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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:

Preclinical and translational R&D insights

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ISSUE SPOTLIGHT:

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CELL & GENE THERAPY INSIGHTS

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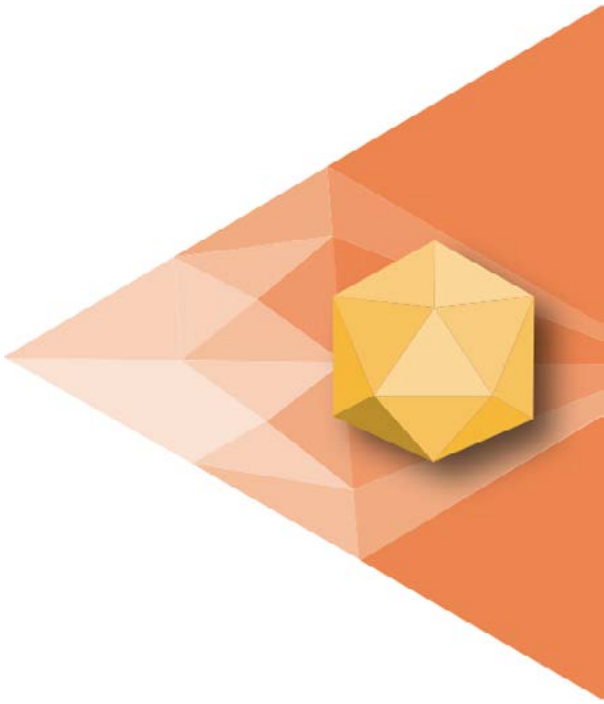
Suspension Culture Systems



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SPOTLIGHT

From laboratory to patients: dissecting obstacles in cell & gene therapy development

EDITORIAL

Daniela Nascimento Silva, Carlotta Spano, Martina Varricchio, Giulia Grisendi, Milena Soares, Anna Pasetto & Massimo Dominici (pictured)

Looking at the cellular therapy landscape, there are more 34,000 clinical studies reported in https://clinicaltrials.gov (searching for 'cellular therapy' closed, past, active, enrolling, non-enrolling) [1]. While this number most probably over-estimates the clinical impact of cells as therapeutics, it is beyond doubt that cells are progressively entering into the

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clinical scenario for a variety of clinical indications [2].

A recent report by The Alliance for Regenerative Medicine (ARM) describes more than 900 cell and gene therapy companies worldwide currently carrying out 1052 clinical trials: 363 phase I, 594 phase II and 95 phase III [3]. While these studies do cover the full spectrum of human diseases, the great majority deals with oncology (65%), compared with only 5% for each of neurological and skeletal diseases.

Among cell-based products (Figure 1), mesenchymal stromal/stem cells (MSC) represent a promising option for a variety of clinical indications, with approximately 1,000 studies overall, 250 recruiting investigations, and 9 MSC-based products with market authorization [2,4,5].

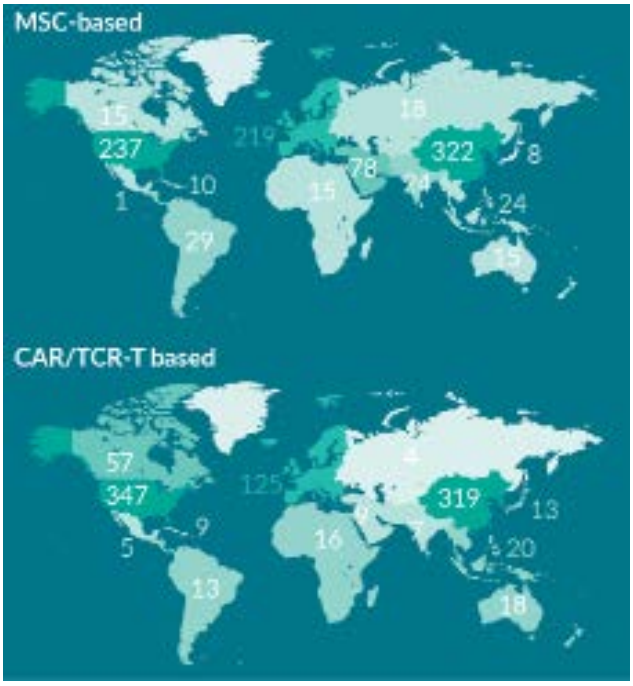
The second cell-based product area that has rapidly emerged as real a driver in cell and gene therapy is the chimeric antigen receptor (CAR)/ T-cell receptor (TCR) engineered cell therapy field; here, approximately 900 studies are reported with about 400 recruiting [6]. However, despite all these increasing numbers only 2 CAR-T cell-based products have so far obtained market authorization [2,7].

Focusing on oncology, we and others uncovered that MSC can be redirected to target tumors, becoming powerful anti-cancer molecules that deliver tools increasing the microenvironment bioavailability upon specific recognitions. Starting from this concept, we developed two main strategies based on both gene modified MSC and lymphocytes.

On the one hand, we aimed to modify MSC inducing expression of ligands capable of generating selective cancer death [8,9], and on the other, to modify lymphocytes by CAR targeting solid tumors [10]. In the first case, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) variants can be delivered to different cancer models, such as pancreatic adenocarcinoma and sarcomas. Here, adipose MSC were armed by lentiviral vectors to target a large variety of tumor lines and primary cancer cells both *in vitro* and *in vivo*. We showed that MSC can successfully deliver TRAIL variants to rapidly induce tumor death thanks also to synergizing chemotherapy agents within a novel combinatory strategy of multimodal chemo-gene therapy.

FIGURE 1

MSC and CAR-T cells reported clinical trials.



https://clinicaltrials.gov, Feb 2020.

Regarding CAR-T and TCR-T, we were able to generate lymphocytes modified to successfully target neuroblastoma, glioblastoma, hepatobiliary diseases and epithelial cancers [10–12]. Finally, in the attempt to further refine the MSC delivering approach, for the first time we included a CAR on the surface of MSC delivering TRAIL variants [13].

Moving these concepts from academic research towards a clinical translation strategy, we have been challenged by a variety of issues including the foundation of a university start-up (Rigenerand srl) that, with more than 500 sqm of classified environment in a cell factory, aims now to produce cell and gene therapy products for solid tumors. For this reason, we wanted to share the challenges that have been faced (and that we are still facing) during the development of MSC

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BOX 1

Key challenges in cell-based translation from laboratory to patients

1. Cells-related obstacles
2. Process and scale-up related obstacles
3. The "original sins" of academia

and CAR/TCR-T projects. We have classified these into 3 main obstacle areas (Box 1). While this may be somewhat reductive, we hope it may have utility for cell therapists around the word.

CELL RELATED OBSTACLES

Cell-based products are classified as drugs accordingly to regulators [14,15]. While this warrants high manufacturing standards and safe clinical translation, it does not completely encompass that cells are living entities coming from human subjects that have both intrinsic and acquired variabilities. These varibilities can impact on cell-based product translation towards a solid clinical scenario, and need to be considered when developing cell-based therapeutic approaches from early laboratory discovery onwards, as is the case for (gene modified) MSC and CAR/TCR-T therapies.

In this sense, there are several issues that can impact on products' clinical translation, as represented in Table 1. In particular, the source of cells can directly and indirectly influence clinical translation. For MSC, different tissue sources are associated with different performance *ex vivo* and *in vivo* [16]. In the case of TRAIL, for instance, the bone marrow derived MSC could not perform as well as adipose MSC while producing TRAIL (unpublished data). Also, for CAR/TCR modified cells, the cell source may make a relevant difference, as is the case when considering peripheral blood or cord blood cytotoxic CD8+ cells or NK cells. This may also impact upon the possible clinical uses (i.e allogeneic, autologous) [17–20].

Similarly, different individuals may see differing performance in terms of *ex vivo* proliferation, viral transduction and *in vivo* therapeutic potential [21]. For MSC, it is known that a fraction of the cells are incapable of generating bone, while for CAR/TCR-T cells, a fraction of patients' lymphocytes may not be efficiently manipulated and transduced [22,23]. Regarding MSC, this calls for ways (i.e., potency assays) to predict the mechanisms of action, either in the unfortunate circumstances of an autologous setting, or in the more dramatic situation of allogeneic cells obtained from a single non-performing donor. The issue of predicting cell performance may also be related to the capacity to predict

TABLE 1

Key issues impacting on MSC and CAR-T clinical translation

Issue	Impact on MSC clinical translation	Impact on CAR/TCR cells clinical translation
Different sources to different cell type	+++	++
Different donors to different performance (variability)	++	++ (some patients cannot generate CAR-T)
Defining MoA to differentiation soluble factor/s secretion, EV	+++	++ (more on side effects)
Animal models to challenge cells performance	++	+++ (more on side effects)
Delivery method: i.v., i.a., i.p. endotracheally etc.	+++	+ (hematological diseases) +++ (solid tumors)

Relevance. +: poor; ++: relevant; +++: very relevant.
MoA: Mechanism of action; EV: Extracellular vehicles.

side effects after CAR/TCR-T infusion, since the current pre-clinical *in vitro* and *in vivo* therapeutic models are not yet able to fully mimic the human clinical scenario.

Finally, the delivery method may also impact clinical translation, not so much for CAR-T in hematological malignancies, but more for solid tumors where the homing of the infused cells has yet to be properly address. Similar situation applies for MSC targeting cancer. In our case, to avoid bias due to cancer homing, we decided during pre-clinical development to move towards an intra tumoral infusion of MSC-TRAIL [8].

PROCESS AND SCALE-UP RELATED OBSTACLES

The translation of cells towards the clinic is not generally dissimilar to any other biotechnological product and involves a series of generic steps.

There is a first step of concept evaluation to generate basic research that can be transferred into pre-clinical investigation (generally *in vitro*). This is then followed by the creation of 'proof-of-concept' *in vivo* studies, where cell survival kinetics/ distribution and toxicology studies are carried out to define a cellular product's desired characteristics.

In this phase, rudimentary methods of cell manufacturing (i.e., for animal studies in the best-case scenario) and product testing are generally conceived, and appropriate laboratory instrumentation and reagents are identified. However, even in this early phase, obstacles in the translation of cells towards the clinic may be apparent, such as the fact that technologies used in early studies may not be optimized or transferrable for larger/clinical-scale cell production. In particular, the selected reagents (i.e., tissue culture media, dissociation and ancillary reagents) in the labs may not be suitable for human uses, and cell features may not be robustly defined. While all these aspects have been generally tolerated in the discovery phase, current advancements in the field call for special attention to be paid for solid translation from the early phases of academic research around cell therapeutics.

In a second step of process development, there is a phase of scale-up and optimization. This is associated with the development of a reproducible, large-scale manufacturing process that parallels the creation of a clinical study design. This also involves translational development into a cGMP laboratory to develop tools, to scale-up the process, and to optimize manufacturing (incorporating regulatory-grade product characterization and QC). In the case of gene therapy, this is also linked with viral vector development and manufacturing, conducted either in house or by CMO. Here, there is also the transition from laboratory experiment book to SOP, and in the best-case scenario, batch production records (BPR) are developed.

This second step is the most critical part of a cell-based product's development. It might be described as the 'teenage years' of the product: lots of excitement for things to come, but also severe uncertainties about the future. Here, research-based methods are frequently not perfectly refined, and the experimental book from "academic wild lab" may be difficult to translate into SOP, hindering a smooth transition into cGMP manufacture. Importantly for gene therapy, viral vector comparability between R&D grade and cGMP grade may become a relevant issue to be accounted for not only for safety reasons, but also for the vector backbone, for the type of promoter, and for manufacturing steps that would allow generation of an adequate quantity of vector to execute early clinical trials in the respect of related regulatory issues (e.g. gene copy number).

Theoretically, this phase has to be carefully designed from the start by cell and gene therapy developers since obstacles here may be so relevant to, and dramatically impair, product development. To underline the critical nature of this moment (generally taking place during Phase I/IIa trial preparation and execution), clinical trial design, patient selection, delivery methods and read-out must also be defined

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and presented to regulatory bodies within pre-IND and IND.

Finally, there is a third very advanced step with methods validation. This begins when a clinically appropriate and optimized method has been defined. Here, SOP are established and a validation plan is generated and executed. In this phase, the CMC section of the investigational new drug application and the validation results are provided to the regulatory body and to the institutional review board for approval. The major obstacle is related to the fact that the CMC of a living cell-based drug is challenging, in particular for autologous products [24].

THE 'ORIGINAL SINS' OF ACADEMIA

Since most cell-based product have emerged from academia, they may carry what we call the "original sin" of academia. In some cases, GMP laboratories in Hospitals/Universities spin out from research and development laboratories, and so may carry the "bugs" of early academic research. This is not necessarily a negative aspect, but it does require a cultural step in the direction of cell manufacturing, cell culture standardization and quality control/assurance.

Aspects related to academic cGMP facilities may negatively impact large cell-based product development in that environment: limited space, small teams and underestimation of long-term sustainability of the maintenance costs and personnel. Other, not inconsiderable aspects relate to regulatory and economic hurdles for phase I/II studies that are generally developed with little consultation with regulatory experts. This may generate obstacles when the product would be moving from a first-in-man study towards advanced Phase II/III studies.

Finally, while academia generally dreams of partnering with biopharmaceutical companies that would empower the process/product with required investment, this tends to take place only very late in the process and scale-up development. This in turn may have a negative impact on the "cGMPification" process and the subsequent industrialization. Therefore, there is the need to combine early academical research with the biopharmaceutical world from the earliest phases of cell (and gene) therapy product development, and in particular, from the beginning of the second step described above.

HOW TO FILL THE GAPS (OR BEGIN TO...)

Having recognized these obstacles during the transition from a research laboratory to a cGMP manufacturing environment, we would like to propose some strategies on how to fill the gaps:

1. to conceive and foster dedicated training programs in collaboration with stakeholders (Universities, Regulators, Scientific Societies, such as ISCT and Pharma Industry) to train scientists to think as cellular therapists from the early steps of concept evaluation;

2. to create dedicated know-how (i.e about media, reagents, instruments) around the translational processes in cell and gene therapy;

3. to continue facilitating and enforcing phase I/IIa studies performed at the academic level by accelerated regulatory pathways;

4. a better (earlier) integration between academia and pharmaceutical industry;

5. to create non-profit national/regional Authority/ies to facilitate the early links between academia and industry;

6. to allocate financial resources for infrastructure and maintenance by national/regional founding bodies favoring

networking between research laboratory and cell factories;

7. to allocate financial resources supporting laboratory services for a number of cGMP facilities;

8. to identify novel *ex vivo* cell manipulation procedures/devices (i.e close systems, bioreactors, isolators) capable of delivering innovative, consistent, safer and sustainable cell manufacturing and gene modification steps.

Recognition of these challenges and the proposed strategies may represent fundamental first steps towards faster, safer development of cell and gene therapies for patients with unmet medical needs.

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AFFILIATIONS:

Daniela Nascimento Silva
Karolinska Institutet, Stockolm, Sweden
and;
Health Institute of Technology, SENAI-CIMATEC, Salvador, Bahia, Brazil

Carlotta Spano
Rigenerand srl, Medolla (MO), Italy

Martina Varricchio
Rigenerand srl, Medolla (MO), Italy

Giulia Grisendi
University-Hospital of Modena and Reggio Emilia, Modena, Italy

Milena Soares
Health Institute of Technology, SENAI-CIMATEC, Salvador, Bahia, Brazil
and
Gonçalo Moniz Institute – FIOCRUZ, Salvador, Bahia, Brazil

Anna Pasetto
Karolinska Institutet, Stockolm, Sweden

Massimo Dominici
Rigenerand srl, Medolla (MO), Italy
and
University-Hospital of Modena and Reggio Emilia, Modena, Italy
Author for correspondence:
massimo.dominici@unimore.it

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CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL INSIGHTS

SPOTLIGHT

EXPERT INSIGHT

The rise of human induced pluripotent stem cell technology in drug development

Chun Liu & Joseph Wu

Drug development has been significantly hampered over the past few decades, partly due to our over-reliance on animal models and immortalized cell lines. Recent advances in human induced pluripotent stem cell (iPSC) technology provide an excellent physiologically relevant approach for disease modeling, compound screening, and preclinical evaluation of drugs. Human iPSCs generated from patients have unique advantages over conventional models, including the potential to differentiate into any cell type and still retain the genetic identity of the patients. The growing interest to adopt iPSC technology for drug development by researchers and pharmaceutical companies has become increasingly evident. Here we discuss in detail the recent advances in iPSC technology relevant to the process of drug development.

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Drug development is a lengthy and complex process that is accompanied by exorbitant costs. Indeed, the passage of a new drug from the bench to the clinic these days costs more than $2 billion and takes around 10–15 years [1]. Moreover, this inefficient process is plagued by a high failure rate as only half of the compounds from the discovery phase progress to the preclinical phase, with the remaining 90% of these compounds failing later during clinical trials [2]. These high drug attrition rates are partly attributed to our over-reliance on non-human cellular and animal models for efficacy and safety studies, especially during the early stages of drug development. Thus, there is a compelling need

to introduce more reliable tools to not only streamline our drug development process but also to produce more safer and more efficacious drugs.

The advent of human induced pluripotent stem cell (iPSC) technology has provided new opportunities to advance the drug development process in a more reliable and efficient way [3]. Since their seminal discovery, iPSCs that can be generated from various human somatic cells (e.g., blood cells, fibroblasts, hepatocytes, and keratinocytes) have been widely used for many purposes, including studies to model diseases, drug toxicity, and regenerative medicine [4,5]. This utility has been mainly due to their capacity to self-renew and differentiate into any cell types in the body, such as neurons, cardiomyocytes, hepatocytes, and islet cells. By providing an invaluable resource of physiologically and clinically relevant human cells with easy access, iPSCs technology has raised great interest in both academia and industry to advance every stage of drug development (Figure 1).

MODELING HUMAN DISEASES FOR A TARGET

Conventionally, cell lines, animal models, and primary patient cells have been heavily relied on for human disease and drug target studies. However, human cell lines typically contain genetic and epigenetic artifacts due to genetic modifications and immortal expansion, factors that prevent the accurate recapitulation of human diseases [6]. Likewise, animal models are limited as faithful human physiological models due to well documented substantial inter-species differences [7]. Finally, primary cells from patients are physiologically relevant but are difficult to acquire and fail to maintain their phenotype in long-term culture [8]. By comparison, iPSCs derivatives such as iPSC-derived cardiomyocytes (iPSC-CMs) offer several major advantages over these conventional models. These include the ability to generate human cells that recapitulate the cellular physiology and function of primary cardiomyocytes, given that the resulting cells are genetic replicas of the patient's own cells. Furthermore, the iPSC technology provides an unlimited supply of cells that allow researchers to conduct long-term studies without the presence of the patients [9]. These superior traits have led researchers to devote a significant amount of effort towards improving methods that can generate pure, scalable, and cost-effective iPSC-CMs [10–12]. Indeed, with these ever-improving protocols, we can now generate iPSC-CMs that are similar to primary human cardiomyocytes with regards to their molecular and metabolic profiling, ultrastructural features, electrophysiological function, and calcium handling properties.

Human iPSC-CMs have been used to model human disease 'in-a-dish' as they can retain the genetic variance exhibited by the patients and thereby serve as an ideal surrogate of the patient for *in vitro* studies. For instance, iPSC-CMs have been used to model inherited heart diseases, such as familial hypertrophic cardiomyopathy (HCM) [13], familial dilated cardiomyopathy (DCM) [14], long QT syndromes [15], catecholaminergic polymorphic ventricular tachycardia (CPVT) [16], and arrhythmogenic right ventricular dysplasia (ARVD) [17]. For instance, the duration of action potential of iPSC-CMs from long-QT syndrome type 1 patients with R190Q-KCNQ1 mutation was found to be markedly prolonged compared to iPSC-CMs from healthy subjects, suggesting that iPSC-CMs can recapitulate the electrophysiological phenotypes of long-QT syndrome 1 [15]. This study further revealed that the reduction of IKs current and altered ion channel activities in iPSC-CMs from long-QT1 were associated with the dominant negative trafficking of mutated KNCQ1 protein. Similarly, iPSC-CMs have also been used to model acquired heart diseases, including Type 2 diabetic cardiomyopathy (T2DM) [18], cardiac ischemia [19], and viral myocarditis [20]. Human iPSC-based disease modeling has allowed researchers to investigate the underlying mechanisms of many heart diseases and

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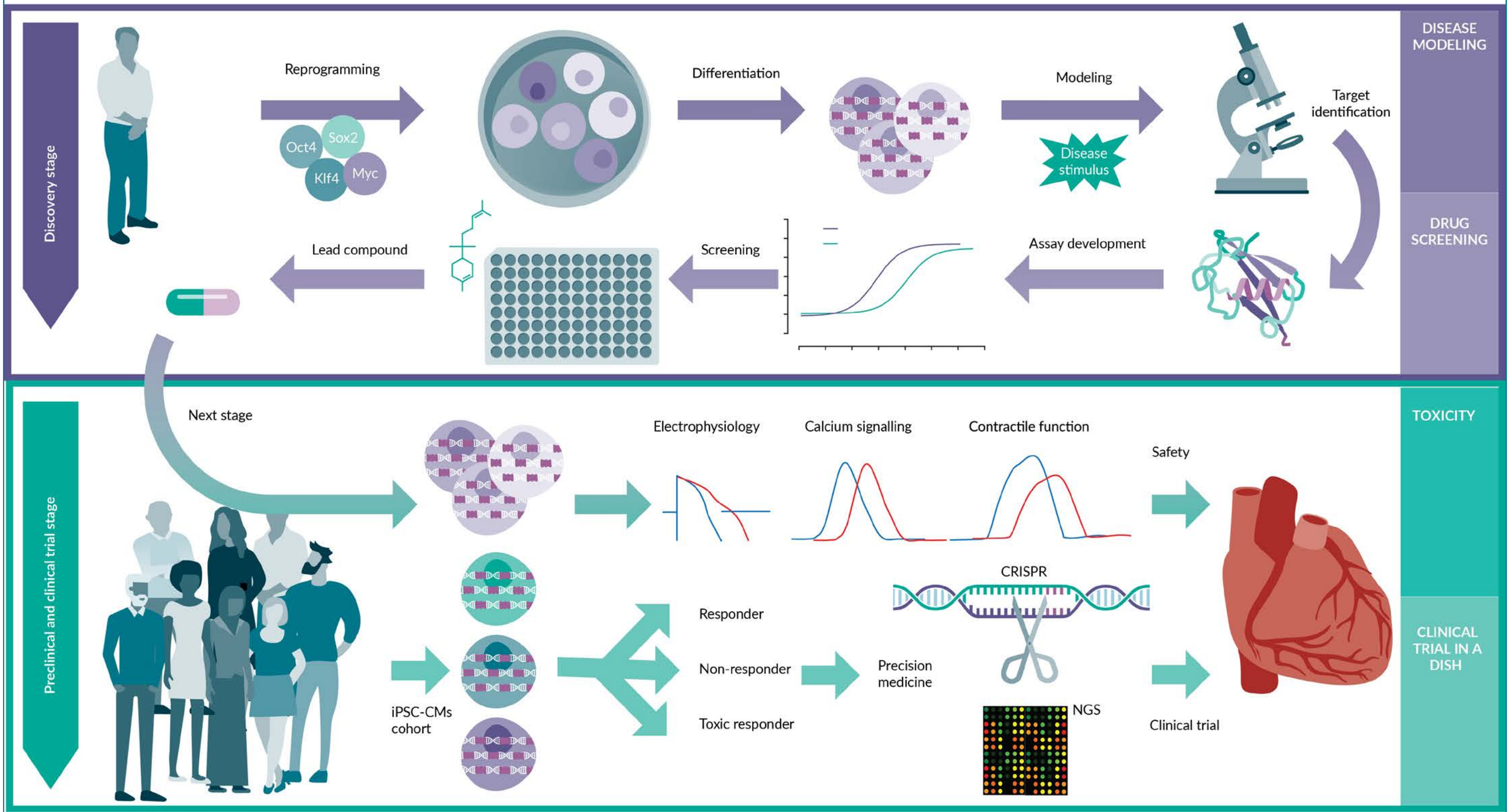
FIGURE 1

Schematic overview of how iPSC technology can be applied in the drug development pipeline.



iPSCs reprogrammed from blood cells or skin fibroblasts can differentiate into clinically relevant cells (e.g., iPSC-derived cardiomyocytes) and be used for modeling human diseases to understand their cellular and molecular mechanisms. By screening compounds targeting the basis of diseases with the iPSC derivatives, leading candidates can be preclinically evaluated for their safety and toxicity. Importantly, iPSC-derived cells from a large cohort of patients may also identify subpopulations of patients who will respond to the drug versus those who will develop adverse effects in advance of clinical trials. Taken together, the growing use of iPSCs can dramatically streamline and reduce the costs of the drug development process.

identify novel drug targets that had remained obscure when using conventional models. A recent study to model familial DCM due to a specific inherited mutation in the lamin A/C (LMNA) gene serves as a good example to illustrate how the iPSC technology can be leveraged to identify novel disease mechanisms and drug targets in an already well-studied cardiovascular disease [21]. In this study, researchers applied genome-editing technology and next-generation sequencing (NGS) to iPSC-CMs derived from LMNA patients to discover that abnormal activation of the platelet-derived growth factor (PDGF) pathway was partly responsible for the observed disease phenotypes in patients. This then allowed them to identify the PDGF pathway as a novel drug target for LMNA-related DCM that may eventually lead to new therapeutic strategies.

SCREENING FOR A DRUG

Target-based screening has been the leading choice in the pharmaceutical industry for the past two decades [22]. A similar approach could be applied when using the iPSC technology. Once a drug target has been validated for a selected human disease, high-throughput drug screening can be initiated using cells derived from patient-specific iPSCs, which serve as an ideal platform for cellular assays for drug discovery studies. Indeed, a recent study conducted a target validation study using iPSC-CMs to demonstrate that MAP4K4 was responsible for the cardiomyocyte death under myocardial infarction stress [19]. After screening ~1,800 compounds, the study identified a novel MAP4K4 inhibitor that could protect human iPSC-CMs from ischemic injury by restoring their mitochondrial function, calcium signaling, and contractile properties. For diseases in which a druggable target has not been identified yet due to lack of mechanistic understanding, a phenotypic screening can be conducted as an alternative approach. This approach allows researchers to screen compounds that can modulate disease-relevant phenotypes without a specific target or mechanism of action [23]. As phenotypic screening requires cellular models that can closely resemble the physiological conditions of the human body, iPSC technology has an undeniable advantage over most other conventional models. Specifically, in heart disease studies, a phenotypic screen can be established based on the measurement of one or more morphological or functional alterations in the iPSC-CMs, such as sarcomere structural, metabolic, calcium handling, or electrophysiological changes. Indeed, a recent study established a phenotypic screening strategy to discover drugs that have the potential to regenerate heart cells following heart failure [24]. The strategy included screening ~5,000 compounds on iPSC-CMs with their readout being cell proliferation. After initial screening, the researchers validated their top 10 hits in iPSC-CMs and discovered that mevalonate pathway activators promoted cardiomyocyte proliferation. Similarly, another study screened 480 compounds to rescue Type 2 diabetic cardiomyopathy using iPSC-CMs [18]. The primary screen was conducted in iPSC-CMs based on their T2DM phenotypes, including loss of sarcomere integrity, elevated BNP release, and increased peroxidization of lipid. Intriguingly, validated hits from the phenotypic screen (e.g., lycorine, bosentan, mifepristone, and H89) can also be translated into rescue T2DM functional defects. With the emergence of fluorescent voltage and calcium sensors, along with advanced automated microscopy platforms and machine learning tools, we can anticipate more high-content phenotypic screens using iPSC-derivatives [25].

PRECLINICAL ASSESSMENT OF TOXICITY & SAFETY

Cardiotoxicity remains as one of the major contributors to the drug attrition rates as many drugs are unable to pass the clinical trial stage due to their low cardiac safety index [26]. Our current safety and cardiotoxicity

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assessment at the preclinical stage is heavily reliant on animal models and human ether-a-go-go related gene (hERG) expressing immortal cell lines such as Chinese hamster ovary (CHO) and human embryonic kidney 293 (HEK293) cells [27]. While animal models are capable of providing some mechanic insights into drug induced cardiotoxicity studies, they are fundamentally limited because they cannot accurately recapitulate human cardiotoxic responses due to considerable variation of the inter-species differences in cardiac physiology and function, such as beating rates (e.g., 500 to 700 beats/min in mice vs. 60 to 70 beats/min in human), electrophysiological activities (e.g., shorter action potentials in mice compared with human), and mitochondrial metabolism [27,28]. Meanwhile, the hERG (potassium Ikr channel) expressing cell lines are commonly employed in current cardiotoxicity safety studies, but these oversimplified cell lines are genetically unstable and lack of complex characteristics of human cardiomyocytes [29]. Therefore, there is an apparent gap between current preclinical assessment methodologies and the translation of drug discovery to their use in the clinic. Human iPSC-CMs have now been comprehensively shown as being capable of filling this gap and serve as a good model for cardiotoxicity studies [30]. Indeed, the FDA Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) initiative has utilized the human iPSC-CM platform to detect potential rate-corrected QT (QTc) interval prolongation and Torsade de Pointes (TdPs) [31]. Moreover, as part of this CiPA initiative, a recent multisite study showed that iPSC-CMs were able to confirm the proarrhythmic potential of 28 drugs with known clinical TdP risk [32]. In another aspect of drug development, iPSC-CMs worked as an excellent platform to detect potential side-effects of drugs that remained undetectable in preclinical animal models. For example, the clinical side-effect of Gilenya, a S1P1/S1P3 agonist that causes bradycardia in patients, was recapitulated in human iPSC-CMs but was not detected in rat CMs [33]. Similarly, recent studies have shown that unexpected dysthymic side-effects due to drug-drug interactions can also be detected in iPSC-CMs, such as the combination of sofosbuvir (a hepatitis C antiviral agent) and amiodarone (a class-III antiarrhythmic drug) [34]. Besides proarrhythmic activities, other types of cardiac toxicity and safety concerns can also be examined using the iPSC-CM platform for preclinical assessment and clinical trials, including the calcium handling, contractile force, mitochondrial function, cell viability, and myocardial damage biomarkers.

CLINICAL TRIAL IN A DISH

Clinical trials play a pivotal role in the process of drug development as the standard for each drug is to achieve the required efficacy and safety in a large group of diverse patients. Even with a potential drug that shows promising results in preclinical studies, the cost and time for recruiting a sufficient number of suitable volunteers for clinical trials can often delay the process of drug development. iPSC technology has the potential to significantly improve our efforts to conduct clinical trials as functional cells derived from patient-specific iPSCs can be used *in vitro* to classify patients who respond (responders) or fail to respond (non-responders) to a drug. These 'clinical trials in a dish' are feasible because iPSC derivatives retain the genetic make-up of the patients and thereby recapitulate safety and efficacy responses to drugs. Moreover, they allow the recruitment of large cohorts of donors without limitations because patient-specific iPSCs can be extensively biobanked and easily derived at a later stage for screening purposes [35,36]. For example, a drug of interest could be tested on a large cohort of patient-specific iPSC-CMs to analyze the efficacy and safety using functional assays. Based on these assays, individual iPSC-CMs' responsiveness to the drug can allow us to identify a subset of patients with specific diseases that are responders, non-responders, or toxic responders. Once classified as a

responder, this specific patient can now be moved to the next phase of the clinical trial. As these patients' iPSC-derivatives respond to the drug *in vitro*, we have a higher chance of running a more successful and faster clinical trial [27].

Despite being in their infancy stage, several pilot studies have shown the potential therapeutic benefits of iPSC-based 'clinical trials in a dish', especially in the cardio-oncology field [37]. Cardiotoxicity is one of the main complications observed in cancer patients receiving anti-cancer treatment. Indeed, iPSC-CMs have been used to successfully recapitulate patient-specific cardiotoxicity phenotypes of various anti-cancer drugs, including anthracyclines, tyrosine kinase inhibitors, and anti-HER2 antibody [38–40]. For instance, in the study of anthracycline-induced cardiotoxicity, iPSC-CMs from cancer patients with clinical cardiotoxicity were found to be more sensitive to anthracycline induced cellular dysfunctions compared to iPSC-CMs from cancer patients without clinical cardiotoxicity [38]. These studies suggest that iPSC-CMs can be used to predict drug toxicity and efficacy in individuals, thereby laying the foundation of iPSC-based 'clinical trials in a dish' and precision medicine.

LIMITATIONS

Although the iPSC technology provides an excellent platform for disease modeling, drug screening, and toxicology studies, they currently have several limitations that can influence the drug development process. One such limitation is the technical variation of obtaining iPSCs and iPSC-derivatives from different methods and resources. Standardized, scalable, and industrialized production of iPSC-derived cells is essential to generate reproducible and reliable data for drug development. Another shortfall is the immature status of these iPSC-derivatives that exhibit more of fetal-like phenotypes and functional characteristics [41]. Indeed, there is an on-going effort to develop methods that can mature these iPSC-derived cells as immature cells may not fully reflect the drug responses as their equivalent cells *in vivo*. For example, strategies to mature iPSC-CMs include mechanical stretch, electric stimulation, metabolic alteration, and genetic modulation [42].

Another major factor limiting the potential use of iPSCs for drug development is the lack of heterogeneity. iPSC-derivatives usually lack tissue- and organ-level organization that may be central to many disease pathophysiology and drug responses [43]. For example, a human heart is a mixed population of many cell types with sophisticated structures. These include atrial myocytes, ventricular myocytes, and cardiac conduction cells that are housed in different chambers. Moreover, the presence of non-myocytes that accounts for around 70% of the total cells (e.g., fibroblast, endothelial cells, and smooth muscle cells) further adds to the complexity of the heart. New developments such as the inclusion of optimal bioengineering tools to construct 3D models of the heart from iPSC-derived cells are therefore much needed to better mimic non-cell autonomous pathogenesis and drug responses.

CONCLUSION

Compared to other therapeutic classes, drugs targeting cancer not only have the most number of applications but also the highest first-cycle FDA approval rate over the last two decades [44]. Cancer cell lines play an instrumental role in the prosperous oncology drug development by providing suitable cellular representatives for drug screening and preclinical evaluation with a human context [45]. Analogous to the utilization of cancer cell lines, iPSC-derived cells have been studied extensively in every step of the drug development pipeline. iPSC-derivatives (e.g., iPSC-CMs as discussed in this review) provide a unique platform for human disease modeling, target validation, drug screening, preclinical assessment, and personalized medicine. Extensive efforts are now

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underway to further promote the industrialized production, adult-like maturation, and organ-like architecture and function using tissue engineering, organoid, and other state-of-art technologies. Importantly, the iPSC platform is shifting the current paradigm of drug development and needs the awareness and input of everyone involved in the industry, regulation framework, and clinics to accelerate this exciting move.

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AFFILIATIONS

Chun Liu
Stanford Cardiovascular Institute, 2Department of Medicine (Division of Cardiology), Stanford University School of Medicine, Stanford, CA 94305, USA

Joseph Wu
Author for correspondence
Stanford Cardiovascular Institute, 2Department of Medicine (Division of Cardiology), Stanford University School of Medicine, Stanford, CA 94305, USA
joewu@stanford.edu

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL INSIGHTS

SPOTLIGHT

EXPERT INSIGHT

Vascular microphysiological systems to model diseases

Qiao Zhang, Xu Zhang & George A Truskey

Human vascular microphysiological systems (MPS) represent promising 3D *in vitro* models of normal and diseased vascular tissue. These systems build upon advances in tissue engineering, microfluidics, and stem cell differentiation and replicate key functional units of organs and tissues. Vascular models have been developed for the microvasculature as well as medium-size arterioles. Key functions of the vascular system have been reproduced and stem cells offer the potential to model genetic diseases and population variation in genes that may increase individual risk for cardiovascular disease. Such systems can be used to evaluate new therapeutics options.

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INTRODUCTION

Complications from cardiovascular disease (CVD) represent the leading cause of death in the United States [1] and other developed countries. The underlying cause for most CVD is atherosclerosis, in which cholesterol-laden plaques on the inner wall of the arterial lumen cause loss of vascular elasticity, reduction in blood flow, and narrowing of the arterial lumen. Risk factors for CVD include age, hypertension, Type 2 diabetes, obesity, hypercholesterolemia, and smoking that induce oxidative stress resulting in modified forms of low-density lipoprotein (LDL), inflammation, and smooth muscle cell proliferation [2–5]. The formation of plaque and oxidation of LDL causes endothelial cell activation and later leads to the recruitment

of monocytes. In the presence of oxidative and pro-inflammatory stimuli, monocytes differentiate to macrophages and promote the formation of foam cells. The plaque may eventually rupture, which results in thrombus formation in the blood vessel, causing ischemia, heart attack, or stroke [4]. Other diseases that lead to vascular damage including thrombotic disorders (deep vein thrombosis and disseminated intravascular coagulation), Marfan syndrome, aortic aneurysm, heart valve disease, congenital defects, and Progeria. Human immune system and inflammatory pathway activation play key roles in the initiation of atherosclerosis [6].

