JUNE 2020

Volume 6, Issue 5

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:

Clinical development strategy, tools and trial designs

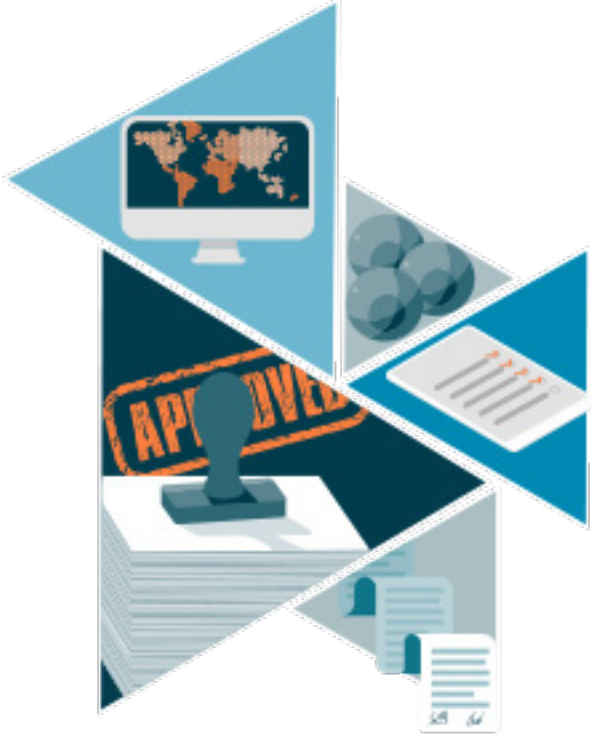
CELL & GENE THERAPY INSIGHTS

Volume 6, Issue 5

Clinical development strategy, tools and trial designs

CELL & GENE THERAPY INSIGHTS

Regulatory Trends



June 2020
Volume 6, Issue 5

REGULATORY PERSPECTIVE

INTERVIEW

CELL & GENE THERAPY INSIGHTS

CLINICAL DEVELOPMENT STRATEGY, TOOLS & TRIAL DESIGNS

SPOTLIGHT

EXPERT INSIGHT

Cellular therapies for solid cancer: clinical experience, challenges and future revolution

Aisha N Hasan, Richard O'Reilly, Annamalai Selvakumar & Ekaterina Doubrovina

Adoptive cell therapy (ACT) is a form of immunotherapy in which cancer-specific T cells are modified and expanded *ex vivo* and re-infused to target and eradicate the tumor. Chimeric antigen receptor (CAR) engineered T cell therapy has shown transformational clinical benefit in hematologic malignancies, but its application to solid tumors has been challenging. This review follows the evolution of ACT from initial insights to the implementation of treatment protocols, focusing on the predicaments during early trials for solid cancers with this treatment. While there is evidence for effective and durable immune rejection of refractory solid malignancies with adoptive cell transfer, the clinical experience disclosed key limitations and provided the impetus for developing the next iterations of cellular therapy products. Future directions of ACT are discussed, in particular with regard to genetic engineering of autologous cells, selection of appropriate targets and optimizing treatment regimens in the era of checkpoint inhibitors.

Cell & Gene Therapy Insights 2020; 6(5), 727–736

DOI: 10.18609/cgti.2020.085

OVERVIEW

Immunotherapy has now emerged as the next frontier in cancer treatment. In 1891, William Coley first established the concept of harnessing the immune system to treat cancer, and since then, this continues

to be applied towards developing novel immune-based therapies in cancer treatment. More than 30 years ago, initial evidence for efficacy of immunotherapy in cancer was demonstrated with clinical responses in 25% to ~40% of patients with relapsed metastatic melanoma or renal cell carcinoma treated with high doses of IL-2 either as single agent or in combination with lymphokine activated killer (LAK), and interferon alpha, respectively [1,2]. Subsequently, multiple clinical successes demonstrated with antibody-based therapies including rituximab in B cell malignancies [3], Herceptin® in breast cancer [4], and more recently with checkpoint inhibitors (anti-PD/(L1), anti-CTLA4) [5–8]. While these therapies can provide durable remissions of disease in a proportion of patients with many cancers, there is an unmet need in relapsed patients. Furthermore, these results are dramatically shifting one of the treatment goals in patients with metastatic malignancy; wherein maintained complete responses have become conceivable for some patients.

Adoptive cell transfer (ACT) to target and treat cancer has emerged as one of the most promising and innovative immunotherapy approaches to treat cancer. Cell therapies are living medicines that have the potential for inducing prolonged remissions after a single dose. ACT is a therapeutic approach which involves the *ex vivo* expansion and reinfusion of antigen-specific (Ag-specific) T cells, and has been used in various forms over the last 25 years [9]. The first recognition that ACT could be a promising treatment for cancer came with the initial reports by Steve Rosenberg *et al.*, describing complete regression of bulky tumors in patients with metastatic melanoma infused with *ex vivo* expanded T cells extracted from surgically resected tumors, also called tumor infiltrating lymphocytes [10,11]. Although TIL-based ACT can induce responses in up to 50% of patients with certain cancer indications like melanoma [12], TIL therapy can only be offered to a limited group of patients based on the need for accessible tumor tissue, the complexity of TIL production procedures, and the very intensive nature of this three-step treatment including both high-dose chemotherapy and interleukin-2 in addition to T cell infusion [13]. T cells used for adoptive cell treatment can also be genetically redirected toward tumor associated antigens by modification with a T cell receptor or TCR or chimeric antigen receptor or CAR. The unprecedented efficacy of CD19 directed CAR T cells and recent approval in B cell malignancies has generated significant momentum for adoptive cell therapies [14,15], with a few other agents due to be approved for hematological malignancies.

Overall thus far, in solid tumors, the clinical activity of cell therapies has been limited to a few tumor types, with majority of responding patients demonstrating short-lived responses. Resistant, metastatic, or recurrent solid tumors represent unmet clinical challenges, since they are seldom surgically resectable, and largely nonresponsive to radiation and chemotherapy (Figure 1). Therefore, driven by patient need and the commercial potential, an increasing number of developers are striving to create safe and effective cell therapies for the treatment of solid malignancies.

As mentioned, the initial academic efforts in this field focused on treatment with TILs or LAK cells in combination with IL-2. [16]. Concurrent efforts by academic experts in bone marrow transplantation demonstrated complete regressions of EBV related lymphomas in recipients of bone marrow transplants with infusion of *in vitro* sensitized transplant donor derived EBV specific T cells [17–19]. The next phase of explorations (1990s–2000s), focused on the genetic modification of T cells to express the α and β chains of a known tumor antigen specific T-cell receptor (TCR) or a synthetic molecule called chimeric antigen receptor (CAR). In the latter approach, the CAR molecules were engineered to contain an extracellular single chain Fv antibody domain targeting a tumor cell surface antigen, linked to a cytoplasmic signaling domain with CD3 ζ chain, and second generation constructs also included a co-stimulatory domain such as CD28 or 4-1BB (Table 1). These investigative efforts

DOI: 10.18609/cgti.2020.085

over 20 years led to the approval of CD19 CART (Kymriah®, Yescarta®) for B cell malignancies, while also helped to elucidate the impediments to the clinical success of cell therapies in solid cancers [20], and develop off the shelf approaches for cell therapy [21–24].

CHALLENGES FOR CELL THERAPIES IN SOLID TUMORS

The key elements hampering the clinical success of cellular therapies in solid tumors include:

1. The targeted antigen;
2. Trafficking of T-cells to the tumor; and
3. The tumor microenvironment and immune evasion.

An ideal target antigen is one that is differentially overexpressed on tumor cells and not on healthy tissue. The selection of target antigen is challenging because the biologic heterogeneity of solid cancers does not lend to an approach of one antigen fits all. This problem is further compounded by the frequent expression of alleged target antigens on normal tissues that can lead to on-target, off-tumor toxicity. Cell therapy trials to date have used numerous tumor expressed antigens that are recognized to be associated with pathogenesis (Table 2). The prominent targets include receptor tyrosine kinases (EGFR, EGFRviii, Her-2, ROR1), tumor associated self antigens (NYESO-1, MAGE A3/A4/A10), membrane glycoproteins, and viral proteins. It is also well recognized that tumor-specific somatic mutations, mostly non-synonymous, can lead to the generation of neoantigens [25]. Analysis of samples from patients treated with vaccines or checkpoint inhibitor approaches confirms the detection of neoantigen specific T cells post treatment and also indicates that the load of neoantigens may help predict responses to these immunotherapies [26–29]. In the context of cellular therapies, TILs were found to contain T cells specific for tumor associated neoantigens, which were cytotoxic. Overall, neoantigens represent attractive targets for adoptive cell therapy approaches because these are exclusively tumor specific antigens, T cells directed against neoantigens are not subject to central and peripheral tolerance and do not target normal tissues.

Approximately 70% of the proteome consists of intracellular proteins, and the bulk

FIGURE 1

SEER Database.

