CELL & GENE THERAPY INSIGHTS

EXPERT INSIGHT

Using serum-free media to streamline and optimize CAR T-cell manufacturing workflows

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CAR T-cell therapies have led to breakthroughs in treating hematological malignancies, but the complexity and cost involved in their production limits their availability to patients. One challenge in CAR T-cell manufacturing outside of efficacy of your CAR-T product is achieving consistent expansion of T cells available after gene transfer. Optimizing the media used to support T-cell proliferation is, therefore, a key consideration—but it has proven difficult to develop media that enable consistent expansion while meeting regulatory quality requirements. However, recent advances in cell culture strategies have improved productivity and performance in CAR T-cell workflows using serum-free media, enabling the development of large-scale regulatory-compliant processes capable of producing billions of T cells within a short timeframe. This article will discuss how serum-free media can streamline CAR T-cell manufacturing workflows, highlighting how this can reduce time-to-market and make these treatments more widely available to patients.

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INTRODUCTION

CAR (chimeric antigen receptor) T-cell therapy plays a central role in the development of innovative therapeutic approaches to battle various cancers and non-cancer targets. Therapies utilizing CAR T cells have led to breakthroughs in treating hematological malignancies, and research is now exploring how to extend their use to treat solid tumors. In such therapies, T cells are extracted from a patient via apheresis and process-specific cell selection and genetically

engineered *ex vivo* to express the CAR, which is an artificial protein receptor capable of binding to specific cancer cell antigens and activating T cell functions. The genetically engineered cells are then expanded using a cell culture medium and returned to the patient for treatment.

Choosing an appropriate cell expansion medium is key to ensuring the health and performance of the relatively small populations of cells present at the beginning of the CAR T-cell manufacturing process, and after gene transfer. Traditionally, serum-containing media are widely used in such processes, but these come with a range of challenges. Serum can vary in quality between batches, and may contain toxic substances and impurities that not only require additional time and investment to remove, but can put the final product—the cells needed for patients—at risk. Moreover, as the industry becomes increasingly competitive, with numerous new CAR T-cell products under development, high-quality serum is becoming more difficult and expensive to obtain.

Due to these complexities and costs, the availability of CAR T-cell therapies to cancer patients is currently limited, despite their significant promise. Serum-free media offer ways to overcome these challenges, providing more reliable and effective ways to produce valuable CAR T cells.

THE LIMITATIONS OF SERUM-BASED MEDIA

The cell therapy industry is heavily reliant on serum derived from either animal or human donors, with demand for serum increasing as more cell therapy products enter clinical phases. However, serum is a complex mixture of a large number of constituents, and the quality of serum varies inherently between individual donors, causing the quality of subsequent products to differ between batches. This fluctuating quality can lead to inconsistency—and runs the risk of compromising CAR T cells produced for oncology therapeutics.

Even serum sourced from healthy human or animal donors may contain pathogens. Serum is usually sterilized by filtration through multiple 0.2 μ or 0.1 μ filters after collection, because heat-treatment may adversely affect its growth-promoting properties. This filter-sterilization procedure is, however, unreliable because small viruses, viral fragments or prions may pass through the filters. As a result, stringent quality control tests must be performed to ensure that serum is free from adventitious agents.

Serum that passes the sterility quality tests may contain undefined cytokines, hormones and growth factors, whose presence may influence the phenotype of the expanded T-cell product. Growth factors such as transforming growth factor beta (TGF-β) cannot be filtered out, and are present in sera at various concentrations in different lots, contributing to lot-to-lot variability. TGF-β induces the generation of regulatory T (Treg) cells that may suppress CD8 T-cell activity, which can diminish the efficacy of the final CAR T-cell product. Hormones such as glucocorticoids that are known for their anti-inflammatory and immunosuppressive actions may be present within sera, again impacting the final CAR T cells produced.

As a further consideration, prior to CAR T-cell infusion, cancer patients are typically pre-treated with lympho-depleting chemotherapy to ensure that endogenous T cells do not suppress the proliferation of infused CAR T cells [1]. Therefore, to safeguard patient health, there is an even greater pressure and need to successfully produce a high percentage of viable CAR T cells—and to ensure that this is done consistently.

