

Back to the Future

The explosion in cell-based therapies has led to new technologies to streamline and standardise their commercial production. These tools could now be used back in the lab to help push the frontiers in biomedical research

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Over the last decade, cell-based therapies have garnered tremendous excitement from researchers, clinicians and the public. These therapies represent the next frontier in drug development. Some cross over into the field of personalised medicine in that they are derived from a patient's own cells, or they are tailored according to what will be optimal for different patient subsets.

With fewer than 40 cell-based therapies in commercial distribution in regulated markets – only half of which have been formally approved – and only a handful making over \$100,000 in annual revenue, the field is still relatively immature, but enjoying a steep growth trajectory (1).

Latest Wave

The pipeline of therapies is maturing, with more than 300 cell therapies currently in development. The latest wave has begun to prove successful in the clinic, with particular attention being paid, of late,

to cell-based immunotherapies (2). This was initially triggered by Dendreon's Provenge approval in the US (2010) and Europe (2013). Recent deals made by Novartis, Celgene and through venture capital investment illustrate that early-stage cell-based immunotherapies continue to gain momentum (see Figure 1, page 12).

Moreover, the explosion of cell-based therapies is having a knock-on effect throughout the biotechnology sector – for example, with the development of new technologies initially designed to streamline the production of cell therapies, but which have much broader applications. Early adopters of these new technologies include biopharmaceutical companies, contract manufacturers, clinics, and even basic researchers.

Cell Therapy Regulations

Traditional small molecule therapeutics must meet benchmarks for safety, purity

and efficacy; so too must biologics, albeit in fundamentally different ways. Many of the tests and terminologies applied to small molecules are not relevant to biologics. As such, international regulatory agencies have responded by developing new guidelines, with recommendations specific to cell therapy drug products (3,4).

The Office of Cellular, Tissue and Gene Therapies – a division of the US Food and Drug Administration (FDA) in charge of overseeing cell therapies – has noted: "The diverse biology and clinical indications, and the rapid and fluid state of the evolving scientific research into these product areas, pose unique scientific challenges in terms of regulatory review" (3). Because every cell therapy is unique, the onus of developing therapy-specific standards, tests and production methods often falls on the therapeutic developer. This inherent variability makes the federal mandate for safety and product standardisation, which has been in effect for more than 50 years, more important than ever.

The European Medicines Agency (EMA) has also established unique classification for biologics, including cell therapies, designating them advanced therapy medical products (ATMPs). Prompted by the highly experimental nature of ATMPs and other emerging therapies, the EMA set up an Innovation Task Force. It recognises that ground-breaking therapies often do not fit existing regulations, and so encourages early dialogue with therapeutic developers to “proactively identify scientific, legal and regulatory issues of emerging therapies and technologies” (5).

Compliance with regulatory agencies requires cell therapy product conformance testing, comparability studies and stability testing. These quality control tests ensure the identity of the cells, their potency, stability and lack of contamination (6).

Good Practice Guidelines

Historically, the FDA has issued regulations for drug development and production to prevent errors or accidents that could harm consumers, beginning in 1962 with Good Manufacturing Practice (GMP) guidelines. In 1978, the FDA issued Good Laboratory Practice (GLP) guidelines, which regulate the preclinical laboratory conditions and organisational system under which experiments are conducted.

As cell therapies began to gain traction in the clinic, the FDA responded by introducing Good Tissue Practice (GTP) guidelines in 2011 to regulate the manufacture of human cells and tissues for clinical use (7). These guidelines are designed to prevent the introduction or spread of communicable disease via cell therapy. GTP regulates the facilities and equipment used for cell product development, plus the ways in which cells are processed, stored and distributed. The guidelines relate to new technologies being developed for the cell therapy field.

Cell Variability

Cell-based therapies are still a very small and emerging niche of the biopharma industry. Developing a successful cell

therapy is difficult, in part because of the large number of variables that come into play when designing human-based biologic drugs, and the lack of standardisation typical of a juvenile industry.