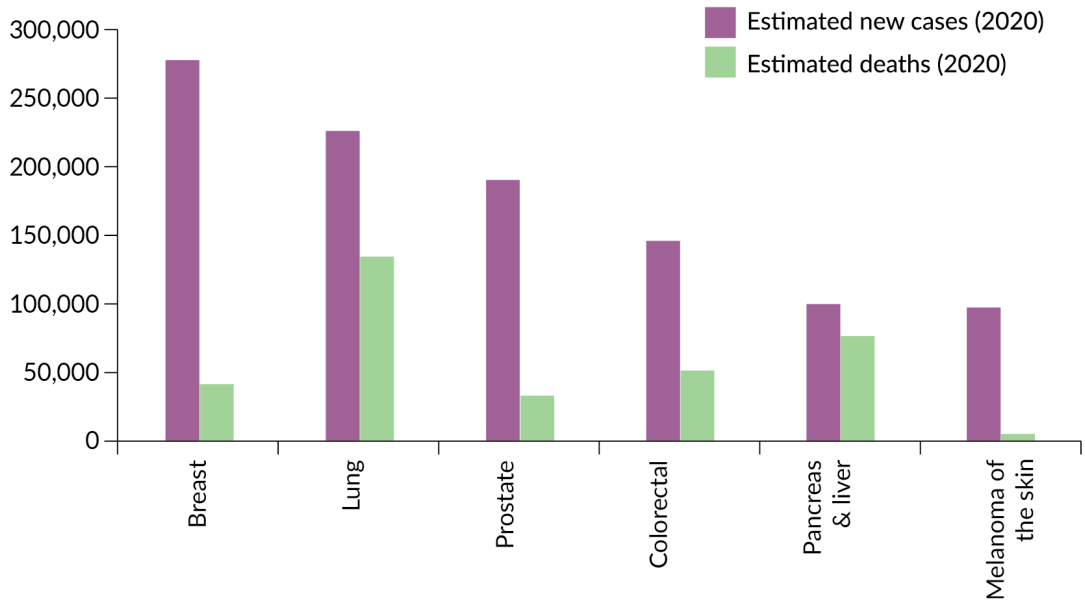


TABLE 1

TCR engineered T cells	CAR engineered cells
Natural TCR α and β chains of a known cancer specific antigen	Synthetic molecule engineered with an antibody binder to cancer antigen
Can target intracellular antigens (70% of proteome)	Can target only extracellular antigens (30% of proteome)
Engagement is physiological, can be very potent and sustained.	Engagement is dependent on binding affinity of ScFv and co-stimulatory domain.
Less prone to T-cell exhaustion due to physiological binding	T-cell exhaustion may occur due to built-in co-stimulatory domain
Require HLA for T-cell binding and activation	Do not require HLA for binding and activation
Requires less antigen density to trigger activation	Higher antigen density required for activation
Immune evasion through downregulation of HLA could compromise activity	Activity of cell product would not be impacted by HLA downregulation

of cancer associated antigens are intracellular. Therefore, TCR engineered cells are particularly valuable among the various cell products since they can target proteins residing anywhere within the cell including the cytoplasm, nucleus and oncofetal proteins, while only 25% of the cellular proteins are extracellular and can be targeted by antibody approaches, including the vast majority of CAR modified cells [30]. Furthermore, TCR stimulation requires lower antigen expression thresholds in comparison to CAR T-cells, which further emphasizes the therapeutic potential of TCR engineered T-cells [31].

Identification of neoantigens, and relevant tumor associated antigens can be challenging. Recent advances in next-gen sequencing technologies as well as bioinformatic analysis have facilitated the efforts towards identifying novel tumor targets. Neoantigens were previously identified in melanoma patients receiving TIL therapy in a peptide-based screening approach using whole-exome sequencing (WES) and peptide-MHC tetramers [32]. Subsequently tandem minigens (TMG) and peptide synthesis were used, all of which were not practicable because they are time and labor intensive [33]. More recently circulating tumor DNA (ctDNA) from patient blood samples has been used to conduct clinical-grade targeted genomic tumor profiling with matched normal samples used to identify nonsynonymous somatic mutations. An *in silico* analysis of identified mutations is then used to predict and prioritize potential high-affinity epitopes, and matched using a neoantigen peptide library assembled using an inventory of shared driver mutation–derived by systematic mining of The Cancer Genome Atlas (TCGA) and Catalogue of Somatic Mutations in Cancer (COSMIC) databases and use of multiple epitope prediction programs [34]. TCRs have been cloned from identified neoantigen specific T cells [35], which can be used to engineer T cells for targeted adoptive immunotherapy approaches. Ongoing clinical trials are exploring personalized neoantigen directed TCR engineered T cells in several malignancies (NCT03970382).

In summary, the choice of antigen and level of expression on tumor versus normal tissue, in conjunction with the type of cell product will inform the clinical activity and risk: benefit of ACT.

The treatment paradigm of cellular therapies largely involves a single dose of cells via infusion. These adoptively transferred cells must traffic the site/s of the tumor to be effective, which can be challenging in advanced stage solid tumors. The location of the tumor, the number and sites of metastasis, and the associated fibrotic response, are all obstacles inhibiting the T cells from reaching sites of tumor and to exert anti-tumor activity. Perhaps the most notable limitation for cell therapies lies in the complex tumor microenvironment, which is often immune inhibitory. Tumors develop mechanisms to evade immune recognition, which include downregulation of tumor antigens or HLA, generation

DOI: 10.18609/cgti.2020.085

of an immunosuppressive microenvironment through secretion of suppressive cytokines and expression of negative immune regulators able to silence immune effectors [20]. For instance, myeloid-derived suppressor cells and tumor-associated macrophages (TAMs) decrease local tryptophan levels in the tumor microenvironment, depriving CAR T cells of an essential amino acid necessary for optimal function [36]. Several approaches are underway to address the inhibitory tumor microenvironment, and antigen escape. These include TCR or CAR constructs co-expressing dn-TGFβ R2 ([37] NCT00889954; or CD8a as well as Tandem CAR with CD19/22 to address antigen escape in CD19$^+$ malignancies [38] or BCMA/TACI CAR to address down-regulation of BCMA in multiple myeloma [NCT 29155426]).

INITIAL CLINICAL EXPERIENCE

Immune cells in various forms have been used for adoptive transfer in the clinic. *In vitro* expanded tumor infiltrated lymphocytes (TILs), T cells sensitized against TAA such as MART-1 or GP100, and more recently TCR and CAR engineered T and NK cells. The first recognition of the therapeutic potential for adoptive T-cell therapy in solid cancer came with the initial reports by Steve Rosenberg et al, describing complete regression of bulky tumors in patients with metastatic melanoma infused with ex-vivo expanded tumor infiltrating lymphocytes extracted from surgically resected tumors [16,39].

The excitement and activity in this space is evident in the number of cellular therapy trials that are dominating within cancer immunotherapy trials approximating over 350 new trials per year [14,40].

EFFICACY

Adoptive transfer studies of TCR engineered autologous T cells specific for NY-ESO-1 have shown objective clinical responses in 50–61% of patients with synovial cell sarcoma and 55% of patients with melanoma [41,42]. Responses corresponded with expansion of infused NYESO-1 TCR modified T-cells and persistence, as previously reported with CD19 CART [43]. With a median response duration of 7 months, and a tolerable safety profile, this therapy is now in Phase 2 development for sarcoma, and pilot studies ongoing in other NYESO-1$^+$ tumors like NSCLC.

Overexpression of EGFR is commonly seen in patients with non-small-cell lung cancer. In a Phase 1 clinical study, two of 11 patients with refractory non–small cell lung cancer experienced a partial response after treatment with second-generation EGFR-specific CAR T cells after lymphodepletion [44]. Infused T cells were detectable in both peripheral blood and tissues in biopsied patients. However, the responses in the two patients were not sustained, lasting only for 2 months and 3.5 months each.

Another Phase 1 study evaluating treatment with CEA targeting CAR T cells in CEA positive metastatic colorectal cancer patients demonstrated disease stabilization in 7 of 10 patients who had rapidly progressive disease to prior therapies. Although no objective responses were observed, the treatment was well tolerated. Disease stabilization lasting 30 weeks and minimal tumor shrinkage on PET and MRI scans were observed in two patients each. Treatment was also associated with diminishing serum levels of CEA in all

TABLE 2

Class	Antigen
Receptor tyrosine kinases	EGFR, EGFR viii, Her-2, met
TAA	NYESO-1, MAGE A3/A4/ A10, MART-1, GP100, WT-1, PRAME, mesothelin
Oncofetal proteins	WT-1, AFP, CEA
Tight junction/adhesion molecules	Claudin 18.2, EpCAM, LiCAM, FAP-Nectin4
Membrane glycoproteins	Muc-1, Muc-16, CD147, CAIX,
Viral proteins	EBV, EBV-LMP2, HPV-E6/ E7, HBV
Neoantigens	

TABLE 3

Selected clinical trials.

Target	CAR/TCR	Indications	Patient no.	ORR	Duration of response (months)	Toxicity/reference
LiCAM	1st generation mRNA	Neuroblastoma	6	0%	SD (1)	Grade 3 pneumonitis (1 pt) NCT00006480
Claudin 18.2	CD28	Gastric, pancreatic	10	20%	3–5	NCT03159819 [60]
HER2/ ErbB2	CD28	GBM	17	6%	1PR (9)	No severe tox. (grade 2 in 2 pts) NCT01109095 [61]
TAG-72	1st generation ɣ-Retroviral	Colorectal cancer	16	0%	–	low grade CRS, no SAE [62]
Mesothelin mRNA CAR	1st generation mRNA	Pancreatic Ca	6	O%	N/A	NCT01355965 [63]

treated patients, and patients receiving higher doses of lymphodepletion seemed to derive longer disease stabilization [45].

Table 3 lists selected cell therapy trials in solid cancers.