Mice are commonly used to study the genetic factors in vascular diseases [7]. While animal models have provided crucial information about the initiation and progression of atherosclerosis, they still possess many shortcomings and cannot produce many of the features of the pathology found in humans. Wild type mice use high-density lipoprotein to transport cholesterol to tissues while humans use LDL [8]. The size of arteries in mice is much smaller compared to humans and the heart rate is much higher [9], leading to very different hemodynamic conditions. The many interacting polymorphisms identified in genome-wide association studies cannot be replicated in mice. Given these limitations, the response of treatments in mice may differ from that in humans [10,11].

To overcome these pitfalls with animal models, human microphysiological systems (MPS) have been introduced to improve the accuracy of experimental predictions, minimize experimental time and cost, and reduce patient risk. Experiments that use MPS are highly reproducible. MPS use advanced fluidic fabrication methods to create 3D models of the functional unit of tissue. These systems can be used for functional assays as well as genomic, metabolomic, and histological analysis. The major advantage of MPS is that they can be modified to test single or combination of hypotheses, which allows identification of the key factors in different model systems.

Vascular MPS use microfluidic devices with 3D culture methods to recapitulate many model systems (Figure 1) [12]. Furthermore, one or more MPS can be combined together to study systematic effects by the key contributing factors. To study the initiation and progression of vascular disease, tissue engineered blood vessels (TEBVs) have been designed to model many vascular diseases, including atherosclerosis, progeria, and thrombotic disorders [13–17]. The versatility of TEBVs allows the use of these models extended to vasculitis in rheumatoid arthritis and lupus or the role of oxidative stress [18]. Given the different features and fabrication strategies of microvascular systems and TEBVs, each will be described separately.

In this perspective we examine the design criteria to build vascular MPS at the level of microvascular networks and as mimics of arterioles and arteries. Next, we address the various cell sources and the properties needed for adequate differentiation. Disease models are an important application area of MPS and may be used to assess the safety and efficacy of novel therapeutics.

DESIGN CRITERIA & FABRICATION OF ARTERIOLE- & ARTERIAL-SCALE MPS

The ultimate goal of designing vascular MPS is to reproduce key vascular functions *in vivo*. The minimum requirements are to allow blood flow under physiological pressures (10–20 kPa) and shear stress (0–2.5 Pa) without inducing thrombosis or inflammation [19]. Given that blood flow is generated by pressure induced by the heart, the large vessel MPS or TEBVs must process two mechanical properties, resistance to rupture (measured by burst pressure) and resistance to plastic deformation (measured by compliance) [19–21]. The burst pressure of the human saphenous vein and artery are around 2000 and 3000 mm Hg, respectively [22–24]. The compliance of human vessels ranges between 1 and 6% [24–28]. Hence, an ideal TEBV should

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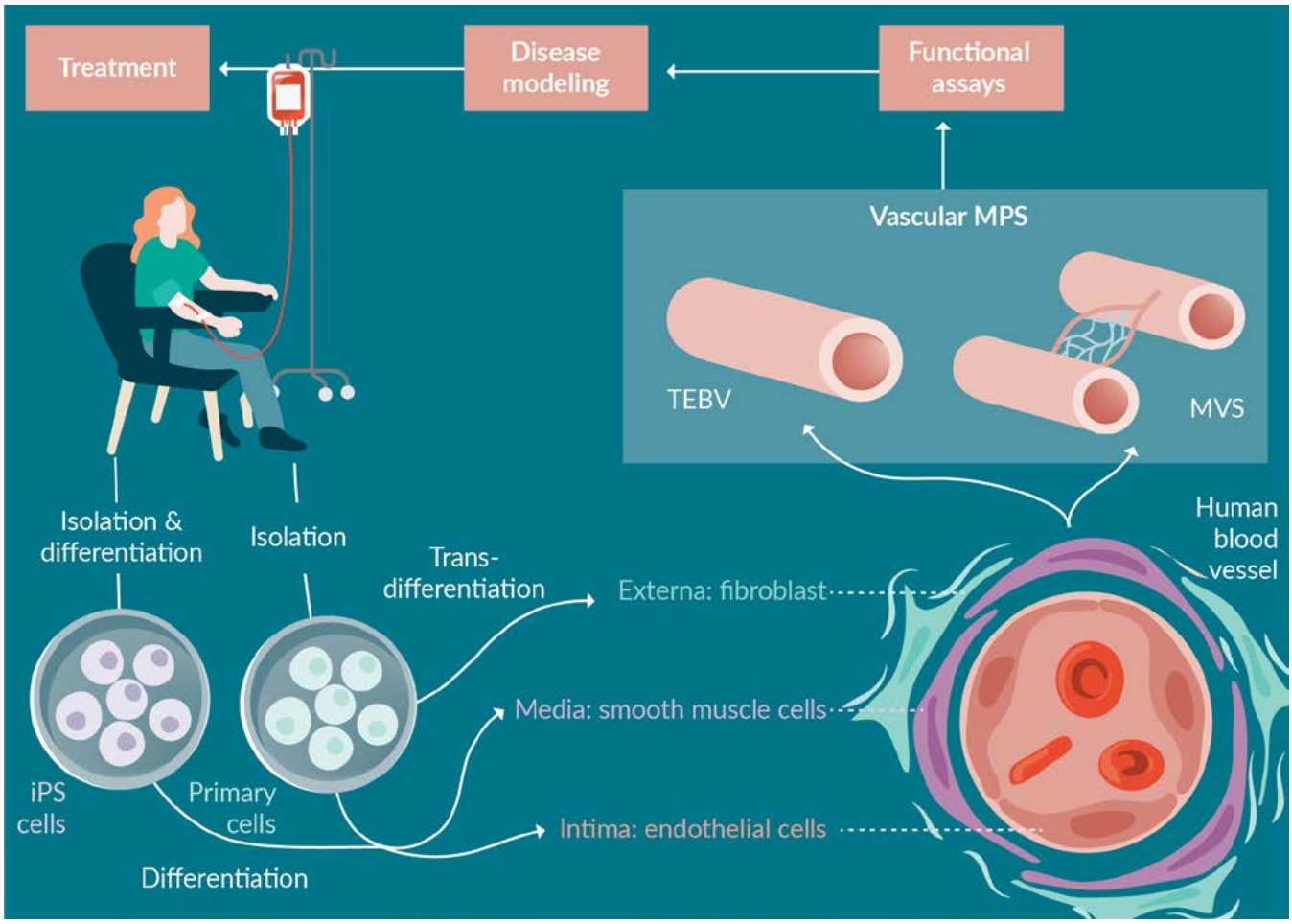
FIGURE 1

Overview of *in vitro* human vascular system.



Fibroblasts, smooth muscle cells and endothelial cells that resemble the externa, media and intima layers, respectively, of human blood vessel could be obtained by isolation, differentiation from stem cells, or transdifferentiation from other cell types. Human vascular MPS including TEBV and MVS manufactured from these cells could be applied for functional assays, disease modeling and treatment discovery. These results could potentially translate back to patients.

possess mechanical properties similar to the natural vessels.

Other than the mechanical properties of vascular MPS, biological properties also contribute to vessel failure. Protein adsorption on the surface of vascular MPS could subsequently lead to blood clotting. Hence, the non-thrombogenic and non-immunogenic behavior is necessary for designing vascular MPS, usually by incorporating a layer of vascular endothelium. The barrier function of vascular endothelium varies considerably from impermeable in the brain microvascular network to relatively permeable in the kidneys and liver [29,30].

Current approaches to fabricate TEBVs that are more or less similar to natural vessels can be divided into two major categories, scaffold based or self-assembly. With scaffold-based method, either synthetic polymer or nature extracellular matrix (ECM) could be utilized as scaffold for *in vitro* cell seeding. The essential requirements for synthetic polymers are biocompatibility and biodegradability. Polyglycolic acid (PGA) and Poly (lactide-co-glycolide) acid (PLGA) are two of the most widely studies biodegradable polymers used in TEBVs. After a period of growth and maturation, the developing TEBVs is placed in a bioreactor with pulsatile flow and burst pressures above 2000 mm Hg can be achieved after 8–10 weeks [31]. Animal studies showed great patency results of these vessels.

Natural biological hydrogels and ECM proteins such as collagen, elastin, fibrin, gelatin, and modified hyaluronic acid [32–34] have been used as scaffold materials. These scaffolds have improved biocompatibility and provide adhesion sites for binding of cell surface integrins or cleavage sites to matrix metalloproteinases (MMPs), which facilitate cell attachment, cell migration and cell proliferation [35]. While fibrin scaffolds also produce high burst pressures [36,37], collagen scaffold TEVBs typically have lower mechanical properties [20,21,38,39].

Although the use of synthetic polymers as scaffold is promising, the long manufacturing time for these vessels poses a great challenge for the use as a disease model system *in vitro*. One way to increase the burst pressure and rapidly fabricate perfusable vessels is by plastic compression of collagen gels embedded with smooth muscle cells, which increase the collagen density and improves the TEBV [14,15,40]. Plastic compression generates TEBVs with burst pressures around 1600 mm Hg in a few hours [14]. After a 1- to 3-week maturation period, these vessels are well-suited for modeling diseases *in vitro* [15].

Another source of natural scaffold is to decellularize TEBVs by depleting tissue and cells from allogenic or xenogeneic sources. Decellularized scaffolds preserved the natural architecture of the ECM of the vessels [34,35]. Complete depletion of cells is required to avoid the host immune response. To overcome this challenge, two strategies have been used, either by advancing decellularization technique or inactivating immunogenic biomolecules [34].

Self-assembly is another approach to manufacture TEBVs. Self-assembly utilizes the ECM produced by seeded cells as the vessel structural supports. With this method, a confluent layer form by cells *in vitro* is rolled into a tubular structure to mimic the vessel [22,24]. These vessels achieve a burst pressure over 2000 mm Hg. The major pitfall for TEBVs generated by this method is the long manufacturing time of several months, which makes it difficult to use as disease modeling purpose. Acellular grafts represent one approach to overcome this challenge.

Extrusion of a Matrigel solution containing endothelial cells and smooth muscle cells enabled self-organized of an arteriole-like structure, termed vesseloids [41]. The vesseloids exhibit key vessel properties including a restrictive endothelial barrier and smooth muscle cell contractility. The vessels respond to inflammatory stimuli causing the endothelial cells to express the leukocyte adhesion molecules VCAM-1 and ICAM-1. The benefit of this novel approach is that vessels can be rapidly produced without a thick layer of extra cellular matrix, in contrast to most methods to produce tissue-engineered blood vessels.

DESIGN CRITERIA & FABRICATION OF MICROVASCULAR MODELS

In addition to models for diseases of large and medium-size arteries, microvascular systems (MVS) have been developed for drug screening, discovery, delivery, and modeling diseases in microvasculature. MVS are often combined with engineered solid organs to allow long-term maintenance [42]. For example, human blood-brain barrier (BBB) tissue chips have been designed to model the BBB dysfunctions in neurological disorders [43] and Alzheimer's disease [44]. Animal models do not recapitulate the whole disease state. The use of *in vitro* MVS with human cells or stem cells allows scientists to model the disease progression and drug response, which would result in better response prediction and reduce the use of live animals for disease modeling and drug testing [45,46].

The structure of human micro-scale vessels (arterioles, capillaries and venules) are quite different from large vessels (arteries and veins). While arterioles and venules contain all three layers, the media and externa layers are very thin compared to arteries and veins, respectively. The capillaries consist of a layer of endothelial cells that function in tissue-vessel material exchanges. Pericytes are attached

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to the endothelium, regulating vessel dimensions and permeability.

Given the structural differences between large and medium size arteries and capillaries, strategies to manufacture MVS are different from those of TEBVs. Current approaches to manufacture MVS are either Top-Down or Bottom-Up [34,47,48]. With the Top-Down approach, the pattern or geometry of the vascular systems are designed and then manufactured by 3D-printing [49], mold degradation [50,51] or multilayer chip [52]. While the pre-designed Top-Down approach could provide a controllable vascular structure and allow perfusions, the major disadvantage is that the resolution of these methods does not yet reach the level of capillaries.

In the Bottom-Up approach endothelial cells, pericytes or pericyte-like cells (e.g., fibroblasts or mesenchymal stem cells) are mixed together with a biological hydrogel and local chemical or physical stimuli from the various cell types induce angiogenesis and vasculogenesis. With these approaches, endothelial cells self-organized to form an interconnected network of microvessels [53–56]. Growth factors such as VEGF and fibroblast growth factors are also supplemented to promote angiogenesis [57]. Pericyte-like cells are needed to stabilize the microvasculature, otherwise the vessels breakdown after 24–48 hours. These methods allow the formation of perfusable capillary-size networks [42,58] but the structures of these networks are hard to control.

CELL SOURCE

Cells are one of the major components in vascular tissue engineering. Primary autologous cells including vascular smooth muscle cells, endothelial cells and fibroblasts, harvested from the patients, showed great successes for manufacturing vascular MPS. However, there are some challenges to use these cells as source for TEBVs. First, these cells required invasive procedure to harvest. Second, primary cells lost the ability to proliferate after prolonged expansion. Third, these cells are not available or not usable in some of the patients. While primary endothelial cells, which can be isolated from blood-derived endothelial colony forming cells, and fibroblasts are relatively feasible to obtain, obtaining functional and proliferative primary SMCs retains a major challenge given the limited accessibility of donors' tissue, limited proliferation rate and donor-to-donor variation [59]. Human embryonic stem cells (hESCs), mesenchymal stem cells (hMSCs) and induced pluripotent stem cells (iPSCs) show great potentials as cell sources in vascular tissue engineering. While the use of hESCs raises ethical issues, hMSCs and iPSCs seem well-suited for clinical translation and regenerative medicine. hMSCs can be easily obtained from various tissues [60] and their multipotent nature allows them to differentiate into many cell types, including smooth muscle cells [61] and endothelial cells [62]. Since the discovery of iPSCs in 2006 by Takahashi and Yamanaka [63], iPSCs have been widely used [64] and showed great potential to develop vascular MPS. iPSCs could be transformed from various adult cells including fibroblasts or blood cells. The pluripotency gives iPSCs the potential to differentiate into cells from all three germ layers (mesoderm, endoderm and ectoderm) [63], which include SMCs [65] and ECs [66]. The main pitfalls with iPSCs cells are their tumorigenic potential and immature differentiation. Progress has been made to reduce the risk of tumorigenicity by using non-integrating methods [67–70]. Current prevailing non-viral and non-integration approaches include adenoviral vectors [68], Sendai vectors [71], episomal vectors [72], minicircle vectors [73], synthetic mRNAs [74] or small molecule cocktails [75].

A challenge with iPS-derived smooth muscle cells has been limited differentiation which reduced mechanical strength of the TEBVs [76,77]. Optimizing the differentiation protocol [65,78] or applying cyclical mechanical stimulation [65] promotes SMC differentiation and increases the TEBV mechanical strength. Self-assembled microvascular networks have been developed using human brain

microvascular endothelial cells and pericytes have been derived from iPSCs and exhibit the low permeability and high levels of tight junction proteins found *in vivo* [79]. Transdifferentiation of human adult cells to ECs and SMCs provides another cell source for vascular MPS. Transdifferentiation eliminates the intermediate step of generating iPSC cells and reduces the risk of tumorigenicity and can be achieved by small molecules [80,81] targeting certain signaling pathways, activation and overexpression of key genes [82,83] or CRISPR/Cas9-based transcriptional activator systems that force expression of key endogenous transcription factors [84]. The introduction of cDNAs or CRISPR/Cas9 systems for gene editing can be achieved by either viral systems or non-viral/non-integration systems [85]. In the context of vascular MPS, several groups have shown success in transdifferentiating human fibroblast cells to ECs [80,82,83,86,87] and SMCs [88] or ECs to SMCs [89].

DISEASE MODELS

iPSC cells and transdifferentiated cells offer great potentials for regenerative medicine and personalized medicine with minimum ethical issues. iPSC differentiated smooth muscle cells have been derived from patients with progeria [15], supervalvular aortic stenosis [90], and fibrillin 1 mutations in Marfan syndrome [91]. Endothelial cells have been derived from individuals with pulmonary hypertension [92]. iPSCs enabled discovery of new biology of these diseases, although only a few have been converted to 3D models. Atchison *et al.*, used Hutchinson-Gilford progeria syndrome (HGPS) patient derived TEVBs and these vessels could reproduce key features of HGPS and the response to drug treatment [15]. A recent study used HGPS patient derived TEVBs and identified the contribution of endothelial dysfunction to the progression of atherosclerosis in HGPS [78]. A tissue-on-a-chip and bottom-up self-assembly model of the neurovascular unit using primary or iPSCs derived from individuals with various neurological diseases, showed that these disease states alter the blood brain barrier permeability and could be suitable testbeds to assess drug candidates [43,44].

TRANSLATIONAL INSIGHT

The development of vascular MPS provides an effective platform for the investigation of vascular development, vascular disease modeling, and evaluation of drug safety and efficacy. The vascular MPS create physiologically relevant microenvironments that closely model the *in vivo* environments. By incorporating recent advances in stem cell differentiation, vascular MPS could also be used in tailored medicine to model diseases individually and provide personalized information for each patient. Microvascular systems have already been used to study angiogenesis [54,93], and the blood–brain barrier [42]. Furthermore, the easy modification of vascular MPS enables deconvolution of the complex *in vivo* systems and testing hypotheses one by one. For example, high levels of LDL, monocyte activation, and accumulation and inflammatory environments all contribute to the initiation and progression of atherosclerosis. However, it is extremely difficult to identify which contributes more to the early stages of atherosclerosis by *in vivo* system. By using vascular MPS, each factor could be tested individually or combined with other factors to give more information about the underlying mechanisms. Gene editing technology allows creation of specific acquired changes to examine complex conditions such as aging [94] and interactions among polymorphisms associated with CVD. The future of vascular MPS relies on new techniques to:

1. Manufacture vascular MPS with shorter manufacturing time and more closely mimicking the natural vessels
2. Promote complete cell differentiate from iPSCs or hMSCs to functional ECs and SMCs
3. Integrate different scales of vessel (artery to capillary) in the same system

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AFFILIATIONS

Qiao Zhang
Department of Biomedical Engineering, Duke University, 1427 CIEMAS, 101 Science Drive, Durham, NC 27708-0281, USA

Xu Zhang
Department of Biomedical Engineering, Duke University, 1427 CIEMAS, 101 Science Drive, Durham, NC 27708-0281, USA

George A Truskey
Author for correspondence:
Department of Biomedical Engineering, Duke University, 1427 CIEMAS, 101 Science Drive, Durham, NC 27708-0281, USA
gtruskey@duke.edu

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL INSIGHTS

SPOTLIGHT

EXPERT INSIGHT

Manufacturing of human iPSC-derived cell therapies: road to the clinic

Raquel Martín-Ibáñez & Dhruv Sareen

One of the major roadblocks in regenerative cell therapies is the lack of appropriate models of human diseases and the availability of relevant patient-specific cells capable of replacing damaged tissues and organs. Induced pluripotent stem cells (iPSCs) derived from patients provide a source of human tissue that addresses this problem and has the potential to create an entire new set of human disease models and ultimately cells for transplantation. However, coordinating the production of quality iPSCs at scale remains a challenge for most academic and industrial laboratories. In this article, we outline key clinical applications of human iPSCs, and challenges associated with at scale clinical manufacturing of iPSCs and their derivatives. Based on the experience of our team in producing, maintaining and distributing iPSC lines for a decade as a global provider of iPSC-derived cell solutions, the progress of the field in developing iPSCs and their derivatives for translating to the clinic has been reviewed here. The development of a scalable iPSC-based biomanufacturing platform will enable innovative and sustainable solutions for translating much-needed iPSC-derived cell therapies into the clinic.

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INTRODUCTION

Regenerative medicine has the potential to heal and replace damaged cells and revolutionize the treatment of individuals with debilitating degenerative diseases and those who sustain severe, complex, and difficult-to-treat wounds. Stem cell therapy has limitless potential applications in

regenerative medicine [1,2]. Generating and expanding new cells from adult differentiated tissues has proven very difficult [3]. Somatic cells are not designed to expand in culture and rapidly lose the ability to generate key tissues of the human body [4,5]. However, there is a way to generate more robust human cells for cell therapies. Human PSCs with their unique self-renewal and differentiation properties provide tremendous promise for use in regenerative medicine [6,7]. An established source of PSCs are embryonic stem cells (ESCs) derived from fertilized human embryos that are generally left over from *in vitro* fertilization treatments [7]. However, these cells have important limitations, including:

1. Ethical issues associated with destroying a human embryo

2. Likelihood of allograft rejection by the recipient's immune system [8]

Induced pluripotent stem cells (iPSCs) are obtained through the reprogramming of adult somatic cells from tissues such as skin or blood [9–11]. iPSCs have similar qualities as ESCs [12,13]. But as they are obtained through the reprogramming of adult somatic cells, they avoid ethical issues and they can also be used for autologous cell transplantation, in which cells are removed from a donor, manipulated, stored, and later given back to that same individual [14]. Unfortunately, a number of obstacles prevent widespread implementation of iPSC-based therapy, including immunogenicity for allogeneic applications and the inability to generate sufficient cell yields of differentiated iPSC derivatives as a therapeutic product [13]. The goal of this article is to outline barriers and potential solutions in iPSC bioengineering and biomanufacturing technologies towards clinical translation of iPSC-based cell therapies.

CLINICAL APPLICATIONS OF IPSCS

Advances within the iPSCs field have shown great promise overcoming some of the limitations associated with other types of cell therapies including low expansion potential or exhaustion after multiple passages, difficulty of scale up in manufacturing processes, low differentiation potential, reduced final product yields and lack of efficacy of the therapeutic product depending on the source/patient, among others. Additionally, human iPSCs have the potential to transform health through different applications such as tissue regeneration, immunomodulation, anti-inflammatory effects, immuno-oncology, tissue/organ replacement and gene therapy, among others. The research community has already shown promising results in applying iPSC technology for regenerating various organs and tissues including the eye [15,16], joints [17], blood [18], brain [12,19], and heart [20]. Further, there is enormous potential for iPSC-derived cellular immunotherapeutic products involving generation of tumor targeting T and NK cells from iPSCs [9,13]. In addition, automated 3D bioprinting technologies (such as extrusion, droplet, Digital Light Processing and laser-based bioprinting), alone or in combination, can be used to engineer tissues derived from iPSCs. Thus, 3D bioprinting can also be integrated with the iPSC technology to produce novel cGMP-grade cell therapy products that, for example, could serve as a skin replacement therapy that is placed onto a wound to accelerate healing by promoting re-epithelization and neovascularization [21,22]. However, the efficiency of iPSC generation using current techniques is still low [23] and suboptimal culturing conditions can alter their identity and their compatibility with downstream differentiation protocols [24]. Thus, novel tools and technologies for iPSC generation, scale-up, harvesting, and preservation are needed so that they can be differentiated into a variety of cell types that can be transplanted into patients for tissue repair with optimal quality.

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Promising iPSC-derived cell therapies in pipeline

Several clinical studies using iPSC-derived cellular products have been launched during the last 5 years (Table 1). The first clinical application of iPSC-derived products was conducted at the RIKEN Institute in Japan using autologous iPSC-derived retinal pigmented epithelium cells to treat age related macular degeneration in 2014 [14]. Since then, at least nine studies have initiated iPSC-based cell therapy clinical studies world-wide including indications for Parkinson's disease, heart failure and spinal cord injury among others. Most of them have been approved in Japan during 2019 due to fast-track facilitating procedure for iPSC technologies. Two private companies have initiated clinical trials based on allogeneic human iPSC-derived cells with indications for graft versus host disease and for solid tumors (Cynata Therapeutics based in Australia) and relapsed/refractory hematologic malignances (Fate Therapeutics based in USA). During next few years many other applications of iPSC-based therapies are expected to follow, especially in the field of adoptive immunotherapies. Use of iPSCs as an 'off-the-shelf' supply of therapeutic lymphocytes constitutes a feasible strategy to overcome current limitations in manufacturing and efficacy over current individual donor-derived cellular immunotherapies [9,13].

Challenges associated with iPSC technology & clinical manufacturing

Immune rejection limits the clinical use of iPSCs. Autologous transplantation of iPSCs is preferable in order to avoid immune reactions and unknown virus infections. However, it is extremely costly, and the time required to prepare and transplant the target cells/tissues can be unacceptably long medically. The alternative is allogeneic transplantation, using iPSC-derived target cells/tissues donated by a third person. With an allogeneic approach, a sufficient number of iPSCs can be generated and comprehensive quality testing can be performed in advance, which can help reduce the cost and time of the iPSC manufacturing process [25]. However, patients who receive cell therapies derived from a single allogeneic iPSC line could become refractory to donor cells due to alloimmunization against mismatched human leukocyte antigens (HLAs). Thus, strategies are needed to attenuate the host immune response to transplanted tissues. The creation of iPSC banks comprising HLA-typed iPSCs that span common HLA haplotypes is a strategy to circumvent this issue by providing HLA-matched cells for the intended recipient population [25]. HLA matched iPSCs-derived cells might not only reduce immune rejection but also increase allograft survival [26]. Such an haplobank could be used to produce countless cell-based products for regenerative medicine, including skin cells, retinal cells, neural progenitor cells, monocytes, and pancreatic cells. Various banks of allogeneic clinical GMP-grade hiPSCs are being created globally [25].

Manufacturing iPSCs and their derivatives at scale is a challenge. Currently, functional iPSC derivatives are largely generated at laboratory scale with varying quality. The biological complexity of cells has hampered the translation of laboratory-scale experiments into industrial processes for reliable, cost-effective manufacturing of cell-based therapies [27]. However, realizing the technology's therapeutic potential will require large-scale expansion and differentiation while complying with current good manufacturing practices (cGMP) guidelines. Current systems for generating large quantities of iPSCs are based on traditional 2D planar cell culture plates. They can be 'scaled-out' by multiplying the culture volume through the use of multilayered flasks, and some robotic platforms that can automate the process providing large-scale quality-controlled manufacture of hPSCs [28]. However, this method of bulk culture is still limited for commercial use by requirements of considerable space, time, cost and operators, while also restricting online monitoring of culture parameters, and thus not

TABLE 1

Clinical trials in Phase 1/2 using iPSC-derived products.

Organ	Target disorder	Reprogramming	Type of transplant	Cell type to transplant	Cell number and product type	Delivery method	Patients enrolled	PI/Institution/ Country	Clinical Trial Identifier
Eye	Age-related macular degeneration	Skin fibroblasts/non-integrative episomal vectors	Autologous	iPSC derived retinal pigment epithelium (RPE) cells	Adherent cells growing as a sheet of 1.3×3.0 mm	Transplant of RPE cell sheet under the fovea	6	Masayo Takahashi/ RIKEN Center for Developmental Biology/Japan	UMIN000011929
Eye	Age-related macular degeneration	$CD34^+$ Peripheral blood cells/non-integrative episomal vectors	Autologous	iPSC derived retinal pigment epithelium (RPE) cells	Monolayer of cells growing on a PLGA biodegradable scaffold	Transplant of RPE+ scaffold at sub-retinal space	12	Kapil Bharti/Ocular and Stem Cell Translational Research Unit at NEI/USA	_
Heart	Ischemic cardiomyopathy, or hearts weakened by narrowed or blocked arteries	Not described	Allogeneic	iPSC-derived cardiomyocytes	~100 million cells in sheets of 0.05 mm thick and several cm in diameter	Two rounds of cardiomyocytes transplanted on the surface of the heart	3	Yoshiki Sawa/Osaka University/Japan	_
Heart	Ischemic heart failure	Not described	Allogeneic	iPSC-derived cardiomyocytes	100 million cardiomyocytes in 2.5–5mL medium	Injection into the myocardium	5	Dongjin Wang/ Help Therapeutics/ Hospital of Nanjing University Medical School//China	NCT03763136
Brain	Parkinson's disease	Peripheral blood cells/ Non-integrating episomal vectors	Allogeneic	iPSC-derived dopaminergic progenitors	$2.4x10^6$ hiPSC-derived dopaminergic progenitors in suspension	Bilateral putamen transplant	7	Ryosuke Takahashi/ Kyoto University Hospital/Japan	UMIN000033564
Blood	Steroid-resistant graft vs host disease	Peripheral blood/non-viral vector	Allogeneic	iPSC derived- mesenchymoangioblast-derived mesenchymal stem cells	Cohort A: $1x10^6$ cells/kg, up to a maximum dose of 100 million cells Cohort B: $2x10^6$ cells/kg, up to a maximum dose of 200 million cells	IV infusion on two administrations (Day 0 and Day 7)	16	Kilian Kelly/Cynata Therapeutics Limited/Australia	NCT02923375
Solid tumor located organs	Advanced solid tumors including lymphoma	Not described	Allogeneic	iPSC-derived natural killer (NK) cells in monotherapy or in combination with immune checkpoint inhibitors	100 million cells/dose and 300 million cells/ dose in suspension	IV administration once weekly for 3 weeks as	76	Wayne Chu/Fate Therapeutics/USA	NCT03841110
Blood	Relapsed/refractory acute myeloid leukemia (AML) and B-cell lymphoma	Not described	Allogeneic	iPSC-derived natural killer (NK) cells engineered with a high-affinity, non-cleavable CD16 (hnCD16) Fc receptor in monotherapy or in combination with monoclonal antibodies for B-cell lymphoma	Escalating doses of NK cells in suspension	IV administration once weekly for 3 weeks	72	Wayne Chu/Fate Therapeutics/USA	NCT04023071
Spinal cord	Spinal cord injury	Not described	Autologous	iPSC-derived Neural cells	$2x10^6$ neural cells in suspension	Injection in the site of injury	4	Hideyuki Okano/ Keio University School of Medicine/ Japan	_
Heart	Chronic heart failure	Not described	Not described	iPSC-derived cardiomyocyte-fibroblast patch	Not described	Implant of the cardiomyo-cyte patch on the infarcted epicardial surface of the heart	Not described	Jordan Lancaster/ Sarver Heart Center-The University of Arizona/USA	–

This table summarizes relevant information about the clinical trials in Phase 1/2 carried out using iPSC derived products for several applications. Information includes, target organ and disorder, somatic cells and plasmids used to reprogram iPSC, type of transplant (allogeneic or autologous), cell type and cell number transplanted, product type, delivery method, number of patients enrolled, PI/Institution and country and clinical trial identifier.
IV: Intravenous; NEI: National Eye Institute; PLGA: poly(lactic-co-glycolic acid).

ready for immediate clinical translation. 3D culture conditions, achieved using continuous bioreactor systems, are essential to realizing high cell numbers and process efficiency for autologous and allogeneic iPSC-based cell manufacturing. While it has been reported different 3D protocols for bulk expansion of hPSCs [29,30] their volume, control of culture parameters, and monitoring system may not be sufficient for human PSC applications that require large-scale production of PSCs. Therefore, along with the establishment of bulk culture systems, the validation of assays that can efficiently and reproducibly monitor growth conditions, cellular stress, spontaneous differentiation signals, gene expression profiles, and chromosomal integrity during production, processing, and storage of human PSCs is absolutely vital to the success of all downstream applications [24].

The therapeutic applications of iPSCs are limited by manufacturing quality control issues and maintaining appropriate genetic integrity. The choice of source tissue (skin fibroblasts, lymphoblastoid cell lines, peripheral blood, hair, urinary epithelial cells etc.) used for reprogramming, their level of expansion prior to reprogramming, methods of reprogramming and the stress of reprogramming process present unique challenges. In particular, reprogramming of iPSCs can be inefficient and can lead to genomic instability [31,32]. Recurrent genomic aberrations have been reported in research studies analyzing human iPSC lines derived from various tissues upon long-term culture [33–46]. Nevertheless, genomic alterations can occur at any stage of iPSC generation and mutations could also arise during differentiation of iPSCs to final cell products to be used for transplantation [31]. Therefore, establishing safe, efficient and reproducible techniques for reprogramming, culturing and differentiating cells are crucial for long-term iPSC line and derived products stability. The development of these technologies should also be accompanied by a clearer and more stringent regulation defining how to reprogram iPSCs and maintain cellular homogeneity in scalable culture systems as well as how to apply thorough screening using standard techniques as well as whole-genome sequencing to determine the genetic stability of the derived iPSCs. In addition, standardized and validated quality control assays for iPSCs and derivatives are also critical for their translation into the clinic.

POTENTIAL SOLUTIONS FOR ACCELERATING CLINICAL TRANSLATION OF IPSCS

Creation of an iPSC haplobank. Since creating autologous iPSC-derived cell therapies for large clinical trials can be a fairly expensive proposition, creation of iPSC haplobanks matched to the population demographics can provide a source of iPSCs for additional purposes including drug toxicity screening and as a model to study disease mechanisms. Such iPSC haplobanks populated with donors homozygous at HLA-A, -B, -C, and -DRB1 alleles selected for maximum utility to match those represented in the various demographics, holds strong promise [47]. Based on simulations to estimate the number of homozygous HLA lines required to provide compatible tissue in various populations, including UK, Japanese, Chinese, and North Americans (northern European, Hispanic, Asian and African ancestry), it was estimated that between 50–150 HLA homozygous donor iPSC lines would provide HLA-compatible tissue for ~50–90% of the population [48]. The generation of iPSC banks present some challenges that are limiting so far the success of these initiatives worldwide [26,49]. Some of the issues to be resolved are: a) The identification of volunteers with the desired homozygous HLA types to donate tissue to populate a global iPSC haplobank would require the random screening of many hundreds of thousands of individuals; b) Extensive international collaboration will be required for the determination of the optimal homozygous human leukocyte antigens (HLA) panel, donor selection, screening and consent, good manufacturing practice

(GMP), standards and quality control and regulatory legislation [48].

A different approach to circumvent immunogenicity is to provide HLA-engineered iPSCs as a source of universal donor cells for applications where the differentiated derivatives scape immunogenic responses. It has been described that HLA-E expressing iPSC are not recognized as allogeneic by CD8(+) T cells, do not bind anti-HLA antibodies and are resistant to NK-mediated lysis [50]. Using a different strategy, Deuse T and collaborators described the generation of hypoimmunogenic iPSCs by inactivation of major histocompatibility complex (MHC) class I and II genes and CD47over-expression. These hypoimmunogenic iPSCs retain their pluripotent stem cell potential and differentiation capacity [51,52]. CRISPR technology is also being used to disrupt HLA genes generating iPSCs with enhanced immune compatibility [53]. Although these engineering techniques are very promising, they are still in a very early developmental stage and additional understanding as well as safety studies are needed before moving to the clinic.

Successful scale-up of iPSCs in bioreactors. Bioreactor systems have the potential to support the development of large-scale iPSC cultures at high cell density by regulating cell expansion in a well-controlled environment. These scale-up systems are necessary for providing standardized and reproducible cell-based products for regenerative medicine. PSC such as iPSCs are typically very difficult to culture in suspension and bioreactors due to loss of pluripotency, high rates of cell differentiation and cell death, and increased genetic instability. Some of the factors influencing the quality and quantity of iPSCs produced in bioreactors include:

1. Colony splitting
2. Cell aggregate formation
3. Inoculation methods (single cells, cell clumps, or aggregates from single cells)
4. Cell inoculation density
5. Agitation rate
6. pH
7. Average size of cell aggregates for inoculation and harvest
8. Expansion period [54]

The optimization of these critical steps and factors allowed scaling up iPSC cultures in stirred-suspension bioreactors that remained pluripotent, karyotypically normal, and capable of differentiating into all three germ layers [54].

Kwok *et al.* demonstrated that iPSC stirred suspension culture system is flexible, fits into existing standard adherent culture workflow and is scalable from a 125 ml spinner to a 3L bioreactor. Using this simple and robust two-step process 16×10^6 iPSCs were expanded into 2×10^9 iPSCs in 14 days for a fold increase per day of 8.93 [55]. Wang *et al.* using the E8 chemically defined and xeno-free medium optimized suspension conditions guided by a computational simulation and developed a method to efficiently expand hiPSCs as undifferentiated aggregates in spinner flasks in xeno-free conditions. The complete elimination of components from animal sources remarkably reduced cost of this system providing a reliable technology for scale-up of hiPSC expansion and take a significant step toward the realization of stem cell therapies [56].

The scale-up of iPSCs in larger volume bioreactors needs to be automated further for closed loop continuous manufacturing and scale up of iPSCs. Non-invasive automated bioanalyzer technologies can be utilized to continuously monitor critical parameters of iPSC quality such as lactate, glutamine, glutamate, ammonium, sodium, potassium, calcium, pH, PCO_2, and PO_2 [24] during the cell expansion process. Such bioanalyzers need to be integrated with high-throughput systems in larger-scale continuous stirred tanks and rocker bioreactor systems in defined cGMP-compatible media. Subsequently, processes to automate cell harvesting by

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connecting these bioreactors to a continuous centrifuge system need to be developed further.