These variables include the source of the originating cells, their differentiation status, and the tissue culture techniques required to maintain the cells. Additional variables include adjuvants in the final drug product, other *ex vivo* manipulations, and any scaffold on which cells are grown for implantation. The potential impact of some of these variables on the predictability of the final product can, in some instances, be largely minimised – for example, by applying best practices in tissue culture. However, challenges arise when trying to minimise all variations, such as those of autologous cell therapies, which inherently involve certain patient-to-patient variability.

From a practical standpoint, both the number of cells required per dose and the method of tissue culture affect cell therapy production. Single cell therapy doses can range from less than one million cells (central nervous system applications) to a few billion cells (cardiac therapy) (8).

Control Measures

Culturing multiple doses of a few million cells at a time is a manageable prospect; sufficient doses can be reasonably produced with existing equipment. However, culturing multiple doses of billions of cells requires uniquely tailored equipment that is currently extremely expensive and operator-intensive. For high-dose cell therapies, generating sufficient numbers of cells can be problematic, especially for small early-stage clinical trials.

Once released from manufacturing, the final drug product must be appropriately preserved to uphold maximum potency, and those conditions must be maintained throughout final product shipment to the clinic. Subsequent handling of the cell therapy drug product, such as cryopreservation

and thawing, are two additional variables that must be controlled (9,10).

The challenge of standardising biologics is relatively new. In response, there is a growing market for products that standardise the techniques and assays common to cell therapy production. New cryopreservation and thawing tools help companies comply with federal regulations, and ensure successful and reproducible cell therapies.

Tools of the Trade

The challenges and mandates mentioned above have resulted in a push for new technologies that streamline, automate and standardise the production of cell therapy products, by minimising contamination and human error in tissue culture, lab practices and release testing. These technologies are also used to fully characterise the cell therapy product, often at the level of RNA, DNA or protein. The same tools being used to standardise the commercial production of cells for the clinic will soon be translated to biomedical research labs, improving the reproducibility of R&D.

For instance, GMP compliance requires that cell therapy products be produced in designated clean environments (cleanrooms), which are subject to environmental monitoring for airborne particles and contaminants. Aseptic production of cell therapy drug products is critical due to their parenteral administration (11). Cleanrooms are expensive to establish and difficult to maintain. Cell processing workstations have been developed to provide a flexible, scalable alternative. They are, in essence, a cleanroom in a box. Designed for the production of cell therapy products, these GMP-compliant contained spaces can be cleaned in as little as 90 minutes. This rapid turnover allows for concurrent development of multiple cell therapy products (12).

High-throughput process automation is another major advance for the commercialisation of cell therapy products. For low-dose cell therapies (millions of cells), automated tissue

culture systems improve product consistency, with the added advantage of speed. Small flasks of adherent and non-adherent cell lines can be cultured in parallel, with no risk of cross-contamination – making them appropriate for early-stage research. The machines provide automated seeding, expansion and sub-cloning. For high-dose cell therapies, other options can fully automate the culture of a single cell line, in T-flasks or roller bottles (8).

Cryopreservation

Standardised cryopreservation techniques are also an important aspect of developing cell therapies. Following the expansion and/or manipulation of cells in culture, some cell therapy products are stored in liquid nitrogen until immediately prior to patient administration. Studies have shown the optimal rate of cryopreservation for maximum cell

recovery is -1°C per minute (13). This can be achieved using a programmable freezer, or a portable, passive freezing device in conjunction with a -80°C freezer. Both deliver consistent freezing profiles, but passive freezing devices eliminate the need for space, money, time and maintenance associated with a programmable freezer.

A recent study showed that such portable devices offer comparable post-thaw cell viability to programmable freezers, providing an inexpensive and scalable alternative to dedicated, expensive cell-freezing machinery. Passive freezing devices are currently being used to maximise the number of eligible Phase 2b clinical trial sites in a cell therapy study involving regulatory T cells (14).