SAFETY

The potential for transformative benefit in high medical need solid cancer patients faces the challenge of safety, which will require early recognition and mitigation of unique toxicities to enable a balanced risk benefit for clinical implementation. For solid tumors, severe toxicities have been observed due to cross-reactivity – either against cancer antigens expressed on healthy tissues or non-target cross-reactivity (off-target). In metastatic colorectal cancer (CRC) patients treated with autologous TCR engineered T cells against the oncofetal protein human carcinoembryonic antigen (CEA), one of three patients treated had an objective response [46], however, severe colitis was associated with this treatment which limited further development. In renal cell carcinoma, targeting carbonic anhydrase IX (CAIX) led to liver toxicity in 50% of patients due to CAIX expression on biliary epithelium [47]. CAR T-cells engineered against ErbB2 given to a patient with metastatic colorectal cancer caused multi-organ failure with acute pulmonary toxicity from antigen expression on lung epithelium resulting in rapid cardiopulmonary distress (15 mins post ACT) and death 5 days post-infusion [48]. Similarly, a study using CEACAM5-CAR T-cells in GI tumors was terminated, in part, due to toxicity from expression of the targeted antigen on lung epithelium [49]. Fatal cross-reactivity SAEs from TCR T-cell therapies have also been documented, with MAGE-A3 TCR-T cross-reactivity observed in several trials. Neurotoxicity observed due to cross-reactivity with MAGE-A12 in the brain resulted in two patient deaths, with mental status changes occurring as early as day one [50]. Cardiac toxicity was observed with cross-reactivity to TITIN-1, expressed in the heart, resulting in two patient deaths within 4–5 days post-infusion [51]. In both trials, toxicity kinetics were rapid due to cross reactivity.

IMPROVING EFFICACY OF CELLULAR THERAPIES IN SOLID TUMORS

Despite enthusiasm for adoptive immunotherapy, many obstacles must be addressed before cell therapy joins the arsenal for treatment of solid cancers. The learnings from initial clinical experience have seen the emergence of novel approaches designed to tackle some of the perceived roadblocks and optimize clinical outcomes.

For discovery of novel antigens associated with tumor mutations, new technologies have been developed that are currently in use for discovery of neoantigens. This knowledge

DOI: 10.18609/cgti.2020.085

is then utilized to clone out reactive TCRs and generate TCR engineered cells targeting tumor specific antigens for adoptive cell therapy. In tumor types that have more than one TAA, the selection of an optimal target is critical to minimize antigen escape via antigen loss or downregulation.

The initial clinical trials have contributed important insights into mechanisms of resistance to cellular therapy, and other challenges with respect to migration, dose, *in vivo* expansion and tumor immune micro-environment. These insights have been incorporated in developing the next generation of cellular therapy trials, Antigen escape is a phenomenon that has been associated with the lack of activity or progression after an initial response to T-cell therapy [51,52]. To address this, advances in cell engineering have qualified approaches to generate dual antigen targeting CAR modified T or NK cells. Such dual CARs are engineered to engage with the alternate antigen if one antigen is downregulated. Such tandem CARs have entered clinical trials in hematological malignancies targeting CD19/20, CD19/CD22 or BCMA/TACI [53,54]. In Preclinical models of breast cancer, a CAR specific for both HER2 and MUC1 had promising *in vitro* results [55], and dual-specific T cells engineered to express both a CAR specific for Her2 and a TCR specific for the melanocyte protein (gp100) demonstrated promising durable complete remissions of $Her2^{+}$ tumors in immunocompetent mice [56]. These observations are soon to be translated into the clinic.

Similarly, approaches to improve innate T-cell trafficking are being explored via co-expression of chemokine receptors or by local/ intracavitary administration of the cell product [57,58]. It is postulated that the route of CAR T-cell administration needs to be tailored to the biology of each solid tumor malignancy for enhanced efficacy. This is being evaluated in clinical trials of intrapleural and intraperitoneal administration of CAR T cells for mesothelioma and ovarian cancer, respectively (NCT02414269, NCT02498912).

Several approaches are also being explored to overcome immune inhibition within the tumor microenvironment. TGFβ is a known immune inhibitory molecule within the tumor microenvironment through binding to its receptor on T and NK cells. TGFβ signaling can be blocked by engineering TCR or CAR modified T cells to co-express a non-signaling dominant-negative TGFBRII (dnTGFbRII) using multicomponent engineering, which enables engineered T cells to function despite the presence of TGFβ [37,59]. Other efficacy enhancing techniques include secretion of PD-1 mini-bodies to enable checkpoint blockade, co-expression of IL-12, or IL-15 within engineered cells to secrete inflammatory cytokines at the site of the tumor to enhance infused cell proliferation and persistence as well as enhance cytotoxic activity.

In the clinic, the treatment regimens are being optimized to facilitate optimal patient condition, as well as T-cell expansion and persistence after infusion. The requirement for lymphodepleting chemotherapy prior to cell therapy can be traced back to studies that informed conditioning regimens for bone marrow transplant. A transient suppression of endogenous lymphocytes is required to achieve favorable expansion and stimulation of infused T cells. Accordingly, different doses of cyclophosphamide and fludarabine, or alternate chemotherapies, immune-modulating agents and different T-cell dosing regimens are being investigated to determine optimal conditioning permitting the highest T-cell expansion after infusion. NK cells either genetically engineered or expanded *in vitro* and re-infused are also being evaluated for potential benefit with or without cytokine supplementation.

In summary, cell therapies are approved medicines for hematological malignancies, and will continue to grow in this space. The promise of transformational benefit with these agents continues to drive further innovation to optimize their development for solid tumors. This will come with the next wave of engineering, to enhance efficacy, prolong persistence thereby providing durable remission of disease.

REFERENCES

1. Rosenberg S, Lotze MT, Muul LM *et al.* A Progress Report on the Treatment of 157 Patients with Advanced Cancer Using Lymphokine-Activated Killer Cells and Interleukin-2 or High-Dose Interleukin-2 Alone. *N. Engl. J. Med.* 1987; 316: 889–97.
2. Rosenberg S, Lotze MT, Yang JC *et al.* Combination Therapy With Interleukin-2 and Alpha-Interferon for the Treatment of Patients With Advanced Cancer. *J. Clin. Oncol.* 1989; 1863–74.
3. McLaughlin P, Grillo-Lo´pez AJ, Link BK *et al.* Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J. Clin. Oncol.* 1998; 16: 2825–33.
4. Sawyers C. Herceptin: A First Assault on Oncogenes that Launched a Revolution. *Cell* 2019; 179.
5. Attia P, Phan GQ, Maker AV *et al.* Autoimmunity Correlates With Tumor Regression in Patients With Metastatic Melanoma Treated With Anti-Cytotoxic T-lymphocyte antigen-4. *J. Clin. Oncol.* 2005; 23: 6043–53.
6. Hodi S, O'Day SJ, McDermott DF *et al.* Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *N. Engl. J. Med.* 2010; 363: 711–23.
7. Topalian S, Sznol M, David F. McDermott DF *et al.* Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J. Clin. Oncol.* 2014; 32: 1020–30.
8. Garon E, Rizvi NA, Hui R. Pembrolizumab for the treatment of non-small-cell lung cancer. *N. Engl. J. Med.* 2015; 372: 2018–28.
9. Restifo N, Dudley ME, Rosenberg SA. Adoptive Immunotherapy for Cancer: Harnessing the T-cell response. *Nat. Rev Immunol.* 2012; 12(4): 269–81.
10. S. Rosenberg, P Spiess, R Lafreniere. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science* 1986; 1318–21.
11. Rosenberg S, Packard BS, Aebersold PM *et al.* Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N. Engl. J. Med.* 1988; 319(25):1676–80.
12. Rosenberg S, Dudley ME. Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. *PNAS* 2004; 14639–45.
13. Weber J, Atkins M, Hwu P. White Paper on Adoptive Cell Therapy for Cancer with Tumor-Infiltrating Lymphocytes: A Report of the CTEP Subcommittee on Adoptive ce;ll therapy. *Clin. Cancer Res.* 2011; 17(7): 1664–73.
14. Sadelain M. CD19 CAR T-cells. *Cell* 2017; 1471.
15. Schuster S, Bishop MR, Tam CS. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *N. Engl. J. Med.* 2019; 45–56.
16. Rosenberg S, Lotze MT, Muul LM *et al.* Observations on the Systemic Administration of Autologous Lymphokine-Activated Killer Cells and Recombinant Interleukin-2 to Patients with Metastatic Cancer. *N. Engl. J. Med.* 1985; 313(23):1485–92.
17. Papadapoulos E, Ladanyi M, Emanuel D *et al.* Infusions of Donor Leukocytes to Treat Epstein-Barr Virus-Associated Lymphoproliferative Disorders after Allogeneic Bone Marrow Transplantation. *N. Engl. J. Med.* 1994; 330: 1185–1191.
18. Heslop H, Ng CY, C Li *et al.* Long-term Restoration of Immunity Against Epstein-Barr Virus Infection by Adoptive Transfer of Gene-Modified Virus-Specific T Lymphocytes. *Nature Med.* 1996; 2(5): 551–5.
19. Doubrovina E, Oflaz-Sozmen B, Prockop SE *et al.* Adoptive Immunotherapy With Unselected or EBV-specific T Cells for Biopsy-Proven EBV+ Lymphomas After Allogeneic Hematopoietic Cell Transplantation. *Blood* 2012; 119(11): 2644–56.
20. Spranger S, Gajewski TF. Tumor-intrinsic oncogene pathways mediating immune avoidance. *Oncoimmunology* 2016; 5(3): e1086862.
21. Hasan A, Selvakumar A, Doubrovina E *et al.* Artificial antigen presenting cells that express prevalent HLA alleles: A step towards the broad application of antigen-specific adoptive cell therapies. *Discov. Med.* 2009; 8(43): 210–8.
22. Doubrovina E, Carpenter T, Pankov D *et al.* Mapping of novel peptides of WT-1 and presenting HLA alleles that induce epitope-specific HLA-restricted T cells with cytotoxic activity against WT-1(+) leukemias. *Blood* 2012; 1633-46.
23. Ahmed M, Lopez-Albaitero A, Pankov D *et al.* TCR-mimic bispecific antibodies targeting LMP2A show potent activity against EBV malignancies. *JCI Insight* 2018; 3(4): e97805.
24. Prockop S, Doubrovina E, Suser S *et al.* Off-the-shelf EBV-specific T cell immunotherapy for rituximab-refractory EBV-associated lymphoma following transplantation. *J. Clin. Invest.* 2020; 130(2): 733–47.
25. Anagnostou V, Smith KN, Forde PM. Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. *Cancer Discov.* 2017; 7(3): 264–76.
26. Rizvi N, Hellmann MD, Snyder A *et al.* Mutational landscape determines

DOI: 10.18609/cgti.2020.085

sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; 348(6230): 124–8.