Generation of iPSCs with low mutation burden & high genetic integrity

While many patient specific iPSC lines have already been derived, most have been generated using genome integrating methods which raises concerns of insertional mutagenesis and continued expression of potentially oncogenic proteins by the integrated transgenes [57]. To evade these safety concerns and derive iPSCs for clinical application, tremendous technological advancements have resulted in the development of non-integrating viral- and non-viral approaches. These gene delivery techniques reduce the risk of genomic alteration and enhance the prospects of iPSCs from bench-to-bedside [58]. Integration-free methods have been reported including episomal plasmids, recombinant proteins, non-integrating viral-based approaches, and synthetic mRNA and miRNA. Although all these methods produced high-quality hiPSCs, substantial variance was observed with respect to aneuploidy rate, reprogramming efficiency, reliability, and workload [39]. Non-integrating viral-based approaches of gene delivery such as adenovirus vectors, AAV vectors and SeV vectors circumvent the dangers posed by integrating viral vectors and have shown commendable potential in ectopically expressing reprogramming genes to generate iPSCs. Generation of modified viral vectors with the desired features is in pursuit to derive integration-free iPSCs. Non-integrating non-viral, DNA-based approaches like plasmid transfection, minicircle vectors, transposon-based vectors, episomal vectors and liposomal magnetofection are also relatively safe due to the absence or minimal integration possibilities, present low immunogenicity and are technically simple but are limited by their low efficiencies (0.01–0.1%) and slow kinetics [58]. The non-viral, DNA-free reprogramming techniques such as recombinant proteins, miRNA, mRNA and small molecules are also promising as there is virtually no possibility of transgene integration and long-term genomic modifications. Therefore, each of these techniques still has to overcome various bottlenecks for its efficient use [58]. Nevertheless, the field of pluripotency induction continues to develop at a rapid pace and further modifications to these approaches are explored in improving the delivery and activation of the pluripotency-inducing transcriptional program in an efficient manner [59]. Our group and others have reliably demonstrated use of episomal plasmids to reprogram iPSCs at scale [34,60].

Our biorepository (https://biomanufacturing.cedars-sinai.org/) at Cedars-Sinai Biomanufacturing Center, has over 800 well-characterized iPSC lines generated with episomal plasmid reprogramming from a spectrum of diseased patients and healthy donors generated in research conditions (Table 2). Experience in iPSC scale-up and scale-out has supported many programs funded by NIH [61–65], CIRM [66,67], Answer ALS, as well as for preclinical regenerative therapies [68–71]. Our team and others have demonstrated in several research projects reliable derivation of neuronal [61,68,72,73], hematopoietic [74], endothelial [62], and skin progenitor cells [75] from iPSCs. iPSCs have also been shown to differentiate into many other cell types including cardiomyocytes [76], muscle cells [77] and pancreatic cells [78]. Thus, iPSC reprogramming technology offers the potential to treat diseases including Alzheimer's disease, amyotrophic lateral sclerosis (ALS), blinding eye diseases, cardiovascular disease, and diabetes [9,13,15,33].

Data from our center show that iPSCs reprogrammed from unexpanded PBMCs have excellent cytogenetic stability and minimal genomic aberrations compared to iPSCs generated from pre-expanded source cells including dermal fibroblasts, lymphoblastoid cell lines or epithelial cells [Revised manuscript in submission]. Aggregate results from cytogenetic analysis of various iPSC

TABLE 2

Current catalog of iPSC lines available to researchers at Cedars-Sinai Biorepository.

Disease of interest	Number of unique iPSC lines
Healthy control iPSC lines	
Healthy controls	155
Lothian Birth Cohort controls	24
Neurological disease iPSC lines	
Amyotrophic lateral sclerosis	474
Parkinson's disease	19
Huntington's disease	16
Alzheimer's disease	8
Spinal muscular atrophy	8
Autism spectrum disorder	7
Charcot-Marie-Tooth	7
Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation	6
Batten disease	4
GATAD2B-associated neurodevelopmental disorder	4
Isogenic controls for amyotrophic lateral sclerosis	4
KAT6A syndrome	4
Monocarboxylate transporter 8 deficiency	4
Fragile X syndrome	3
Huntington's disease like	3
Spastic paraplegia 11 (SPG11)	3
Spinocerebellar ataxia type 12 (SCA12)	3
Isogenic controls for Charcot-Marie-Tooth	2
Leukoencephalopathy with brainstem and spinal cord	2
Primary lateral sclerosis	2
Shashi-Pena syndrome (ASXL)	2
Vanishing white matter disease	2
Dravet's syndrome (Epilepsy)	1
Epilepsy	1
Familial diabetes insipidus (FNDI)	1
Intraventricular hemorrhage (IVH)	1
Kleefstra syndrome	1
Moyamoya disease	1
NFASC mutation	1
X-linked adrenoleukodystrophy	1
Other disease iPSC lines	
IBD/ fibrosis	14
Crohn's disease	13
Type 2 diabetes	11
Spondelometaphyseal dysplasia- Kozlowski type	7
Chronic pancreatitis	8
Obesity	5
Breast cancer	3
Erdheim-Chester disease (MEC)	2
Keratoconus	2
Chromosome translocation associated ectrodactyly (SFM)	1
Inclusion-body myositis (IBM)	1
Incontinentia pigmenti	1
Lynch syndrome	1
TOTAL	844

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lines over a period of a decade in our laboratory highlight that optimizing techniques of reprogramming as well as the quality and the period of pre-culture prior to reprogramming are crucial for long-term iPSC line stability and suggest that deriving iPSCs from unexpanded PBMCs could be a preferred method for iPSCs in research and therapeutic clinical applications. PBMCs present several advantages:

1. They can be isolated by routine venipuncture with minimal risk to the donor and can be obtained in enough numbers to enable reprogramming;
2. PBMCs are not only minimally invasive to harvest, but more often accessible through the large numbers of frozen patient samples stored in blood biorepositories;
3. They also provide flexibility as they can be cryopreserved for reprogramming at a time when convenient thus allowing for iPSC 'future-proofing'.

However, some limitations associated with the blood derivatives might restrict usage of their derived iPSCs for some applications. First, the main parent cells are mature T cells bearing specific T cell receptor (TCR) re-arrangements [79,80] which yield iPSCs with germ line IgH and TCR alleles, that could be undesirable for potential applications that require a broad T-cell repertoire to be derived from an iPSC lines. However, various groups have utilized this characteristic of T lymphocyte reprogrammed iPSCs as an advantage in developing targeted cytotoxic tumor targeting T cell populations with specific rearrangement [81–83]. Further, if a non-T cell derived iPSC clone is more desirable from PBMCs as a cell source, it is often easy to enrich for non-T and non-B cell iPSC clones (derived from monocytes) that do not contain any lymphocyte specific gene rearrangements by growing cells in specific cell growth media suited to enriching non-T iPSC clones, either prior or the iPSC reprogramming process [57]. Additional concerns about using PBMCs as a cell source for iPSC reprogramming are those cases where blood infections are involved (e.g., hepatitis C virus, and HIV), and using PBMCs from patients with blood diseases (e.g., hemophilia and leukemia) [84].

QUALITY CONTROL OF IPSC-BASED CELLULAR PRODUCTS

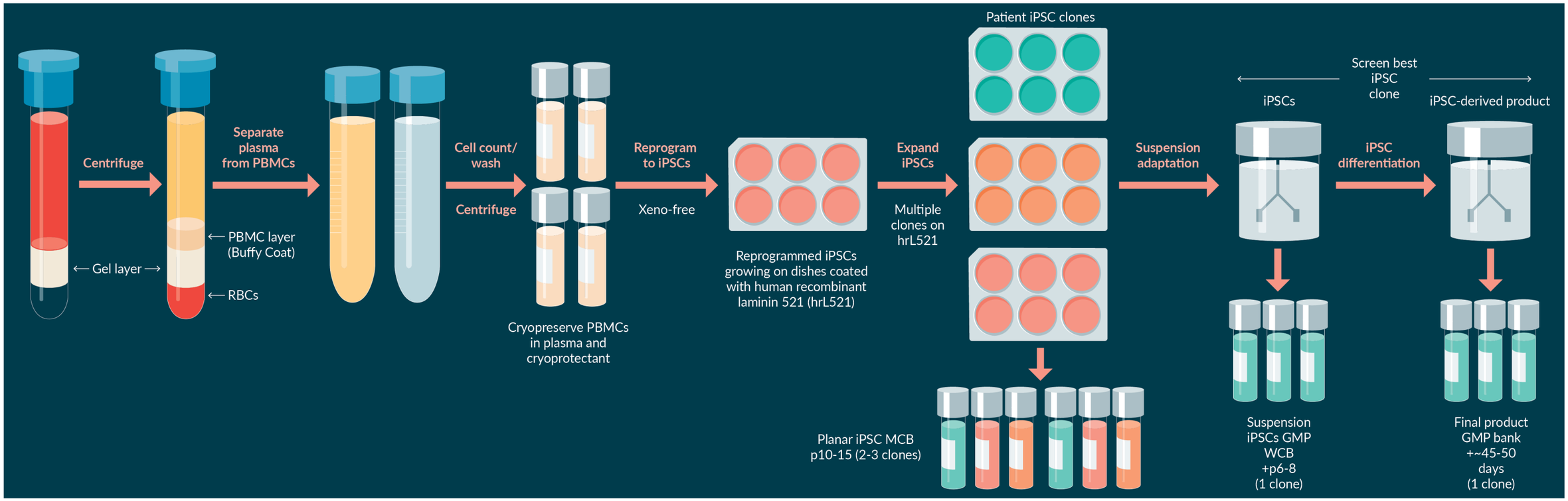
In order to exploit the potential of iPSC-derived therapeutics, it is critical that the starting materials (the clinical-grade iPSC lines), the manufacturing processes and the cellular therapy products themselves are extensively characterized. Variations may still exist during iPSC expansion, reprogramming, colony selection, culture system selection, iPSC differentiation, and the purification process within different iPSC cell lines generated from different individuals and iPSC core facilities. Therefore, the development of automation, closed cell systems, standardized manufacturing processes and validated testing protocols will support the reduction of variability and the scale-up of manufacture of clinical-grade iPSC lines and their derived cell products.

There is a general agreement in the field that the successful generation of iPSCs and their derivatives relies on quality attributes to produce consistent, high-quality iPSCs and their derived products. Thus, a QC guideline for producing clinical grade iPSCs has been reported in 2018 by the Global Alliance for iPSC Therapies [85,86]. Critical Quality Attributes (CQA) in respect of iPSCs include identity, microbiological sterility, genetic fidelity and stability, viability, characterization and potency [87]. The type of assay has to be defined for each CQA as well as the appropriate limit, range or distribution to ensure quality and safety of the product.

In our laboratory, identity of the iPSC line is validated with the short tandem repeat (STR) analysis to genotype the original source cells and the iPSC to certify the absence of cross contamination during generation or

FIGURE 1

Xeno-free PBMCs reprogramming protocol to generate a cell-based therapeutic product.



Cell type	White blood	Isolated PBMCs	iPSCs MCB (planar)	iPSCs WCB (suspension)	IPSC-derived product
Criteria (COA)	Clinical screening	Cell viability	Normal karyotype	Normal karyotype	>XX% marker 1
	Serology tests	Number of vials	Clearance of residual episomal vectors	Clearance of residual episomal vectors	>XX% marker 2
	OncoPanel	Sterility and mycoplasma (negative)	Pluripotency	Pluripotency	>XX% marker 3
			Sterility, endotoxin & mycoplasma tests (negative)	Sterility, endotoxin & mycoplasma tests (negative)	<0.05% Oct 4 (by PCR)
			Adventitious agent testing (negative)	Adventitious agent testing (negative)	Functional assay
			Interspecies contamination		Normal karyotype
			Cell identity (16 human STR loci)	Cell identity (16 human STR loci)	Sterility, endotoxin and adventitious agents, mycoplasma tests (negative)
			Viability	Viability	Cell identity (16 human STR loci)
					Viability

Figure (top): Xeno-free protocol developed to reprogram PBMCs to iPSCs in xeno-free conditions. After planar iPSC Master Cell Bank generation, IPSCs are adapted to suspension expansion in bioreactors for the generation of a Working Cell Bank that will be used for the differentiation towards the therapeutic final product.
Table (bottom): Critical quality attributes (CQA) for the starting material, intermediate and final product.
PBMCs: Peripheral blood mononuclear cells; STR: short tandem repeat; PCR: polymerase chain reaction; MCB: master cell bank; WCB: working cell bank.

maintenance process. Since stem cell-based products cannot be sterilized, sterility determination is highly important not only in the final product, but also in intermediate ones. This should include the mycoplasma, endotoxin, bacterial and fungal sterility tests, viral testing for human adventitious agents and in the case of non-xeno-free culture reagents used, include also appropriate nonhuman adventitious agents. It is advised to use USP (US pharmacopeia) recommended tests when available.

Genetic change in iPSCs, or a product derived therefrom, is of concern as it raises potential hazards around cell transformation and the risk of causing tumors in patients. Residual vector testing and karyotype are the tests used in our group for the genetic stability and fidelity of the iPSC lines evaluation. Residual episomal vector copies used to reprogram PBMCs have to be ≤1plasmid copy per 100 cells in seed and master cell banks. In house assays for residual vector determination have to be validated when no accredited laboratories offer this assay. Genomic integrity and mutational burden of iPSCs should be determined by G-band karyotyping standard methods (should be normal on more than 20 metaphases) in conjunction with other methods inducing array comparative genomic hybridization (aCGH) and next generation sequencing. In order to give an appropriate dosage of cells to the patient, viability determination should be carried out using a validated method. Calculation of doubling time and detection of cell debris are not required but could provide useful information. Characterization of iPSC intermediate cell banks have to be determined by the expression of a minimum of two markers from the standard human pluripotent stem cells panel (OCT4, TRA-1-60, TRA-1-81, SSEA-3, SSEA-4, Sox2, Nanog). A combination of one intracellular and one extracellular marker should be used and should be >70% of the total population. Finally, embryoid body formation can be carried out to measure propensity for differentiation into the embryonic germ layers (ectoderm, mesoderm and endoderm). TaqMan array plates (PluriTest from ThermoFisher Scientific) are used to assess pluripotency after EB formation to standardized and improve characterization efficiency as described by others [87].

iPSC-derived differentiated therapeutic products include the same CQAs as well as:

1. The phenotypic characterization of the final differentiated product by the expression of specific markers
2. The absence of pluripotent stem cell markers, to avoid tumorigenicity
3. Purity of the final product
4. Potency assay

Figure 1 shows an example of PBMCs reprogrammed to iPSCs that are differentiated to an iPSC-derived product and CQA are defined for starting cellular materials, intermediate and final cellular products.

SUMMARY

The iPSC field has advanced tremendously in the last 10 years with some therapies being already in the clinic. However, there are still several challenges that must be overcome for iPSCs to reach their full potential. Development and implementation of large-scale GMP-compliant protocols for the generation and maintenance of human iPSC lines is crucial to increase the application safety. Reproducible differentiation protocols generating complex 3D engineering tissues or organs must be developed and standardized without compromising quality of iPSCs. Quality and safety controls are also challenging. Thus, further advancement in iPSC automated technology is still needed to improve quality, scalability, reduce variability and costs. Importantly, a better understanding of the immunogenicity and tumorigenicity of iPSC-based products will greatly help to translate to the clinic effective and safe products to treat unmet clinical needs.

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AFFILIATIONS

Raquel Martín-Ibáñez
Cedars-Sinai Biomanufacturing Center, West Hollywood, CA, 90069, USA

Dhruv Sareen
Corresponding author:
Board of Governors-Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, 90048, USA.
and;
iPSC Core, The David Janet Polak Foundation Stem Cell Core Laboratory, Cedars-Sinai Medical Center, Los Angeles, CA, 90048, USA.
and;
Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, 90048, USA.

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CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL INSIGHTS

SPOTLIGHT

COMMENTARY

Complexity be damned: the need to better use biology to achieve more impactful cell therapies

Nancy L Parenteau

Biological complexity is both a benefit and a bane of cell therapy. An astute partnership with nature is required to achieve a reliable clinical outcome from cell therapy. To accomplish it, we must dig deep enough to acquire knowledge crucial to translation, while maintaining a perspective that will prevent misdirection. Not every bit of information will be of equal importance, yet we can't skip over or miss what could be pivotal. Dealing with the biological complexity surrounding cell therapy may seem like a risky balancing act. However, luck favors the prepared, and there are practices we can employ to reduce translational risk and form a clinically impactful collaboration between science and nature.

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BIOLOGICAL COMPLEXITY IS A FORMIDABLE CHALLENGE, BUT IT IS NOT AN EXCUSE TO IGNORE IT. INSTEAD, WE HAVE TO UNDERSTAND IT AT THE MOST INSIGHTFUL & USEFUL LEVEL

Biology is complex but goal-oriented and, in humans, remarkably determined to fulfill evolved developmental, physiological, and protective objectives. Yet we want to correct genetic abnormalities, persuade nature to do things differently to achieve a better outcome,

and give the immune system an advantage against cancer. To form an effective partnership, we have to understand the objectives of the cell population, the more complex tissue, and the functional organ. They provide vital context to make wiser decisions. If we think we are smarter than mother nature, we'll lose. To be effective, we must appreciate that we are never really the controllers but rather the enablers. Therefore, one of the most powerful ways to work with complex biology is to find a way to foster a process and then, preferably, get out of the way to let innate programs add the details. For example, a cell therapy healing a chronic wound interacts with the patient's tissues at multiple levels that we are only beginning to understand through genomic expression and network analysis [1,2] even though we've observed its functional impact in the clinic for over 20 years in the USA.

The beauty of cell therapy is that we don't have to get deep into the weeds to produce an effective treatment. It doesn't mean we don't need enabling knowledge, which can still be substantial. If we understand the processes and systems at work for us and against us, we are more likely to gain understanding we can use to design biologically savvy therapies, robust manufacturing processes, and achieve more reliable and impactful clinical applications. The more we can work with nature, at all levels, the better chance we have to be able to improve outcomes.

TECHNOLOGY IS ONLY AS GOOD AS THE STRENGTH OF ITS APPLICATION

Genetic engineers may think that cell behavior is not their problem. We know that single genetic mutations can have far-reaching consequences. If we can fix it, then we cure the disease. However, the questions then become: where is the correction of the mutation needed, can we reach it, and at what efficiency? For decades we have known how we might cure Duchenne's muscular dystrophy (DMD). Yet correcting the genetic deficiency with the capability to create a lasting clinical impact is a significant challenge. Despite advances in the clinical application of gene therapy for DMD and knowing what to target, the corrected dystrophin levels achieved are disappointingly low, suggesting that some biological obstacles may still exist. However, cures are achievable when there are enough enabling knowledge and experiences, e.g., in the use of hematopoietic stem cells and the process of hematopoiesis. In my own experience, the development of a therapeutic organotypic skin construct over 20 years ago, was enabled by a preceding decade of epidermal cell research that created a useful scientific foundation. In attempting to cure DMD, is there something we are inadvertently missing about satellite cells and muscle fiber generation that might better enable a DMD gene therapy? The same type of scrutiny is probably overdue for other applications of cell and gene therapy.

The idea that we'll get to a definitive therapy faster by getting into humans earlier without crucial foundational work is wishful thinking and, worse, slows real progress. We have to guard against premature leaps to development that plague many attempted applications of cell therapy. It does not mean we shouldn't be bold and innovative, just smarter and more willing to identify, illuminate, and face the limitations head-on. New science and technology have exciting therapeutic possibilities until reality sets in as we attempt to translate it in a safe and clinically meaningful way. We know the reasons why biological hurdles are often back-burnered: careers, ability to publish, limiting dogma, grants, appeasing venture capitalists, patents on the technology and so on. However, to achieve the best technology can offer and biology will allow, and deliver it to patients in the fastest way, we have to deal with reality early and often. Also, the practices and institutions stymying this must change to allow it and support a fast fail approach.

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WE CAN HAVE TECHNOLOGY PROGRESS WITHOUT A THERAPEUTIC ADVANCE

There are now many ways to induce pluripotency, design a chimeric antigen receptor (CAR), or print cells into a 3D form. The limitations beyond the technological innovation lie in the biology: challenging us to reliably differentiate pluripotent cells into functional cells with high fidelity, direct T cells engineered with CARs to curative solid tumor targets, or clinically translate 3D printing.

IT IS ONE THING TO HIGHLIGHT THE CHALLENGES & SUGGEST THAT WE HAVE TO DO BETTER, BUT HOW CAN WE DO IT?

I offer a few suggestions for how we can achieve more fruitful translation:

Work to an applied standard, which is focused on gaining actionable knowledge

There is a misconception that applied research is simply the application of knowledge gained through academic research. Working to an applied standard is much more; it is a more demanding research approach and use of knowledge. Genetic characterization can now dig deeper and be better understood using network analysis tools. It becomes even more powerful combined with functional measures at different levels of experimentation, from single-cell analysis, the culture of a single population in a dish, organotypic, or organoid culture, to grafts in animals. Each level gives us a different insight into the dynamics of a cell population, its interaction, and its innate genetic program. Attention to processes and tissue objectives provides insight that can lead to practical application. Relying on the academic process, which is driven by the need to publish, can be slow and inadequate. Academic data on tumor biology is particularly problematic. Ideally, to speed translation, industry and academic colleagues should work in concert to create research plans that are complimentary – guided by the right questions, a broader integration of information, and a deeper dive in the right places.

Maintain a proper perspective

While there is a need to dig deep to tackle challenges, we also have to step back from the data to gain perspective on the processes at work. Think of an impressionist series like Monet's Japanese Bridge, which depicts a bridge over a pond – the defining element being the bridge. Focusing too closely on a brushstroke or its color is not very informative. However, when we step back, we now see what the brush strokes and their color are trying to achieve even as the bridge in the series becomes increasingly obscure. The colors impact our ability to see the bridge, but the composition is more informative than a single color. Do elements in the rest of the garden add information and impact to the painting? Yes, but the bridge defines the series and, once we see it, we understand that it is needed to cross a pond. How can we apply these principles to big data?

Use biological priorities to hone therapeutic objectives

Cell and gene therapies to eliminate cancer, correct biochemistry, administer hormones, cure metabolic diseases, support or redirect failures in regeneration, and repair get muddy in search of key elements as our access to big data increases. The biology of our tissues and organs has evolved to be interactive. An organ's differentiated parenchyma, stromal component, and its vasculature each have a role to play. Those roles set biological priorities. The parenchyma that defines the function of the organ is the most important component and, through my experiences,

the most self-contained or self-directed. While a stromal response leading to fibrosis may limit epithelial regeneration, it does not drive the parenchyma. Likewise, while angiogenesis enables regeneration, it does not drive the regenerative response in the parenchyma. That means that if the problem lies within the parenchyma, that is the primary concern. Then, if the lack of persistence is due to inflammation working against us, the inflammatory response is the next priority. Successful engraftment and establishment of functional parenchymal cells enabled by the control of inflammation then work against fibrosis.

Interpret wisely

The challenges of working with complex biology lie in the gray areas, where many things have some effect. We're rarely entirely wrong, but frequently a bit off the mark. It is challenging to stay on the most direct and most effective path. Part of this stems from how we interpret a biological result. An example, and a potentially far-reaching one, is in the interpretation of the meager therapeutic effectiveness of adoptive cell transfer in solid tumors. The microenvironment, T cell biology, and fibrosis, much of it rooted in stages of the wound healing process, have been proposed as reasons for disappointing results. However, first and foremost, we lack good targets for the tumor cells, particularly with CAR-T, which requires surface molecules of reliable expression and biological significance. The microenvironment, myeloid cell composition, and T cell biology do have a role and an impact, they just aren't the first step. Also, it suggests that technological advances of CAR-T processes while needed will only go so far. The primary shortfall, in this case, will not be a technological inadequacy but more a biological one. If it turns out that surface targets are all that biology permits for a certain type of cancer, then, by all means, we should pull out the stops where we can, cognizant of their limitations. However, it shouldn't replace dealing with first things first.

Form a comprehensive knowledge base for savvy product design & strategy

Effective translation will mean administering cells at the right point of their lineage commitment or differentiation to achieve functional significance in the patient. It requires knowledge of their developmental program, reaction to regenerative challenge, and attention to cell lineage and resulting heterogeneity. A reluctance to dig deep where there are inconsistencies, gain proper perspective, and integrate what organogenesis, normal injury, regeneration, and repair (nature) telegraph will continue to stall clinically meaningful advances.

NO WEAKLINGS ALLOWED: ROBUSTNESS IN THE FACE OF NATURE'S ODDS IS KEY TO A CELL THERAPY'S CLINICAL UTILITY

When do we know our efforts are ready for clinical translation? Experiences with the development of wound healing therapies teach us that even the 'same' cellular components, delivered differently, can cause one approach to fail when another succeeds. The use of dermal fibroblasts and keratinocytes to change the course of a venous leg ulcer is an example. To date, there have been many approaches, yet only one has succeeded clinically in this application. Sometimes, the seemingly 'small' things matter. In the bilayered organotypic skin construct Apligraf® (Organogenesis Inc., Canton MA, USA), preclinically, the development of a barrier was pivotal to its ability to engraft and persist on an athymic mouse [3], suggesting it was something to pay attention to. Also, the physical strength of the stratum corneum facilitated the handling of the material in the clinic. Thus, the stratum corneum was likely enabling in ways from physiological to physical. I believe it added a critical level of robustness that contributed to the material's clinical utility in the chronic wound. Yes, it required the skill to create an epidermal cell

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population with sufficient regenerative capacity and protocols to manufacture the construct reliably. However, the differentiation program of the epidermal keratinocyte and how it was used, made it an effective therapy.

BEWARE OF A 'GOOD ENOUGH' MENTALITY; NATURE DOESN'T CARE WHAT IS EASIER OR HARDER FOR US TO ACHIEVE

To redirect or activate a biological course of events in a patient will require all the robustness we can muster in our design.

- The less required of the cells to get to a state that helps the patient, the more reliable and robust the results will be;
- The more directly a therapy connects to the primary element of the problem, the more potent the treatment will be;
- The more 'natural' the design, the more enabling it will be for cell function, survival, and effect.

No matter what your expertise is in 3D printing, you are unlikely to form an organized tissue as well as the right cells can through growth, lineage progression, and interaction, so be sure to enable that with or without a 3D printer.

For cell therapies that require the implantation of a stem cell or progenitor cell, we should look to how processes in the body will enable or thwart their development. However, first and foremost, we need sufficient insight into the lineage and behavior of the cell population, gained through experimentation at several levels (alluded to earlier). The more we can develop a cell population along the path to the desired function as far as is feasible, the more robust the therapy will be, and the more reliable the outcome will be. The development of a pancreatic islet transplant is a good test case for those considerations.

QUESTIONS FOR THE FUTURE MIGHT BE

- Can we leapfrog the limitations that chronic inflammation or fibrosis place on regeneration by engineering a robust regenerative phase through the administration of the right progenitor population at the right time?
- Could we enable more effective impact or engraftment where necessary with anti-inflammatory or anti-fibrotic agents?
- Can we improve the efficiency and permanent integration of genetic modifications through a more robust use of biology?

Let's curb the urge to do premature 'product development', acquire enabling knowledge to an applied standard, maintain a proper perspective, be mindful of biological priorities, and use some brass tacks to nail things down.

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AFFILIATIONS

Nancy L Parenteau
Parenteau BioConsultants, LLC

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CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL INSIGHTS

SPOTLIGHT

INTERVIEW

Programming superior cells: translational progress at the Altius Institute for Biomedical Sciences and Umoja Biopharma

SHON GREEN is trained at UC Berkeley and UCSF where she developed and employed preclinical models of cancer to study tumor development and potential therapeutic approaches. She utilized these expertise to drive the preclinical development of T cell-based immunotherapies targeting both solid and liquid tumors at Eureka Therapeutics Inc. (Emeryville, CA, USA), where she successfully guided two novel programs through preclinical proof-of-concept and safety studies to support investigational new drug applications and clinical testing. Since July 2018, she has been directing the development and clinical translation of novel genetic and epigenetic editing tools to enhance cellular therapies for cancer at Altius Institute for Biomedical Sciences (Seattle, WA, USA). She currently also serves as the head of Translational R&D at Umoja Biopharma, a new startup in Seattle, where she is leading the preclinical strategy to obtain proof-of-concept for potentially disruptive new approaches to cancer immunotherapy.

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Q Please can you give us some background on the Altius Institute for Biomedical Sciences – how and why did it get its start, and what are its chief areas of activity?

"...when you have disease-associated variance, or SNPs, or any genetic element associated with a disease, it's still very difficult to know what is linked to it..."

SG: The Altius Institute for Biomedical Sciences was formed as a non-profit partnership between Dr John Stamatoyannopoulos, a pioneer in regulatory genomics, and GlaxoSmithKline. It was originally founded to understand how genes are regulated, specifically in disease, and develop the translational potential of regulatory genomics.

This is still a big problem in biology and medicine in general: when you have disease-associated variance, or SNPs, or any genetic element associated with a disease, it's still very difficult to know what is linked to it, which proteins are actually affected by, or have an effect on, a disease.

So Altius has developed genome-wide assays around DNase-seq to map chromatin accessibility and the location and activation state of regulatory elements at high resolution, as well as various types of large data analysis tools that help to decode these regulatory elements.

One of the other things Altius has done in order to understand disease variants and how they link to specific genes and their functions is to develop tools for gene control via genome and epigenome editing. This happened in parallel, out of the need to understand and validate some of the findings. Altius developed a TALEN-based platform for very precise gene knockout and also for gene repression or up regulation using synthetic transcription factors based on the TALE DNA-binding domain. That is the work that's perhaps most relevant to our conversation today.

As the technology continued to develop, Altius realized it obviously had a lot of implications for the enhancement and improvement of cell therapies. The life sciences field is very invested in cell therapy right now, but we lack ways to actually programme and engineer cells very precisely and safely. That is where I came in: my role at Altius is to direct that translation. Altius was very much a basic science kind of research institute without any clinical programmes, so I came in to apply gene editing and epigenetic editing technology to T cell therapies - in particular, CARTs for oncology – in order to develop this platform towards clinical application.

Q How and with whom does the Institute seek to collaborate?

SG: One of our major funders and collaborators is GlaxoSmithKline. They were the entity that gave the founding funding for the Institute, and we remain in various different collaborations with them.

In addition, we have collaborations with other companies in the cell therapy field, and also several collaborations that are not related to cell therapy, but more towards regulatory genomics. Altius is an integral part of the ENCODE Consortium and is also a Center of Excellence in Genomic Science, and there are other academic collaborations in that particular area.

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"We use an adaptor in order to direct [the] universal CAR to different solid tumor antigens ... We are also working on a vaccine approach to help boost expansion of our CARs in patients, and a method that will potentially allow us to directly 'paint' tumors with the antigen so they can be targeted with this universal CAR."

Q You've newly joined Umoja Biopharma – what can you tell us about the work you're doing there to enhance the preclinical-clinical translation of their product candidates?

SG: Umoja is quite an exciting and potentially disruptive company. It's still in stealth mode, but I can give you a little overview.

The scientific founders are quite well known. One is Mike Jensen, who was one of the co-Founders of Juno Therapeutics here in Seattle. Another is Andy Scharenberg, who has been a successful scientist and entrepreneur in the cell therapy space. And there's Philip Low from Purdue. The meaning of the word 'Umoja' is 'unity' in Swahili, and their idea is to unify several different technology platforms into something that will actually work in solid tumors.

The platform involves several elements, including a CAR approach that is universal. There are other companies doing this, of course, but we believe our CAR design has some advantages. We use an adaptor in order to direct this universal CAR to different solid tumor antigens, which allows the flexibility of targeting a range of antigens and receptors on tumors to help overcome the heterogeneity of solid tumors. We are also working on a vaccine approach to help boost expansion of our CARs in patients, and a method that will potentially allow us to directly 'paint' tumors with the antigen so they can be targeted with this universal CAR. And what is actually the most exciting to me, is a technology Umoja is developing that we think can overcome many of the limitations of current CAR manufacturing practices. This last piece is potentially the most disruptive, and the other elements offer a lot of versatility in terms of how they can be applied to solid tumors.

Q There are still many barriers to success in preclinical-clinical translation of cell and gene therapies, but which ones stand out for you as the most significant?

SG: I would have to say that particularly for cellular immunotherapy, which is where I've been focusing a lot of my energy, the lack of appropriate models is probably the biggest barrier to success.

It's not an issue that is exclusive to this field, but it is particularly difficult to create preclinical models, either *in vitro* or in animals, that will give you any real idea about the safety or efficacy in a human of a cell or gene therapy. And that's especially the case when the immune system is involved.

With small molecules, for example, you obviously have a lot more flexibility, since there's a great deal of homology in how different organisms respond to small molecules. But when it comes to a gene therapy or cell therapy, you typically have to use immune-compromised mice in order to avoid rejection of your drug. If you're doing gene therapy, the genetic homology between mice and human is not high enough for these complex interactions. Plus of course, there's the issue of the immune system – once you go into immune-compromised models, you're not really getting the full picture of what the interactions produce in an immune-intact human patient.

So all these things combine to make it extremely challenging and really not predictive at the present time. We all do preclinical development, we try really hard, we're trying to develop new models all the time. It's something the field is very focused on. But at the end of the day, we arrive in a clinical trial in humans without really knowing for sure if our drug will be efficacious or safe. The trials have to be designed around that fact – the risk–benefit ratio has to be appropriately factored in and patient selection is also restricted, obviously. It has been and is a significant limiting factor for the field as a whole.

Q Focusing in on gene editing, what are your thoughts on how the field should continue to meet the challenges in clinical translation of engineered cellular immunotherapeutics in particular?

SG: I will focus on *ex vivo* gene therapy here – we're not currently using *in vivo* gene therapy, and those technologies have a great many issues, the biggest being immunogenicity and actually getting enough activity *in vivo*. But if you have a cell population *ex vivo* that you can manipulate in a lab, you can actually achieve very effective and efficient editing.

I also think it can be quite safe. I'm not super concerned about off-target effects (even though they're clearly not something we can ignore) in the context of editing specific types of cells that have less tumorigenic potential. I think the field as a whole can definitely learn a lot from those types of editing – for instance, editing T cells *ex vivo*, which are pretty safe relative to stem cells. And as the field progresses, we will learn more about off-target effects and whether or not they're detrimental. So for me, it's not necessarily the off-target effects or immunogenicity that are the greatest concern – it's the actual on-target effects of gene editing, which are

> "I am glad to see the field moving towards base editing, and different modalities that could be safer and also offer some tunability."

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permanent and give an 'all or nothing' type of outcome.

I guess this stems from my work at Altius where we have done a lot of gene editing, but where we have started shifting towards using transcription factors and chromatin remodeling in order to create time-limited changes. While they are very potent and can really change the ways in which cells behave for a certain amount of time, they are not permanent, which will greatly increase safety.

"In the future, I imagine that we'll be able to actually programme superior cells that really do have the biology that we want."

I think the other aspect that's a problem for anyone manipulating any type of cell *ex vivo* – and particularly with gene editing, because it does require multiple steps and lots of time in culture – is that you're altering the biology of the cell. Ultimately, you're infusing back into the patient something that is not optimal.

It's not optimal because of the time spent in plastic dishes in the lab, and also the time spent with the various activating molecules and cytokines (particularly in the case of T cells). You're differentiating the cells, you're introducing pathways that then activate exhaustion or apoptosis, and so the final product is not ideal.

In summary, while I think gene editing is a great tool, all the associated steps and manipulations of cells *ex vivo* takes a toll on the biology of those cells. I also think that knockouts are not the most appropriate tool. I am glad to see the field moving towards base editing, and different modalities that could be safer and also offer some tunability. And I also see transcription factors being used more and more. It is good to see quite a few companies pursuing these alternative options to edit cells instead of just cutting out a piece of DNA and permanently eliminating the expression of a gene.

Q Looking to the future, how will both the Altius Institute and Umoja Biopharma continue to develop and evolve to drive novel biological innovation into and through the clinic?

SG: Altius is embarking on additional collaborations to get more transcription factors created - for any gene that is interesting in the biology of a T cell, for example – and doing directed evolution in order to enhance the activity of these enzymes.

In the future, I imagine that we'll be able to actually programme superior cells that really do have the biology that we want. I use the word 'programme' because we'll be able to create a complex pattern of gene expression, which is really what determines cell fate. If we can create that through chromatin manipulations and transcription factors, without actually chopping up the genome, we'll have a superior therapeutic product. We could potentially reverse the effects of *ex vivo* manufacturing, if we block those pathways that get turned on in the artificial setting of manufacturing with transient transcription factors.

As for Umoja, I have faith that this is an engineering problem and we will overcome it - that we will be able to create an effective immune response against solid tumors. I believe we'll be able to overcome the serious issues with potency and scale currently associated with CAR-T manufacturing, overcome tumor heterogeneity, and some of the other hurdles that solid tumors present.

Q What's your personal vision for the role that computational science may one day play in cell & gene therapy R&D?