To ensure high levels of sterility and an absence of unwanted substances or impurities, scientists must qualify all the raw materials and components they use in the serum-based expansion media. However, the need to check the quality of each serum lot before it can be used increases the overall cost of the CAR

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T-cell expansion process, and can delay CAR T-cell manufacture if there is a shortage of high-quality serum. With increasing numbers of new CAR T-cell therapies under development, competition for good quality serum is becoming steeper and more prevalent—and this may eventually increase the cost of CAR T-cell products.

SERUM-FREE MEDIA: OPTIMIZING CAR T-CELL EXPANSION

The use of serum-free media offers a potential way to address the challenges, limitations and costs associated with serum-containing media—including concerns around fluctuating quality, the need for extensive supplementation and the complexity of regulatory requirements. Serum-free media bring reliability, flexibility and versatility to the CAR T-cell expansion process, facilitating large-scale and regulatory compliant processes that are capable of producing billions of T cells in a short timeframe [2].

Improved quality & consistency

Serum-free media may use proteins (such as serum albumin) that are purified from plasma, and the additional processes involved in purifying these proteins help to eliminate some of the undesirable contaminants mentioned above. Using serum proteins derived from human plasma instead of materials derived from cattle also minimizes the risk of including transmissible bovine spongiform encephalopathy in the end products.

To eliminate inconsistency and ensure that vein-to-vein time is achieved within expectations, scientists require greater control. This control can be provided by serum-free media, which eliminate much of the variability that may compromise CAR T-cell production. Lower levels of variability also reduce the need for lot-by-lot qualification, which in turn helps the final product reach the market faster.

Streamline the supplementation process

Many available media are offered in the form of a liquid base that requires the addition of one, two, three or more components—such as serum, critical amino acids and other supplements—before use to stimulate cell growth and maintain high cell viability [3]. However, these supplements are often stored separately and under different conditions to the base media, and have different shelf lives, making their storage and maintenance a challenge. Some supplements must be stored in freezing conditions and thawed before use, adding time, inefficiency and complexity to the overall cell culturing process.

Serum-free media are 'complete' and ready to use, reducing the risk of contamination by eliminating the need for additional supplementation, which streamlines the overall cell expansion process.

This high-performance serum-free media is applicable not only to T-cell therapies, but also many other cell types, such as natural killer (NK) cells and dendritic cells. Due to their high versatility, serum-free media form highly suitable bases for a wide range of therapeutic applications (either with or without cytokine supplementation, depending on the specifics of the process).

Current Good Manufacturing Practice (cGMP) release criteria

Regulatory compliance and cGMP processes underpin the successful production of CAR T-cell therapies, and are, therefore, essential in ensuring effective therapeutic treatment reaches those in need. To protect patients, release criteria are strict. For example, the criteria for commercially manufactured Tisagenlecleucel (CTL019; Kymriah®), a medication used to treat B-cell acute lymphoblastic leukemia, which usually demand at least 80% viability before the therapy can be used to treat patients. Achieving high levels of T-cell viability, though, requires consistency and

high quality: even small changes in processes and raw materials, such as the composition of the growth media, can introduce inconsistency that puts the quality of the CAR T-cell product at risk.

By bringing the control and definition needed to eliminate inconsistencies and variability, serum-free media facilitate the production of CAR T cells that meet rigorous cGMP criteria. These media are a safe, high-quality alternative to serum-based media for effective CAR T-cell expansion in-line with regulatory requirements. They enable effective scale-up and are available in versatile formats—bottles or bags with plug-and-play connectors—that can be tailored to meet the specific needs of various cell production platforms.

TRANSLATION INSIGHT

Serum-free media made of non-animal origin materials hold great promise—and as the cell therapy industry moves away from serum and its galaxy of undefined, inconsistent components, more therapies will move towards serum-free media expansion. Media that do not rely upon blood-derived supplements offer increased regulatory compliance and process control, and will bring many more specific choices and opportunities regarding quality and performance in the development of novel cell therapies.

A notable area for future advancement regards chemically defined media. The introduction of recombinant versions of proteins and growth factors that are derived from blood will further eliminate the need for undefined, animal origin constituents from cell culture media and bring even-more consistent performance. Not only will this facilitate compliance with regulatory quality requirements, it will also enable the development of therapies that will present less risk to patients, while removing the variability associated with human sourced components.

While moving to this higher quality level of chemically defined media, manufacturers of expansion media should always ensure that performance remains top priority to safeguard cell viability. Overall, this will contribute to reaching the ultimate goal of cell-based therapeutics—ensuring patient safety and product efficacy—in a timely, efficient and reliable way.

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