27. Schumacher T, Schreiber RD. Neoantigens in Cancer Immunotherapy. *Science* 2015; 69-74.

28. Carreno B, Magrini V, Becker-Hapak M *et al.* Cancer Immunotherapy. A Dendritic Cell Vaccine Increases the Breadth and Diversity of Melanoma Neoantigen-Specific T Cells. *Science* 2015; 803–8.

29. Johanns T, Miller CA, Connor J Liu CJ *et al.* Detection of Neoantigen-Specific T Cells Following a Personalized Vaccine in a Patient With Glioblastoma. *Oncoimmunology* 2019; e1561106.

30. Chandran S, Klebanoff CA. T cell Receptor-Based Cancer Immunotherapy: Emerging Efficacy and Pathways of Resistance. *Immunol. Rev*. 2019; 290(1): 127–47.

31. Harris D, Kranz DM. Adoptive T Cell Therapies: A Comparison of T Cell Receptors and Chimeric Antigen Receptors. *Trends Pharmacol. Sci.* 2015; 220–30.

32. Robbins P, Lu Y-C, El-Gamil M. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat. Med.* 2013; 747–52.

33. Tran E, Turcotte S, Gros A *et al.* Cancer immunotherapy based on Mutation-Specific CD4+ T Cells in a Patient with Epithelial Cancer. *Science* 2014; 344(6184): 641–5.

34. Chen F, Zou Z, Du J *et al.* Neoantigen identification strategies enable personalized immunotherapy in refractory solid tumors. *J. Clin. Invest.* 2019; 129(5): 2056–70.

35. Parkhurst M, Gros A, Pasetto A *et al.* Isolation of T cell receptors specifically reactive with mutated tumor associated antigens from tumor infiltrating lymphocytes based on CD137 expression. *Clin. Cancer Res.* 2016; 23(10): 2491–505.

36. Ninomiya S, Narala N, Huye L *et al.* Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. *Blood* 2015; 125(25): 3905–16.

37. Kloss CC, Lee J, Zhang A. Dominant-Negative TGF-b Receptor Enhances PSMA-Targeted Human CAR T Cell Proliferation And Augments Prostate Cancer Eradication. *Mol. Ther.* 2018; 26(7): 1855–66.

38. Fry T, Shah NN, Orentas RJ *et al.* CD22-targeted CAR T Cells Induce Remission in B-ALL That Is Naive or Resistant to CD19-targeted CAR Immunotherapy. Nat. Med. 2018; 24(1): 20–8.

39. Dudley M, Wunderlich JR, Yang JC *et al*. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J. Clin. Oncol.* 2005; 2346–57.

40. Tang J, Pearce L, Jill O'Donnell-Tormey J *et al.* Trends in the global immuno-oncology landscape. *Nat. Rev. Drug Discov.* 2018; 17: 783–4.

41. Robbins P, Kassim SH, Tran TLN *et al.* A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor:long-term follow-up and correlates with response. *Clin. Cancer Res*. 2015; 21(5): 1019–27.

42. D'Angelo S, Melchiori S, Merchant MS *et al.* Antitumor Activity Associated With Prolonged Persistence of Adoptively Transferred NY-ESO-1 c259 T Cells in Synovial Sarcoma. *Cancer Discov.* 2018; 8(8): 944–57.

43. Fraietta J, Lacey SF, Orlando EJ. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat. Med.* 2018; 24(5): 563–71.

44. Feng K, Guo Y, Dai H *et al.* Chimeric antigen receptor-modified T cells for the immunotherapy of patients with EGFR-expressing advanced relapsed/refractory non-small cell lung cancer. *Sci. China Life Sci*. 2016; 468–79.

45. Zhang C, Wang Z, Yang Z *et al.* Phase I Escalating-Dose Trial of CAR-T Therapy Targeting CEA+ Metastatic Colorectal Cancers. *Mol. Ther.* 2017; 25(5):1248–58.

46. Parkhurst M, Yang JC, Langan RC *et al*. T-cell targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer. *Mol. Ther.* 2011; 19(3): 620–6.

47. Lamers C, Sleijfer S, van Steenbergen S *et al*. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. *Mol. Ther.* 2013; 21(4): 904–12.

48. Morgan R, Yang JC, Kitano M *et al.* Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol. Ther.* 2010; 18(4): 843–51.

49. Thistlethwaite F, Gilham D, Guest R *et al.* The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. *Cancer Immunol. Immunother.* 2017; 66: 1425–36.

50. Morgan R, Chinnasamy N, Abate-Daga D *et al.* Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J. Immunother.* 2013; 36(2): 133–51.

51. Linette G, Stadtmauer EA, Maus MV *et al.* Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood* 2013; 122(6): 863–71.

52. Shah N, Fry TJ. Mechanisms of Resistance to CAR T-cell therapy. *Nat. Rev. Clin. Oncol.* 2019; 16(6): 372–85.

53. Pan J, Zuo S, Deng B *et al.* Sequential CD19-22 CAR T therapy induces sustained remission in children with r/r B-ALL. *Blood* 2020; 135(5): 387–91.

54. Yan Z, Cao J, Cheng H *et al.* A combination of humanised anti-CD19 and anti-BCMA CAR T cells in patients with relapsed or refractory multiple myeloma: a single-arm, phase 2 trial. *Lancet Hematol.* 2019; 6(10): e521–e529.

55. Wilkie S, van Schalkwyk MCI, Hobbs S *et al.* Dual targeting of ErbB2 and MUC1 in breast cancer using chimeric antigen receptors engineered to provide complementary signaling. *J. Immunol.* 2012; 32(5): 1059–70.

56. Slaney C, von Scheidt B, Davenport AJ *et al.* Dual-specific Chimeric Antigen Receptor T Cells and an Indirect Vaccine Eradicate a Variety of Large Solid Tumors in an Immunocompetent, Self-antigen Setting. *Clin. Cancer Res.* 2017; 23(10): 2478–90.

57. Moon E, Carpenito C, Sun J *et al.* Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor. *Clin. Cancer Res.* 2011; 17(14): 4719–30.

58. Koneru M, O'Cearbhaill R, Pendharkar S *et al.* A Phase I clinical Trial of adoptive T cell therapy using IL-12 secreting MUC-16(ecto) directed chimeric antigen receptors for recurrent ovarian cancer. *J. Transl. Med.* 2015; 13: 102.

59. Bollard C, Tripic T, Cruz CR *et al.* Tumor-Specific T-Cells Engineered to Overcome Tumor Immune Evasion Induce Clinical Responses in Patients With Relapsed Hodgkin Lymphoma. *J. Clin. Oncol.* 2018; 36(11): 1128–39.

60. ASCO 2019.

61. Ahmed N. Human Epidermal Growth Factor Receptor 2 (HER2) –Specific Chimeric Antigen Receptor–Modified T Cells for the Immunotherapy of HER2-Positive Sarcoma. *J. Clin. Oncol.* 2015; 33:1688–96.

62. Hege KM. Safety, tumor trafficking and immunogenicity of chimeric antigen receptor (CAR)-T cells specific for TAG-72 in colorectal cancer. *J. ImmunoTher. Cancer* 2017; 5: 22.

63. Beatty GL. Activity of Mesothelin-Specific Chimeric Antigen Receptor T Cells Against Pancreatic Carcinoma Metastases in a Phase 1 Trial. *Gastroenterology* 2018; 155: 29–32.