SG: 'Computational' is a broad term. Computation is used to understand genetics, genomics. It's also used to provide the information and algorithms for machine learning, and to advance what we understand about regulatory genomics.

Personally, I think this last area is where computation can help the most. Altius's core work – to understand the 'regulatome' – is still a work in progress, but once we understand that, we will know what patterns we want to create with the programming tools that we've developed. And we'll actually have enough knowledge about what is the phenotype or genotype that we want.

That will inform both gene therapy and cell therapy: knowing exactly what we want to change in the genome, or in the cell, in order to achieve disease resistance or any other type of outcome.

So I'm hoping that the development of computational skills and of additional machine learning and bioinformatics tools to understand how genes are regulated will contribute that knowledge that we're still lacking in the cell and gene therapy field.

AFFILIATIONS

Shon Green
Altius Institute for Biomedical Sciences
and
Umoja Biopharma

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INTERVIEW

Streamlining preclinical-clinical translation processes for an expanding CGT pipeline

JOHN MOSCARIELLO joined Celgene in 2018 and serves as the Senior Director of Viral Vector and Gene Editing Process Development. Prior to joining Celgene, John was the Vice President of Process Development at AGC Biologics (formerly CMC Biologics) where his organization was responsible for all cell line development, upstream and downstream process development, analytical and formulation development, and technology transfer and technical manufacturing support. Prior to AGC Biologics, John held director-level positions at Amgen, focused on process development and characterization, clinical and commercial technology transfer and process validation. John has a PhD in chemical and biological engineering from the University of Wisconsin-Madison and a bachelors of chemical engineering from the University of Delaware.

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Q What are you working on right now?

JM: We're working on a lot of things! One of the things that's really exciting about Bristol-Myers Squibb is we're getting very close to commercializing our first round of cellular therapies – from my standpoint, that's about making sure we have commercial viral vector ready to go when we need it.

"Having robust platforms allows you to be very streamlined in terms of the amount of development you need to do before you have a process that's fit for GMP manufacturing."

But we're also now transitioning to a situation where we have a nice earlier-stage pipeline coming through from R&D. In this area, my group is focused on process development and the raw materials needed for cellular processing – that's both the viral vectors and some of the gene editing raw materials we're now interested in. We're now thinking about our portfolio and how to make sure we can meet the needs of all the new, exciting projects coming in from the research pipeline.

Q Can you tell us about the challenges in translational R&D that come with a rapidly expanding cell and gene therapy pipeline as you perceive them?

JM: My background is in the protein therapeutics world, where we had the luxury of many, many years to establish good systems and good handoffs between research and the CMC and process development organizations. We also had well-established platforms and criteria to inform our colleagues in research as to when a molecule was ready to go into clinical development. Many of the key challenges relate to the fact we are still building all of this in cell and gene therapy, and because the technology is so exciting, we need to do so very rapidly.

Some of the things we're working on in this regard are around firming up the interfaces with research and making sure we're taking the right amount of risk.

If you think about a cellular product, there's a lot of things needed to manufacture it and you need to consider end-to-end requirements. This includes plasmids, gene editing raw materials, viral vectors and the cellular process. Traditionally, it might take you over 2 years to make your viral vector, and then even more time to develop your cellular bioprocess. Those timelines are way too long – it's simply not realistic to tell the clinical programmes it might take that amount of time before you can take a molecule into a clinical study to see if it's as effective as the research data indicates.

We're really thinking about end-to-end aspects of cellular therapy and where we can be more aggressive. Identifying activities we can begin while research is still engaged in their own work, where we can do certain workstreams in parallel, and what are the true requirements to go from one step to another. In this way, we aim to really streamline our development and accelerate the transition from research and preclinical proof of concept into clinical studies.

Q Can you go into more depth on the risk-based approaches you employ – for example, in overlapping different elements of bioprocess R&D for cellular immunotherapy product candidates?

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"Traditionally, it might take you over 2 years to make your viral vector, and then even more time to develop your cellular bioprocess. Those timelines are way too long – it's simply not realistic to tell the clinical programmes it might take that amount of time before you can take a molecule into a clinical study to see if it's as effective as the research data indicates."

JM: One example would be starting to make some small investments, both in terms of internal resourcing and external costs, before research has its entire data package ready. This can cut a significant number of months off your timeframe for moving into a clinical study.

There are also calculated risks that can be taken from the more technical standpoint. For example, you might not want to wait until you have a full GMP-released vector in order to start a lot of your development activities – a platform vector process, which is still undergoing minor optimizations, can be adequate for beginning both your cellular bioprocess development and your analytical development work. You're taking something of a technical risk in taking this approach – that the changes that happen as you optimize your viral vector process aren't going to impact the work you've done on the cellular and analytical sides. Obviously, you would only do so if there were a lot of strong data and rationale behind using a highly representative platform process, whilst also acknowledging that things might change slightly as you proceed along the pathway to GMP manufacture. And again, the potential benefit would be significant: a considerable chunk of time saved off your cellular process development that would otherwise have been spent waiting for a final GMP vector product to be ready.

Q What enabling tools are available – and what's still needed – to support this work?

JM: I'm a technical person and so I would naturally think about new technologies and equipment that is needed, but I must say that the thing that's perhaps most important is having really strong and robust infrastructure in place. It's about understanding with research that when a certain point is reached then other activities can begin, and for all parties to acknowledge any potential risk in going ahead.

We have very strong functional timelines at Bristol-Myers Squibb. Each function knows exactly how many resources are needed to take a project from the beginning to the end – the

amount of time, the amount of material, etc. That's across both the viral vector and the cellular bioprocess functions, allowing us to create an overall, integrated timeline that means we all understand exactly when different functions need material, and when the handoffs between one function and another can occur – for example, a handoff between process development to a tech transfer or a manufacturing group.

You need that infrastructure in place to make sure you're able to act very quickly and enable seamless transitions as a project goes from one step to the next.

Q What are the best practices you've identified to date in terms of streamlining and accelerating the target validation and clinical translation processes?

JM: Focusing on the CMC side, I think most of the best practices we have relate to establishing very robust platforms for our viral vectors – that goes for both the lentiviral and other vectors we work with. Having robust platforms allows you to be very streamlined in terms of the amount of development you need to do before you have a process that's fit for GMP manufacturing.

Q What will be the next steps moving forward for you and BMS as you continue to drive this pipeline expansion and translational R&D streamlining process?

JM: We are thinking about how we can leverage things like gene editing technology – how we can look into specific areas of the genome and use gene editing tools in association with our products to knock in or knock out particular genes. We will continue to consider that technology and how it might be most effective in relation to cellular therapies and in terms of how best to approach various different indications. This not only includes liquid and solid tumors for cancer, but the various diseases for which a cellular or gene therapy product could be the right approach

We're really excited about the utility of these technologies from a process development standpoint, in terms of their potential to open up avenues that might not be accessible with traditional lentiviral vectors and the other types of viral vector that are out there today.

AFFILIATIONS

John Moscariello
Senior Director, Viral Vector and Gene Editing Process Development, Juno Therapeutics

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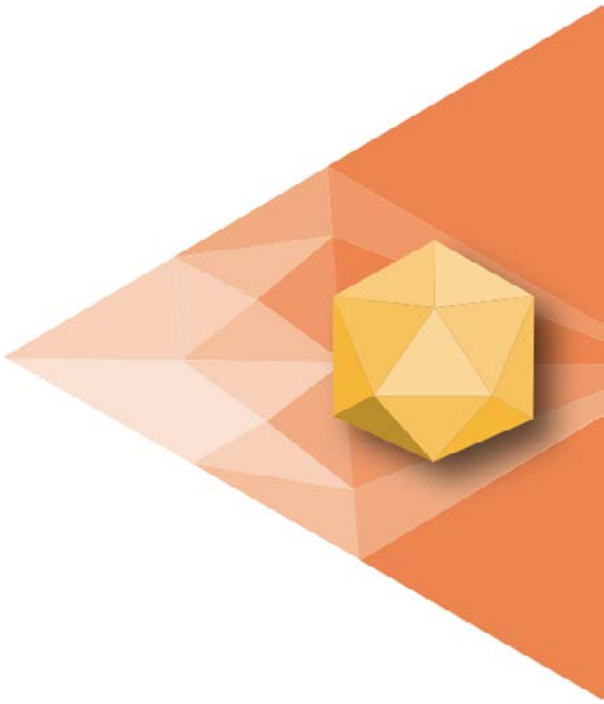
CELL & GENE THERAPY INSIGHTS

Vector Channel

SUSPENSION CULTURE SYSTEMS EDITION

CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL: Suspension Culture Systems



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INTERVIEW

CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL: SUSPENSION CULTURE SYSTEMS

EXPERT INSIGHT

Development and scale-up of suspension culture processes for viral vector manufacturing: challenges and considerations

Helen Young & Philip Probert

Viral vectors are currently the preferred vehicle for delivery of DNA for cell and gene therapies, which have the potential to revolutionize the treatment of human diseases. Developing high yielding, robust, scalable and commercially viable processes to manufacture sufficient quantities of viral vectors that also meet the stringent standards of purity, identity, potency and safety presents challenges. This article will discuss some of these challenges and considerations for the development of viral vector production processes. We conclude that although the industry will see a variety of production technologies employed for the manufacture of viral vectors, suspension culture will become the predominant method of production due to its scalability and it likely being the only method able to generate sufficient quantities of product at commercial scale. We also anticipate that the industry will move away from using transient transfection-based processes and move towards stable producer lines in common with conventional biologics manufacture.

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INTRODUCTION

Cell and gene therapies have the potential to transform the treatment of human diseases including metabolic, cardiovascular, infectious, ocular, immunologic disorders and cancer. These therapies predominantly exploit viral vectors as a vehicle to deliver therapeutic DNA to the patient. The global viral vector and plasmid DNA manufacturing market is growing rapidly and is expected to reach a value of $1.8 billion by 2026 [1] with the number of candidates entering clinical trials increasing. The ARM's Quarterly Regenerative Medicine Global Date Report for the third quarter of 2019 showed over 1000 cell and gene therapy clinical trials were in progress as of the end of Q3 [2]. Viral vector manufacturing, on a per dose cost, is significantly more expensive than manufacturing of conventional biologics due to low yields and challenges in product purification and characterization [3]. These factors will need to be addressed if the full potential of these therapies is to be realized.

Cell and gene therapies are classified into two groups, depending on the method of delivery of the therapy to the patient; *ex vivo* or *in vivo*. *Ex vivo* therapies involve administering the therapy to cells *in vitro* before the cells are transfused back into the patient. These therapies use either the patient's own cells (autologous therapies) or donor cells (allogenic therapies). *In vivo* therapies involve direct delivery of the therapy into the patient. Viral vectors are currently the preferred delivery vehicles, with the two viruses used most frequently as therapeutic vectors by cell and gene therapy developers being adeno-associated virus (AAV) and lentivirus (LV). Additionally, of the relatively few approved cell and gene therapy products, AAV and LV are the predominant viral vectors in use [4]. Therefore, this article will predominantly focus on these viruses. AAV is a small, non-enveloped virus with a diameter of ~25nm comprising an outer icosahedral protein capsid housing a single stranded DNA genome of ~4.7kb [5,6]. It is non-pathogenic, replication-defective and each AAV serotype has its own characteristic capsid providing a broad host and cell type tropism range. Due to its small size however AAV has limited transgene capacity. LV, part of the retrovirus family, is larger than AAV with a diameter of ~80–100 nm comprising an enveloped capsid containing a single-stranded RNA genome of ~7–12kb in length [5,6]. LV therefore has a larger transgene capacity and it has the ability to reverse transcribe its own genome into double-stranded DNA that is incorporated into the host genome. This ability makes it the main delivery vehicle for *ex vivo* gene therapies. Different virus types will continue to be used in the cell and gene therapy field with their diverse properties reflecting the diverse indications that they are used to treat, therefore selection of the appropriate viral vector is critical for the success of the therapeutic.

Developing commercially viable processes to manufacture sufficient quantities of viral vectors for clinical applications presents challenges across all virus types, some of which will be considered in this article. The article will discuss the choice of viral vector production mode; transient transfection or a stable producer cell system ('Viral vector production modes: transient transfection vs stable producer cell systems' section). The former will include a review of one of the most critical parts of a transfection-based process; the supply of GMP-grade DNA, which is at risk of becoming a bottleneck in supply of new gene therapies to the market [7]. CPI's experience of developing AAV and LV processes will inform a discussion on the upstream scale-up challenges and considerations for suspension culture ('Upstream process development and scale-up challenges: suspension culture' section) and the article will conclude with considerations for translation ('Translation insights' section).

VIRAL VECTOR PRODUCTION MODES: TRANSIENT TRANSFECTION VS STABLE PRODUCER CELL SYSTEMS

The two main production modes employed for viral vectors are transient transfection and

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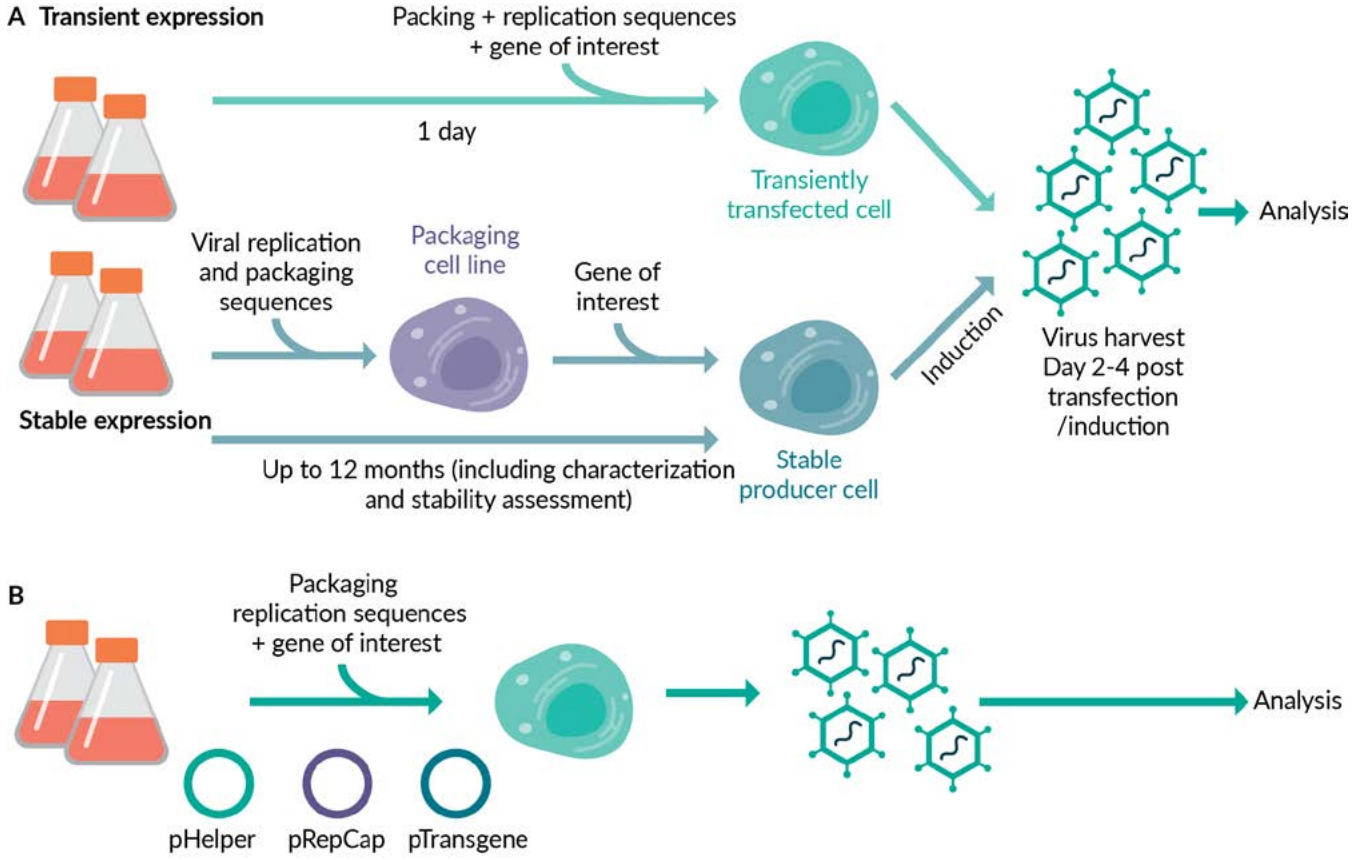
stable producer cell lines as shown in Figure 1A. Transient transfection is presently the gold standard for both AAV and LV production. It is quick, flexible and does not require the substantial upfront cost and effort required to produce a stable producer cell line. It also allows for successful viral vector production where a gene product arising from production is toxic to cells. The key raw starting material is plasmid DNA that is transfected into cells and encodes the transgene of interest and sequences required for virus production. The quality, stability and purity of this material is critical for manufacture of the final viral vector product. Traditionally plasmid DNA is microbially derived, typically by *E. coli* fermentation, involving a full manufacturing process with its own final product specifications. Plasmid DNA manufacturing is typically subcontracted to manufacturers routinely generating GMP-grade plasmid DNA using their own platform process. Manufacture is costly however and currently there is a lack of organizations providing this service hence manufacturers are experiencing long lead times for GMP manufacture of their plasmid DNA.

Although the FDA has not specifically outlined guidelines for plasmid DNA for viral vectors, for plasmid DNA vaccines the FDA recommends that the supercoiled plasmid content should exceed 80%, the level of host cell DNA, RNA and proteins be below 1% and the endotoxin level should not exceed 40 EU/mg plasmid [8]. If plasmid batches fail to meet these specifications the plasmid manufacture must be repeated at significant cost and time. For microbial vectors for gene therapy the FDA recommends not to use antibiotics for bacterial selection and

FIGURE 1

AAV production workflows.



(A) Transient versus stable AAV expressions systems. **(B)** Process and key variables for manufacture of AAV.

if antibiotics are used, the amount of residual antibiotics in the final drug product needs to be quantified as well as microbially-derived contaminants [9]. Viral capsids have been documented to not only package the virus genome but also other unwanted DNA fragments including host cell DNA and plasmids used for transfection [10], which may present safety concerns. For example, antibiotic-resistance genes present on microbially-derived plasmids could potentially be mispackaged in the final product.

Given the challenges associated with plasmid DNA manufacturing via microbial systems, it would be pertinent to consider synthetic DNA as an alternative for this critical starting material. The risks associated with the presence of antibiotic resistance genes and microbial-derived contaminants are consequently eliminated and, in addition, the synthetic DNA manufacturing process has the potential to be more cost-effective, rapid, of higher fidelity and have the ability to amplify complex DNA sequences that are difficult to amplify in bacteria. For example, Touchlight Genetics has developed an *in vitro* DNA amplification technology suitable for the large-scale production of GMP DNA, known as their doggybone DNA™ (dbDNA™) platform, which has been demonstrated to produce AAV particles with total and genomic titers comparable to those made using plasmid DNA [11]. The technology has also been used to successfully produce lentiviral vectors yielding transgene expression comparable to those made using plasmid DNA [12]. Whilst differences, if any, between synthetic and plasmid DNA are yet to be fully characterized, adoption of synthetic DNA technologies has the potential to improve availability of DNA and could therefore alleviate some of the challenges currently faced in the manufacture of viral vectors.

Using stable producer cell lines however negates the requirement for DNA in the viral vector production process therefore eliminating the above issues. It also makes for a simplified downstream process due to the absence of contaminating DNA from transfection in the process. Producer systems are also easier to scale, however generating stable cell lines is an expensive, complex and time-consuming task that many companies have avoided to date. It requires lengthy cloning and validation steps, and there are also concerns over longer term genetic stability that could affect product quality. Another challenge in creating stable producer cell lines is that some of the gene products arising from vector production are toxic to cells [13]. Through modification of potential cytotoxic viral elements, some success has been achieved in production of stable producer lines [14,15]. Inducible systems have also been used to circumvent vector toxicity, but given the number of sequences involved, these systems are time-consuming to develop and tune for optimal expression. In addition, evidence that the inducing agent had been removed from the final product would be required. Nonetheless inducible systems have been developed by various groups for AAV [16,17] and LV [18–20]. In many cases stable lines are produced as packaging lines, in which the gene of interest is transfected into a stable clone already expressing the other required viral sequences. This enables a platform approach to expression, since only a single gene needs to be introduced for different products and therefore the packaging line can be more comprehensively characterized, and process established. Importantly, yields from stable production/packaging systems historically fell behind transient approaches, however with advances in technology in some instances yields are now reaching comparability with transient systems. Commercially available packaging cell lines entering the market such as CEVEC's CAP-GT platform for production of AAV [21] and OXGENE's lentiviral packaging cell lines [22] are such systems.

The decision to use transient transfection or stable producer cell lines is currently made on a case-by-case basis considering budget, timelines and need for flexibility. Without time and upfront budget constraints, investing in a stable producer cell line for production in the long term we believe would be the most cost-effective approach based on current

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DNA costs. It would allow for easier scaling and downstream purification, increased reproducibility and, if the inducing agent could be effectively removed from the final product, be clinically safer. There is significant progress being made in this space and as more data is generated highlighting comparable titers to transient transfection systems, we are likely to see more manufacturers adopting stable producer systems.

UPSTREAM PROCESS DEVELOPMENT & SCALE-UP CHALLENGES: SUSPENSION CULTURE

Whilst adherent cell culture systems have historically been widely used for viral vector production, suspension cell culture systems are becoming much more commonly used; being accurately scalable from small laboratory scale systems to large industrial scale systems. Suspension cultures are also easier to monitor and control, and process development is more amenable to high-throughput approaches. Many adherent culture processes still use media containing serum; however, data has shown that these processes are able to be adapted to one that is suspension and serum-free including for both AAV [23–25] and LV [26]. Using chemically defined media is especially important for viral vector processes, which cannot include a viral inactivation step typically found with more conventional biologics. In terms of scalability, the largest currently available adherent bioreactor system is the iCELLis, which is available in up to 500m^2 capacity. For the sake of comparison, assuming a yield of 4 x 10^4 genomic particles/cell and either 1 x 10^5 cells/cm^2 or 1 x 10^6 cells/ml for adherent and suspension processes respectively (though titer and cell counts will vary depending on the particular process), a 500m^2 iCELLis and 500L stirred tank bioreactor would both produce 2 x 10^{16} genomic particles. For further scale up, larger scale stirred tank bioreactors are available, however this is not the case for adherent reactor systems. Additionally, at this large scale, preparation of the inoculum requires significant laborious culture in numerous hyperflasks, cellstacks or equivalent for adherent processes whereas for suspension the later seed train stages are typically composed of single Wave-bioreactor type systems, which are more consistent and less laborious to run. Suspension manufacturing is the current main mode for production of recombinant protein biologics and therefore experience gained with these processes can be readily transferred to new gene therapy products. With continuous processing also being considered as a promising approach for viral vector manufacture, this technology is more suited to adoption with suspension systems [27]. Where cells are unable to be cultured in suspension, microcarriers enable the suspension culture of adherent cells with associated benefits [28,29].

One of the biggest challenges with the development of upstream viral vector processes is that there are multiple steps to optimize; initial cell growth (seed train and expansion), transfection (if a transient-transfection production process), virus production and virus harvest. The media optimal for cell growth is not necessarily optimal for virus production and exchanging media is challenging, especially as processes are scaled up. A media and supplement cocktail should therefore be identified that is conducive to both cell growth and virus production as we have found that the initial cell growth step is important for high productivity and therefore titer. At each of these process stages there are a plethora of variables that need to be optimized. These include cell density, harvest method, harvest time and, for transfection-based processes, ratio of plasmids as detailed in Figure 1B, which outlines a typical suspension AAV production process utilizing a triple PEI-mediated transfection and HEK293 cells cultured in shake flasks. CPI has found, as an organization working with multiple processes from different viral vector developers, that process development is required for each product. Generating a

platform process for AAV for example is possible and could be used to generate multiple different products but titers could be compromised without additional process development. We have found that achieve the highest titers possible for each product, and importantly the highest full to empty capsid ratio, conditions for each set of plasmids and cell line need to be fully optimized.

When scaling up processes from shake flasks to bioreactors, process conditions such as pH, dissolved oxygen (DO) level and agitation rate also need to be optimized for each cell line and product. While shake flasks are useful for early development and can be used to screen multiple process variables (Figure 1B) we have observed differences when processes are scaled up from shake flask scale to bioreactor scale. For example, cell culture media that performs well at shake flask scale does not necessarily perform well at bioreactor scale. Further, process conditions such as pH and DO cannot be controlled at shake flask scale and therefore there is limited benefit to intense process development at this scale when parameters such as pH and DO could interact with conditions such as plasmid ratio. High-throughput screening platforms, for example the Sartorius ambr® systems, are therefore invaluable when performing successful early process development work and are amenable to DoE studies. Having an effective process development strategy is key, which includes access to this type of high-throughput equipment, however for some small start-ups this expense can be prohibitive. CPI has developed a suspension process development and scale-up workflow (Figure 2) that can be effectively applied to viral vector products to develop scalable, cost-effective and high-yielding processes. This involves transferring and demonstrating processes at shake flask scale, then moving to small-scale high throughput ambr® systems before scaling up to 2L, 10L, 50L and 200L vessels. The ultimate challenge is to develop scalable processes that produce high yields of high-quality vector whilst keeping costs as low as possible. For transient transfection-based manufacture, the largest costs are often attributed to the DNA and transfection reagent so, in order to minimize costs, processes are optimized to use the lowest amount of DNA and transfection reagent possible without impacting titer. Maximizing the full to empty capsid ratio of viral vectors is also a challenge and we have observed that optimizing the feeding strategy is important for this. For some processes, too much feed can result in more empty capsids being produced therefore ensuring the cells are in the optimal state for virus production is imperative.

Another point to consider when scaling up a viral vector process is when to perform more in-depth analytics. Process improvements demonstrated by an increase in physical titer (total capsid number and number of viral genomes) might not necessarily correlate with an increased functional titer that quantifies how much virus can transduce the target cell. Determination of functional titer is much more time consuming than determination of physical titer and is not practical to perform throughout process development, for example with an ambr®15 DoE experiment, yet must be considered before taking a process to large scale. Similarly, for transient transfection-based processes, optimization of plasmid ratio and amount of plasmid used could impact on the level of DNA misincorporation whereby nucleic acid impurities are incorporated in the viral capsid. The FDA recommends that the level of residual DNA in final viral vaccine products should not exceed 10ng/dose [30]. Viral vector encapsulated nucleic acid impurities may arise from any of the sources of DNA in the production process including DNA from producer cells or the plasmids used for transfection. Unlike residual nucleic acid impurities that can be removed by in process steps, packaged impurities can't be removed by nuclease treatments such as benzonase. Even slight changes in upstream processing can significantly affect downstream processing and analytics and this must be considered at the appropriate time(s) during process development.

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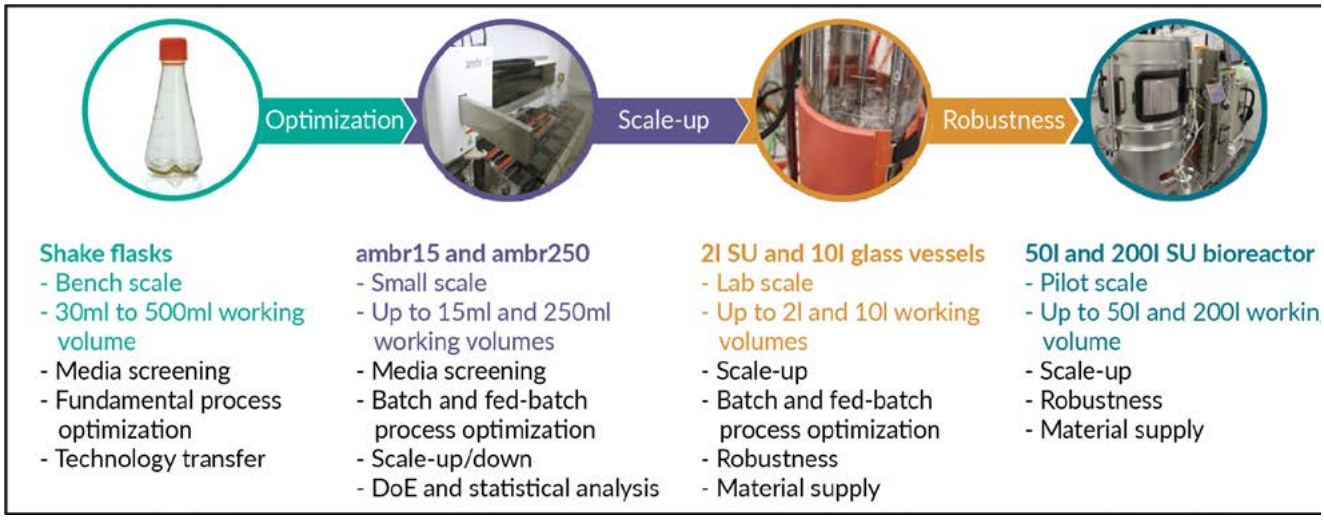
FIGURE 2

Suspension process development workflow at CPI from flask process to pilot scale.



TRANSLATION INSIGHTS

The major hurdle for commercialization of gene therapies utilizing viral vectors is the ability to produce sufficient quantities of product at commercial scale. For Duchenne muscular dystrophy for example using AAV as the delivery vehicle, the annual projected viral vector requirement is approximately $5x10^{20}$ viral genomes, assuming ~10^{15} vector genomes per patient and 500,000 patients per year [27]. Depending on titers achieved and recovery in the downstream purification process, on average one 200L bioreactor run would generate enough material for only two doses assuming $8x10^{15}$ viral genomes produced per reactor and a 30% recovery from the downstream purification process. For eye diseases, the annual requirement is lower at approximately $1.6x10^{10}$ for age-related macular degeneration, assuming $8x10^{5}$ LV transducing units per eye and 10,000 patients per year [27] highlighting why many cell and gene therapies target the eye. We predict that although a variety of production systems will be exploited for viral vector generation depending on cost, timelines and dose requirements it is likely that suspensions systems are going to be the only means of generating the amount of product required. Since one of the key challenges in adoption of suspension systems is that transfection at large scale (>200L) is challenging, we speculate that viral vector manufacturers will move towards employing stable producer cell systems. Not only would it be more amenable to scale-up, it would also come with the added benefit of a simplified downstream process through elimination of the risk of contamination from transfection materials. It would also reduce the risk of microbial-derived contaminants being present in the final product. Once stable lines are defined, it also enables more thorough characterization of material through increased standardization. For companies opting to use transient transfection for production; for convenience, flexibility and time constraints, there will need to be significant progress in plasmid DNA manufacturing to prevent manufacture becoming cost prohibitive as processes are scaled. We believe that the use of synthetic DNA for transfection is promising but the technology has yet to be demonstrated at large scale and any differences in product quality attributes compared to plasmid DNA have yet to be determined. Irrespective of production method, the need for product-specific process development necessitates that manufacturers have access to an effective, high-throughput process development workflow that allows for the development of high yielding, robust, scalable and commercially viable processes. We and others believe that

to address these challenges, collaboration is key [27]. CPI has developed unique expertise in developing scalable viral vector manufacturing processes through collaborative projects with Cobra Biologics, Gyroscope Therapeutics, Freeline Therapeutics and Puridify/GE, and through these projects have made progress attempting to address some of the challenges discussed in this article through sharing knowledge and working collaboratively. Despite the challenges discussed, gene therapies are undoubtedly moving towards fulfilling their potential and the future will see exciting and rapid advances in the field.

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AFFILIATIONS

Helen Young
CPI, 1 Union Square, Central Park, Darlington, DL1 1GL, UK

Philip Probert
CPI, 1 Union Square, Central Park, Darlington, DL1 1GL, UK

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL:
SUSPENSION CULTURE SYSTEMS

CHANNEL CONTENT

PODCAST INTERVIEW with **Emmanuelle Cameau**, Senior Bioreactor Applications Scientist within the Scientific Laboratory Services at Pall Biotech, and **Joseph Capone**, Senior Global Product Manager at Pall Biotech.

Evolution of culture systems for viral vector production: advantages, challenges and cost considerations

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Q What is the current state of play in terms of the evolution of both adherent and suspension culture systems for viral vector production?

JC: The real question here is about the expression systems that are used to produce viruses. There are several expression systems in use that are in constant evolution.

The three main expression systems are transient transfection using HEK 293 cells, an insect cell based system based on infection by baculovirus of the Sf9 cell line, and packaging/producer cells, where some of the genes required for virus production are stably integrated into a host cell genome, and a helper virus is used to trigger virus production. The majority of virus production currently takes place using transient transfection.

To circle back to the question of adherent versus suspension: transfection systems can be done in either suspension or adherent culture, while both the insect cell and packaging/producer cell systems are done in suspension. The majority of viral vector production still occurs in adherent cells, and we have seen strong movement towards using bioreactors for either adherent or suspension, as this provides greater control over batch productivity. This means you require less labor and less overall resources for upstream processing. Over the past 5 years, production has gradually shifted towards suspension cells – a trend which appears to have accelerated in the past 12 months. There is a still a space for adherent culture though. It remains the gold standard with at least two FDA approved drugs (Luxturna® and Zolgensma®) made using adherent systems. Zolgensma® requires large quantities of virus and the iCELLis® bioreactor system is used to make this drug.

Q What are the pros and cons of each of the different expression systems?

> "There is a perception that cost of goods would be lower for a suspension process, but this may not be accurate in practice."

EC: At the moment, more than 70% of clinical trials use transient transfection systems. This system is generally easy to get up and running, and can be scaled easily in either suspension or adherent mode. It suffers from the fact that the transfection process can be quite difficult to control, which can result in batch to batch differences in viral vector yield. It also requires plasmid DNA (pDNA) as an input, which is both expensive and sometimes in short supply. Despite these drawbacks, the relative speed to market it can provide will likely mean it will continue to dominate in coming years.

The Sf9 baculovirus system is well adapted to suspension culture, but can be quite challenging to get right, particularly the ratio of the different types of baculovirus. The baculovirus also has to be separated from the therapeutic virus, which can be a challenge. Additionally, there is some evidence that virus produced in insect cells is less potent than what we would get from mammalian cells.

Finally we have packaging/producer cell lines. Theoretically these cells should have the most consistency, as most of the genes requires to generate the virus are still integrated in the host cell genome. Viral vector production is stimulated by infecting with a helper virus which has to be separated from the therapeutic virus, as with the baculovirus system. Unfortunately, this system can take as long as 2 years to generate, with no guarantee of success at the end. This is simply too long in the gene therapy world.

> "...therapy developers need to weigh the need to get products to market as quickly as possible against the need to develop more productive expression systems."

Q What are the important advantages and limitations of the current state of the art in bioreactors for suspension and adherent systems?

EC: There is still a widespread industry belief that it is faster to bring a product to market if it is produced in adherent cells. This has been demonstrated with the very rapid development and approval of Zolgensma®, which is manufactured using the iCELLis® fixed-bed bioreactor. With the acceptance of the iCELLis® as a platform for adherent cell manufacturing, scale up and process intensification are made easier.

There is a perception that cost of goods would be lower for a suspension process, but this may not be accurate in practice. On average these cell lines produce less virus than their adherent counterparts, which in turn increases the cost per dose both in terms of cell line and plasmid raw material requirements.

JC: The industry simply has a deeper understanding of suspension bioreactors, and the seed train for suspension bioreactors is much simpler and better understood.

Scalability of adherent platforms is limited, as the larger fixed-bed bioreactor is only 500 m^2. On the other hand, a suspension bioreactor could scale up indefinitely, although many additional challenges are encountered when transfecting suspension cells at volumes greater than 200 L.

Overall, therapy developers need to weigh the need to get products to market as quickly as possible against the need to develop more productive expression systems. Unfortunately, these two objectives are often directly opposed – this is one of the major dilemmas facing the industry.

Q What are the key requirements and considerations for scalability?

JC: A representative scale down model is needed for upstream and downstream unit operations, whether in a fixed-bed or suspension bioreactor. Greater titer productivity must be obtained by identifying critical operating parameters and maintaining these conditions when scaling up.

It's also vital to consider the cost of raw materials – particularly the amount of transfection plasmid required per batch using the bioreactor – and the ability to maintain transfection efficiency when scaling up. Technologies that enable a streamlined and reliable seed train at production scale are also important when focusing on scalability.

> "The majority of viral vector production still occurs in adherent cells, and we have seen strong movement towards using bioreactors for either adherent or suspension..."

Q What potential impact can your choice of expression system have on downstream bioprocessing? How can you seek to minimize any negative repercussions?

EC: As we discussed above, if you're using a helper virus such as adenovirus or baculovirus, these will need to be separated from the therapeutic virus, which is generally adeno-associated virus (AAV). There are large size differences between these viruses, so techniques such as filtration can be used, as long as the larger virus is in relatively low concentration. If the helper virus is in too high concentration the viral filter will clog, and alternative methods must be found to reduce the amount of virus prior to filtration. In packaging/producer cells, where the helper virus and therapeutic virus are produced 1:1, another virus removal step must be introduced prior to filtration, using methods such as chromatography.