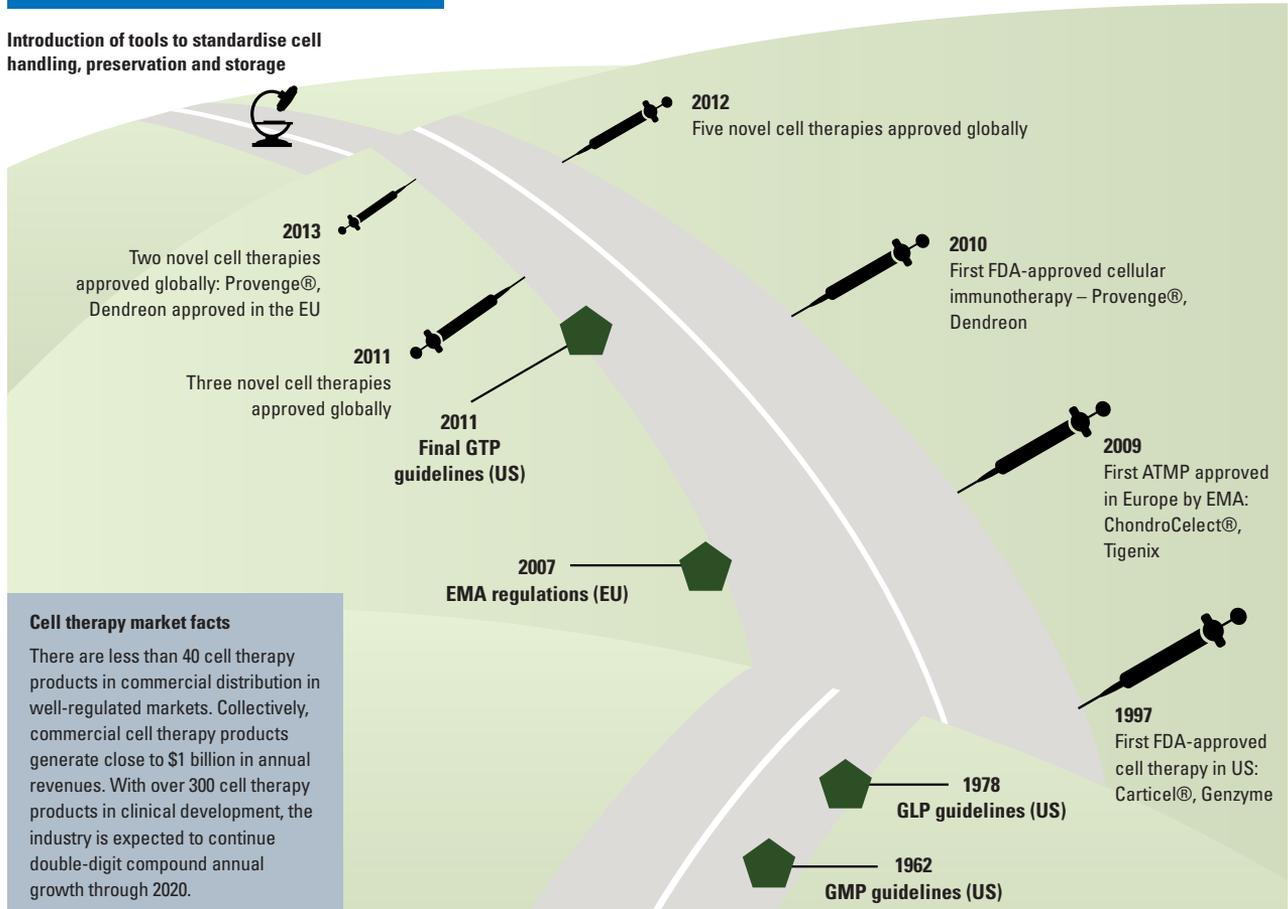
Other Developments

More devices are coming down the pipeline to assist in the standardisation

of cell-based therapies, all of which can be translated to research labs. Notable examples include cell washing devices (for elimination of cryopreservation excipients and subsequent volume reduction) and an automatic cell thawing device, which will bring the same level of standardisation to thawing as currently exists for cell freezing.