AFFILIATIONS

Aisha N, Hasan
Memorial Sloan-Kettering Cancer Center, Department of Pediatrics, and Bone Marrow Transplantation, NY, USA
hasana@mskcc.org

Richard O'Reilly
Memorial Sloan-Kettering Cancer Center, NY, USA

Annamalai Selvakumar
Memorial Sloan-Kettering Cancer Center, NY, USA

Ekaterina Doubrovina
Memorial Sloan-Kettering Cancer Center, NY, USA

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Hasan, Dr O'Reilly and Dr Doubrovina have received royalties from Atara biotherapeutics. Dr Hasan is an employee of and holds stocks in GlaxoSmithKline.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *May 4 2020* ; ***Revised manuscript received:*** *Jun 2 2020*; ***Publication date:*** *Jul 1 2020.*

DOI: 10.18609/cgti.2020.085

CELL & GENE THERAPY INSIGHTS

CLINICAL DEVELOPMENT STRATEGY, TOOLS & TRIAL DESIGNS

SPOTLIGHT

EXPERT INSIGHT

Consideration of clinical translation of cardiac AAV gene therapy

Kelly P Yamada, Serena Tharakan & Kiyotake Ishikawa

Advancements in conventional cardiac care have significantly reduced mortality from coronary heart disease and acute myocardial infarction. However, the prevalence of heart failure continues to increase in an aging population with profound social and economic consequences. Cardiac gene therapy with adeno-associated virus (AAV) vectors is emerging as a potential modality for addressing this desperate clinical need. After showing initial promise in extensive preclinical studies and an early clinical trial, disappointing results of large-scale clinical trial derailed the progress of AAV-mediated cardiac gene therapy. However, it appears that knowledge gained from previous failures coupled with developments in targeted gene delivery have set the stage for a new frontier in cardiac AAV gene therapy.

Cell & Gene Therapy Insights 2020; 6(5), 609–615

DOI: 10.18609/cgti.2020.073

INTRODUCTION

Heart failure and ischemic coronary disease remain among the most prevalent causes of morbidity and mortality worldwide [1]. Improvements in acute cardiac care have increased the likelihood that patients will survive acute cardiac episodes. Ironically, this has resulted in a greater number of patients with chronic cardiac disorders. These patients remain at high risk of repeat hospitalizations

and sudden cardiac death. New therapies are urgently needed to reduce the social and economic burden of treating such patients in an aging world. Gene therapy is a modality that can potentially be a game changer for chronic cardiac disorders by modifying the cellular signaling pathways that have been difficult to target using traditional approaches. Among numerous gene delivery vectors, adeno-associated virus (AAV) vectors possess several unique features that render them an ideal option for delivering genes to the heart. These features include efficient gene transduction compared to non-viral vectors, minimal risk of acute inflammatory response allowing for the safe delivery of genes, long-term expression in non-dividing cells including cardiomyocytes, and cardiac tropism in some serotypes that improves cardiac specificity. On the other hand, the high prevalence of pre-existing anti-AAV neutralizing antibodies in the general population [2] can preclude patient participation in clinical trials and is a formidable impediment to gene delivery to the myocardium. In this article, we provide a concise review of the current status of cardiac AAV gene therapy with a focus on clinical translation and discuss challenges and areas needing refinement.

CARDIAC AAV GENE THERAPY: WHERE DO WE STAND?

Beginning in the late 20th century, several clinical trials examined the efficacy of angiogenic cardiac gene therapy for treating ischemic heart disease using plasmid DNA and adenovirus [3]. Targeted delivery of genes promoting vascular growth such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF) demonstrated promising efficacy in preclinical and early phase clinical trials, but the larger late-phase trials mostly failed to show consistent benefit. None of these trials led to changes in routine clinical treatment [3]. As AAVs emerged with the above-described features, the choice for cardiac gene therapy shifted toward this vector, especially in the research field.

Led by Dr Roger J Hajjar, the AAV1-based delivery of Sarco/endo-plasmic reticulum $Ca2^{+}$-ATPase (SERCA2a) program was the first to enter a clinical trial using AAV for heart failure. Supported by extensive data from *in vitro*, small animal, and large animal studies that showed improvement of cardiac contractility by AAV1.SERCA2a gene therapy [4], the CUPID Phase 1/2a trial (Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease) began in 2007 in the United States. This early trial demonstrated a reduced number of clinical events accompanied by favorable functional parameters [5] and pushed the trial forward to Phase 2b, which was a randomized, double-blinded, placebo-controlled, international trial. Results were announced in 2015 with disappointment: there was no significant benefit of AAV1.SERCA2a gene therapy in patients with NYHA class II–IV heart failure [6]. Sister trials that were also studying AAV1.SERCA2a were terminated shortly after this announcement.

Since then, there had been no cardiac-specific AAV gene therapy clinical trials. However, one clinical trial launched very recently. The NAN-101 trial, which is sponsored by Asklepios Biopharmaceutical, Inc. (ClinicalTrials.gov Identifier: NCT04179643) started in November, 2019 and is examining the effect of chimeric AAV (BNP116) based gene delivery of constitutively active inhibitor-1 for patients with congestive heart failure. This is a Phase 1 open-label, dose-escalation study using intracoronary delivery in 12 patients. The company announced dosing of first patients in February 2020. In addition, a few AAV gene therapy trials targeting muscular diseases are ongoing. Because many muscular diseases accompany cardiomyopathy, cardiac function is also an important outcome of these studies. Gene Therapy for Male Patients With Danon Disease Using RP-A501 (ClinicalTrials.gov Identifier: NCT03882437) is an ongoing trial sponsored by Rocket Pharmaceuticals that began in April 2019 and is

DOI: 10.18609/cgti.2020.073

testing AAV9-based systemic LAMP2B gene delivery in male patients with Danon disease. The vectors are injected systemically through the intravenous route, targeting the heart as well as skeletal muscle. Two other trials targeting Duchenne Muscular Dystrophy also use intravenous AAV9 delivery with different gene constructs. IGNITE DMD (ClinicalTrials.gov Identifier: NCT03368742) is a study sponsored by Solid Biosciences and delivers microdystrophin in 16 patients. A Study to Evaluate the Safety and Tolerability of PF-06939926 Gene Therapy in Duchenne Muscular Dystrophy (ClinicalTrials.gov Identifier: NCT03362502), sponsored by Pfizer, delivers mini-dystrophin in 15 patients. Positive results in muscle gene transduction and functional improvement have been reported in the Pfizer trial [7], but its impact on cardiac function has not been revealed. A Phase 3 study is expected to begin in 2020. Because these trials targeting muscular diseases deliver modified genes (truncated or engineered), immune response to the transgene remains a concern. In fact, IGNITE DMD trial is currently on clinical hold due to the occurrence of treatment-related serious adverse events in treated patients. Anti-immune drugs are given to these patients to avoid immune reactions, and how that might affect gene transduction is of interest.

IMPLICATIONS FROM CUPID TRIAL FAILURE

While the initiation of new trials is exciting and fuels our enthusiasm for realizing clinical translation of cardiac AAV gene therapy, it is important to learn from previous failures. To seek possible explanations for failure of the CUPID trial, the hearts of subjects who unfortunately died or underwent cardiac transplant were examined. Unexpectedly, there was little vector genome found in analyzed tissues, suggesting deficient gene transfer rather than ineffective function of transgene [8]. This result indicates that our current challenge remains in delivery and, until this issue is overcome, we will not be able to examine the therapeutic effect of transgenes. Box 1 summarizes the problems we currently face for successful clinical translation of cardiac gene therapy.

WHAT ARE THE FACTORS THAT DETERMINE CLINICAL EFFICACY OF CARDIAC AAV GENE THERAPY?

Clinical efficacy of cardiac AAV gene therapy is influenced by numerous factors but can be classified to three main categories: factors that regulate gene transduction, factors associated with transferred gene, and factors associated with the recipient of gene therapy.

Factors that regulate gene transduction

Gene transduction efficacy is a key parameter that determines the success of gene therapy. Importantly, consistent evaluation of gene transduction efficacy in clinical trials is extremely challenging because cardiac tissues cannot be easily obtained and there is currently no established method to non-invasively track transgene expression. Compared to functional gene assessments, the characterization of cardiac transduction efficacy for pre-marketing production testing studies is often not very extensive and is usually limited to dose-determination studies.

The key factors that regulate cardiac gene transduction include the vector, dose, and delivery method. These factors are inter-related and their optimal combination may also depend on the target disease, transgene and studied animal species. For example, AAV serotype tropism may differ depending on the route of delivery. Endothelial barrier might inhibit transduction more in certain serotypes after intravascular delivery. While AAV9 has been shown to be most cardiotropic in rodent studies [9], large animal studies that used direct injection of AAVs generally

BOX 1

Problems encountered by cardiac gene therapy

- Limited experience in human cardiac gene therapy
- Optimal combination of AAV serotype, promoter, and delivery method for human heart is unknown
- No evidence of sufficient gene transduction in human heart
- Difficulty in evaluating transgene expression *in vivo*
- Difficulty in detecting decreased expression of target gene prior to therapy
- High prevalence of patients with neutralizing antibody
- High cost for producing sufficient amount of AAV vectors for human heart
- Unknown influence of concurrently administered anti-immune drugs, if indicated

showed higher transduction using AAV6 [10,11]. Whether this was due to differences in the delivery method or animal species remains unclear. It is of note that although direct intramyocardial injection overcomes endothelial barrier, distribution of gene expression is generally around the peri-injection sites only [12]. The effective dose of a given vector is also likely to be influenced by the method of delivery. The choice of promoter is another important factor regulating gene transduction. The relationship between AAV dose and gene expression can be influenced by promoter efficiency. However, the way this interaction operates in the human heart remains unknown, even for commonly used promoters. New clinical studies to implement transduction analysis using MRI or endomyocardial biopsies might improve our understanding of these elements. Finally, gene delivery method can also influence gene transduction and distribution. Current AAV gene therapy technology does not allow 100% transduction of the heart and various degrees of heterogeneity can be seen after gene delivery, by which distribution is largely influenced by the delivery method. For therapeutic efficacy, the percent of cells in the heart that must be transduced likely depends on the therapeutic gene. For AAV1.SERCA in CUPID, estimated expression was <1% compared to preclinical studies in rodents.