There are also implications for the early part of the downstream process; a recent study we performed has shown that different clarification strategies are required depending on whether the virus is manufactured in adherent or suspension format [1].

Q What about cost? How do the available expression systems compare?

EC: The cost of transient transfection systems is primarily driven by the cost of pDNA. For packaging/producer cells where no pDNA is required, batch costs can be significantly cheaper. However, the delay in getting product to market caused by moving to a packaging producer cell generally means these are not currently favored.

There may be some cost advantages to using an adherent system compared to a suspension system, given that pDNA is used more efficiently. Another recent study we performed showed that there are clear benefits to moving away from flatware systems and into bioreactor systems [2]. The study also showed that in some conditions, there are potential cost benefits to adherent methods.

Q How are Pall Biotech's solutions for upstream viral vector processing continuing to evolve with the key issues and challenges facing today's gene therapy sector in mind?

JC: In the upstream space, the iCELLis® bioreactor has become the most widely accepted technology for the manufacture of viral vectors in an adherent sub-platform. Both the bench-scale iCELLis® nano bioreactor and production-scale iCELLis® 500 bioreactor have undergone recent improvements to their vessel design, software, hardware, and automation. For an adherent cell seed train, Pall also supplies the Xpansion® multiplate bioreactor.

The iCELLis® bioreactor system is an automated single-use fixed-bed bioreactor providing excellent conditions to manufacture high quality viral vectors. It is linearly scalable up to 500 m^2; equivalent to greater than a 1000L bioreactor. We also have a robust and secure supply chain associated with the iCELLis® to service the installation base of more than 130 systems. Furthermore, the iCELLis® Nano provides a scale down model to the iCELLis® 500, and consists of an install base of over 400 systems globally.

To accommodate suspension cell platforms, Pall has the Allegro™ STR suspension bioreactor portfolio. This is available in sizes ranging from 50 to 2000 L. The Allegro™ STR range has been designed with enhanced usability in mind: its novel cubicle design concept allows for quick and easy installation, while maximizing mixing efficiency and mass transfer to optimal performance. We have shown the ability to culture insect cells to ~$7x10^6$ cells/mL in the Allegro™ STR [3]. To round out the upstream offering, Pall also has an Allegro™ XRS rocking platform bioreactor that is available to support a seed train from 2 to 25 L.

In the downstream space, Pall has the MVP system, and multiple filtration media available for product clarification. The Mustang® Q anion exchange membrane chromatography technology, has been proven effective at purifying both adeno and lentiviral vectors.

We therefore feel that in both the upstream and downstream space, Pall has the ability to provide technologies to support key challenges in gene therapy suspension and adherent applications.

> "There may be some cost advantages to using an adherent system compared to a suspension system, given that pDNA is used more efficiently."

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BIOS

Emmanuelle Cameau is a Senior Bioreactor Applications Scientist within the Scientific Laboratory Services (SLS) at Pall Biotech. Highly skilled in the field of cell culture applications and with 10+ years of experience, Emmanuelle previously held positions as a Bioprocess Specialist at Pall, and a Biotech Process Sciences Upstream Process Development Engineer at Merck Serono. She received her Biotechnology Engineer diploma from the former Ecole Supérieure d'Ingénieurs de Luminy (now Polytech Marseille).

Joseph Capone has been a Senior Global Product Manager at Pall Biotech since 2014, with over 15 years industry experience in the upstream space. He has held various cross functional roles in marketing, sales and technical application, as well as a cell culture associate in a commercial manufacturing in a cGMP environment.

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CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL:
SUSPENSION CULTURE SYSTEMS

INTERVIEW with **Rénald Gilbert,** Senior Researcher at the Human Health Therapeutics Research Centre at the National Research Council Canada

"I would advise starting to optimize your process at small-scale, because it's cheaper and faster to make changes at that point."

Key considerations for the use of suspension culture systems for viral vector manufacturing

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Q Ensuring the consistency, quality, and performance of viral vector production is a long-standing challenge for the space – where do you see real progress being made in this area?

RG: When looking specifically at viral vectors such as adeno-associated virus (AAV), or lentivirus, these vectors are produced by transient transfection. Recent developments in relation to their manufacturing have centered around the development of efficient, robust, scalable processes for large-scale transient transfection, ideally with chemically defined medium. In addition to this, there has been a push to ensure that these processes are, of course, cGMP-compliant to enable commercial manufacture.

We've also seen promising progress in the development of improved resins or membranes for the concentration and purification of viral vectors such as AAV and lentivirus, which ultimately has a positive impact on final recovery and purity.

Q Can you speak to the challenges relating to making the transition from adherent to suspension cultures during scale-up? What are some of the key considerations for making this as seamless and efficient as possible, for you?

RG: Right now, this transition is not quite seamless or pain-free. At the National Research Council Canada (NRC), strategically we made the decision to use cells in suspension culture, rather than adherent, for the production of viral vectors, due to the complexity and labor intensive issues around scale-up of adherent cells.

If you are working with adherent cells and wish to transition to scale-up in suspension culture, you need to adapt the cells, and this can take many months and not be always successful. One of the key aspects of this transition is that the adherent cells are cultured using serum and when you decide to move to suspension culture, the cells may have to be adapted to grow in serum-free medium first.

> "[We] use cells in suspension culture, rather than adherent, for the production of viral vectors, due to the complexity and labor intensive issues around scale-up of adherent cells."

Then you have to adapt the cells to grow in suspension conditions. Often you may observe a loss of productivity, a slower growth rate and potentially genetic instability. It's also not uncommon to see a change in the cells' gene expression pattern.

Because this transition to suspension is challenging, I would advise starting this process early, factoring in around six months before you are able to adapt your cells to the new culture conditions. Additional time will be required to characterize your cells again, and for the possibility of creating another cell bank. For these reasons, at NCR, we decided from the outset to work with cells that are already adapted to suspension culture and using serum-free medium which has simplified our production process considerably.

Of course there are options for scale-up whilst maintaining adherent culture – using roller bottles, cell factories, or fixed bed bioreactors, for example. I would advise that you either use a production process that is anchorage dependent all the way from small scale to larger scale, or start from the outset with cells already adapted to suspension culture because the transition step as outlined is currently not challenge free.

> "...either use a production process that is anchorage dependent all the way from small scale to larger scale; or start from the outset with cells already adapted to suspension culture."

Q What or where are the chief lingering safety concerns in viral vector manufacture in your view?

RG: In terms of the manufacturing process, one of the issues in working with replication incompetent lentivirus vector is that, for many countries such as Canada, it's considered as a risk group 2 agent, therefore, meaning it must be used in a BSL-2 laboratory. If you then consider manufacturing in large volumes and at higher virus concentrations, that can of course present a safety issue as well as presenting challenges regarding disposal of potentially hazardous waste. We also have to be cognizant of the possibility of generating replication-competent virus and putting in place measures to address that.

It's also important to mitigate the risk of cross-contamination of products by working in a closed environment, with minimal opportunities for contamination with bacteria or mycoplasma.

Looking at safety concerns from the final product perspective, of course it's essential that you ensure your final product for the patient is pure and that there is batch-to-batch consistency. Furthermore, if you are using plasmid to produce your viral vector, such as you do with AAV and lentivirus, you should use plasmid with a low level of endotoxins.

To help mitigate these safety concerns and optimize your end product, there's a great deal of effort to develop relevant assays, such as for example one to detect the presence of empty or non-functional virus particles, which can affect the efficacy of your product. And of course during purification you have to make sure you have a low level of contamination of genomic DNA, as well as RNA and host cell proteins.

Q What are the most important considerations for you when choosing the right cell culture platform and media, particularly in the suspension systems realm?

RG: For anyone producing material for clinical application it is important to ensure that media are serum-free and also free of animal-derived components. With serum in particular, there's a great deal of lot-to-lot variation, and it could be contaminated with adventitious agents.

Ideally, you would want to work with a medium that is chemically defined, as this would reduce lot-to-lot variation and also minimize the risk of adventitious agent contamination. And because lentiviral/retroviral vectors or AAV are produced by transient transfection, it's important your medium allows transient transfection (with adenovirus vector production this is not an issue as you do not use transient transfection).

> "Ideally, you would want to work with a medium that is chemically defined, as this would reduce lot-to-lot variation and also minimize the risk of adventitious agent contamination."

It is also important that the medium should allow cell growth to high cell density, and support the production of viral vectors at high cell density, with rapid doubling time of around 24 hours.

Q Can you speak more to the benefits of having a chemically defined system from the early stages of bioprocess development? And what is the related impact on raw materials and the supply chain in general?

RG: The issue of lot-to-lot variability really can be a limitation in your manufacturing process for viral vectors. If you choose a serum-free, but not necessarily chemically defined medium, such as one made using plant hydolysates for example, whilst it is free from animal-derived products, it's not a well characterized product and therefore can be prone to variation from lot-to-lot.

By having a chemically defined system you are ultimately improving the safety and purity of your end product, because all components that enter into its manufacture or final composition are well characterized.

As such, I would advise looking at optimizing your manufacturing process early on, at small-scale, ideally by choosing the medium formulation that you will want to use as you progress to large-scale manufacture. In this respect it's important to, where possible, have more than one supplier of this critical component to reduce possible supply continuity issues as you scale up, or ensure your sole provider has risk mitigation plans in place to minimize any potential delays or problems with media supply.

Q Can you talk to us through your approach to identifying suspension culture system-based bioprocess parameters, highlighting any key learnings from your experience in this area?

RG: As mentioned, selection of a cell line that will meet your requirements in terms of scalability in suspension culture within serum-free medium with good doubling time, is critical as you move to scale up. It's also important to understand if the cell line has a good history and whether there is a cGMP cell bank available, because if you have to create a cGMP bank yourself, you will need to factor in additional time until that material meets the requirements to be used for clinical applications.

Once you've identified your optimal cell line, you can start to assess which media works best with your cells. If, for example you want to produce a third-generation lentivirus vector, this involves transfection with four plasmids and therefore an important early step is to identify which ratio of plasmid will give you the highest titer of vectors. At the NRC our preferred transfection agent is PEIpro (PolyPlus Transfection); because this is GMP ready and in our hands gives highly reproducible titers. At the start of a project, we will determine the best concentration of PEIpro and the ratio to plasmid concentration, which can take several weeks as you have to test several conditions. But once you have optimized your conditions, you can then start to scale up.

We initially work with 20 ml in shaker flasks, before scaling up to 300–500 ml working volume in larger shaker flasks, to ensure the process we develop is robust, scalable, and reproducible, delivering the same titer at each step. Following the shaker flask scale-up, we move to a small bioreactor, of up to 3 L. We perform several runs at this scale before moving to different sizes of bioreactor, such as 50 L, 200 L, or even 500 L, again to assess that the yield and productivity are maintained at each scale-up step.

This approach has worked well for us when producing lentiviral vectors and AAV, and we've found that if you start with a robust cell line, a good medium formulation, normally what you observe in the shaker flasks in terms of yield, you will then replicate in the bioreactor.

Q Can you summarize the chief pros and cons in suspension culture terms of the four viral vectors most extensively used in clinical trials (adenoviral, adeno-associated, retroviral and lentiviral)?

> "...we've found that if you start with a robust cell line, a good medium formulation, normally what you observe in the shaker flasks in terms of yield, you will then replicate in the bioreactor."

RG: Let's look at adenovirus, which I would say is probably the easiest viral vector to produce, namely because you don't have to perform transient transfection. It's also a well understood virus, having been used in gene therapy applications for over 20 years. It's also a very stable virus, unlike the envelope viruses, so you can use chromatographic methods to purify it.

However, one of the challenges in using adenovirus produced through HEK293 cells – which is a very common method of adenovirus production – is that you are likely to generate replication competent virus particles.

Similar to adenovirus, AAV is a fairly stable virus, and when considering its manufacturing "strengths," there are

effective methods and commercially available resins to purify AAV via affinity chromatography. Therefore, the purification step is particularly strong. However, with AAV you can be impacted by the presence of empty or partially filled capsids, which reduces potency of your end product, and removing these empty capsids is not a straightforward process at present.

In addition, as AAV is produced via transient transfection, often with three plasmids, the process is more complex and you must ensure you don't have residual plasmid in your final product.

A current limitation with transient transfection, which impacts the production of AAV, retroviruses, and lentiviruses, is the lack of efficient process to transfect at higher cell density. Presently, most of the transfection processes are conducted at cell densities not higher than 2 million cells per milliliter, which still is a challenge to overcome.

The added complexity of utilizing retroviruses and lentiviruses, is that in contrast to AAV they are not stable and this complicates downstream processes, in particular purification, because if your process lasts too long the fragility of the virus can lead to loss of infectious particles. Furthermore, these viruses are produced in the presence of exosomes or extracellular vesicles, and these are very difficult to purify and separate from your end product.

That said, a big advantage of retroviruses and lentiviruses is the availability of packaging cell lines, which removes the need for transfection, thus simplifying the production process. Whilst the yield is often lower than that achieved with transient transfection, if you have packaging cell lines you could produce the vector using perfusion mode which will allow you to perform continuous-harvest at high cell density. This is a very important point for lentivirus, or retrovirus, because the virus is not stable, by performing continuous-harvests this enables you to use the optimal amount of virus that your system would be able to make. So you may harvest the virus via perfusion during the period of three to four days. Because this process is complex however, you will need specialized equipment and trained personnel.

Furthermore, due to the aforementioned fragility of these viruses, it's more difficult to sterilize them using ultrafiltration, often with losses of up to 50% of your virus during this step. Perfusion is a less viable option for AAV, given that most of the virus is intracellular, whereas, lenti- and retro-viruses are secreted into the culture medium, from where the budded virus can be harvested.

"...a big advantage of retroviruses and lentiviruses is the availability of packaging cell lines, which removes the need for transfection, thus simplifying the production process."

Q What is your view on the current prevalence of HEK293 use? What other cell types should people consider for larger-scale suspension system production?

RG: We, and other groups around the world, have developed HEK293 cells that were adapted to suspension culture with a serum-free medium, and they provide a very good yield for AAV and lentivirus. Owing to this good yield, HEK293 is currently the most popular cell line used for the production of AAV, retrovirus, and lentivirus.

In the past, HEK293 cells were widely used to produce adenovirus, but one of the issues that arose, is that due to homology between the HEK293 cells, previously transformed using sequence derived from adenovirus, and the adenovirus vector, that cell line generates replication-competent adenovirus by the process of homologous recombination. For this reason, many groups, including ours, have developed cells to specifically produce adenovirus. Some cell lines, such as the one developed by NRC are derived from A549 cells. Other cell lines include PER.C6 (Crucell) and CAP-GT cells (Cevec).

Another option for AAV is to use insect cells, a process that involves first producing the baculovirus stocks to supply different components of AAV production by infecting the insect cells. But if you want to keep with mammalian cells, I think HEK293 cells are ideal for the production of AAV as well as lentivirus.

Q What for you have been the key technological advances that have contributed to the current state-of-the-art in viral vector production platforms?

RG: Certainly, the development of chemically defined media that allow efficient transient transections and supports cell growth at high cell density over 4 million cells/mL is a big step forward. About twenty years ago, we were using serum and undefined medium components which undoubtedly complicated the process, with challenges arising around reproducibility because the composition of the medium would vary from batch to batch.

Another development would be the marked improvement in filtration and separation membranes to remove the cells and cell debris from the virus.

Q And what should be, or will be, the next steps for innovation in this field?

RG: Where the field needs to make advances is in the ability to culture cells at a higher cell density. For example, if you are working with CHO cells to produce recombinant proteins, you can work at a cell density of say 20 to 30 million cells/ml.

Unfortunately, in the field of viral vector production, this is not yet possible. It would be a key advancement if we could develop a process whereby you could transfect cells at high cell density while maintaining the cell specific yield as this would greatly improve volumetric yield.

Further to this, I feel that innovation to improve our analytical capabilities is also key to advancing this field – such as in-line measurement of metabolites and cell growth, which we expect to be developed in the near future.

Q Lastly, can you summarize what for you are the key elements for a successful approach to viral vector bioprocess scale-up?

RG: You need to start with a good cell line, that is GMP compatible, to set yourself in a strong position for future scale-up of your processes.

In parallel to optimizing your cell lines and manufacturing processes, from the outset you need to develop good analytical assays that enable you to measure the purity, integrity, and functionality of your vector. Whilst you need assays for your final process, they are also essential in the scale-up process, allowing you to assess whether a process change or improvement, for example, impacts the final product.

And finally, I would advise starting to optimize your process at small-scale, because it's cheaper and faster to make changes at that point and once you have developed the process at small scale, you have to make sure it is robust and can be scaled up.

BIO

***Rénald Gilbert** is a senior researcher at the Human Health Therapeutics Research Centre of National Research Council Canada (NRC) in Montreal. Throughout his career at the NRC, he has held various leadership roles including the Program leader of the Vaccines Program, which delivered on the Government of Canada's mandate on innovation, fostering Small Medium Enterprise (SMEs) and public well-being. Currently as the lead of the NRC Biomanufacturing Research Initiative, he manages a portfolio of projects aimed at developing new and improved proprietary platforms enabling biomanufacturing of scalable, cost-effective and safe complex biological products. Dr Gilbert is also an adjunct professor in the department of Bioengineering at McGill University. His research interests include the optimization of viral vectors for the development of vaccines and for cell and gene therapy applications, as well as their methods of production. He is currently directing research projects aiming at increasing the yield and efficacy of vectors derived from adeno-associated virus (AAV) and lentivirus using suspension cultures of mammalian cells. Dr Gilbert completed his PhD in Biochemistry at McMaster University (Hamilton Ontario) in 1994. Before starting his career as a research officer at NRC in 2002, he got trained as a postdoctoral fellow in the department of Cell Biology of the Weill Cornell Medical College (New York City) and in the department of Neurology in Neurosurgery of McGill University.*

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CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL: SUSPENSION CULTURE SYSTEMS

INTERVIEW

Suspension systems in continuous viral vector production processes

FELIPE TAPIA is a chemical engineer with a PhD in bioprocess engineering carried out at the Max Planck Institute in Germany. He specializes in continuous virus production and is a co-Founder of ContiVir, a startup project that develops fully continuous processes of gene therapy viral vectors. ContiVir received a €1.1 million grant from the European Union, the German government and the Max Planck Society and its development is taking place at the Bioprocess Engineering Group of the Max Planck Institute in Magdeburg (Director: Prof. Udo Reichl).

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Q Tell us what you are working on right now

FT: I am a postdoctoral researcher at the Max Planck Institute (MPI) in Magdeburg, Germany. I am co-founder of an MPI-research project called ContiVir, where our aim is to develop the fastest and easier-to-scale viral vector manufacturing platform. To do that we plan to build a fully integrated continuous viral vector manufacturing platform using suspension cells. Our technology will contribute to closing the gap between lab- and commercial-scale viral vector production.

Q Can you give us some more background on the ContiVir project – how and why did it begin?

FT: ContiVir is the result of two PhD projects of the Max Planck Institute. One of these projects developed a continuous tubular bioreactor that enables stable virus production over weeks, much faster than batch bioreactors and with high productivity levels. The second project developed a highly efficient membrane-based virus purification platform with yields beyond 95%. Hence, the idea of ContiVir is to adapt and integrate both technologies to the needs of the gene therapy industry: the development of a much faster, more scalable and cost-efficient viral vector manufacturing platform.

Q Take us on a journey through the continuous viral vector production processes you are developing – what are the particular areas/steps of focus for you?

FT: Current viral vector manufacturing processes are operated in batch mode and one engineering approach to make vector production more efficient is developing continuous processes. However, viral particle production in continuous mode has been historically not easy to implement because 1) undesired viral mutations can occur over time, and 2) virus production yields might drop due to the presence of 'defective viral particles' (naturally present in viral populations).

These two main issues were overcome by our research team at the MPI by developing a continuous tubular bioreactor where virus production takes place inside a tubular bioreactor operated under laminar regime. In this system, suspension cells and reagents (infectious viruses or plasmids) are mixed at the entry of the tubular reactor and pumped along the tube where the biological reaction takes place for several hours. As a result, and over weeks, a harvest with constant product quality attributes is collected at the end of the tubular bioreactor. Our continuous technology was already proved for influenza virus production and we plan to adapt it for adeno-associated virus (AAV) production.

Q Zooming in on suspension culture systems in particular, can you firstly frame for us the challenges and limitation with current systems, as you see them?

FT: Gene therapy is seen as a tool that will open a new era of medicine. However, significant technical challenges need to be solved to make it feasible for commercialization. The main limitation with

"Our technology will contribute to closing the gap between lab- and commercial-scale viral vector production."

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"...the production of viral vectors for genetic diseases with thousands, or even millions, of patients worldwide will necessarily need the development of more efficient processes. We believe that the solution to this issue can be found in continuous processes and this can only be implemented if cells are grown in suspension cultures."

current viral vector production systems is that they were developed for relatively small volumes for which lab-scale technologies are suitable. This includes processes based on adherent or suspension cells. The problem arises when larger volumes are needed for late clinical phases and commercialization, since the number of theoretical batches needed to fulfill local and global demand is high. While current production platforms might be suitable for genetic diseases with a relatively small number of patients, the production of viral vectors for genetic diseases with thousands, or even millions, of patients worldwide will necessarily need the development of more efficient processes.

We believe that the solution to this issue can be found in continuous processes and this can only be implemented if cells are grown in suspension cultures. Challenges associated with suspension cultures in continuous mode are associated with diminishing process-induced batch to batch variation and characterization of cell (and product) genetic stability over time.

Q How is ContiVir seeking to further optimize/develop suspension culture systems for gene therapy vector production?

FT: We are currently focusing on the development of a continuous production system (reactor and purification column) that enables AAV production with suspension HEK cells. However, since different animal cell lines have different metabolic rates, our most challenging goal is to construct a universal bioreactor system that can be used with different suspension cell lines.

Q Finally, what are your and ContiVir's chief goals and priorities for the months and years ahead?

FT: In the short term (2020), our main goal is to build the fastest and most efficient viral vector manufacturing technology and prove its feasibility. Moving from

stainless-steel to single-use materials is part of our mid-term goals. Finally, we plan to have a commercially available technology within 2 years.

AFFILIATIONS

Felipe Tapia
Max Planck Institute, Germany
and
Co-founder, ContiVir

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CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL: SUSPENSION CULTURE SYSTEMS

INTERVIEW

Emerging upstream bioprocessing and analytical tools for vector manufacture

MICHAEL J WHITE is a Scientist in the BioProcess Development group at Ring Therapeutics. He has been working with/on viruses since 2012 as a postgraduate fellow at Yale University School of Medicine in Brett Lindenbachs's Lab where he worked on virus assembly and viral protein interactions for Hepatitis C Virus. From there he went to Purdue University to pursue his doctorate in the laboratory of Richard Kuhn studying assembly and entry/infectivity mechanisms of flaviviruses such as Dengue and Zika, where alongside purifying virus, he developed expression platforms and purification processes for viral proteins. From here, he broke into industry and gene therapy as a postdoctoral fellow at Medimmune/AstraZeneca within the BioProcess Development Group. It was here that he worked on end to end bioprocess development of AAV vectors focusing on how upstream cultivation and harvest conditions from suspension cultures can influence downstream purification recoveries and vector potency. He is currently at Ring Therapeutics as team lead for upstream process development for a novel gene therapy vector.

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Q Can you give us some brief background on your career to date in viral vector bioprocessing, and tell us what you are working on right now?

MJW: My viral vector experience started at MedImmune/AstraZeneca, in Gaithersburg, MD, where I was essentially sitting between upstream and downstream within the bioprocess department. I focused on AAV production, looking at new ways to purify them and at how certain upstream processes can influence downstream recoveries.

I'm now at Ring Therapeutics in Cambridge, MA, working as a team lead overseeing the upstream process development for a number of our novel viral vectors.

Q How do you reflect upon the range of upstream bioprocess technologies available for viral vector production today? Firstly, what are the chief remaining issues with the current state of the art?

MJW: We've entered into an amazing era of bioprocess tech improvements. The range of bioreactor options for vector production allow easier scale-ups and more process control – I'm thinking of the suspension-based bioreactors such as the Wave bioreactor and more recently, the ICELLis, which is clearly a huge improvement for adherent systems through its capability to allow an increased density scale-up of adherent cultures within a smaller volume.

I think some of the challenges now come down to the fact we're hitting a bottleneck with the DNA required for transfections, and also the general transfection efficiencies you get at larger volumes. Both of these are real bottlenecks in the process of viral vector manufacturing, at least in my experience. Transfections at smaller amounts tend to yield you higher efficiencies in transfection, whereas at larger amounts you don't necessarily get the same result. That means you're going to have to actually increase your volumes or cell densities at larger amounts to compensate for that loss. That really is an ongoing issue for many of us in this field in terms of scalability.

Q Focusing in on suspension culture systems specifically, what do you see as the likely/desirable future directions for further evolution?

MJW: The bioreactors are generally good, although as I've mentioned, the transection efficiencies are a little on the low side for optimal scale-up.

What I'd like to see in the future is perhaps a better understanding of, and more focused, cell line engineering to increase productivity. Of course, it would be ideal to develop some stable producer cell lines which would overcome several of these issues, some of which is already being done. I'd particularly welcome technology that's focused on the production of stable

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> "...some of the challenges now come down to the fact we're hitting a bottleneck with the DNA required for transfections, and also the general transfection efficiencies you get at larger volumes ... Transfections at smaller amounts tend to yield you higher efficiencies in transfection, whereas at larger amounts you don't necessarily get the same result."

producing cells, and also on cellular engineering to increase productivity per cell, meaning you don't have to use such large volumes when you're trying to scale-up your processes.

Q Tell us about the process analytical tools (PAT) side of things – are there any emerging technologies or approaches that show particular promise for you, particularly in-line?

MJW: I think one of the PAT tools that's really interesting is the NOVA FLEX bioanalyzer, which essentially allows in-line measurement of some metabolites, density, viability, dissolved oxygen, temperature, and so on. You can use this tool to closely examine upstream processes for vector production, and how changes in them can really influence your metabolites, your growth strategies, media optimizations, and things of that nature. All of these factors will have a direct effect on your viral vector of choice and more-so the recovery in downstream operations and subsequent activity.

In terms of the more recent technologies now emerging, one thing that's now being looked at is the analysis of post-translational modifications (PTMs). That essentially provides structural information on your drug substance. What I'd like to see in the future of in-process analytics – and I know it's been done to some extent already – is the in-line use of mass spec to identify PTMs resulting from extended cultivations or other things of that nature. Being able to conduct this more in-depth analysis of your PTMs as your vectors are being produced, as opposed to after the production has happened, is going to be really useful and will add a lot of value to bioprocess designs in general. Designing ways in which you culture or process your vectors around minimizing these PTMs can really improve a process and result in a higher quality vector product.

Q How does the team at Ring Therapeutics seek to ensure a coordinated approach to bioprocessing as a whole, particularly in terms of minimizing any potential repercussions for downstream bioprocessing of upstream vector production?'

MJW: We're seeing this more and more recently – this link between upstream and downstream. It's really a balancing act.

Traditionally in biopharma, upstream and downstream have been seen as two separate, completely individually operating elements of the bioprocess scheme – that was certainly the case with some of the bioprocess activities for antibodies back in the day. However, with viral vectors, we're starting to see how upstream processes – when you do or don't harvest your vector, for instance – directly affect your downstream results.

At Ring, we try to focus on a more balanced approach. As the team lead for upstream process development, I work extremely closely with the downstream lead. When we're assessing some of our main candidates and trying to screen some out to move forward with, we produce them in the upstream and then the yields we're getting off are verified with downstream processes. We then look at what our final recoveries are. So we're tying them extremely closely together. When you look at your upstream processes, you really need to think about when you're harvesting, how you're harvesting, how your cell viability is, and how any supplements you're adding might each be influencing your downstream process.

I think there are some things that are often neglected a little when we talk about upstream and downstream, such as particle heterogeneity, charged ion production and vector half-life. All of these aspects are going to have an influence on your downstream processes, and a lot of them are actually going to be driven through how you manage your upstream process.

Q Finally, can you sum up the priorities both for yourself in your role and for Ring Therapeutics as a whole over the coming 12–24 months?

MJW: Basically, our goal is to expand our current capabilities in the production and scalability of these novel viral vectors.

These viruses are notorious in terms of the difficulties in growing them *in vitro*, never mind reaching levels at which it becomes feasible to move into a larger scale. However, we've had great success so far, and over the next 1–2 years our focus is going to be on further improving on the current key cell lines we're utilizing. We'll be seeking both to boost further productions and to further expand our preclinical candidate pool, eventually leading to production of material for IND-enabling studies.

"Designing ways in which you culture or process your vectors around minimizing post-translational modifications can really improve a process."

AFFILIATION

Michael J White
BioProcess Development Group
Ring Therapeutics
Cambridge, MA, USA

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CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL: SUSPENSION CULTURE SYSTEMS

INTERVIEW

Establishing a suspension culture-based lentiviral vector bioprocessing facility

GABRIELLE HUMPHREY, Associate Director is the lead of the Vector Manufacturing Science and Technology group at Adaptimmune, UK. In 2018, Gabrielle established the team responsible for starting-up Adaptimmune's manufacturing capabilities at the Catapult CGT Manufacturing Centre in Stevenage. Her focus is currently on the production of GMP lentiviral vector for TCR T-cell therapy. Previously she has been involved in the GMP manufacturing of lentiviral vectors for CAR T-cell therapy and for gene therapy applications. Her area of expertise is the design and delivery of cleanroom bioprocesses for novel breakthrough therapies focusing on single-use disposable flow paths, aseptic processing and data monitoring.

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Q What you are working on right now?

GH: Adaptimmune recently completed production of our first GMP batch of lentiviral vector. This batch is to support our internal cell therapy supply strategy.

"One of the technological issues with adopting a platform technology as a 'plug and play' tool is that we may encounter issues with functionality each time we try to integrate a new genomic material."

Adaptimmune's TCR engineered T cells are specifically targeted for hard-to-reach tumors in patients with advanced cancers. One of our objectives is to enable rapid turnaround of the T-cell manufacturing in our facility, thus helping reduce the time patient have to wait for their own engineered T cells to be re-infused, to a technically and safely feasible minimum. Obviously, the internal supply of vector directly supports this strategy, and having our own vector manufacturing capability gives us greater agility within the supply chain to respond to evolving technical and clinical objectives.

Q What can you tell us about your experiences with suspension culture systems for vector production to date?

GH: Given the challenges in managing the supply chain, producing lentiviral vector (LV) at a scale that can reliably provide a number of vector doses commensurate with the size of a given clinical trial is a primary priority for us.

In addition to this, we have extensive and time-consuming testing activities to complete for each batch, which are driven by the regulatory requirements for this type of vector product. The costs associated with this testing programme provide further incentive to try to produce larger scale batches from an early stage of development.

Suspension culture systems are very well suited to addressing both these challenges, particularly in circumstances where the timescale for vector development is critical.

This has to be balanced, though, because although this is definitely the system of choice for us, the downstream considerations are often a limiting factor – particularly for producing the yields we're expecting and the number of doses per batch. (Of course, it's worth mentioning that this is an issue we've seen with both adherent and suspension cell lines).

Q What for you are the chief remaining challenges for the suspension culture technology area? How would you like to see it evolving further?

GH: There is a growing field of single use closed system solutions out there, and the manufacturing technology suppliers are extremely supportive of the development of a platform approach for vector manufacturing. However, we still have challenges remaining around the specificity both of each cell line and of the vector construct. For instance, we may select raw materials that are fully defined, but even with that level of control, biological interactions remain difficult to characterize.

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"The main objective when scaling-up a lentiviral vector bioprocess is really to determine the optimized culture conditions and the best vector expression at a small scale, and to then try to maintain this strategy linearly throughout the scale-up ... It's important right from the beginning ... that you select suppliers or manufacturing partners that really can support the process development..."

One of the technological issues with adopting a platform technology as a 'plug and play' tool is that we may encounter issues with functionality each time we try to integrate a new genomic material. In order to succeed, we need to run feasibility studies for each vector construct, and to ensure our yields are appropriate and the product functionality is satisfactory. This is the case for every vector manufacturer. Each one must define and understand their own critical parameters in order to demonstrate the robustness of the platform for new products.

The point I'm making here is that it leaves a large field to explore, particularly in terms of offering solutions to better control the gene integrations into the cells and the vector expression from those cells. As a company, Adaptimmune is always keen to learn more about new technology and new areas of development relating to both of these aspects.

Q How are Adaptimmune's GMP vector manufacturing operations at Cell and Gene Therapy Catapult's facility developing? What can you tell us about how this model operates in practice?

GH: By way of a brief summary, Adaptimmune has a GMP manufacturing platform for lentiviral vector production, which is located in one of the modules at the Catapult Cell and Gene Therapy (CGT) Manufacturing Centre in Stevenage, UK.

This is a modularized GMP facility with the overall building serving as a host or GMP 'umbrella'. What this has meant in practice is that Adaptimmune has been able to focus on developing a successful LV vector production platform, whilst working hand-in-hand with the Catapult team who are responsible for the building envelope, the supporting functions (such as the warehouse, QC and QA) and the facility management.

One of the main benefits of this concept is how it speeds up learning curves for the host and the company, as each can focus respectively on the GMP envelope and the processes. In turn, this has accelerated progress towards setting up the actual operations. I can confidently say that we have accelerated our GMP capability programme thanks to this collaboration.

The communications interface between the Catapult team and any given collaborator is somewhat different to that employed internally by an integrated company, which has all the functions under the same leadership or company name. Typically, we have more formal communication via QA systems. The operator training consists of core training, which is provided by the host entity, but alongside that we also carry out training within our own systems. There is also a lot of attention given to ensuring that the systems flow between the host and the collaborator – we spend a lot of time and effort making sure the Quality Management and the communication systems correspond across both organizations.

We have a slightly different way of operating on a daily basis, but the workflow is still very reminiscent of what would be employed with an external CMC function, for instance. I would expect any biotech professional working with an external CMC organization, a CDMO, or even a CMO, to find many similarities between their ways of working and communicating and ours.

Regarding the cleanroom itself, we do have additional crosschecks in place – for instance, with material movements – and we carefully coordinate our biological transfer as well.

In the warehouse, we rely on short cycle delivery. One of the challenges there is to ensure the procurement and supply chain systems are robust enough to allow those short cycle times to be successful.

One of the great advantages at the CGT Manufacturing Centre is that we have a very dynamic network of subject matter experts, suppliers and equipment providers close to hand. As a small company with a small facility, we do get a faster response time in this regard than we would have if we were using an external facility based at any other location. It also provides greater visibility in terms of who is out there as a solution provider – both for us and for all the other collaborators in the manufacturing center.

Q Would you have any words of advice for anyone thinking about building a suspension system-based viral vector production facility in the future?

GH: Our lentiviral vector (LVV) is a membrane-based and cell expressed vector. With the vector present in the suspension medium, this means that vector titration doesn't rely on cell destruction to express vector. So due to that feature, LVV is a very good model to investigate optimum cell culture conditions, and we could do this in various ways. Obviously, titration needs sampling, but this is a lot easier when you don't rely on external processing of the cells.

The main objective when scaling-up a lentiviral vector bioprocess is really to determine the optimized culture conditions and the best vector expression at a small scale, and to then try to maintain this strategy linearly throughout the scale-up. To achieve this, it's important right from the beginning – from when you are still developing the cell line and the vector

> "decision-makers within the various stakeholders responsible for manufacturing development programmes really need to be flexible..."

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expression – that you select suppliers or manufacturing partners that really can support the process development and scale-up efforts from the lab bench-scale of a few milliliters right up to a very ambitious 500 or 1,000 liters.

I think that partnering with this type of bioreactor company or CDMO that has experience with well-developed scale-up pathways will definitely give an advantage to any developer. The reason for this is the decision-making can sometimes be accelerated just as soon as a positive indicator of a candidate therapy is observed. When you start a bioprocess development programme, the definition of the optimal scale might not be obvious. And it's also challenging to decide whether your final scale is going to be 50 liters or 500 liters when you have a brand-new breakthrough therapy and there is no development template available, either within your organization or through close collaboration. Therefore, decision-makers within the various stakeholders responsible for manufacturing development programmes really need to be flexible in order to succeed in being supportive of the clinical output.

"We recently announced the demonstration that our SPEAR T-cell platform can deliver positive responses..."

Q Finally, can you sum up the priorities both for yourself in your role and for Adaptimmune as a whole over the coming 12–24 months?

GH: Adaptimmune's mission is to transform the lives of people with cancer. We do this by designing and delivering cell therapies and however challenging this may seem, I do use this mission as a means of keep my efforts fresh every day.

We recently announced the demonstration that our SPEAR T-cell platform can deliver positive responses, and we also have previous positive data in synovial sarcoma – that has been very exciting for the company and particularly, for the CMC function – I'm very proud that the team at the Catapult Cell and Gene Therapy Centre has been able to complete GMP batch within 18 months of starting at a completely new facility with an unequipped cleanroom.

