – the same motion, the same force, the same volume. Standardize for less waste and better data!

## DO MORE, FASTER

Cell culture workflows are time and labor intensive, with scaling-up creating logistical bottlenecks as throughput struggles to match supply.

Automation improves throughput by performing multiple workflows simultaneously and by continuing to operate during all hours of the day and days of the week.

## STAY ORGANIZED

When culturing cells for cell therapy, each cell has its own individual set of optimized parameters. Scaling-up means more cells, more reagents, more equipment, and more confusion

Automated systems can link to database and planning software. File away sample information upon acquisition and link it to data acquired down the line. Maintain detailed records of the experimental parameters used for every step of every experiment.

## MEET REGULATORY STANDARDS

Logistical pressures caused by scaling up create inconsistencies, meaning that the end product can fail quality control and quality assurance evaluations.

Automation not only relieves logistical pressures and bottlenecks, but also improves precision, consistency, and record-keeping simplicity through production and QA/QC workflows, helping you become compliant with regulations and stay compliant.

# Checking it Twice: Compliance and Regulatory Issues for Cell Therapy Workflows

In order to bring any agent from the bench to the clinic, scientists must ensure not only the safety and efficacy of the product, but also the consistency of both product and production. This is especially pertinent for cell therapy candidate cells given their dynamic nature. Small inconsistencies in any aspect of the workflow could potentially cause variation in the final product that deleteriously affects efficacy, selectivity, and/or safety. In order to prevent such situations from arising and presenting a risk to public health, regulatory agencies enact strict regulations on all aspects of agent generation, from production, to quality assurance/control practices, to storage and transport, and to data storage and record keeping.

## Top Notch Therapy: Staying Compliant

Good manufacturing practice (GMP) guidelines were not written with cell therapy products in mind, but that does not mean that these processes are exempt from GMP, although the strict definition of GMP does vary from jurisdiction to jurisdiction.[1] Regulations pertaining to cells and cell therapy-related products for use in the United States are issued by the United States Food and Drug Administration (US FDA) and can be found under Code of Federal Regulations Title 21, Sections 600-699 (21 CFR 600-699),[2] which covers FDA regulations for biological products as a whole. In particular, §600.10 contains regulations on the necessary qualifications and protections for personnel involved in product manufacture,[3] while §600.11 details the requirements for the work area, equipment used during the workflow, and animals used during manufacturing processes.[4] §600.11 also provides information regarding required procedures for workflows occurring in multiproduct manufacturing spaces and for contamination.[4] Researchers working with cell therapy-candidate cells should pay close attention to Section 610, which contains regulations concerning testing for product potency, purity, sterility, and identity, as well as regulations for cell culture (§610.18).[5]

A product that passes all quality control/assurance tests upon leaving the production line is not necessary one that retains that state throughout its shelf life, especially if stored and/or transported under harsh environmental conditions. To evaluate product consistency over time, FDA regulations mandate that a representative sample of each product is kept in storage for six months beyond the expiration date for potential examination and testing (§600.13).[6] Specific mandatory transport conditions are also provided in §600.15,[7] although these pertain largely to vaccine products and blood products.

Proper record keeping is an essential element to the commercialization of a cell therapy product. Detailed records are not only critical to identifying potential issues within the workflow before they evolve into major problems, but also are necessary in case an event occurs that compromises the integrity of the product and/or the production workflow. The FDA provides regulations on record keeping practices in §600.12,[8] on dating and labeling products in §610.50 and §610.60-68,[5] and mandates that all deviations from GMP and/or unexpected events be detailed and reported in §600.14.[9] Specific guidelines pertaining to electronic record acquisition, maintenance, storage, and security can be found in Section 11 of Title 21 of the CFR.[10]

## Carrying the Load with Automation

Achieving regulatory compliance can initially appear to be a lofty task, especially if the production workflow involves multiple sites (*e.g.*, a clinical site and a manufacturing laboratory) – all of which must be GMP compliant so that product equivalence can be demonstrated.[11] Here, automation can be useful in alleviating logistical burdens, not only by minimizing practical manufacturing variability in the workflow, but also through the rapid and seamless collection, organization, and centralization of monitoring and testing data. Making regulatory-related processes routine can help scientists achieve and maintain regulatory compliance.

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# Complete your **cell therapy workflow** with Beckman Coulter Life Sciences

| Parameters collected during cell therapy production and the Beckman solution | | |
|---|---|---|
| **Viable Cell Count** | Used throughout the process to monitor cell viability and the impact of any cell manipulation events |  2 |
| **Cell Size and Volume** | Label-free method to monitor cell population during expansion, differentiation and/or transduction stages |  2 |
| **Cell Count** | General population monitor, particularly during expansion stages and when sample volume is manipulated (concentration, packaging, etc.) |  |
| **Aggregate Detection** | Primarily a concern during packaging and prior to patient administration |  2 |
| **Gene Expression** | Looking for specific surface marker or reporter gene expression |  1 |
| **Metabolic Indicators** | General monitoring during expansion stages when cells are under continuous culture |  2 |
| **Immunophenotyping** | Critical to ensure correct cell types are isolated and enriched in therapy population |  |

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