Factors associated with transferred gene

These factors are associated with the function of the transgene and host immune reaction to transgene. Obviously, the gene delivered to the heart needs to have a therapeutic effect in human disease. Commonly, the function of the transgene is well characterized before moving into a clinical trial and the efficacy and safety of gene transfer are supported by preclinical studies. Nevertheless, animal models are limited in their ability to provide translatable information about the immunoreactivity of a vector. Furthermore, differences in intracellular signaling and protein functions/interactions between humans and animals might cause unexpected effects that were not seen in preclinical studies. It is important to note that changing the cellular properties of cardiac cells might also affect electrophysiological properties of the heart and lead to increased arrhythmias. Immune reaction to transgenes might also cause arrhythmias. Detection of arrhythmias can be difficult in animal models, as arrhythmia monitors are not always implanted and preclinical studies tend to be of relatively short duration and could fail to identify longer-term effects. Additionally, gene expression in off-target organs can also induce unanticipated effects. As mentioned above, lack of evidence in successful cardiac gene transduction in the clinical studies precludes assessment of whether the transferred gene function was ineffective in humans.

Factors associated with recipient of genes

The basic concept of gene therapy is to intervene in the intracellular signaling process by supplementing endogenously low expression of genes or inhibiting highly active genes in a disease setting. This is more straightforward

DOI: 10.18609/cgti.2020.073

for genetic diseases, where we know that endogenous genes are absent or defective. In contrast, when targeting more prevalent diseases like heart failure, the rationale of gene therapy relies on an assumption that endogenous expression of target genes is low (or high), based on previous studies. However, confirmation of gene expression levels is challenging in the heart and the actual protein level in a specific patient may not be dysregulated. In a patient of this type, increasing expression of a certain gene from normal to high may not have much benefit. This might have been the case for SERCA2a gene therapy in the CUPID trial since a milder patient population was included in the later phase study (NYHA class II), in contrast to the inclusion of a more severe patient population in the early phase trial (NYHA class III–IV). There is limited evidence that NYHA class II patients have low endogenous SERCA2a expression, and highlights the importance of a well-designed study. For gene supplementation therapy, the abundance of endogenous expression relative to the expression level achieved by gene transfer should be taken into consideration. The same degree of overexpression could result in ten-fold supplementation or add little depending on the abundance of existing endogenous gene and protein expression. Other factors associated with the recipient (patient heart) include immune responses during and after AAV delivery and type of cardiac disease. Immune responses typically minimize the efficacy of gene transfer, both acutely and chronically. A heart failure with a large infarction may not benefit from gene therapies targeted at improving cardiomyocyte function in the absence of remaining viable myocardium.

ASSURING SAFETY OF CARDIAC AAV GENE THERAPY

In addition to optimizing the above factors for effective gene transduction, assuring the safety of therapy is another important aspect of realizing clinical application. The delivered gene should not induce side effects such as arrhythmias, vectors and transgenes should not induce a severe immune response, and the delivery method should not compromise already impaired cardiac function in gene therapy candidates. In this regard, AAV vectors have showed excellent safety profiles in previous clinical trials including those targeting the heart. However, it is likely that some modification of vector, dose, or delivery method will be required to overcome current problems in low cardiac gene transduction. Therefore, any modification should be thoroughly evaluated for safety in available systems before actual testing in humans.

REMAINING CHALLENGES FOR SUCCESSFUL CLINICAL TRANSLATION OF CARDIAC AAV GENE THERAPY

As discussed above, there is little evidence that we have been able to overexpress genes successfully in the human heart using AAV. The current major challenge is to overcome low gene transduction efficacy without compromising safety. The chimeric vector being tested in NAN-101 is certainly promising and we look forward to more of these vector modification approaches. Prior to enrollment in clinical trials, patients should be screened for pre-existing anti-AAV antibodies that could neutralize vector before gene delivery to the myocardium. Cardiac targeted gene delivery methods need to be refined and this is one of the focused topics in our lab. Difficulties in detecting gene expression in the heart might be overcome by using sophisticated imaging modalities. Similar strategies for refinement in angiogenic gene therapy have been reviewed in an earlier publication [13]. Not exempt from other gene therapies that are already in clinical arena, cost and production of vectors is another issue once we become able to transduce the heart effectively. We believe improving gene transduction efficacy will also allow reduction of total vector dose required to exert therapeutic effect, promoting cost containment.

TRANSLATION INSIGHT

Many studies report successful correction of cardiac pathology using AAV gene therapy in isolated cells and small animals. These results indicate that gene therapy can significantly improve the fate of patients with chronic cardiac disorder, once AAV gene transduction efficacy can be improved. We believe that more emphasis on research focused on refining vectors and gene delivery methods is the current key to realizing clinical translation of cardiac AAV gene therapy.

REFERENCES

1. Benjamin EJ, Virani SS, Callaway CW *et al.* Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. *Circulation* 2018; 137: e67–e492.
2. Hulot JS, Salem JE, Redheuil A *et al.* Effect of intracoronary administration of AAV1/SERCA2a on ventricular remodelling in patients with advanced systolic heart failure: results from the AGENT-HF randomized phase 2 trial. *Eur. J. Heart Fail.* 2017; 19, 1534–41.
3. Yla-Herttuala S, Baker AH. Cardiovascular Gene Therapy: Past, Present, and Future. *Mol. Ther.* 2017; 25: 1095–106.
4. Kawase Y, Hajjar RJ. The cardiac sarcoplasmic/endoplasmic reticulum calcium ATPase: a potent target for cardiovascular diseases. *Nat. Clin. Pract. Cardiovasc. Med.* 2008; 5: 554–65.
5. Jessup M, Greenberg B, Mancini D *et al.* Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2⁺-ATPase in patients with advanced heart failure. *Circulation* 2011; 124: 304–13.
6. Greenberg B, Butler J, Felker GM *et al.* Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial. *Lancet* 2016; 387: 1178–86.
7. Curtis M, Philipson R. Cell and Gene Therapy Commercial Insight – June 2019. *Cell Gene Ther. Ins.* 2019; 5: 983–97.
8. Hulot JS, Ishikawa K, Hajjar RJ. Gene therapy for the treatment of heart failure: promise postponed. *Eur. Heart J.* 2016; 37: 1651–8.
9. Pacak CA, Mah CS, Thattaliyath BD *et al.* Recombinant adeno-associated virus serotype 9 leads to preferential cardiac transduction *in vivo*. *Circ. Res.* 2006; 99: e3–9.
10. Gabisonia K, Prosdocimo G, Aquaro GD *et al.* MicroRNA therapy stimulates uncontrolled cardiac repair after myocardial infarction in pigs. *Nature* 2019; 569: 418–22.
11. Gao G, Bish LT, Sleeper MM *et al.* Transendocardial delivery of AAV6 results in highly efficient and global cardiac gene transfer in rhesus macaques. *Hum. Gene Ther.* 2011; 22: 979–84.
12. Vassalli G, Bueler H, Dudler J, von Segesser LK, Kappenberger L. Adeno-associated virus (AAV) vectors achieve prolonged transgene expression in mouse myocardium and arteries *in vivo*: a comparative study with adenovirus vectors. *Int. J. Cardiol.* 2003; 90: 229–38.
13. Rubanyi GM. Mechanistic, technical, and clinical perspectives in therapeutic stimulation of coronary collateral development by angiogenic growth factors. *Mol. Ther.* 2013; 21: 725–38.

AFFILIATIONS

Kelly P Yamada
Cardiovascular Research Center, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Serena Tharakan
Cardiovascular Research Center, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Kiyotake Ishikawa
Author for correspondence
Cardiovascular Research Center, Icahn School of Medicine at Mount Sinai, New York, NY, USA
kiyotake.ishikawa@mssm.edu

DOI: 10.18609/cgti.2020.073

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *This work was supported by NIH R01 HL139963 (K.I.). The authors would like to acknowledge the Gene Therapy Resource Program (GTRP) of the National Heart, Lung, and Blood Institute, National Institutes of Health for their vector support.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

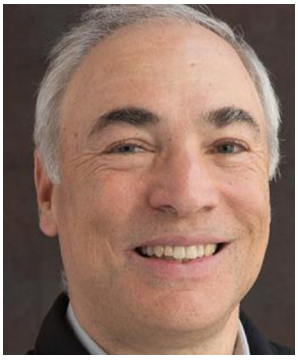
ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Mar 3 2020;* ***Revised manuscript received:*** *Apr 28 2020;* ***Publication date:*** *May 14 2020.*

INTERVIEW

Progressing Bristol Myers Squibb's clinical-stage pipeline of cellular cancer immunotherapies