My personal goals for the near future are to make sure we can sustain our vector manufacturing, supporting our ongoing and future clinical trials with the same degree of success we've had in the past year and a half, but also to support my regulatory colleagues in trial applications and preparing our first commercial launch. There's obviously a lot of technical information that needs to be shared between the CMC function and other functions in the company.

AFFILIATION

Gabrielle Humphrey
Associate Director
Vector Manufacturing Science and Technology Group
Adaptimmune, UK

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CELL & GENE THERAPY INSIGHTS

February 2020

Clinical Trends

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Clinical Trends

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Feburary 2020
Volume 6, Issue 1

COMMENTARY

INTERVIEW

CELL & GENE THERAPY INSIGHTS

Clinical Trends

-

COMMENTARY

Clinical Update: Breaking New Ground

***Cell and Gene Therapy Insights* proudly present the inaugural edition of Clinical Trends – a quarterly snapshot of key breakthroughs, challenges and areas of evolution in cell and gene therapy clinical development. Clinical Trends is specifically designed to provide an efficient means of updating yourself on the global trials landscape in general, whilst gaining more in depth insights into clinical progress in specific therapeutic areas and indications.**

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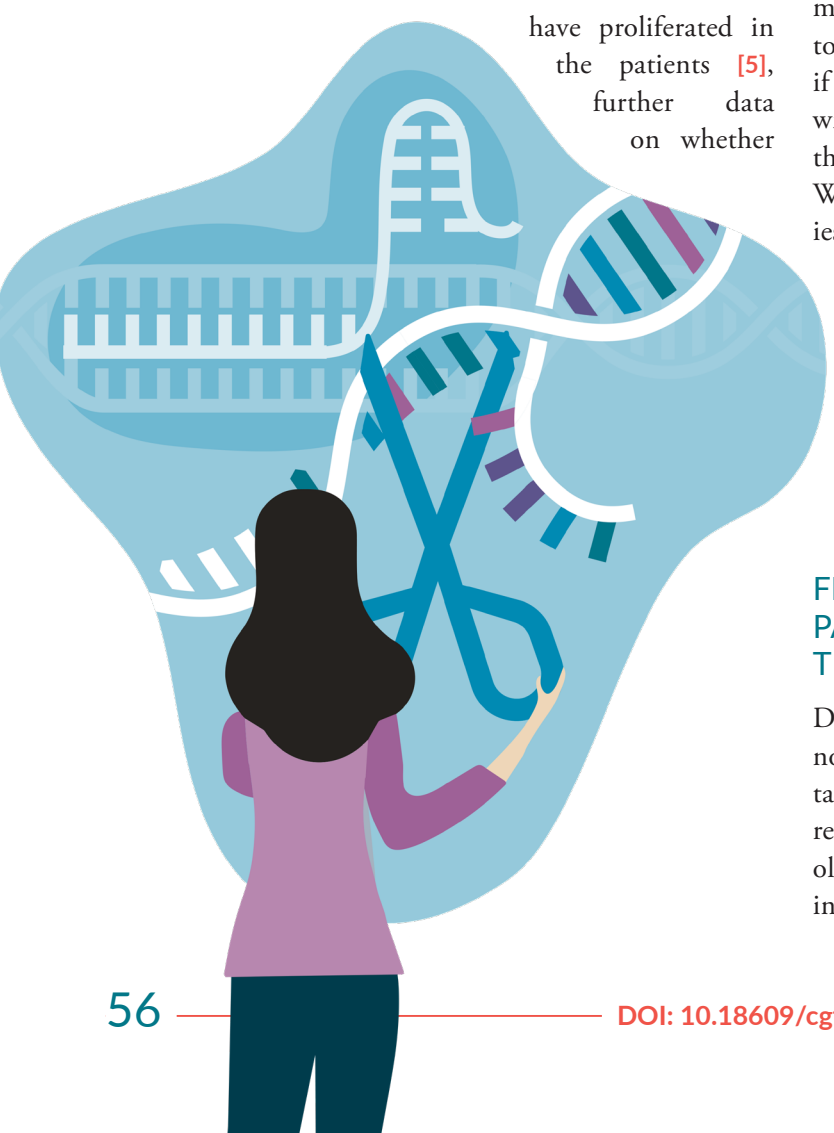
As a new year and decade begin, the cell and gene therapy clinical development space is clearly in rude health. The Alliance for Regenerative Medicine (ARM) recently reported that there were 1066 ongoing clinical trials in the global field at the end of 2019, including 94 in Phase 3, with the R&D community as a whole targeting some 500 indications worldwide [1]. Of course, with this growth comes an increase in competition, both from within and outside of the cell and gene therapy field. For example, one of the major talking points at the American Society of Hematology (ASH) Annual Meeting last December was the growing threat to the CAR-T cell immunotherapy that is carried by the bispecific antibody field [2]. It will be interesting to see how these two technology areas evolve in clinical application through the remainder of this year, and if they find a way to work synergistically or step on each other's toes. Another of the more intriguing aspects of cell and gene therapy clinical pipeline expansion, which seemingly only points to continued growth in future, is the fact that some of the most promising and disruptive novel technologies and approaches are only just beginning their journeys as clinical-stage drug candidates. Cell and Gene Therapy Insights' first Clinical Trends edition of 2020 focuses in the main on some of the most significant amongst them.

CRISPR GENOME EDITING IN CLINICAL EVALUATION

Following early clinical testing in China stretching back to 2015 (which has not been without its controversy, of course, although up to seven studies are still ongoing in the country) [3], last year saw the first US patient dosed with a CRISPR genome editing-based therapeutic. This ongoing study by the University of Pennsylvania [4] is testing HLA restricted NY-ESO-1 cells with edited endogenous T-cell receptor (CAR-T) and PD-1 in liquid cancers. Three patients have been enrolled to date (two with multiple myeloma, one with sarcoma) with the study currently closed to further enrollment. The UPenn approach theoretically removes some of the 'safeguards' which have previously limited CAR-T or TCR cell therapies' access and ability to bind to cancer cells. The fundamental questions are if these edited T-cells can still do their job, and are they safe? With initial data suggesting that these cells are indeed safe and have proliferated in the patients [5], further data on whether they work or not (and if so, how well) is eagerly awaited. While it should be noted that patient eligibility for this initial study was restricted to a very specific sub-set of patients, meaning future use in this exact form may be somewhat limited, the general approach is potentially very exciting as it may open up a way to better access hard-to-treat malignancies, including solid tumors – the current 'holy grail' for cellular cancer immunotherapy. Much work remains: identifying both the best targets for editing and the patients most likely to respond in a positive way are both key points for future investigation. With current CRISPR approaches, a number of edits are being made – each of which is relatively untested in humans – and due to the nature of the therapy, we are presently unable to test one at a time. Consequently, the number of variables becomes very large, both in terms of the location and accuracy of the edits, and of their clinical (including off-target) effects. Additionally, bioprocessing and bioanalytics will need to step up and meet the challenge if these new therapies are to be industrialized successfully. However, if all these challenges can be managed, we will very likely have an incredibly powerful therapy against most cancers on our hands. With other high-profile first in human studies involving CRISPR either underway – as is the case with Vertex/CRISPR Therapeutics' studies of CTX001 in patients with beta thalassemia and sickle cell disease [6,7] – or in the planning, this is clearly a key technology area to watch in 2020 and beyond.



FIRST TRIAL IN THE US OF A PATIENT-SPECIFIC IPSC-DERIVED THERAPY GETS UNDERWAY

Dr Kapil Bharti and colleagues' recent announcement of their first in human study targeting geographic atrophy of the eye as a result of dry AMD in patients aged 65 and older marked another US clinical trial 'first' in a highly touted novel field of cell and gene

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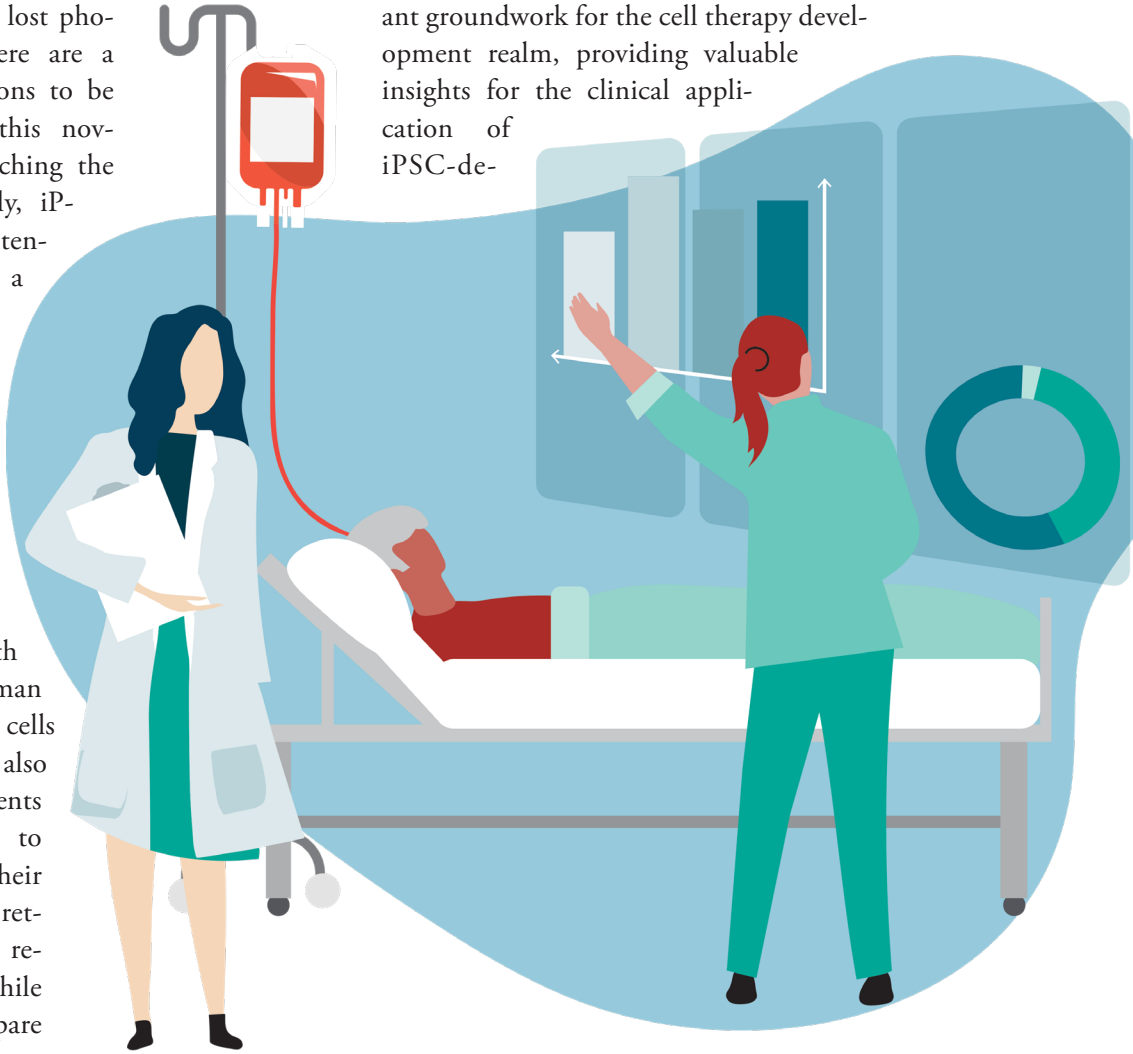
"...while iPSCs seem destined to become another valuable component in the cellular and molecular toolbox for treating and curing disease, there are several reasons for caution. The most obvious of these is the risk of malignant teratoma transformation."

therapy [8]. Dr Bharti's group at the National Eye Institute's Unit on Ocular Stem Cells and Translational Research (OSCTR) begin with autologous induced pluripotent stem cells (iPSCs), which are converted into retinal pigment epithelium (RPE) cells before being implanted on a biodegradable scaffold in order to replace lost photoreceptors. There are a number of reasons to be excited about this novel approach reaching the clinic [9]. Firstly, iPSCs have the potential to provide a stem cell product of high purity and consistency, which offers the benefits without any of the ethical challenges involved with harvesting human embryonic stem cells (hESCs). iPSCs also allow adult patients to gain access to hESCs and their (still largely theoretical) powers of regeneration. (While on this 'compare and contrast' topic, it may be a while yet before we can say with certainty whether hESCs and iPSCs are truly equivalent – but equally, one might ask whether that is really important?) [10]. Secondly, iPSCs are already in widespread use in non-clinical disease modeling and drug selection applications [11]. This may prove to be important groundwork for the cell therapy development realm, providing valuable insights for the clinical application of iPSC-de-



rived cells. Thirdly, our improving ability to 'manufacture' iPS cells opens up the possibility of developing very specific cell types, which may otherwise be challenging to locate, harvest, and culture in the lab. Indeed, this increasing degree of control may ultimately lead to the ability to identify and create altogether novel types of cell that allow certain functions to be carried out in the body (for example, local enzyme production and secretion – a realization of the in vivo 'drug factory' concept). However, while iPSCs seem destined to become another valuable component in the cellular and molecular toolbox for treating and curing disease, there are several reasons for caution. The most obvious of these is the risk of malignant teratoma transformation (mutagenesis). This may be related to vectors initially used, genetic reprogramming, or even the lack of local control mechanisms to inhibit transformation once implanted. All of these are the target for current iPSC optimization efforts aimed at alleviating safety concerns. There is also a theoretical risk in using integrating vector delivery systems with iPSCs, especially with the random insertion of genes. Moving forward, choosing the correct starting cells in the best possible state of differentiation will be vitally important to enabling optimized GMP manufacturing at large-scale. Clinical application in larger indications will require much larger cell batches and doses. Furthermore, as we come to better understand the transformation process, it will be critical to develop assays to form an integral part of quality control for the iPSC process and cell assurance.

THE RISE OF EXOSOMES

Dr. Gauri Varadhachary of MD Anderson Cancer Center is running one of a number of ongoing clinical trials assessing the clinical potential of exosome-driven therapies [12]. Dr Varadhachary is investigating dose and safety of mesenchymal stromal cell (MSC)-derived exosomes loaded with KrasG12D-targeting siRNA (iExosomes) for treatment of patients with various forms of pancreatic cancer with the KrasG12D mutation that has metastasized to other locations in the body. Patients will receive the exosomes intravenously on days 1,4 and 10, and treatment may continue for up to a total of 4 courses, 14 days apart, based on disease progression or toxicities. Follow up will be for up to a year. Challenges in targeting and exosome loading are two of several that lie ahead for the field in clinical application (limiting off-target delivery and identifying optimal cell source are others). Despite this particular therapeutic being aimed at metastatic disease, there still seems to be no inherent targeting capability attached to the exosomes. (Pancreatic cancers typically metastasize via the lymphatics to the liver, peritoneum and lungs). The mechanisms for targeting specific tissue types are still generally poorly understood and likely need further work. In order to work around this issue, researchers may employ a number of mechanisms, including local administration or administration of very large doses. These bring with them some additional challenges, especially with the administration of very large doses where off-target effects may occur causing unexpected and potentially problematic effects. The loading mechanisms

> "Exosomes both allow the delivery of much larger payloads and are amenable to repeated dosing. But...more directed work and research is needed to fully understand the best application and limits of this technology. "

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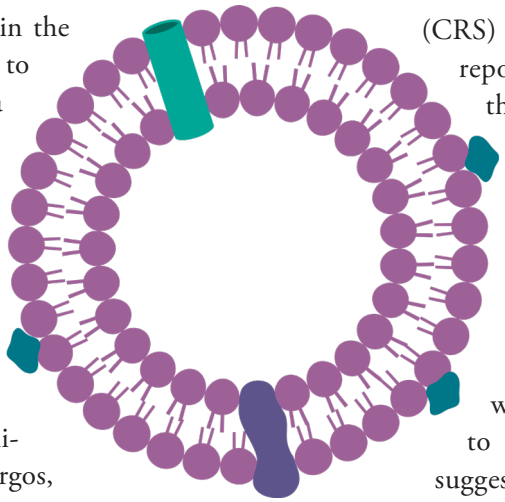
have been well described in the literature, but in order to be used therapeutically, a mechanism will need to be defined to standardize the loading to ensure reproducible and accurate loading concentrations for therapeutic use. However, Exosomes do show huge promise as delivery vehicles for a wide range of cargos, from plasmids through to proteins, and there is a rapidly increasing number of clinical trials underway involving them in indications ranging from sepsis to autoimmunity, and from wound healing to stroke [13–15]. Exosomes both allow the delivery of much larger payloads and are amenable to repeated dosing. But as with other vector delivery mechanisms, more directed work and research is needed to fully understand the best application and limits of this technology.



A NEW GENERATION OF OFF-THE-SHELF CELLULAR CANCER IMMUNOTHERAPY BEGINS TO READ-OUT

Fate Therapeutics recently provided an update on their phase 1 trial for its allogeneic, iPSC-derived NK cell therapies, FT516 and FT500 [16]. It is very exciting to see no incidents of cytokine release syndrome (CRS) and no DLTs being reported, especially as these therapies were administered more than once (three once-weekly doses) per treatment round. There were no signs of immunogenicity, and it was also very positive to see strong signals suggestive of cellular response in both liquid and solid tumors. Points of caution might include the fact that this was first in human study with a very limited number of participants, plus the fact that the best results seem to have been achieved with adjuvant therapy. It remains to be seen whether these therapeutic candidates will deliver long-term efficacy and become established as single administration treatments – the questions of what are the ideal co-treatments needed for efficacy, and what is the most effective dose, will no doubt be explored over the coming months and years. However, an off-the-shelf solution such as this would clearly open up the cellular cancer immunotherapy field in terms of convenience and cost, especially in instances where more than one round of therapy might be needed. This iPSC platform approach, utilizing an iPSC master clonal cell line, theoretically also allows for larger numbers of doses to be manufactured at lower costs whilst maintaining product consistency.

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AFFILIATION

Sven Kili
Principal, Sven Kili Consulting Ltd, UK

SVEN KILI provides specialist strategic consulting services to innovative Regenerative Medicine companies. His clients include small and medium sized companies from company formation through to clinical development and commercialisation. He was previously the Head of Development for the Cell and Gene Therapy division of GSK Rare Diseases where he led teams developing ex-vivo Gene Therapies for a variety of rare genetic disorders including Strimvelis®, the first *ex-vivo* gene therapy to be approved for children with ADA-SCID; Wiskott – Aldrich syndrome (WAS); Metachromatic Leukodystrophy (MLD) and Beta-Thalassemia. Prior to this, he was Senior Director, Cell Therapy and Regenerative Medicine for Sanofi (Genzyme) Biosurgery where he led the clinical development, approval and commercialisation activities of the first combined ATMP approval in the EU for MACI®. His team also prepared and submitted Advanced Therapy regulatory filings for Australia and the US, including health technology assessments and he was responsible for late stage developments for Carticel® and Epicel® in the US. Before joining Genzyme, Sven led the cell therapy activities and oversaw all UK & Irish regulatory functions and was the QPPV for pharmacovigilance for the Geistlich Pharma. Sven trained as an Orthopaedic surgeon in the UK and South Africa and since leaving full-time clinical practise has developed expertise Cell and Gene Therapy in clinical development, regulatory compliance, value creation, risk management and product safety, product launches and post-marketing activities. He sits on the board of CCRM in Canada; Xintela – a Swedish Stem Cell company and is the chair of the CGTAC as part of the UK BIA and the VP of the Standards Co-ordinating Body for Regenerative Medicine. Additionally, he still maintains his clinical skills in the UK NHS and serves as an ATLS Instructor in his spare time.

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CELL & GENE THERAPY INSIGHTS

Clinical Trends

-

INTERVIEW

Analysing clinical trends for cell & gene therapy in neurodegenerative diseases

ROGER BARKER is the Professor of Clinical Neuroscience at the University of Cambridge and Consultant Neurologist at the Addenbrooke's Hospital Cambridge. He is a PI in the Wellcome – MRC Cambridge Stem Cell Institute and Director of the MRC funded UK Regenerative medicine hub working on Pluripotent Stem and Engineered cells.

He trained at Oxford University and St Thomas Hospital Medical school and after completing his medical training took up an MRC Clinician Scientist Fellowship. For the last 25 years he has run research that seeks to better define the clinical heterogeneity of two common neurodegenerative disorders of the CNS- namely Parkinson's (PD) and Huntington's disease (HD). This has helped him define the best way by which to take new therapies into the clinic. In this respect he has been heavily involved in gene and cell based trials for patients with these conditions and currently co-ordinates an EU funded transplant programme using human fetal tissue for patients with PD, following on from an earlier MRC funded trial using similar tissue in HD. He is part of a new EU project (STEM-PD) and a global initiative (GFORCE-PD) that is seeking to take stem cells to trial in these disorders.

He is currently is Co-Editor in Chief of the Journal of Neurology and sits on the editorial board of many other journals. He is on the research advisory board of the Cure PD Trust and Parkinson's UK. In 2015 he was elected a Fellow of the Academy of Medical Sciences.

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Q Tell us what you are working on right now.

RB: So we work on a number of different projects, but I guess they would probably coalesce around three major areas.

One is the work around trying to take a stem cell-derived dopamine cell therapy to clinic in patients with Parkinson's disease.

The second major area is trying to develop better predictive models of Parkinson's disease in the lab, whether they be *in vitro* cell models or *in vivo* animal models.

Then the third major area is in Huntington's disease, where we're looking at the role of sleep: when does sleep go wrong and what contribution does sleep make to the symptomatology and disease progression in Huntington's disease?

Q Can you take us through the key current trends in cell/gene therapy clinical development in the neurodegenerative diseases area as you see them, beginning with the chief challenges currently faced by sponsors and clinicians in trial design and implementation?

RB: There's a lot of activity in this area at the moment, but I would say the two main clinical areas that I am aware of are in eye disease – in particular, age related macular degeneration and cell and gene therapies – and the brain, with the obvious target being Parkinson's disease. This is by virtue of the fact there's a very clear, core pathological event (albeit not the only pathological event) in Parkinson's, which is the loss of the dopamine cells – that creates an obvious rationale for using a cell or gene therapy that tries to put dopamine back to normal in the brain.

Thinking about the key challenges (which I do think are all being steadily resolved) I would perhaps begin with the regulators. I think gene therapy has a much longer history in this field, so the regulatory authorities are much more familiar with gene therapies and how they can best be monitored when put into patients. I think cell therapies have proven to be a little trickier in this regard, insomuch as it's a little unclear exactly how best to come up with criteria and guidelines for allowing people to take them into clinical trials. That revolves around the fact that a cell therapy is not really a drug and it's not a device either – it is a biological agent. It is a therapy that remains there forever and has the potential therefore to transform at any time, as for any cell. It's a little unclear at the moment quite what information you actually require from these cells before you can go into clinical trials, given that when you put them into patients they will last, hopefully, for decades, whereas any preclinical studies will only be done over months, or at most, a year or two.

So there are challenges in terms of how we can negotiate the regulatory landscape to take cell therapies into the clinic. And to some extent, this will also have to take into account the slight differences that exist in this area between the various regulatory authorities in Europe and America.

I think the other key challenges faced by the sponsors and people taking these therapies forward generally relate to the positioning of them in the existing therapeutic landscape and thus their commercial potential.

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There are lots of very good therapies out there for treating the dopaminergic aspect of Parkinson's disease – one of the main diseases in which I specialize. There are lots of drugs, there's deep brain stimulation, there's Duodopa®, where you give dopamine directly into small intestine, there are apomorphine pumps, and such like.

So there's a lot of activity and plenty of good therapies already out there for this aspect of Parkinson's disease. Therefore, the question isn't 'do these therapies work?' but 'can your cell or gene therapy actually do something that none of these other therapies can do, and can it do it in a way that gives it a competitive advantage?' Coupled with this are the challenges of making your therapy and then delivering it to the brain in a commercially viable way.

"Sponsors are therefore faced by a twofold problem: proving that they have got a commercially and economically viable and competitive therapy, which can outdo those that already exist...whilst negotiating a somewhat uncertain regulatory landscape."

Sponsors are therefore faced by a twofold problem: proving that they have got a commercially and economically viable and competitive therapy, which can outdo those that already exist for Parkinson's disease, whilst negotiating a somewhat uncertain regulatory landscape.

Q What are the key 'tools of the trade' for clinicians in this field today, particularly in terms of identifying/monitoring biomarkers?

RB: The history of biomarkers in neurodegenerative disorders is long and largely unsuccessful, but as to what is needed, that really depends on what your therapy is trying to do.

A lot of the cell and gene therapies that are currently going into clinical trials in Parkinson's disease are based around dopamine replacement. In some ways, biomarkers are quite straightforward in that area: you need to have a marker that looks at the dopaminergic aspects of Parkinson's disease in the brain, which you can do using PET imaging and specific ligands that bind to dopamine cells. There are some questions about exactly what these PET ligands (which are said to be specific for the dopamine system) are actually labeling, but I think most people would be fairly confident in the combination of markers that currently are used to look at the dopaminergic system reliably, and that have been proven to map disease course in Parkinson's. As such, these would be good markers with which to start looking at whether your cell or gene therapy is working in this condition by restoring dopamine levels back to normal.

I think the problem becomes more difficult when you start to think about using a disease modification approach and in this respect, people have used gene therapies to deliver growth factors to the brain for Parkinson's disease, for example. Now again, you could look at the dopaminergic system – that's a perfectly reasonable way to show that it's not degenerating in

the way that it normally does. But if you start to think about going beyond that by introducing genes that have some more fundamental aspect around cell death, then trying to monitor disease progression outside of the dopaminergic system is a challenge.

But I do think that as things stand, given that most novel therapies are designed around the dopaminergic nigrostriatal pathway, that we can just concentrate on that system. That's in Parkinson's disease. In the eye, you obviously have a lot more tools available, so that's relatively easy to look at. However, as we move into other conditions, it becomes more and more problematic to ascertain exactly what one needs in order to show that you have got a therapy that's actually working in repairing the brain or slowing disease progression.

Q Where else is innovation most needed to improve the design and/or implementation of effective cell/gene therapy clinical studies in this area, for you?

RB: I would say there are a number of areas that I think are challenging and where different forms of innovation would benefit the field.

One is delivery. To some extent, people have thought that the key is simply to develop an effective cell or gene therapy, and that is certainly true – you need to have something that works preclinically. However, while preclinical experiments are obviously very helpful, you ultimately have to deliver your therapy into a human brain. And the human brain is obviously much bigger than the brains of other animals. I think that getting proper delivery of these agents across the area of the human brain that you're interested in restoring or regenerating has been slightly overlooked.

A lot of the therapeutic trials that have happened to date, which have failed, have failed in part because the therapy is not as effective as we thought it was, but also partly because the delivery has not been optimal. I think that a lot of the trials with growth factors of the dopaminergic system – such as with neurturin, for example – and some of the trials of other gene therapies designed to replace dopamine, have used volumes of delivery which were too small to cover the target area. Therefore, the lack of response has not necessarily been due to an ineffective agent, but an ineffective dose delivered across only part of the target area.

So I do think delivery is a key factor, which I think people need to think about more.

There are two other areas for increased focus, for me. Firstly, I would like to see a greater desire within the field to set up central registers so that we can look more critically at all of these therapies that are being taken to clinic in small numbers of patients. We've been trying to do this with cell therapies and obviously, it's difficult when there are competitive and commercial interests involved. But I believe trying to pool the combined expertise as people move these products forward would help the field in general.

> "...I think that making it mandatory to follow-up patients ...for years, will give us much more information."

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"...for me, many of the greatest success stories in modern medicine involve combined therapies ... There's no reason to believe that the future of neurodegenerative disorders will be any different, so that we should pursue a combination therapy that deals effectively with the symptomatic elements along with something more disease modifying."

The third area is not related to technological innovation, but rather is a conceptual idea. I think we need longer-term follow-ups on these patients. Now, there are already certain regulatory requirements if you've given a gene therapy to someone. But I think that making it mandatory to follow-up patients not for 6, 12 or 24 months after you've intervened, but for years, will give us much more information. This is because some of these therapies, especially some cell therapies, can take 3–5 years before they have an optimal maximum effect. (This is based upon some of our previous experience with fetal dopamine cell transplants in Parkinson's).

So I think trying to build in long-term follow up for patients rather than the more short-term follow-up that is currently mandated would also be very helpful for the field.

Q When you look across the range of technology platforms and approaches that comprise the cell and gene therapy field today, where in particular do you see the greatest promise in terms of potentially game-changing approaches to tackling neurodegenerative diseases such as Parkinson's?

RB: This is a tricky question because until we have proven results with a more simplistic approach, I think it will always be a bit of a challenge to make such predictions. However, assuming that these therapies we've already discussed do work, I would say there are two areas or approaches that are going to compete in the future.

These diseases that we consider to be focused around certain networks – dopaminergic cell loss in Parkinson's disease, for instance – are clearly much more distributed than that. The pathology is much more extensive across the brain and even involves sites outside of the brain. This means there's going to be a need to deliver therapies that have systemic effects, or at least a more diffuse CNS effect. And I think that is the key challenge moving forward with any cell or gene therapy. The two approaches I have in mind will both speak to that challenge.

One is to develop some sort of peripherally delivered cell or gene therapy that can target areas of pathology. Such a therapy will likely have to compete with a repurposed drug and/

or a novel small molecule approach where you're delivering systemic agents designed to undo pathology in cells that have disease. I think there are emerging technologies that have the potential to allow for these new cell and gene therapies to be delivered systemically with the ability to target areas of pathology.

The other area I can see emerging as a very interesting approach is the idea of *in situ* reprogramming. This approach doesn't get around or deal with the systemic, more diffuse pathologies in these diseases. Instead, and again taking the dopaminergic system in Parkinson's as an example, the idea is rather than injecting a cell or gene therapy that makes dopamine, one simply reprogrammes resident astrocytes into dopaminergic neurons *in situ*. This could be achieved in theory by using the same technologies as have been developed in the lab to turn stem cells into dopamine cells. You would effectively be hijacking a relatively small population of the patient's own cells; you don't need many in Parkinson's – perhaps converting 100,000 to 200,000 of the patient's own cells to dopamine cells at the right site to reverse all the clinical features that normally respond to dopamine drugs.

This approach is exciting because it gets around a lot of the ethical and immunological problems. There are obviously concerns about safety to address, and there will always be the question as to whether these new reprogrammed cells will also succumb to the same disease process?

But I do think these are both exciting areas for future exploration.

Q Finally, can you share your vision for what clinical care of patients with Parkinson's disease might look like in future, and what role cell and gene therapies will play in this?

RB: I would say that whilst finding a cure or something that's disease modifying, that slows disease progression, is obviously something we should strive for, I can see a very clear role for dopamine replacement cell and gene therapies in the future. One could imagine their role being around becoming the first line treatment for newly diagnosed Parkinson's disease. That's assuming we can say we're pretty confident a patient has Parkinson's disease, which goes back to the discussion on biomarkers – if we could identify a biomarker that is specific to Parkinson's disease, it would greatly help.

Why would this early intervention with a cell and gene therapy be a useful approach? Well, assuming it works, it's a one-off procedure. And while it wouldn't cure patients with early-stage disease – other aspects of the disease would catch up with them – you could imagine them getting 10–15 years of benefit with a single therapeutic intervention at the point of diagnosis or soon afterwards. This would mean they would

a) not need to take any other medication over that period

b) avoid the side effects that those same therapies generate over time.

These effects include neuropsychiatric problems with lots of the dopamine agonists, and also the motor fluctuations: the so-called on/off phenomena you see in Parkinson's disease and the involuntary movements with chronic levodopa use (levodopa-induced dyskinesia)

In other words, these therapies could transform the natural history of treated Parkinson's disease without actually curing it. You'd also have the advantage of being able to treat patients

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as they are diagnosed, and then have a further 10–15 years for someone to come up with a disease modifying therapy to help those patients further down the line!

I think this last point is an important one because a lot of people regard these two main therapeutic options as being mutually exclusive – you either have one or another. However, for me, many of the greatest success stories in modern medicine involve combined therapies. In HIV/AIDS, for example, the really transformative work has involved the use of combination treatments. Treatment of TB was by combination therapy in years gone by. And today, heart failure is treated with combination therapy and so the list goes on.

There's no reason to believe that the future of neurodegenerative disorders will be any different, so that we should pursue a combination therapy that deals effectively with the symptomatic elements along with something more disease modifying. This sort of combined approach would transform the field. If we could put cells in and give a drug that slows down the disease even by 50%, then for Parkinson's disease, we would have essentially cured the vast majority of people who have the condition.

AFFILIATIONS

Roger Barker
Professor of Clinical Neuroscience, University of Cambridge, Cambridge, UK
and
Consultant Neurologist, Addenbrooke's Hospital, Cambridge, UK

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COMMENTARY

State of the Industry: The Financial, Clinical, and Scientific Landscape for Cell and Gene Therapies

Janet Lynch Lambert

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2019 was a year of significant growth in the regenerative medicine sector. Thousands of patients are now benefitting from commercial regenerative medicines, and the impact of the early cell and gene therapies is dramatic. The clinical pipeline is robust, with nearly a hundred Phase 3 trials underway, several late-stage products poised for approval, and next-gen technologies such as gene editing beginning to enter the clinic. Therapeutic developers are increasing their focus on solving manufacturing challenges. The sector continued to attract billions in investment, further fueling our scientific, clinical and commercial progress.

Each year, the Alliance for Regenerative Medicine (ARM), the international advocacy organization for the cell and gene therapy and broader regenerative medicine sector, hosts a Cell and Gene State of the Industry briefing, presenting global sector data at the Biotech Showcase at the JP Morgan Healthcare Conference. The industry overview is followed by two panels focusing on the latest developments in the sector. Recordings and slides are available on the ARM website [1]. This year, for our tenth annual briefing, I spoke to over 1,000 attendees in-person and online. This group of people – scientific and industry experts, regulators, payers, investors,

and patient advocates – are incredibly knowledgeable and motivated to bring these therapies to patients across the globe, and we at ARM are grateful to be spearheading efforts to get safe and effective medicines to patients.

Since I came to ARM in 2017, this sector has really come of age – and there is still so much more to come. While 2019 was very successful, we still have considerable work to be do together to ensure that the early clinical promise and commercial successes of these therapies translates to widespread patient access. With that in mind, I wanted to share some of the major trends we've seen in 2019, as well as the outlook for 2020.

GLOBAL FINANCINGS

Regenerative medicine sector financings trended positively in 2019 compared to five- and ten-year data, with this past year finishing as the second-strongest year on record. Investment activity, corporate partnerships, and mergers and acquisitions flourished as the nearly 1,000 therapeutic developers active in this space worked to bring their products to patients worldwide. These 987 developers tracked by ARM raised $9.8B, including $7.6B in gene therapy, $5.1B in cell therapy, and $442M in tissue engineering [Footnote 1].

> "These 987 developers... raised $9.8B, including $7.6B in gene therapy, $5.1B in cell therapy, and $442M in tissue engineering."

In particular, 2019 was a strong year for venture capital activity in the cell and gene therapy space, with developers raising $4.1B in venture financing – a 32% increase year-over-year from 2018. Notable venture financings from 2019 include Century Therapeutics' launch with $250M to develop iPSC-based allogeneic cell therapies; Maze Therapeutics $191M debut; and Poseida Therapeutics' $142M Series C financing.

In addition, corporate partnerships in the cell and gene therapy space totaled to $1.5B in upfront value, with potential royalties and milestone payments worth up to $17.6B [Footnote 2]. Many of these partnerships focused on developing therapies for non-monogenetic disorders, reflecting increased clinical development in indications with larger patient populations. These partnerships included a $150M upfront partnership between Grunenthal and Mesoblast to develop Mesoblast's cell therapy for lower back pain; REGENXBIO and

1. Financings by companies active in gene-modified cell therapies are counted in both the gene therapy and cell therapy categories; as such, these categories do not add up to the total financings figure. The total financings figure does not include M&A, which is calculated separately.

2. Only upfront payments are included in the total financings figure.

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Neurimmune's collaboration to develop gene therapies for tauopathies; Verve Therapeutics and Beam Therapeutics' agreement to develop gene-edited therapies for cardiovascular disease; and AbbVie and Voyager's $65M agreement to develop gene therapies for Parkinson's disease.

There continued to be significant M&A activity in the sector, as large- and mid-cap pharma look to expand their regenerative medicine portfolios by acquiring smaller developers. Upfront payments for mergers and acquisitions in 2019 totaled $11.3B – not including Astellas Pharma's $3B acquisition of neuromuscular gene therapy developer Audentes, which closed early in Q1 2020. Other notable acquisitions this year include Roche's $4.3B acquisition of Spark Therapeutics, Vertex's $950M acquisition of Semma Therapeutics, Biogen's $877M acquisition of Nightstar Therapeutics, and Bayer's $240M payment to acquire the remaining stake in BlueRock Therapeutics.