Also in development are high-throughput systems for standardised cell characterisation. These devices use microwells and microfluidics to do high-throughput analyses of DNA and RNA. They also have the capability to do small molecule screens (15). It is anticipated that any such material contribution to standardisation has the potential to improve patient-to-patient reproducibility and efficacy within clinical trials, by improving and standardising quality assurance efforts and increasing potency.

Figure 1: Road to standardisation of cell therapies



Industry Informs Academia

Irreproducibility is an issue of growing concern both in the academic community and among individuals in the pharmaceutical industry who rely on basic science to inform their decisions. The tools that bring standardisation to the clinic will serve the same purpose in research labs, resulting in more reliable results and a faster pace of research, as more techniques become automated.

These technologies will change the way biomedical research is done as they move from industrial and clinic settings into research labs. In other words, cell therapy has a real chance to improve standardisation and reproducibility, not just in development but also in basic research.

Standardised protocols for cell culture, cell freezing and cell handling will ensure that experiments can be repeated, and that cells are comparable from one experiment to the next. These are the same mandates that clinical cell therapies must meet, and integrate solutions for standardised preclinical and clinical research.

References

1. Buckler RL, Cellular therapy wave finally cresting, *Genetic Engineering News* 32, 2012
2. Buckler RL, Rise of cell-based immunotherapy, *Genetic Engineering News* 33, 2013
3. FDA, Guidance for industry: Preclinical assessment of investigational cellular and gene therapy products, 2013
4. Ancans J, Cell therapy medicinal product regulatory framework in Europe and its application for

MSC-based therapy development, *Front Immunol*, 2012

5. EMA Innovation Task Force. Visit: www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000334.jsp&mid=WC0b01ac05800ba1d9
6. FDA, Guidance for industry: Potency tests for cellular and gene therapy products, 2011
7. FDA, Current Good Tissue Practice and additional requirements for manufacturers of human cells, tissues, and cellular and tissue-based products, 2011
8. Prieels J, Lesage F, Argentin D and Bollen A, Mastering industrialization of cell therapy products, *BioProcess International* 10: pp12-15, 2012
9. Daniele N *et al*, T(reg) cells: collection, processing, storage and clinical use, *Pathol Res Pract* 207: pp209-215, 2011
10. Golab K *et al*, Challenges in cryopreservation of regulatory T cells for clinical therapeutic applications, *Internat Immunopharm* 16: pp371-375, 2013
11. World Health Organization, Environmental monitoring of cleanrooms in vaccine manufacturing facilities, 2012
12. Colmenares C, SANYO equipment puts UAB beyond the state-of-the-art for cell manufacturing, 2011. Visit: www.uab.edu/news/latest/item/1323-new-sanyo-equipment-propels-uab-cell-therapy-toward-clinical-care-potential-sickle-cell-cure-among-the-first-to-be-processed
13. Yokoyama WM and Ehrhardt RO, Cryopreservation and thawing of cells, *Curr Protoc Immunol*, Appendix 3:3G, 2012
14. Foussat A *et al*, Use of a low-cost passive-freezing device in the effective cryopreservation and recovery of human regulatory T-cells for use in cell therapy, *BioProcess International* supplement: Cell therapies, commercialisation, compliance, scale-up and biopreservation, March 2014
15. Fernandes TG, Clark DS, Dordick JS and Cabral JMS, High-throughput cellular microarray platforms: applications in drug discovery, toxicology and stem cell research, *Trends Biotechnol* 27: pp342-349, 2009

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