STANLEY R FRANKEL Following the acquisition of Celgene in 2019, Stan Frankel joined Bristol Myers Squibb as Senior Vice President, Cellular Therapy Development. Prior to his role at Bristol Myers Squibb, Stan served as Corporate Vice President, Head of Immuno-oncology, Clinical Research and Development, at Celgene for nearly five years. He oversaw the durvalumab alliance with Medimmune/AstraZeneca, the tislelizumab alliance with BeiGene, and the initial Celgene clinical development alliance with Juno Therapeutics. In addition to serving as co-chair and representative for various hematology/oncology development committees and leadership teams, Stan was the Head of the Cellular Therapy Center of Excellence and chaired the Celgene Protocol Review Committee. Earlier in his career, Stan led hematology and oncology development programs in all phases of clinical development at Genta Therapeutics, Merck Research Labs, Roche, Micromet, and Amgen, and was instrumental in the approvals of Blincyto® and Zolinza®. Stan has internationally recognized clinical expertise across hematologic malignancies including acute promyelocytic leukemia (APL), acute lymphocytic leukemia (ALL), lymphoma and Waldenstrom's macroglobulinemia. He has served as an academic investigator for the development of more than a dozen approved oncology drugs and has authored more than 80 peer-reviewed scientific papers. Previously, he had an academic practice in stem cell transplantation and hematologic malignancy clinical trials at Roswell Park Cancer Center, Georgetown University and the University of Maryland. Stan is a Diplomate of the American Board of Internal Medicine with subspecialty credentials in Hematology and Medical Oncology. He is also an Adjunct Associate Professor of Medicine at the Vagelos College of Physicians and Surgeons at Columbia University and is licensed to practice in New York. He is a Fellow and member of the American College of Physicians (ACP), and a member of the American Association for Cancer Research (AACR),

American Society for Transplantation and Cellular Therapy (ASTCT), American Society of Clinical Oncology (ASCO), American Society of Hematology (ASH), European Hematology Association (EHA), and European Society for Medical Oncology (ESMO). Stan received his BA from Harvard College and his MD from Northwestern University. He received his post-graduate training at Mount Sinai Hospital in New York, and Memorial Sloan-Kettering Cancer Center, where he was Chief Fellow.

Cell & Gene Therapy Insights 2020; 6(5), 738–744

DOI: 10.18609/cgti.2020.086

Q What are you working on right now?

SRF: We have three candidates currently in late stage clinical trials. The first of these is lisocabtagene maraleucel, known as liso-cel or JCAR17. This is an autologous CD19-directed cell therapy product. What's different about liso-cel from either of the already-approved CD19 constructs is that the manufacturing process is distinct. We control the ratio of the CD4 and CD8 lymphocytes in a very precise fashion, so that we look to deliver to the patients a one-to-one ratio of the CD4 and CD8 subtypes.

The second product is directed against B-cell maturation antigen, or BCMA; it's known as idecabtagene vicleucel, or ide-cel for short, and is being studied in patients with relapsed and refractory multiple myeloma.

The third product candidate is known as orvacabtagene autoleucelor, or orva-cel. This is also directed against BCMA but has a distinctive design with a fully human scFv binder (the part that recognizes the target) and other design features that allow it to potentially have better persistence, which may lead to more durable responses in patients. This is being studied in a similar population to ide-cel in relapsed and refractory multiple myeloma.

Looking across the portfolio, we've now treated more than 1,100 patients. We have approximately 16 open clinical trials, two of which have now matured to the point where the pivotal data are under review by regulatory authorities for approval.

Q There is obviously no getting away from COVID-19 at the moment – what has been the impact on the clinical development pipeline, and how you are seeking to minimize it as far as possible?

SRF: It became very clear as the WHO declared the pandemic in March that the intricacies and the demands on the medical systems, and the risk to the patients who would get a cellular therapy, were different than for the general population requiring medical care, or even the general cancer patient population. We went through a series of additional safety measures with our investigators early on in order to encourage testing and provide some guidance on how we thought patients could be safely screened and managed during periods of peak demand on the system.

We put patients, our employees, and our clinical investigators and staff first, and so following those discussions, we made the tough decision to temporarily suspend enrolment in our ongoing trials. There were just too many uncertainties in terms of whether patients would be able to be treated safely and whether study sites would be able to actually comply with the requirements of the clinical trials. Ultimately, that would affect the integrity of the data that had been gathered from other patients who might not have been impacted by the pandemic.

> "...how do we design the constructs and manufacture them in a way that we think will offer the maximal benefit for the product?"

So we went on a temporary pause. We have lifted that pause now and are open for business again. However, it is not quite business as usual, because many of our sites are in cities, states, or countries where there are still shelter-in-place orders. That type of disruption doesn't allow for safe conduct of clinical trials.

Q There has been much excitement in the immuno-oncology field around the potential of CAR T cell immunotherapy-checkpoint inhibitor combinations – what can you tell us about BMS's current and future plans in this particular area?

SRF: We are in a very fortunate place that we have leadership in both domains – in checkpoint inhibitors with Yervoy® (ipilimumab) and Opdivo® (nivolumab), and with the three cell therapy products I mentioned earlier. We are carefully considering what new studies we would like to initiate now that we are one company, and cell therapy is integral to BMS.

However, we will only go where the science takes us. We have actually done this experiment already – with Imfinzi® (durvalumab) in our earlier Celgene-AstraZeneca alliance – and while we saw some hints of interesting activity when combined with liso-cel, it was not such a dramatic improvement in outcomes that we are prioritizing a huge investment now in moving forward with nivolumab.

There will be some continued work looking at why patients don't have good responses to the cellular therapies, or lose those responses, to see if we can find a way to match these assets in our overall immuno-oncology portfolio. That involves not only the approved agents, but several clinical-stage compounds that are currently undergoing trials. We'll aim to profile the defects in the patients who aren't having an optimal response to the CAR T cells, and do this in a thoughtful, precise manner.

We're working on revising our protocols to go in that direction. But our emphasis has been more on combinations with other agents in our portfolio, and with the cereblon modulators in particular. For example, Iberdomide (CC-220) has published data on its activity in relapsed refractory multiple myeloma, but this compound also has activity in lymphoma.

We have validated the potential of this combination in preclinical studies and are actively enrolling patients in our platform trial, where patients will receive liso-cel with an overlap of iberdomide during the first month or two of therapy in order to augment the activity of the CAR T cells. We will be looking for any increase in the cells' potency and persistence, as well as any direct anti-lymphoma activity that iberdomide may exert. We are also in the planning stage to do exactly the same thing with ide-cel in multiple myeloma patients, again with iberdomide.

We are also engaged in an academic collaboration generating some very interesting, promising results in modulating the surface expression of b-cell maturation antigen in myeloma patients. This is through inhibition of an enzyme known as gamma secretase, which cleaves off BCMA from the membrane and then enters the circulation. If you inhibit gamma secretase you actually increase the antigen expression of BCMA on target cells. It's an interesting hypothesis we will test further in an upcoming combination trial with ide-cel.

So these studies have been a bit more of a priority on the myeloma front than other I-O combinations. On the lymphoma front, in addition to testing iberdomide, we are looking at what a BTK inhibitor, ibrutinib, may do both to the quality of the cells we produce, as well as to their expansion and persistence.

Q Are there any particular issues or challenges relating to such combinations that need working through?

SRF: Like any other set of combinations in drug development, you have to have a reasonable understanding of each of the individual components: their dose, schedule, and toxicity. You then have to be very thoughtful in terms of how you design the studies to combine the agents, making sure that as you escalate doses or change schedules, you are watching closely for safety signals.

We've done this with the checkpoint inhibitors and liso-cel, and safety was not an issue. Really, we were just disappointed that we didn't see more dramatic efficacy.

So I think we know what to do. We know how to do it in a safe manner. But it does require time – you can't expose more than a handful of patients over a period of a month or two, in order to make sure that any delayed toxicities are accounted for before you increase the exposure to a larger number of subjects.

Q What are the major trends you see in terms of trial design and endpoint selection for cellular immunotherapies?

SRF: There has been a great opportunity, which is now going to turn into a challenge for the cellular immunotherapy field – particularly for those products that are not first to gain a regulatory approval for a given target.

The first two CAR T cell therapy approvals – and indeed, our own first two filings – are based on single-arm clinical trials. That was acceptable for the first two compounds, and

 DOI: 10.18609/cgti.2020.086

"We have three candidates currently in late stage clinical trials ... Looking across the portfolio, we've now treated more than 1,100 patients. We have approximately 16 open clinical trials, two of which have now matured to the point where the pivotal data are under review by regulatory authorities for approval."

hopefully will be acceptable for ours, because of the extreme magnitude of benefit that was demonstrated in each case. If you take a population where you would expect a response rate (any type of response) to be in the region of 10–20% of patients, and suddenly you're getting high quality responses in 75–90% of patients, and if those responses are durable, you likely don't need a randomized trial to show this is different to anything else we have – it addresses an unmet medical need and it clearly needs to be considered rapidly by the regulators without doing a larger, longer, more expensive randomized clinical trial.

Q However, once the first few such products are on the market, the challenge will be how do you bring a next-in-class compound for that target through the regulatory process without a randomized trial?

SRF: We've piloted this for our own filings by creating synthetic clinical trials where we use real world evidence in order to match the characteristics of the patients and show the benefit. We are really excited to be showing that data at ASCO and at European Hematology Association. We've been able to do a matched comparison to the patients in the KarMMa registration study for ide-cel, providing additional assurance that the dramatic benefit we see with ide-cel is indeed statistically significant when compared to what those patients might get in a synthetic clinical trial, where they've exhausted all the other available therapies.

Q What are the key areas of emerging enabling technology for the cellular immunotherapy space, in your view?

SRF: I think everything starts with high quality science, followed by making sure you're collecting the relevant data, and not being biased in thinking you know the answer until you're able to interrogate that data appropriately.