PATIENT IMPACT

Already, regenerative medicine products are providing a significant positive clinical benefit to patients with severe diseases and disorders, many of whom previously had few or no treatment options available. In the USA, we estimate that approximately 4,500 to 5,500 patients have already been treated with FDA-approved gene therapies and gene-modified cell therapies, and thousands of additional patients treated with early generation cell and tissue products.

In oncology, CAR-T therapies Yescarta and Kymriah are providing 40 to 80% complete response rates for patients suffering from what would have once been terminal hematological malignancies. Novartis's gene therapy Zolgensma is durably treating spinal muscular atrophy (SMA), including SMA Type 1, a serious genetic disorder that, in the past, was nearly always fatal by age 2. bluebird bio's Zynteglo promises an alternative to patients with severe beta thalassemia, who have historically relied on regular transfusions – which often had long-term impacts on their overall health – to control their disease. And Luxturna has drastically improved the vision of patients with a rare inherited blindness-causing retinal disease.

The number of patients expected to benefit from regenerative medicines will only increase. Globally, regenerative medicine clinical trials have a combined target enrollment of over 60,000 patients, suffering from a diverse array of rare and prevalent indications. MIT NEWDIGS predicts that by 2030, over 500,000 patients will have been treated with a cell or gene therapy in the USA alone [2]. In 2019, developers

filed for marketing authorizations for 10+ regenerative medicines, many of which we expect to be approved in 2020. These include the first gene therapies for hemophilia A (BioMarin) and metachromatic

leukodystrophy (Orchard Therapeutics), two additional CAR-T products (Bristol Myers Squibb's liso-cel and Kite/ Gilead's KTE-X19), Mesoblast's cell therapy for graft-versus-disease as well as their cell therapy, co-licensed with JCR Pharmaceuticals, for epidermolysis bullosa, and Enzyvant's tissue-engineered product for pediatric congenital athymia. Officials from the US FDA [3] and European Medicines Agency (EMA) [4] have said that by 2025, they expect to be approving 10–20 cell and gene therapy products each year.

CLINICAL & SCIENTIFIC ADVANCES

2019 was a particularly significant year for the sector with immense scientific and clinical progress, particularly in gene-modified cell therapies and in gene editing products. Therapeutic developers are increasingly turning to indications with large patient populations, including cardiovascular disorders,diabetes, and age-related neurodegenerative diseases.

The regenerative medicine clinical pipeline is robust, looking to provide a durable or even curative therapeutic benefit for patients in more than 500 indications. As of the end of 2019, there were 1,066 ongoing clinical trials in regenerative medicine, including 94 in Phase 3, and the clinical landscape is continuing to expand.

In particular, gene-modified cell therapies are entering the clinic in record numbers, making up more than half of Phase 1 trials. On a panel on Emerging Cell Therapies at ARM's State of the Industry Event, CRISPR Therapeutics CEO Samarth Kulkarni said that, "Cell therapies are here to stay in cancer," predicting that cell therapies would make up at least one third of the market for therapies for liquid tumors within the next 5–6 years.

Allogeneic cell-based immunotherapies in particular are reaching clinical viability as developers improve strategies to deal with immunogenicity. In April 2019, Fate Therapeutics announced that they had dosed the first patient with their FT500 allogeneic NK cell therapy and Precision BioSciences announced that they had dosed the first patient in their allogeneic CAR-T clinical trial. Many researchers are continuing to explore iPSCs and gene editing technologies as strategies to deal with immunogenicity and allow for the development of additional 'off-the-shelf' therapies. When Claudia Mitchell, Senior Vice President of Product & Portfolio Strategy at Astellas Pharma, was asked during the Emerging Cell Therapies panel if she thought allogeneic therapies would replace autologous therapies, she replied: "Absolutely." Therapeutic developers are also looking to expand the application of CAR-Ts and other adoptive cell therapies outside of oncology. In 2019, Cartesian initiated the first CAR-T clinical

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trial for an autoimmune disorder (generalized myasthenia gravis), and Sangamo Therapeutics received UK authorization to begin a clinical trial of their CAR-Treg product TX200 to prevent immune rejection following kidney transplantation.

Traditional gene therapies also make up a large percentage of the regenerative medicine clinical pipeline, with approximately one third of ongoing trials utilizing this technology. Researchers drove progress in gene therapy delivery in 2019, with many of these advances focused on improving efficiency of gene delivery methods, as well as on driving vector manufacturing processes. Non-viral gene therapy delivery also continues to advance. There are currently 57 ongoing gene therapy clinical trials utilizing non-viral delivery methods, and the first non-viral gene therapy, Colletagene,was approved in Japan to treat critical limb ischemia this past spring. Though it makes up a much smaller proportion of the clinical pipeline, genome editing had a watershed year in 2019. There are currently 31 early stage clinical trials ongoing worldwide utilizing genome editing, including trials in oncology (20 trials), inherited disorders (8 trials), and HIV (3 trials). CRISPR joined ZFNs and TALENs in the clinic this year, with early signs of positive clinical benefit reported by Tmunity / Penn Medicine for their CRISPR-edited CAR-T for patients with multiple myeloma and sarcomas, and by Vertex /CRISPR Therapeutics for their gene-edited product for beta thalassemia and sickle cell disease. In addition, Sangamo reported evidence of successful *in vivo* editing in their Phase 1/2 trial utilizing ZFNs. Looking forward, Editas plans to treat the first in vivo CRISPR patient in a clinical trial this year.

As this technology advances, it continues to be the focus of international dialogue on bioethics. While genome editing has proven itself to be a powerful tool in the search for cures for many serious diseases, germline editing, which makes heritable changes in the human genome – in contrast to somatic cell editing, in which the effects are limited to the patient treated – continues to present important safety, ethical, legal, and societal issues. In August 2019, 15 leading therapeutic developers active in gene editing signed on to ARM's Statement of Principles, asserting that germline modifications are currently inappropriate for in-human use, and the World Health Organization launched an advisory committee to implement international mechanisms for oversight of clinical gene editing.

> "...gene-modified cell therapies are entering the clinic in record numbers, making up more than half of Phase 1 trials."

There is also an increased focus on clinical development for regenerative medicines for indications with larger patient populations. In the panel on Next Generation Cell & Gene Technologies, Senti Bio CEO Tim Lu said,

> "We need to try to figure out how to enable greater access to cell and gene therapies into other indications [...] how do we go beyond making single changes, single modifications, which I think are inherently limited to certain types of diseases [...] I think it's pretty clear now from the basic research side that it's possible. The design cycle for modifying and making these sort of therapies is only going to accelerate over the next decade and it's a matter of how do we then take that pattern, match that with the right indications, and really drive those into the clinic."

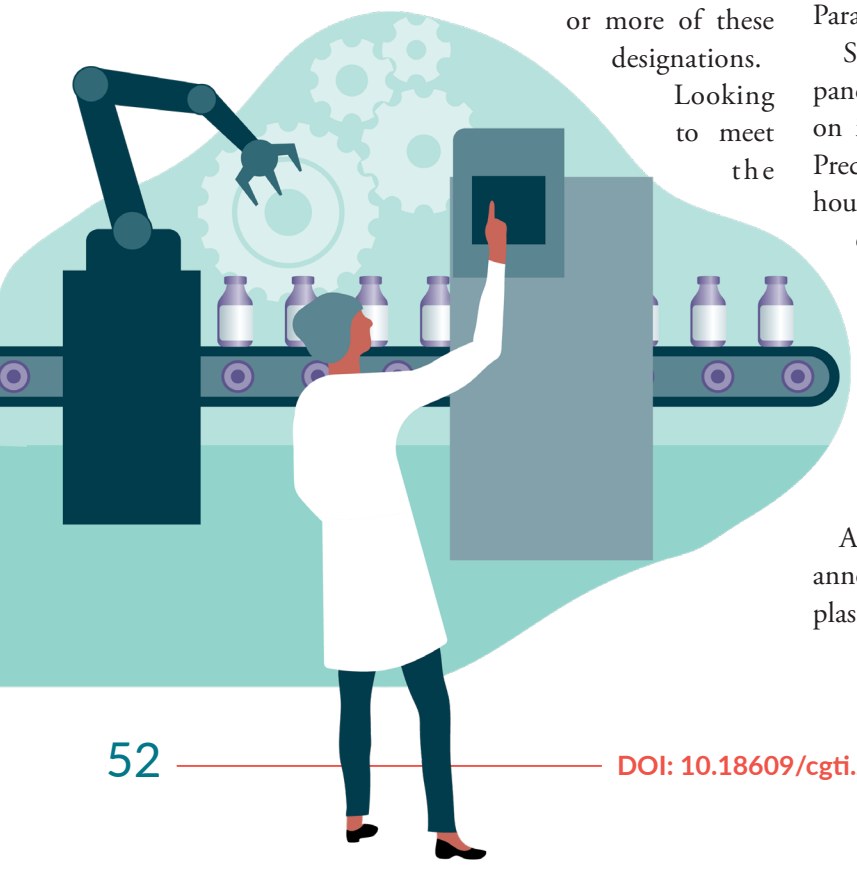
While much of the clinical development landscape is dominated by oncology and rare monogenetic disorders, an increasing number of clinical trials are ongoing in more common indications. These include

common cardiovascular indications, such as myocardial infarction, peripheral artery disease, and critical limb ischemia (40 ongoing trials); diabetes and related complications (23 ongoing trials); aging-associated neurological disorders such as Parkinson's, Alzheimer's, and macular degeneration (19 ongoing trials); common musculoskeletal injuries and disorders (15 ongoing trials); and stroke and stroke recovery (10 ongoing trials). It is likely that these indication areas in particular will drive increased uptake of regenerative medicines going forward.

"Cell and gene therapy manufacturing will continue to play a prominent role in the sector as these therapies expand from orphan indications into indications with larger patient populations."

MANUFACTURING

As the number of patients receiving regenerative medicines increases, both through clinical trials and approved products that have come to market, companies across the clinical development timeline are implementing strategies to deal with manufacturing, CMC requirements, and scale up. This is particularly true as expedited approval programs, such as FDA's RMAT designation, EMA's PRIME designation, and Japan's SAKIGAKE designation, provide pathways for earlier approval, shortening development timelines. In 2019, 17 regenerative medicine products received one or more of these designations. Looking to meet the growing supply needs for cell and gene therapies, many larger companies made headlines in 2019 with plans for expansive facilities to improve their in-house manufacturing capabilities. In particular, Pfizer and Novartis have shared plans to invest a combined $2 billion on gene therapy production. For larger companies, CMOs are attractive acquisition targets to further increase their manufacturing capabilities. In 2019, Novartis completed their acquisition of French CDMO CellforCure; Thermo Fisher announced a $1.7 billion acquisition of Brammer Bio; Hitachi completed their acquisition of European CMO apceth; and Catalent acquired CMO Paragon Bioservices for $1.2 billion.



Smaller developers are also looking to expand their manufacturing capabilities earlier on in the development timeline. This year, Precision BioSciences opened the first in-house cGMP manufacturing facility dedicated to genome-edited allogeneic CAR-Ts in the US; gene therapy developer REGENXBIO announced a new manufacturing facility to be operational by 2021; Elevate Bio uncloaked with $150 million to provide centralized R&D and manufacturing resources to a suite of cell and gene therapy companies; and Audentes, recently acquired by Astellas, announced the launch of their new cGMP plasmid manufacturing facility.

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Cell and gene therapy manufacturing will continue to play a prominent role in the sector as these therapies expand from orphan indications into indications with larger patient populations. When discussing the outlook for cell and gene therapies in the next decade, Sheila Mikhail, CEO of AskBio, said

"I think there will be a lot of interesting developments as we move into pathway diseases, we have a lot of potential. AAV gene therapies for monogenetic diseases – we've made a great impact – but I think there's a lot more that will be happening outside of that space."

THE OUTLOOK FOR 2020

Looking forward in 2020, we expect many of these trends to continue. There is a strong demand for financing in the regenerative medicine sector globally; while the IPO market may continue to be constrained by US elections, financings prospects are generally strong.

As an increasing number of products are entering pivotal trials, we expect a large number of significant Phase 3 data readouts. The extensive late-stage progress in many clinical programs in 2019 is expected to translate to several regenerative medicine approvals worldwide in 2020; in particular, we expect the number of approved gene therapies to more than double over the course of the next one to two years. Researchers will continue to progress the technology forward, with advances expected in both viral and non-viral gene therapy delivery methods and in addressing immunogenicity for off-the-shelf therapies.

On the policy side, we have seen a lot of excitement and willingness from legislators and regulators to advance this sector and ensure patients can access these therapies. As an increasing number of products begin to come to market, however, it is essential that policymakers enact the systematic changes needed to allow patients timely access to safe and effective therapies. In particular, ARM has worked with CMS in the US, as well as payers in Europe, to identify barriers to patient access as well as potential solutions. This will almost certainly be an area of focus for all stakeholders this year. In the context of regenerative medicine, drug pricing legislation in the US in 2020 may enable value-based payment models.

Other expected policy activity in 2020 includes additional FDA enforcement activities against clinics advertising unapproved stem cell therapies (the period of enforcement discretion comes to end in November), increased international dialogue on point-of-care therapies, including the Hospital Exemption in the EU. ARM will continue to work with stakeholders to develop and promote the necessary policy frameworks for these innovative therapies.

Regenerative medicine is on the rise. The scientific, clinical, and financial milestones are the evidence of a strong and growing sector, bolstered by strong stakeholder support from not only industry experts, but regulators, providers, and patient advocates. With tens of thousands of patients poised to receive and benefit from regenerative medicines in the coming decade, it is vital that we continue to work to build the infrastructures

"With tens of thousands of patients poised to receive and benefit from regenerative medicines in the coming decade, it is vital that we continue to work to build the infrastructures necessary to develop, deliver, and reimburse these therapies."

necessary to develop, deliver, and reimburse these therapies. The past decade saw phenomenal advances in the science of regenerative medicine, and I am excited to continue to work with sector stakeholders in 2020 and beyond on the steps still needed to bring that amazing science to patients.

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AFFILIATIONS

Janet Lynch Lambert,
CEO, Alliance for Regenerative Medicine

AUTHORSHIP & CONFLICT OF INTEREST

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Investor Insight

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INTERVIEW

Building for future success: the Syncona Partners model

MARTIN MURPHY is Chief Executive Officer of Syncona Investment Management Ltd. He co-founded Syncona in 2012 with The Wellcome Trust. Since then, Martin has been closely involved in the foundation and development of five Syncona companies: Achilles, Autolus, Blue Earth, OMass and Quell Therapeutics. Previously, he was a partner at MVM Life Science Partners LLP, a venture capital company focused on life science and healthcare, where he led their European operations. Martin has also held roles with 3i Group plc and McKinsey & Company. He has a PhD in Biochemistry from the University of Cambridge.

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Q What's occupying most of your time at Syncona Partners at the moment?

MM: The bulk of our time is spent on building our portfolio companies. We're very hands-on in that regard – what we do is found companies, often in the cell and gene therapy space, then put in operational resource and build them over the long-term. So overwhelmingly, the activities that occupy us are on

the operations side: working with a founder, setting up the company, writing a business plan, putting people and capital around that plan, and then operating the business.

We are always looking to make new investments, too – we make about two new investments a year, on average. That's obviously a time-intensive activity because you're building everything from scratch. Of course, the more mature companies now have management teams and operations of their own, so they have their own momentum.

It's really those two things – that mix between growing the existing portfolio of companies (we have nine today) and adding new companies to the book.

Q Can you give us some highlights from Syncona's current portfolio of investments in the cell and gene therapy area?

MM: We currently have three cell therapy companies: Autolus, which is a very well-known business, and two that are younger but working in very interesting spaces.

One of those is a company called Achilles Therapeutics, which is a neoantigen-specific T cell immunotherapy business. About 4 or 5 years ago now, we became very convinced that tumor infiltrating lymphocytes (TILs) was the way to go, particularly in the solid tumor space. The subsequent progress of companies such as Iovance has been pretty supportive of that view. However, we feel that the Iovance procedure, which is effectively a non-selective expansion of TILs, could be improved with a selective expansion that focused on selecting neoantigen-specific T cells. Achilles is just now entering the clinic, so we're excited about the data those studies will deliver.

The other cell therapy company is a company called Quell Therapeutics, which is an engineered T regulatory (Treg) business. One of the trends we see is an expansion from the first wave of cellular immunotherapy companies, which were focused on cytotoxic effector T cells for oncology applications. We're now seeing a new wave of cell therapy companies targeting a variety of different cell types, including Tregs. I think Tregs are going to clearly be interesting in autoimmune disease and organ transplantation applications, whilst being able to leverage much of the knowledge that's been grown in the effector T cell area, particularly in terms of how to engineer T cells for improved activity.

I think we'll see more and more cell types being engineered in this field. The NK cells space has now become very active, for example, but I think there will be more cell types beyond that.

On the gene therapy side, we're very AAV-focused – that's where our expertise has traditionally resided. We founded Nightstar Therapeutics, which is a business we sold, and now we have a whole wave of further AAV gene therapy businesses following on, each of which targets different tissue compartments: we have SwanBio targeting the central nervous system, Freeline Therapeutics targeting the liver, and Gyroscope Therapeutics targeting the retina for dry AMD.

We're very excited about all of these companies and moving forward, we think there will be more opportunities in both cell therapy and gene therapy. Again, though, our model

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is to do a small number of investments per year and then focus on really building those businesses.

> "...the activities that occupy us are on the operations side: working with a founder, setting up the company, writing a business plan, putting people and capital around that plan, and then operating the business. We are always looking to make new investments..."

Q What differentiates Syncona's approach to fostering innovation in this field in particular?

MM: I think it's the hands-on approach. We don't really think of ourselves as investors – we're company builders. The vast majority of our portfolio has been founded by us, so sourcing for us is not about finding an investment to make in a pre-existing company – it's talking to an academic with a technology, with intellectual property, and ideally with clinical knowhow. We then build a company around that individual on the strategy we favor, which is this long-term strategy that targets setting up companies that have the capability to take their technology late into development and preferably, all the way to approval. I think that's a second point of difference for us – the fact that we prefer to focus on those companies we feel can take their own products all the way to market.

Q How do you reflect upon the changes you've seen in the cell & gene therapy industry and financing environment from the early days of Syncona's involvement leading up to the present time? Where is life easier for you today, and where is it harder?

MM: It's obviously changed enormously. When we started back in 2012/13, we were really among the very first in terms of focusing on cell and gene therapy. What we saw was the remarkable clinical efficacy that these technologies offered – particularly in diseases where existing modalities, whether small molecule or biologics, didn't really offer potential for efficacy.

So we were initially attracted by their remarkable utility and promise. Offsetting that appeal was the complexity of the products – of manufacturing, for example, with the lack of GMP capabilities. However, we believed that ultimately, the strong efficacy argument would win out - that if you delivered that efficacy, you would see regulatory and market adoption of these products.

I think it's fair to say that back in 2012, there was a fair bit of skepticism around whether that efficacy would be achieved. If you look at the field now, that skepticism has vanished. What you see now is multiple gene therapy products approved, cell therapy products approved, commercial traction in those products. You see extreme large company interest in

the space. You see large acquisitions involving the likes of Spark Therapeutics, Nightstar, Audentes… the list goes on.

There's been an incredible change in the whole environment. I think these therapies are now here to stay, and there will be multiple therapies approved in the relative near-term that will deliver remarkable outcomes for patients with diseases that have been poorly served, historically speaking.

In terms of where things are easier and harder for us: certainly, easier in that we've now built many cell therapy and gene therapy businesses. We know how to build them and we're getting better at it.

Where it's harder is that there's a lot of competition in the field now, of course. I think you have to be very focused on differentiation and why you believe the particular company you're backing has a potentially unique approach.

Q Looking across the broad cell and gene therapy space today, how would you rank the traditional areas of concern from the investor's point of view (e.g., clinical, regulation, manufacture and supply chain, market access, IP, etc.)?

MM: I think it's three things.

It's certainly manufacturing – I'd put right at the front of the list. These products are complex to make, there is a very significant shortage of CDMO supply, and a lack of opportunity to easily source plasmids, vector, cell handling, etc. – these are very difficult things to do. There are so many manufacturing-related aspects for investors to think through in this field: can you physically make this product? Does the expertise exist to make it? Does the capacity exist? Do you have to invest ahead of the data curve in order to build that capacity? And then when you've finally got all of those things sorted, can you reliably make it batch in, batch out? That requires incredible investment in CMC.

I would say the second thing is adoption, which again is really a question that's related to manufacturing – to cost of goods and robustness. Ultimately, can these products be scaled to serve the demand beyond the niche markets? We know that manufacturing AAV vector for the retina is thankfully straightforward but manufacturing it for muscular dystrophy is very demanding given the much higher dosage required. Can they be scaled to meet supply?

"I think we'll see more and more cell types being engineered … The NK cells space has now become very active, for example…"

And then thirdly, there is the pricing model for these products. There's a lot of work being done by companies like Novartis, for example, in terms of how they're moving forward with Zolgensma, how they're pricing that both in terms of the headline price and the pricing model as a whole. All of those things need working out.

So I think this isn't really about clinical anymore, which perhaps is where a biotech

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> "We're going to see significant developments in promoter technology to lead to more specific expression. We're going to see an increase in our ability to turn expression off, to regulate expression. I think there will be discrete technology components that will allow you functionally to have more flexibility in how you deliver therapies."

investor might have focused previously, taking for granted the manufacturing piece. I think the lens is now very much on manufacturing, scale-up and the in-market model.

Q Are there any emerging issues or threats to the sector's success that particularly draw your attention?

MM: No, I think it's a data question, really. These therapies undoubtedly offer remarkable promise. In hemophilia and the cancer cell therapy space, for example, we've seen products that offer remarkable benefits for patients.

As a consequence of this, we've seen many companies enter the space and a lot of investor interest. Ultimately, what we need to ensure is that the flow of new clinical data continues to excite investors, because what allows these products to be successful is remarkable clinical data: the reality is that an autologous cell therapy is an expensive product to make – the prices will come down, but it is an expensive product for the system to support. However, it is supportable with exceptional clinical efficacy.

So I think the challenge for the sector is and will be to continue to deliver the remarkable efficacy that got everyone excited in the first place.

Q Looking to the future, what are the key things (beyond delivering that stellar clinical data) that the cell and gene therapy space needs to do to continue enjoying the favor of investors?

MM: In the gene therapy space, I think it's going to be about moving out of the rare monogenic diseases and into the larger chronic diseases. We started in the monogenic space for very good reasons, because they typically combine very severe needs, very few therapeutic options, and relatively straightforward genetics. With monogenic recessive disorders, the biological hypothesis of using an AAV vector to put a copy of the gene back in order

to complement that deficit is very compelling. But ultimately, I think the opportunity here is considerably broader than that.

We're working on that in our own portfolio. If you look at the retina space, for example, Nightstar Therapeutics was a business that had choroideremia as its lead indication, an excellent recessive monogenic disorder disease target. Gyroscope, which is a company in our portfolio now that we're very excited about, is working in the dry AMD space and targeting complement inhibition. That involves moving into a disease with many more patients – millions of them versus perhaps a relatively few thousand with choroideremia. You're moving into diseases where there are millions of patients with a more complex genetic background, so I think that transition from monogenic to polygenic will be important for the sector as well.

In the cell therapy space, I think it's going to be about data and format. There is the big question there around autologous and allogeneic approaches – how that will play out is at the moment unclear. I think it's a data question – which product will reliably generate the best data – allied to a supply chain question, which is what is the point in having the best data if the product can't reliably be made and delivered to patients?

Those are the two questions that I think the cell and gene industry has to answer.

Q What else can you tell us about any particular indications or emerging technology areas in the ATMP realm that you expect to see coming to the fore over the course of the new decade?

MM: In gene therapy, I think we're going to see significant developments in vector technology. We're going to see significant developments in promoter technology to lead to more specific expression. We're going to see an increase in our ability to turn expression off, to regulate expression. I think there will be discrete technology components that will allow you functionally to have more flexibility in how you deliver therapies.

On the cell therapy side, I think we're right at the tip of the iceberg in how we're engineering the cells. The first products we delivered, the approved products, are relatively simple products, yet they're still efficacious. The focus will be on increasing our ability to add modules of programming that will lead to better trafficking, better persistence of those cells, better efficacy in terms of their ability to survive (in the adverse tumor microenvironment, for instance), and better safety, of course. So I think we will see many years of successive cellular engineering advances. We'll be adding properties into them with the goal of delivering better, safer medicines.

Q Finally, can you tell us about your and Syncona Partner's key targets and priorities moving forward?

MM: As I mentioned earlier, our portfolio is nine companies today. We're going to grow that number to around 15–20 companies. What we're looking to do over a roughly

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10-year period is to deliver a number of those companies – three to five of them – all the way through to approved products.

We don't exclusively invest in the cell and gene therapy space, but it is an area of extreme interest for us and the majority of our portfolio is in that space. I think that will be the situation moving forward.

But regardless of technology area, our core model and focus will remain the same: to found these companies, put operational resource into them, and fund them over the long-term with the goal of delivering approved products.

AFFILIATION

Martin Murphy
Syncona Investment Management Ltd

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INTERVIEW

Tackling the plasmid supply bottleneck

MARCO FERRARI has a degree in Law at Luigi Bocconi University of Milan (Italy), with a specialization in Health Management at Imperial College Business School of London. Today Marco Ferrari is CEO of Anemocyte Srl, company of Nine Trees Group Spa, a private holding with five affiliated companies operating in the Life Science sector.

STEFANO BAILA received his PhD in 2007 based upon translational research and development of gene therapies for hemophilia at the Children's Hospital of Philadelphia. Since that time he has been actively involved in the process development and manufacturing of advanced therapeutic medicinal products through business development and strategic marketing roles at Areta International, a CDMO, and by leading field implementation and commercialization activities for the cell processing unit of Terumo BCT. Stefano also worked as Industrialization Manager at Celyad where he led process development and automation efforts for CAR-T therapeutics. Now he serves as Director of Operation and Business Development for Anemocyte .

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Q The supply of plasmid to the burgeoning gene therapy sector has become a significant bottleneck – can you firstly frame for us the background to and scale of this issue?

MF: The recent and current tremendous increase in demand for plasmid is very much linked to the cell and gene therapy as a whole – and gene therapy in particular – finally achieving really consistent growth. Obviously, gene therapy in its various different forms generally has a viral vector as one of its main components, and viral vector production relies on plasmids.

It is a true bottleneck that we are currently witnessing. Perhaps the growth of the gene therapy field could have been predicted, but then again, with highly dynamic industries such as ours, it's always the case that some things occur differently to what was expected. It was probably natural in years gone by for us to think 'OK, let's take it step by step and not rush into an increase in production capacity'. But suddenly, everyone is asking for this particular kind of technology to be readily available on demand.

The resultant backlog in production and long waiting lists are certainly generating a lot of difficulties for the industries we serve – and it is a situation that is likely to become more severe as demand only increases for starting materials, the intermediates of production and the final means of transduction or transfection.

I would say that the major risk we face today as a sector is an inability to serve the industry properly and at the right time with specific compounds like plasmids. This could result in a slowing of progress in R&D pipelines worldwide, and it can negatively affect the expectations both of the market and more importantly, of the patients who are waiting for new products and solutions for their specific needs.

"...the major risk we face today as a sector is an inability to serve the industry properly and at the right time with specific compounds like plasmids. This could result in a slowing of progress in R&D pipelines worldwide, and it can negatively affect the expectations both of the market and more importantly, of the patients..." - MF

SB: While we've recently seen several players investing in new facilities, new capacity, plasmid was neglected for a number of years. This may have been the case because in a sense, it is not part of the cell and gene therapy industry - it's more similar to a standard biological in many respects. Everyone was looking into the cells, looking into the viral vectors, but plasmid was not something that people in this space really thought about too much.

For many years, there were just a couple of providers that could offer true GMP quality plasmid, plus many others supporting laboratory and early clinical demand. Today, the industry is shifting towards the commercial

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sphere very quickly, and I think there are many companies deciding to invest in commercial-quality production even at the earliest stages of R&D. Hence, there is this gap between need for high quality plasmid and what the supply side can support. We regularly hear from suppliers of 6-to-12-month backlogs, which is an issue.

We are a relatively new player on the plasmid side. We're coming with a lot of background in biologics, and we're now trying to leverage this knowledge in the plasmid space, recognizing these key needs related chiefly to time and quality.

"With production, I think it's important to stress that currently, manufacturing capabilities are not equivalent to those in the biologics realm." - SB

Q Tell us about some of the key considerations in plasmid DNA production today

SB: The first thing to bear in mind is an IP issue, really, relating to how the plasmid is created. There are several components that come from different sequences that were identified (and patented) separately in years gone by, which have been pulled together to form the backbone of what has become the packaging plasmid to make lentiviral vector, or the different serotypes of AAV, or the many other viral vectors that are used.

Plasmid production begins with R&D aimed at creating a plasmid that is optimal for a specific use. In the past, plasmids were mainly created for internal academic approaches, whereas today, there are companies that specialize in making their own plasmid and then selling it to a third party.

The next stage is process development: identifying the best manufacturing strategy for the given plasmid. Again, there's a lot of knowledge applied here that comes from the wider biologics world, but equally, every product has its own unique characteristics and that is something to account for.

Finding a process that is fully closed and automated is of course highly desirable and important for meeting quality requirements, which are stringent - for example, there is the 'triple c', which is a standard of quality for the plasmid. Obviously, it's important at the end of the process to have a plasmid that can be considered fit for purpose.

R&D is really the foundation on which to build the production strategy, with ease of transfer to production a further key responsibility of the process developer. With production, I think it's important to stress that currently, manufacturing capabilities are not equivalent to those in the biologics realm. For example, whilst biologics fermentation is measured in the thousands of liters, with plasmids, you would be looking at hundreds of liters for a big production run. So the scales of production are different but just because you're making less of the plasmid, it doesn't necessarily mean the process is any easier.

Q Where specifically can/should further improvements and development be sought in this area?

SB: From my perspective, the area that needs the greatest attention is the regulatory aspect, in the sense that plasmid is yet to be properly accommodated within the regulatory framework.

There's always this vague reference to 'high quality', but there are still no real guidelines that explain exactly what 'high quality' should look like. I think the picture here is complicated to a degree by a sense of expectation that plasmid should tie in somehow with the same quality progression that occurs with the drug product, as it proceeds through R&D and towards full GMP. But of course, we're not talking about the drug product - we're talking about a raw material that will contribute to the generation of the final product, but that will not be a part of it by any means.

In our opinion, we need a clear statement from regulators on how the plasmid should be assessed by everyone, creating a level for all to work to. It would provide much-needed clarification and simplification relating to the lingering question of what GMP actually means in the context of plasmid produced for viral vector manufacture.

On the technological side, I think there's room of improvement to specific steps that were originally designed for other purposes. The technology does already exist - it's more a question of working together with a supplier in order to maximize the use of existing technologies – so I would say the technological aspect is perhaps less of an issue. Again, what remains the area of greatest concern is how to properly frame the plasmid used for viral vector manufacture in regulatory terms.

Q What are the keys to maintaining high quality and consistency of plasmid DNA production?

SB: The keys to maintaining high quality are really the quality assays.

We as a company decided to apply all of the GMP standards that would be applied to final drug product to our plasmid production. In other words, we have in place a quality system that is exactly the same as it would be if we were manufacturing a drug. That has allowed us to work with batch records, operate a system to notify clients if something goes wrong with a batch, etc. It's really raising the bar in terms of the quality framework within a company doing plasmid manufacturing, and of course, the analytics are absolutely key to achieving this.

The other important aspect is selecting the proper analytical panel. Again, there's no 'right' panel or standard way of doing things at the moment. However, there are some guidelines in the pharmacopeia relating to plasmid where the plasmid is the actual drug product, so if you're going to physically inject the plasmid into a human being, you know what you have to do. One exercise we did was to review that guidance, selecting the different analyses that are required in the context of a drug product. We then put them in the right context for plasmid that is not going to be a drug product, but that will be a building block in the manufacture of something else.

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We also decided to internalize all analytical development, meaning we gained both in terms of time but even more importantly, in terms of control of the analytical side. We believe that's a plus not just for us as a plasmid provider, but for the eventual end user, too.

So to summarize, the keys from my perspective are to create the proper quality framework - to create the proper quality panel for both the master cell bank used to produce the plasmid, and also the plasmid itself - and to maintain full control of the analytical side. Whether you're looking at release criteria or in process controls, you really have to know what you're doing and be able to do it properly.

> "the keys...are to create the proper quality framework... and to maintain full control of the analytical side. " - SB

MF: I would just emphasize Stefano's comment on the importance of being in control of the process not just from the plasmid provider's viewpoint, but from the customer's, too.

This leads me back to the topic of current regulatory uncertainty. A customer expecting a certain grade of production, but there being no clear guidance on how the quality aspect will be interpreted on the regulatory side, can lead that customer to be misled in terms of understanding exactly what is happening in the facility where the plasmid is being produced – what is actually being done by the provider to ensure the desired grade is being reliably achieved. Having the capability to be very clear and transparent in this regard is in our view a major plus.

So it's really, really important to be capable of maintaining this level of control. That doesn't mean being capable of controlling everything, but it does mean being able to answer specific requests that come to us, and to provide rationale and viable solutions to end users.

Q It is notable how significant a role Italian organizations play in gene therapy manufacture on a global scale – can you share your thoughts on why this is the case, and what benefits this phenomenon brings to Anemocyte in particular?

MF: I think this role that the Italian cell and gene therapy community has created for itself is fundamentally related to the resilience demonstrated during the past 20-30 years of strong activity in the field. I believe it is a resilience that is quite unparalleled worldwide. It's very much testament to the efforts and belief of the many researchers and other stakeholders who always strongly believed in the opportunities that lay beyond the scientific and technical complexity of cell and gene therapy, and who kept investing in it year after year.

This resilience and willingness to continuing investing throughout difficult and uncertain periods like the '90s led directly to the creation of today's world-leading Italian facilities and pool of expertise. And I think that we as a country and community kind of deserve to take

> "This resilience and willingness to continuing investing throughout difficult and uncertain periods like the '90s led directly to the creation of today's world-leading Italian facilities and pool of expertise." -MF

a leading role in cell and gene therapy today - or perhaps a better was to put it is we insist upon it - because we truly contributed to its creation. But of course, it's not enough to say we have been true believers and innovators in this field: we now have to demonstrate that we are able to master the knowledge and expertise that we developed in order to maintain and potentially increase our contribution to cell and gene therapy moving forward.

Anemocyte benefits in the main through the ecosystem that exists in Italy today, which is very lively and competent. It comprises excellent research centers, leading hospitals on the clinical application side, ambitious start-ups, and also competent authorities. All of these together create and foster an environment geared to accelerate the growth of our sectors. This ecosystem is key for Anemocyte as it facilitates exposure to innovation and knowledge, helping nurture talents and competencies that are core for us. Of course, it also creates a favorable environment for investment, which is such an important part of the story for each and every actor in this field.

So in my opinion, I think Italy did a great job!

Q What are your expectations for the growth of demand for plasmid moving forward?

SB: As we mentioned earlier, the cell and gene therapy industry is growing, and not just in terms of early clinical trials, but there are and will be more and more products in phase 3 and on the market. Furthermore, we've recently started to see more and more products jumping directly from first safety assessments in man to pivotal trials, simply because they are aimed at rare diseases where the unmet medical need is high. That means that you have to very quickly address all these GMP-related question marks around the plasmid you're using. All of this speaks to a growing recognition of the importance of securing a robust, high quality plasmid supply from the earliest stages of product development. So we obviously expect demand from the maturing gene therapy industry to continue growing substantially, and the onus is on plasmid suppliers such as Anemocyte to find ways to increase both the number and size of the batches we produce while maintaining the highest quality standards.

However, I think it is important to also stress the fact that plasmid demand is not restricted solely to viral vector production. In parallel, you have tools like transposons and gene editing

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platforms that in some cases require plasmid as well. So we also expect to see considerable growth in demand from other fields, and different uses of plasmids continuing to emerge.

Q How is Anemocyte mobilizing to meet this demand?

SB: For the past 12 months or so we've been really digging into reports, but perhaps more importantly, we've also been asking questions directly of the players that have reached commercialization.

The aim of these interviews was really to understand what challenges they face from a plasmid supplier perspective. These boil down to time and quality, basically - those were the two key points there were mentioned.

So we've aimed to build a facility that addresses these particular aspects. For example, we've created spaces where we can easily manufacture multiple batches in parallel, without creating specific bottlenecks. That was the first phase of the solution that we identified

We were also able to build a footprint that was very scalable so that if we realize that the existing facility is forming a bottleneck, it's relatively easy to 'copy and paste' what we have designed into another manufacturing unit. And each unit is designed to include everything needed for plasmid production, making them very self-sufficient. This combination of features allows us to meet current demand, whilst also affording us the flexibility to quickly replicate our footprint next door - or if necessary, elsewhere in the world – in order to cater for a growing market.

We've also started a collaboration with a player that has knowhow on specific areas relating to plasmid manufacturing, such as having IP around a specific plasmid. That's an area where it's really much easier for us to collaborate with third parties that have already established this knowledge and their position as a supplier of plasmid as starting material. We are also in other collaborative discussions – for example, with a transposon provider – so that if there is a need to enter into other emerging spaces, we have access to possible solutions.

I think the interviewing process and these collaborative interactions have combined to provide a really good foundation for us to create the right sort of flexible, scalable manufacturing environment.

Q EXELLULA is a particularly exciting initiative – can you go into more depth on that and what it will bring to the cell and gene therapy space?

MF: EXELLULA is an extremely exciting and fascinating project. It was built from a strong base, which began with the very challenge we've been talking about - how to meet rapidly increasing demand in the field of cell and gene therapy.

As we've discussed, quality, innovation and capacity are all key considerations. The basic idea behind EXELLULA is to bring all of them together, and importantly, to do so at just the right time for the industry.

EXELLULA is a modular project, the first step of which was the creation of the new plasmid unit that Stefano mentioned earlier - our key move in the plasmid space. It is an initiative specifically aimed at answering a real need with a state-of-the-art solution. So we're delivering a solution to something that was and is a genuine pain point for the industry, which of course is the bottleneck in the production of plasmids. We are offering something that is real and tangible. It's not just a dream or a marketing tool; it has walls, people working in it, technology that is actually available in order to provide services. And it's something that was conceived of purely with the immediate and future needs of industry in mind.

AFFILIATIONS

Marco Ferrari
CEO, Anemocyte

Stefano Baila
Director of Operations and Business Development, Anemocyte

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CELL & GENE THERAPY INSIGHTS

COMMERCIAL INSIGHT: DEC 2019

Commercial Insight: cell & gene therapy

Providing a critical overview of the sector's commercial development: M&As, licensing agreements & collaborations, financial results, IPOs and clinical/regulatory updates, with commentary from our Expert Contributors.

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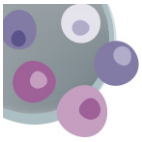

CELL THERAPY – Mark Curtis.

Astellas finished 2019 with the acquisition of Xyphos in December for $120 million upfront, giving the company access to Xyphos's convertible-CAR platform, which is aimed at safer and tunable T cell immunotherapies. The acquisition came weeks before the announcement of a deal with Adaptimmune, indicating that Astellas is ready to make a move into the T cell space. Astellas will get to pick three allogeneic T cell programs to develop that are complementary to Adaptimmune's existing programs and has an opportunity to deploy the cell cloaking technology it gained from its acquisition of Universal Cells in 2018 to mitigate risk of rejection following infusion. Also on the T cell front, there was a string of announcements at ASH related to data on CAR-T cells in the competitive multiple myeloma space, with Juno, J&J, and BMS all providing updates on their respective programs.

GENE THERAPY – Richard Philipson. Chief Medical Officer, Trizell Ltd, UK

The year of 2019 ends on a high with another late phase clinical trial success for Alnylam, this time in primary hyperoxaluria Type 1 with its RNAi therapeutic lumasiran. At a much early stage, good news also for patients with leukocyte adhesion deficiency type-1, with early success in the first patient treated with RP-L201, Rocket Pharmaceuticals' lentivirus-based *ex vivo* gene therapy. Progress also continues apace in haematology, with Sangamo announcing encouraging interim results from its ongoing Phase 1/2 study. The year of 2019 ends on a high with another late phase clinical trial success for Alnylam, this time in primary hyperoxaluria Type 1 with its RNAi therapeutic lumasiran. At a much early stage, good news also for patients with leukocyte adhesion deficiency type-1, with early success in the first patient treated with RP-L201, Rocket Pharmaceuticals' lentivirus-based *ex vivo* gene therapy. Progress also continues apace in haematology, with Sangamo announcing encouraging interim results from its ongoing Phase 1/2 study in severe hemophilia A, and bluebird bio continuing the positive news stream with evidence of durable transfusion independence in patients with β-thalassaemia treated with its LentiGlobin gene therapy. The prospects for 2020 look excellent.

Clinical Regulatory

SANGAMO'S GENE THERAPY OFFERS HOPE TO HEMOPHILIA PATIENTS

Sangamo Therapeutics has announced encouraging interim results from its ongoing Phase 1/2 study evaluating an AAV-based gene therapy approach to treat severe hemophilia A.

Data presented at the American Society of Hematology (ASH) meeting in Orlando, Florida reported encouraging results from Sangamo's and Pfizer's Phase 1/2 Alta gene therapy trial.

The Alta study of SB-525 is designed to evaluate the safety and kinetics of a single intravenous in¬fusion of SB-525 in hemophilia A patients. SB-525 is a recombinant adeno-associated virus vector 6 (AAV6) encoding the complementary deoxyribonucleic acid for B domain deleted human FVIII.

The study evaluated 11 patients across four ascending dosage cohorts; two patients in the first three cohorts and 5 patients in the highest dose (3e13 vg/kg) cohort. Patients demonstrated a dose-dependent increase in FVIII levels and dose-dependent decrease in the use of FVIII replacement therapy. Patients in the highest dose cohort achieved normal FVIII levels starting at 5–7 weeks following the therapy. The treatment was generally well tolerated.

2 patients in the 3e13 vg/kg cohort continue to have normal FVIII levels through 44 and 37 weeks of follow-up. The next two patients in this cohort are at 7 and 4 weeks of follow-up and have demonstrated FVIII activity kinetics similar to that of the previous patients in the same cohort. The two patients most recently treated in this cohort are at 22 and 12 weeks of follow-up, respectively, and have demonstrated a similar pattern of FVIII expression. One patient who is currently at week 24 of follow-up had achieved normal FVIII expression at 7 weeks following treatment, but the levels fluctuated at week 13. However, at week 18, FVIII levels began to increase, and as of the latest measurement at week 24, it is stable. No patient in this cohort experienced bleeding events up to 44 weeks of follow-up.

Sangamo and Pfizer entered into a global collaboration and license agreement in 2017 for the SB-525 program. Later the collaboration was also extended to developing gene therapies for amyotrophic lateral sclerosis and frontotemporal lobar degeneration using Sangamo's proprietary zinc finger protein transcription-factor technology.

SB-525 received FDA's Orphan Drug and Fast Track designations and EMA's Orphan Medicinal Product designation. FDA had also granted regenerative medicine advanced therapy designation for SB-525 gene therapy to treat severe hemophilia A, a designation which allows the company to interact with FDA more frequently.

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Sangamo has completed the manufacturing technology transfer and initiated the transfer of the Investigational New Drug (IND) Application to Pfizer, which is expected to be completed in the first quarter 2020.

Dr Bettina Cockroft, Sangamo's CMO commented:

"The updated results from the Alta study suggest that SB-525 may represent a differentiated gene therapy for patients with severe hemophilia A. The results continue to suggest that if sustained over a longer duration, SB-525 has the potential to be a predictable, reliable, and safe treatment that may bring clinical benefits to patients with severe hemophilia A."

BRISTOL-MYERS SQUIBB SUBMITS BLA FOR ITS CAR-T CELL THERAPY

Bristol-Myers Squibb has announced that it has submitted a Biologics License Application (BLA) to the US Food and Drug Administration (FDA) for its autologous anti-CD19 chimeric antigen receptor (CAR)-T cell immunotherapy, lisocabtagene maraleucel (liso-cel).

Liso-cel comprises of individually formulated $CD8^+$ and $CD4^+$ CAR-T cells to treat adult patients with relapsed or refractory (r/r) large B-cell lymphoma (LBCL) after at least two prior therapies.

The submission is based on the safety and efficacy results from the Phase 1 TRANSCEND NHL 001 trial, evaluating liso-cel in 269 patients with r/r large B-cell lymphoma, including diffuse large B-cell lymphoma (DLBCL).

Liso-cel targets CD19, a surface glycoprotein expressed during normal B-cell development and maintained following malignant transformation of B cells. Liso-cel aims to target CD19-expressing cells through a CAR construct that includes an anti-CD19 single-chain variable fragment (scFv) targeting domain for antigen specificity, a transmembrane domain, a 4-1BB costimulatory domain hypothesized to increase T-cell proliferation and persistence, and a CD3-zeta T-cell activation domain.

Liso-cel has been granted Breakthrough Therapy and Regenerative Medicine Advanced Therapy designations by the FDA for r/r aggressive large B-cell non-Hodgkin lymphoma (NHL), including DLBCL, not otherwise specified (de novo or transformed from indolent lymphoma), primary mediastinal B-cell lymphoma (PMBCL) or Grade 3B follicular lymphoma (FL) and Priority Medicines (PRIME) scheme by the European Medicines Agency (EMA) for r/r DLBCL.

LYSOGENE'S GENE THERAPY SHOWS PROMISE IN PRECLINICAL MODELS OF MPSIIIA

Lysogene has published promising preclinical data on its gene therapy drug, LYS-SAF302, for treating Mucopolysaccharidosis Type IIIA (MPS IIIA/ Sanfilippo A). The therapy is currently been tested in a Phase 2/3 clinical trial in the US and Europe.

MPS IIIA is a rare and lethal inherited neurodegenerative lysosomal storage disorder caused by mutations in the *SGSH* gene, which encodes an enzyme called Heparan-N-sulfatase necessary for heparan sulfate recycling in cells. Lack of SGSH results in a build-up of sugars in the body, particularly the brain, leading to severe neurodegeneration. It affects approximately 1 in 100,000 newborns and currently there are no treatments available.

Lysogene's LYS-SAF302 is an AAV10-mediated gene therapy which has the potential to replace the faulty *SGSH* gene with a functional copy of the gene in the brain. The ongoing Phase 2/3 trial, AAVance, is a single-arm trial designed to assess the efficacy of one-time delivery of LYS-SAF302 in improving or stabilizing the neurodevelopmental state of MPS IIIA patients. The trial is expected to complete in 2022.

Data from the preclinical study published in *Molecular Therapy Methods & Clinical Development* has provided long-term effects of LYS-SAF302 on the lysosomal pathology in MPS IIIA mice as well as SGSH expression and distribution in the brain of two large animal species, dogs and monkeys.

LYS-SAF302 was administered to 5-week-old MPS IIIA mice at three different doses and was injected into the caudate putamen/striatum and thalamus. LYS-SAF302 was able to dose-dependently correct disease pathology in mice models at 12-weeks and 25-weeks post-dosing.

To study SGSH transgene distribution in the brain of large animals, LYS-SAF302 was injected into the subcortical white matter of dogs and monkeys. SGSH enzyme activity was found to be increased by at least 20% above endogenous levels in the brains of both dogs and monkeys, suggesting AAV administration as a promising method to achieve widespread enzyme distribution and disease correction in MPS IIIA.

Ralph Laufer, Lysogene's CSO commented:

"Extrapolating the results of the dog and monkey studies to the human brain, it appears that the current clinical dose and volume should be able to restore at least 20% of normal SGSH activity throughout the brain of a MPS IIIA patient, which is predicted to have a significant positive impact on disease progression".

ALNYLAM'S RNAI THERAPY MEETS EFFICACY ENDPOINTS

Alnylam, a Cambridge, MA-based biopharmaceutical company specialized in the developing RNAi-based therapeutics, has reported positive topline results from Illuminate-A, its Phase 3 study of lumasiran, an investigational RNAi therapeutic targeting glycolate oxidase for treating primary hyperoxaluria type 1 (PH1).

PH1 is an inborn error of metabolism and is a rare genetic disease characterized by excessive oxalate accumulation in plasma and urine, resulting in calcium oxalate crystal formation and deposition in the kidney and many other tissues. It arises from mutations in the enzyme alanine-glyoxylate aminotransferase.

Illuminate-A was designed to enrol approximately 30 patients with PH1 aged six and above, at 16 study sites, in eight countries around the world. Patients were randomized 2:1 to lumasiran or placebo, with lumasiran administered at 3 mg/kg monthly for three months followed by quarterly maintenance doses. The primary endpoint for the study was the percent change from baseline in 24-hour urinary oxalate excretion averaged across months 3 to 6 in patients treated with lumasiran as compared to placebo. At 6 months, lumasiran met the primary endpoint in patients with PH1 and achieved statistically significant results for all six tested secondary endpoints.

The study also achieved statistically significant results for all six tested secondary endpoints. There were no serious or severe adverse events in the study, and results showed that lumasiran was generally well tolerated with an overall profile generally consistent with that observed in Phase 1/2 and open-label extension studies of lumasiran.

Based on the positive results, the company plans to submit a New Drug Application

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(NDA) and file a Marketing Authorisation Application for lumasiran in early 2020.

The company is also conducting ILLUMINATE-B – a global Phase 3 study of lumasiran in PH1 patients less than six years of age, with results expected in mid-2020, and ILLUMINATE-C – a global Phase 3 study of lumasiran in PH1 patients of all ages with advanced renal disease, with results expected in 2021.

Expert Pick

Alnylam rounds off the year with a hat-trick of positive Phase 3 studies, with the announcement of positive outcomes from its Phase 3 Study of lumasiran in primary hyperoxaluria Type 1 (PH1). In the study of 30 patients with PH1, a statistically significant change from baseline in 24-hour urinary oxalate excretion was observed in patients treated with lumasiran, with the proportion of patients achieving near-normalization or normalization of urinary oxalate levels also achieving statistical significance when compared to placebo. PH1 is an ultra-rare disease where excessive oxalate production causes calcium oxalate crystals to deposit in the kidneys and urinary tract, eventually leading to end-stage renal disease in some patients. Lumasiran, which is an RNAi therapeutic, targets glycolate oxidase and prevents the over-production of oxalate. The very encouraging data on lumasiran in PH1 rounds off the year on a high for the company, following recent successes in TTR amyloidosis (patisiran) and acute hepatic porphyria (givosiran).

– Richard Philipson

AVROBIO DOSES FIRST PATIENT USING ITS AUTOMATED PLATO™ PLATFORM

The clinical-stage gene therapy company Avrobio has announced that the first patient has been dosed using the company's plato™ gene therapy platform, which is intended to support the worldwide commercialization of Avrobio's gene therapies.

The plato platform is a closed, automated vector system for $CD34^+$ cell-based therapies. developed to enable worldwide commercialization of the company's gene therapies. It includes a state-of-the-art lentiviral vector (LV2) designed to optimize vector copy number, transduction efficiency and resulting enzyme activity; a personalized conditioning regimen with precision dosing via therapeutic drug monitoring intended to enhance safety and engraftment; advanced cryopreservation to extend shelf life and enable flexible scheduling for patients; and an automated, closed-manufacturing process intended to improve consistency and predictability of the drug product. The company expects that plato's self-contained manufacturing pods will facilitate global manufacturing site expansion.

The first patient dosed using plato is enrolled in FAB-201, the company's Phase 2 trial of AVR-RD-01, an investigational gene therapy for Fabry disease. That trial is evaluating

the safety and efficacy of AVR-RD-01 in 8 to 12 treatment-naïve patients, with ongoing recruitment in the US, Canada and Australia.

plato has received regulatory clearance for clinical use and will be used for all patients going forward in FAB-201, AVROBIO's Phase 2 Fabry disease trial, and in GAU-201, AVROBIO's Phase 1/2 clinical trial of AVR-RD-02, an investigational gene therapy for Gaucher disease.

DURABLE RESPONSE OBSERVED IN BLUEBIRD BIO'S CAR-T THERAPY FOR MULTIPLE MYELOMA

bluebird bio and its partner, Bristol-Myers Squibb, have provided updated safety and efficacy results from their ongoing Phase 1 study (CRB-402) of bb21217 in patients with r/r multiple myeloma. Results were presented at the 61st ASH Annual Meeting and Exposition in Orlando, Florida.

CRB-402 is the first-in-human study of bb21217 in patients with r/r multiple myeloma designed to assess safety, pharmacokinetics, efficacy of the therapy. CRB-402 consists of two parts; a dose escalation (completed) part and a dose expansion (ongoing) part.

bb21217 is an investigational BCMA-targeted CAR-T therapy that uses the ide-cel CAR molecule and is cultured with the PI3 kinase inhibitor (bb007) to enrich for T cells displaying a memory-like phenotype to increase the *in vivo* persistence of CAR-T cells. It is being developed in partnership between bluebird bio and Bristol-Myers Squibb.

Results were obtained from 38 treated patients. Twenty-four patients received bb21217 in the dose escalation cohort at three dose levels. Fourteen additional patients received bb21217 in the dose expansion

Expert Pick

GILEAD POISED FOR LAUNCH OF SECOND CAR-T THERAPY

Gilead has filed for FDA approval of KTE-X19 for use in patients with Mantle Cell Lyphoma. While KTE-X19 uses the identical anti-CD19 CAR construct found in the marketed drug product, Yescarta, the manufacturing process for KTE-X19 involves a lymphocyte enrichment step that Gilead believes will position the therapy to treat B cell cancers with circulating lymphoblasts. Data presented at ASH shows the therapy had a complete response rate of 67% patient in a Phase 2 study of 60 patients. Durability data suggests that a number of heavily pre-treated patients could benefit from the therapy, as 43% of the subset of patients that reached 24 months since treatment remained in remission.

– Mark Curtis

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cohort at two dose levels. The patients had a median of six prior lines of therapy.

33 of the 38 patients were evaluable for clinical response. Ten of 12 patients in the 150 x 10^6 CAR+ T cells dose cohort demonstrated clinical response. Among the ten confirmed responders, the median duration of response was 11.1 months. Follow-up within the two higher dose cohorts (300 x 10^6 and 450 x 10^6 CAR+ T cells) remains early and none of the confirmed responders have experienced disease progression.

In the 300 x 10^6 CAR+ T cells cohort, 14 patients were evaluable for response and six of the 14 evaluable patients demonstrated clinical response with a median follow-up of 4 months. In the 450 x 10^6 CAR+ T cells cohort, seven patients were evaluable for response and four of the seven evaluable patients demonstrated clinical response with a median follow-up of 3.3 months.

CAR-T cell persistence was observed in eight of ten patients with ongoing response and evaluable at six months, and two out of two patients with ongoing response and evaluable at 18 months.

The dose expansion part of the study is ongoing to further recruit patients and explore bb21217 at the 450 x 10^6 CAR+ T cells dose cohort, assess functional persistence of bb21217 and durability of response.

The adverse events observed with bb21217 were consistent with known toxicities of CAR-T therapies. This includes, neutropenia, leukopenia, thrombocytopenia, anemia, lymphopenia, hypophosphatemia, hyponatremia and febrile neutropenia.

Ones to Watch

News of the successful treatment of one infant with leukocyte adhesion deficiency type-1 (LAD-1) brings hope for families of children with this very rare disease. Affecting around 1 in 1 million of the population worldwide, infants with LAD-1 experience bacterial and fungal infections affecting the skin and mucous membranes, often spreading to involve large areas. Due to recurrent infection, life expectancy is often severely shortened and affected individuals may not survive past infancy. Underlying mutations in the ITGB2 gene give rise to leukocytes that lack integrins, and which cannot therefore attach to or cross the blood vessel wall to contribute to the immune response. Data from the treated patient are limited, but it appears that engraftment of ex vivo stem cells transduced using the company's lentiviral gene therapy was successful, and there was visible improvement of multiple disease-related skin lesions. A second patient will be enrolled in the Phase 1 portion of the trial, followed by 7 patients in the Phase 2 portion, where overall survival will be evaluated.

– Richard Philipson

KITE SUBMITS BLA FOR ITS SECOND CAR-T CELL THERAPY

Kite, a Gilead Company, has announced that it has submitted a BLA to the FDA for KTE-X19, its investigational CAR-T therapy designed to treat adult patients with r/r mantle cell lymphoma (MCL), a rare form of non-Hodgkin lymphoma (NHL) that arises from cells originating in the "mantle zone" of the lymph node.

The BLA submission is based on data from the Phase 2 ZUMA-2 trial, which demonstrated an overall response rate of 93%, including 67% with complete response, as assessed by an Independent Radiologic Review Committee (IRRC) following a single infusion of KTE-X19. In the safety analysis, Grade 3 or higher cytokine release syndrome (CRS) and neurologic events were seen in 15% and 31% of patients, respectively. No Grade 5 CRS or neurologic events occurred. Detailed findings from this trial were recently presented at the ASH Meeting & Exposition in Orlando.

Kite plans to submit a Marketing Authorization Application for KTE-X19 in the European Union in early 2020. KTE-X19 has been granted Breakthrough Therapy Designation (BTD) by the FDA and Priority Medicines (PRIME) by the European Medicines Agency (EMA) for relapsed or refractory MCL.

KTE-X19 is an investigational, autologous, anti-CD19 CAR T cell therapy. KTE-X19 uses the XLP™ manufacturing process that includes T-cell selection and lymphocyte enrichment. Lymphocyte enrichment is a necessary step in certain B-cell malignancies with evidence of circulating lymphoblasts. KTE-X19 is currently in Phase 1/2 trials in acute lymphoblastic leukemia (ALL), mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL).

ROCKET'S GENE THERAPY SHOWS PROMISE IN THE FIRST TREATED LAD-I PATIENT

Rocket Pharmaceuticals has provided preliminary data from its Phase 1/2 trial of RP-L201 for Leukocyte Adhesion Deficiency-I (LAD-I).

LAD-I is a rare, autosomal recessive pediatric disease caused by a mutation of the ITGB2 gene that encodes for the beta-2 Integrin component CD18. Absence of CD18 leads to decreased leukocyte adhesion and extravasation from blood vessels to combat infections. As a result, children with severe LAD-I are often affected immediately after birth. Without a successful bone marrow transplant, mortality in patients with severe LAD-I is 60–75% prior to the age of 2 and survival beyond the age of 5 is exceedingly rare.

RP-L201 trial is designed to evaluate the safety and efficacy of the infusion of autologous hematopoietic stem cells transduced with a lentiviral vector encoding the ITGB2 gene. In November last year the FDA had accepted its IND application. The study is expected to enrol nine patients globally. The Phase 1 portion of the trial is expected to enrol two patients and will assess the safety, tolerability and preliminary efficacy of RP-L201. The Phase 2 portion of the trial will evaluate overall survival at several leading US and EU centers.

Data from the first patient treated with RP-L201 has demonstrated early evidence of safety and potential efficacy. The patient also displayed visible improvement of multiple disease-related skin lesions after receiving therapy. No safety or tolerability issues related to RP-L201 administration were identified. These data are consistent with Rocket's preclinical studies, which demonstrated that

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administration of RP-L201 in murine models resulted in stable engraftment and phenotypic correction with restored neutrophil migration capability.

In additional news this month, the European Medicines Agency (EMA) has granted PRIority MEdicines (PRIME) status to Rocket Pharmaceuticals' RP-L102, a lentiviral vector (LVV)-based gene therapy for the treatment of Fanconi Anemia (FA).

With this milestone, Rocket's FA program has received all accelerated regulatory designations in the US and EU, including FDA Regenerative Medicine Advanced Therapy, Fast Track and Orphan designations.

RP-L102 is currently been tested in a Phase 1/2 trial, FANCOLEN-I, to assess the therapeutic safety and preliminary efficacy of a hematopoietic cell-based gene therapy consisting of autologous $CD34^+$ enriched cells transduced with a lentiviral vector carrying the FANC-A gene in patients with FA subtype A.

ANIXA BIOSCIENCES TO FURTHER OPTIMIZE CAR-T THERAPY FOR OVARIAN CANCER

Anixa Biosciences has provided an update on its CAR-T therapy intended to treat ovarian cancer. The company is developing the therapy with its partner, Moffitt Cancer Center.

Anixa's CAR-T technology was developed by Dr Jose Conejo-Garcia, Chair of the Department of Immunology at Moffitt Cancer Center. Early experimental results indicate the potential of this technology as a treatment for ovarian cancer and the teams are hopeful that the therapy could be improved further through additional genetic engineering.

The company's CAR-T therapy is designed in a way that the T-cells are transformed to express the follicle stimulating hormone (FSH) on their surface, enabling it to target cells that are expressing follicle stimulating hormone receptor (FSHR). Although early evidence with the current setting shows promise, researchers are working to increase the potency of the transformed T-cells so that they could express much higher levels of FSH. To achieve this, researchers at Moffitt will create an improved viral vector and verify experimentally that it will result in a more powerful cancer therapy.

Anixa therefore plans to optimize the therapy first and then file an Investigational New Drug (IND) application with the FDA in late 2020.

Dr. Amit Kumar, President and CEO of Anixa Biosciences stated:

"While this new development may be disappointing to shareholders, we have decided that we want to go into the clinic with the best possible therapy. CAR-T technology has not worked in a clinically meaningful way for solid tumors. Making this change would result in a radically superior therapy and give us a dramatically greater chance of success against ovarian cancer, a solid tumor."

BLUEBIRD BIO'S GENE THERAPY: LONG TERM SAFETY AND EFFICACY DATA PROVIDES HOPE

bluebird bio has provided an update from its LentiGlobin™ gene therapy trials for β-thalassemia (betibeglogene autotemcel) in pediatric, adolescent and adult patients who have transfusion-dependent β-thalassemia (TDT). Data was presented at the

61st ASH Annual Meeting and Exposition last month.

Results from three studies were presented at the meeting: Phase 3 Northstar-3 (HGB-212) study in patients with a β0/β0 genotype or IVS-I-110 mutation, Phase 3 Northstar-2 (HGB-207) study in patients who do not have a β0/β0 genotype, and 5 years follow-up from the completed Phase 1/2 Northstar (HGB-204) study.

52 pediatric, adolescent and adult patients with TDT who do not have a β0/β0 genotype or have a β0/β0 genotype have been treated with LentiGlobin for β-thalassemia in the Northstar, Northstar-2 and Northstar-3 studies.

TDT is an inherited blood disorder caused by a mutation in the beta-globin chain resulting in ineffective red blood cell production. Anemia caused by TDT is corrected by blood transfusions, however, regular blood transfusions leads to iron overload.

Lentiglobin is a cell-based gene therapy where autologous $CD34^+$ cells from patients are transduced *ex vivo* with a lentiviral vector encoding βA-T87Q-globin gene. Following transplantation of these gene-corrected stem cells into patients, patients are monitored for the production of gene therapy-derived hemoglobin (Hb) which increases Hb levels.

Major highlights include:

- **Phase 1/2 Northstar (HGB-204) trial:** More than 4 years of durable transfusion independence (TI), stable total Hb levels and reduced liver iron concentrations were observed in treated patients who do not have a β0/β0 genotype. The trial is completed.
- **Phase 3 Northstar-2 (HGB-207) study:** 90% of evaluable patients who do not have a β0/β0 genotype achieved TI, with median average total Hb levels of 12.2 g/dL.
- **Phase 3 Northstar-3 (HGB-212) study:** Two patients with β0/β0 genotype or IVS-I-110 mutation evaluable for TI achieved it with Hb levels of 13.2 g/dL and 10.4 g/dL at last visit. The trial is ongoing. Nine of 11 patients with at least 6 months of follow-up in HGB-212 have not had a transfusion for at least 3 months.

Therapy related non-serious adverse events reported were hot flush, breathing difficulty, abdominal pain, pain in extremities and non-cardiac chest pain. Thrombocytopenia was one serious adverse event reported which was related to lentiglobin. With more than five years of follow-up, there have been no new unexpected safety events, no deaths, no graft failure and no cases of vector-mediated replication competent lentivirus or clonal dominance.

These results highlight the therapy's potential benefits and consistent safety profile across a broad range of TDT genotypes and patient populations and the outcomes demonstrate the long-term disease-modifying potential of lentiGlobin for people living with TDT.

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Licensing agreements & collaborations

GENEDIT COLLABORATES WITH EDITAS MEDICINE FOR NANOPARTICLE-BASED GENE THERAPY

GenEdit a developer of a novel polymer nanoparticle technology platform for non-viral- and non-lipid-based delivery of gene therapies, has announced that it has entered a worldwide, exclusive license and collaboration agreement with Editas Medicine.

GenEdit has developed a nanoparticle-based delivery system for CRISPR-based therapeutics, including gene knockout and gene repair therapies, to enable safer delivery options with improved efficiency.

Under the terms of the agreement, GenEdit has granted Editas Medicine an exclusive worldwide license, with rights to sublicense, to GenEdit's Cpf1-based technologies. In return for these rights, GenEdit will receive undisclosed upfront and development milestone payments, including royalties on net sales of products incorporating the licensed intellectual property. In addition, GenEdit and Editas Medicine will collaborate on evaluating delivery of Cpf1-based technologies with GenEdit's nanoparticle platform. Editas Medicine will provide research funding and have an option to continue development after the initial collaboration period.

GenEdit's nanoparticle platform consists of a proprietary non-viral, non-lipid library of polymers that efficiently encapsulate and deliver cargo [RNA, DNA, protein and/or ribonucleic acid-protein complexes (RNP)] to specific tissues. The company screens the library to identify initial hits and then uses computational analysis and medicinal chemistry for iterative lead optimization. The company has used this platform to identify multiple candidate polymers for efficient and specific delivery of gene editing to a range of tissues.

Dr Timothy Fong, GenEdit's CSO, commented:

"Compared to viral vectors and lipid-based nanoparticles, our approach has the potential for better targeting, more cargo, and lower manufacturing cost. In particular, our approach has the potential to enable in vivo gene editing of multiple tissues with CRISPR and expand the potential of gene therapies to treat more diverse sets of diseases."

DICERNA TO PARTNER WITH ROCHE TO TREAT HEPATITIS B VIRUS INFECTION

Dicerna, a pharmaceutical company developing RNAi-based therapies, has announced the successful closing of the research collaboration and licensing agreement with Roche following expiration of the waiting period under the Hart-Scott-Rodino Antitrust Improvements Act of 1976, as amended.

The companies entered into an agreement to develop novel therapies for the treatment of chronic hepatitis B virus infection using Dicerna's proprietary GalXC™ RNAi platform technology. The collaboration will focus on worldwide development and commercialization of DCR-HBVS, Dicerna's investigational therapy in Phase 1 clinical development, and includes the discovery and development of therapies targeting multiple additional human and viral genes associated with HBV infection using the technology platforms of both companies.

Under the terms of the agreement, Dicerna will receive an upfront payment of $200 million, which is due early in the first quarter of 2020. It is also eligible to receive up to an additional $1.47 billion over time for the achievement of specified development, regulatory and commercial milestones for DCR-HBVS. In addition, Dicerna may be eligible to receive royalties on potential product sales of DCR-HBVS. Dicerna also retains an option to co-fund the development of DCR-HBVS worldwide and, if exercised, would receive enhanced royalties on net sales in the USA. If Dicerna exercises this co-funding option, it shall also have an option to co-promote products including DCR-HBVS in the USA.

Dicerna and Roche also agreed to collaborate on the research and development of additional therapies targeting multiple human and viral genes implicated in chronic HBV infection, using technology from both companies, for which Dicerna is eligible to receive additional milestones and royalties on any potential products.

GalXC™ technology aims to advance the development of next-generation RNAi-based therapies and is designed to silence disease-driving genes in the liver and other tissues. Liver-targeted GalXC-based compounds enable subcutaneous delivery of RNAi therapies that are designed to bind specifically to receptors on liver cells, leading to internalization and access to the RNAi machinery within the cells. The GalXC approach seeks to optimize the activity of the RNAi pathway so that it operates in the most specific and potent fashion. Compounds produced via GalXC are intended to be broadly applicable across multiple therapeutic areas, including both liver and non-liver indications.

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Finance

SANGAMO EARNS $25 MILLION FROM PFIZER FOR TRANSFER OF IND

Sangamo Therapeutics has announced the completion of the transfer to Pfizer of the SB-525 Hemophilia A gene therapy Investigational New Drug application (IND). Pfizer is advancing SB-525 into a Phase 3 registrational clinical study in 2020 and has already commenced enrolling patients into a Phase 3 lead-in study. Sangamo has now earned a $25 million milestone payment, per the terms of a December 2019 amendment to the parties' collaboration agreement for the global development and commercialization of gene therapies for Hemophilia A.

Sangamo entered into a global collaboration and license agree¬ment with Pfizer in 2017 for the SB-525 program. Under the terms of the agreement, Sangamo has been responsible for Phase 1/2 clinical development. Pfizer will be operationally and financially responsible for subsequent research, development, manufacturing and commercialization activities for SB-525. Sangamo is eligible to receive total potential milestone payments of up to $300 million for the development and commercialization of SB-525, and up to $175 million for additional Hemophilia A gene therapy product candidates that may be developed under the collaboration. Sangamo will, additionally, receive tiered royalties starting in the low teens and up to 20% of annual net sales of SB-525.

Sandy Macrae, Sangamo's CEO commented:

"I want to congratulate our team for their success in developing SB-525 through to this important milestone where we have handed over the IND to Pfizer for Phase 3 development. We are thrilled to be in a partnership where both parties have cooperated to accelerate study timelines, resulting in completion of the IND transfer ahead of schedule. Pfizer and Sangamo are united in our common interest to help patients with Hemophilia A and will do everything that we can to safely and expeditiously advance this promising gene therapy candidate for patients in need."

ASTELLAS ACQUIRES XYPHOS BIOSCIENCES FOR $665 MILLION

Japan-based Astellas Pharma has announced that it has acquired Xyphos Bioscences to strengthen its immuno-oncology pipeline. With the acquisition Astellas will gain Xyphos' novel and proprietary ACCEL (Advanced Cellular Control through Engineered

Ligands) technology platform, as well as its immuno-oncology expertise, to develop next-generation cancer immunotherapy.

Xyphos' synthetic biology platform is designed to direct cells of the immune system to target single or multiple tumor antigens while controlling the immune cell proliferation and endurance. Xyphos's proprietary molecules can be delivered to natural immune cells or to engineered CAR cells to generate immunotherapies for oncology. Xyphos' patented CAR technology is based on an engineered modification to a natural human receptor named NKG2D.

Through protein engineering, several natural ligands of NKG2D have been modified to bind exclusively to the otherwise inert NKG2D receptor. Various functional molecules are attached to the modified ligand. The ligand-directed functional molecules then bind exclusively to immune cells expressing the inert CAR on their surface – the proprietary convertibleCAR®-cells. The CAR-cells can be directed by the ligand-bound antibody to seek, become activated and attacks a targeted cancer cell. Xyphos' first convertibleCAR-T cell product candidate is in preclinical development and scheduled to be tested in a first-in-human clinical study in 2021.

As part of the acquisition deal, $120 million was paid upon closing of the acquisition, and Xyphos became a wholly owned subsidiary of Astellas. In addition to this payment and potential future development milestone payments, it will provide a total transaction value of $665 million.

James Knighton, CEO of Xyphos commented:

"At Xyphos, we are driven to advance our innovative cell therapy technology platform as an exciting new approach to potentially manage and cure cancer. Astellas' commitment to immuno-oncology makes them an ideal partner to advance our proprietary NKG2D-based NK-cell and T-cell platform to the next stage of clinical exploration. Further, we look forward to becoming part of Astellas to accelerate this immuno-oncology research and development in the vibrant South San Francisco community."

Ones to Watch

Astellas finished 2019 with the acquisition of Xyphos in December for $120 million upfront, giving the company access to Xyphos's convertible-CAR platform, which is aimed at safer and tunable T cell immunotherapies. The acquisition came weeks before the announcement of a deal with Adaptimmune, indicating that Astellas is ready to make a move into the T cell space. Astellas will get to pick three allogeneic T cell programs to develop that are complementary to Adaptimmune's existing programs and has an opportunity to deploy the cell cloaking technology it gained from its acquisition of Universal Cells in 2018 to mitigate risk of rejection following infusion. Also on the T cell front, there was a string of announcements at ASH related to data on CAR-T cells in the competitive multiple myeloma space, with Juno, J&J, and BMS all providing updates on their respective programs.

– Mark Curtis

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Movers & Shakers

DAVID MEEK JOINS FERGENE AS CEO

FerGene, a new gene therapy company launched by Ferring Pharmaceuticals and Blackstone Life Sciences in November, has announced the appointment of David Meek as President and CEO.

Mr Meek has over 30 years of industry experience. Most recently, he was the CEO of the biopharmaceutical company, Ipsen. He transformed Ipsen into a global biopharma growth leader with initiatives to transform external innovation, Ipsen's R&D operations and build out the company's footprint in countries like the US and China. In addition to his time as CEO of Ipsen, Mr Meek's prior leadership roles have included serving as EVP & President of Oncology at Baxalta from 2014 to 2016, following its spin-off from Baxter. He was also Chief Commercial Officer of Endocyte from 2012 to 2014. Prior to that, Mr Meek served in various executive leadership roles at Novartis Pharma and Novartis Oncology after beginning his career at Johnson & Johnson and Janssen from 1989 to 2004.

FerGene was launched in November 2019 by Switzerland-based Ferring Pharmaceuticals Blackstone Life Sciences. The company's main focus is on the global development and marketing of nadofaragene firadenovec, a gene therapy currently in Phase 3 trial for high-grade, Bacillus Calmette-Guérin (BCG) unresponsive, non-muscle invasive bladder cancer.

- Written by Dr Applonia Rose, *Cell and Gene Therapy Insights*