For us, technical innovation begins with the question of how do we design the constructs and manufacture them in a way that we think will offer the maximal benefit for the product? That can involve everything from the binder, to the backbone, to the vector, to the spacer, to the activation regions of the CAR. All of these things are in play, because any improvement along the way in the construct design may pay off as a benefit downstream when the product actually goes into the patient. You have to be able to learn across compounds, across constructs, and from both your preclinical work and clinical data, as to which of these things might be the most important as you change a variable. We're really looking at all of them – better binders, better spacers, better design, additional activators – in order to come up with a better overall construct.

The next step is how do you actually manufacture your product. This is not only a matter of quality and control, but also a matter of speed. Shorter processes, serum-free processes, reduced risk processes, processes that are able to generate a higher yield or higher quality of cells – all of these have value. We are very interested in what we do in improving manufacturing and we've invested heavily in our Seattle-based team. We are looking at every type of new enabling bioprocessing technology to see if we can take the same construct and just by changing one of the steps in the manufacturing process, can dramatically improve the yield or the speed of production, or the actual activity of the final product.

I think you can broadly see where we are heading by the technologies that we have brought in to date. For example, we've brought in new ways in our collaboration with Immatics to screen for neoantigens that might be targeted by T cell receptors. We're really excited about moving beyond CAR Ts to looking at these neoantigens as an approach for engineered T cell receptors, and we look forward to bringing the first of those into the clinic shortly. We're also very interested in gene editing techniques, through our announced collaborations with Editas - that will be for allogeneic-based products moving forward.

We're interested in what we can do with enhancers. This will include our controllable element deal with Obsidian, in which we are looking at IL-12 and CD40 as ways of increasing signaling and activity, and attacking the microenvironment where the CAR Ts need to recognize and kill the target tumor cells.

We're also looking at different cell sources, moving beyond autologous and considering allogeneic cells. We're looking at things besides T cells, including NK cells and pluripotent stem cells – all areas in which there is a great deal of current activity.

"Although I do think a median progression free survival of more than a year with ide-cel is a major accomplishment ... we still want it to be longer."

We're interested in dual constructs – bicistronic constructs – and what we might do there in order to raise that overall survival curve that we're seeing in our patients now.

So while we're gratified that we're seeing a plateau in the lymphoma durable response curve at about 50–60% in terms of those who get a complete response, that still leaves a substantial proportion of patients who aren't getting to a functional cure. And in myeloma that proportion is a little bit larger,

DOI: 10.18609/cgti.2020.086

and although we are seeing great responses there, they're not as durable as those we have in lymphoma. Although I do think that a median progression free survival of more than a year with ide-cel is a major accomplishment, especially in a group of patients who would be counting their response duration from other agents in weeks, we still want it to be longer. Let's get it out to 2, 3, 4, 5, and 10 years. That's what we're looking to these new technologies to help us achieve.

Q Can you comment further on how liso-cel is differentiated from Kymriah® and Yescarta®?

SRF: Obviously, if you're not first-to-market, your clinical data needs to be competitive. We think the data we have with liso-cel shows a positive benefit–risk profile. We have efficacy of 73% overall response rate and 53% complete response rate, and because of the difference in manufacturing, potentially, the safety profile is quite different.

42% of liso-cel patients develop cytokine release syndrome (CRS). There are no head-to-head studies to directly compare but based on available data, the incidence of CRS is much higher with the other commercially approved products. Additionally, time to onset of cytokine release syndrome when it does occur is at five days with liso-cel, whereas generally it is within the first 24–48 hours with the other products.

I think that when products are approved, prescribers will look at how the clinical behavior is different in order to make the choice of what they think is the best option for their patient. We feel we will have a competitive profile with liso-cel based on the data we have generated.

But as I've said, we will continue to innovate. We think liso-cel is a great drug, but we are looking at two of three ways we can improve it even now. One is to come up with a shortened manufacturing process which further skews to a more naive T cell population, and we're looking forward to generating clinical data with that construct very soon.

AFFILIATION

Stanley R Frankel
Senior Vice President, Cellular Therapy Development, Bristol Myers Squibb

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Stanley R Frankel is an employee and shareholder of Bristol Myers Squibb.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *May 29 2020;* ***Publication date:*** *Jun 30 2020.*

DOI: 10.18609/cgti.2020.086

CELL & GENE THERAPY INSIGHTS

CLINICAL DEVELOPMENT STRATEGY, TOOLS & TRIAL DESIGNS

SPOTLIGHT

EXPERT INSIGHT

Delivery methods for cardiovascular cell-based therapies: tools and clinical strategies

Ruben A Alexanian & Amish N Raval

The regenerative capacity of the adult mammalian heart is limited, hindering effective repair and recovery of myocardial tissue after ischemic and non-ischemic injury. Heart failure is a common, lethal, disabling, and costly disorder with rising prevalence and poor prognosis. Numerous human clinical trials are underway to test the potential therapeutic benefit of cells and cell-derived agents for myocardial repair, using an assortment of systemic and local delivery tools and clinical trial strategies. In this review, we highlight the advantages and limitations of emerging tools and trial strategies and provide insights into future tissue engineered biomaterials to enable cell delivery.

Cell & Gene Therapy Insights 2020; 6(5), 559–568

DOI: 10.18609/cgti.2020.065

INTRODUCTION

Heart failure is one of the leading causes of death worldwide, with rapidly rising prevalence and now an epidemic in industrialized nations. Despite advances in pharmaceutical and device therapies, the prognosis remains poor, and often worse than that for some forms of cancer [1–3]. The most common cause of heart failure in the United States is coronary artery atherosclerosis [2]. Apart

from rapid coronary artery reperfusion in the setting of acute coronary syndrome, there are currently no available therapies to prevent the loss of cardiac tissue. There is significant interest in the use of bone marrow derived cells, pluripotent cells and extracellular matrix constructs to repair or remuscularize the myocardium, and thus alleviate the underlying cause of heart failure. The optimal delivery method for these therapies has been as perplexing as the source of cells or biomaterials. Transcatheter intramyocardial injection, coronary artery infusion and open chest surgical methods have evolved as the prevailing routes of cell and cell derived biomaterial delivery in contemporary human trials, although intravenous infusion and cytokine mobilization approaches have been attempted in the past [4–7]. Tissue engineered constructs to deliver cells has emerged as an alternative delivery method that has shown tremendous promise [8,9]. Investigators have also contended with clinical trial considerations such as administering autologous versus allogeneic cells, the optimal control group(s), adaptive trial designs, and novel statistical analysis methods that combine patient centered functional outcomes with traditional major adverse cardiovascular events [10]. Herein, we review modern cell delivery methods used for clinical investigation, highlight emerging technologies and discuss clinical trial design considerations.

DIRECT TRANSCATHETER DELIVERY SYSTEMS

Intracoronary catheter delivery systems

Intracoronary catheter infusion delivers cells through patent coronary arteries to localized areas of the myocardium. Over-the-wire coronary infusion and coronary balloon angioplasty catheters have been employed for intracoronary infusion. The central guidewire lumen is used for infusing the investigational agent to the distal coronary bed. Temporary interruption of antegrade coronary blood flow can be accomplished by inflating the balloon to low atmospheres, which increases dwell time, albeit with unclear benefit in regards to acute cell retention [7,11]. Microvascular obstruction, worsening ischemia and edema are concerns for intracoronary infusion with certain cell types. Currently, there are no coronary balloon angioplasty catheters with FDA approval for cell-based therapies [4,7,12]. In instances where coronary artery revascularization is not an option, retrograde coronary venous infusion has been trialed [13,14], although this method is limited by site-specific targeting. In either case, low cell retention has been a problem.

Transendocardial catheter delivery systems

Investigational transendocardial catheters used in human trials have included the Helix™ (BioCardia Inc, San Carlos, CA), MyoCath™ (Bioheart Inc. Sunrise, FL), Myostar™ (Biologic Delivery Systems, Irvine, CA), C-Cath® (Cardio3 Biosciences, Mon-Saint-Guibert, Belgium) and Stiletto™ (Boston Scientific, Marlborough MA). These deflectable catheters are steered via a peripheral artery, and advanced retrograde across the aortic valve into the left ventricle. In the case of the Helix™, the helical tipped injection needle is telescoped within a deflectable guide (Morph®, Biocardia, San Carlos CA). They all have a distal beveled injection needle with diverse shapes [7]. Intramyocardial delivery occurs by penetrating the myocardium using the needle and infusing the investigational agent through a proximal port. Most transendocardial injection systems were developed in parallel with cell products in clinical trials and consequently have undergone extensive biocompatibility testing with regulatory approval [7,15]. The Myostar™ system is tracked using an electromechanical mapping technology (NOGA) that permits delineation of viable and nonviable myocardium. The remaining catheters utilize X-ray based roadmap

DOI: 10.18609/cgti.2020.065

images for targeting [16]. Transendocardial injection offers increased cell engraftment but can risk myocardial perforation.

Surgical direct injection

Direct myocardial injection, primarily at the time of coronary artery bypass surgery, provides a direct route for administration of cells localized to site of injury [4,17,18]. As with other forms of intramyocardial injection systems risks include arrhythmias [19] and ventricular wall injury with an additional caveat of invasive open-heart surgery. Surgical intramyocardial injection has shown great variability in delivery efficiency and cell retention compared to catheter approaches [20].

SYSTEMIC DELIVERY METHODS

Intravenous infusion

Intravenous infusion is the least invasive, readily available, and potentially most economical method of cell delivery for cardioregenerative therapy. This approach is generally considered safe and has been tested primarily with cells of hematopoietic origin [6,21,22]. More recently, some studies have highlighted the paracrine effects of intravenous stem cell therapy [23–25]. Yet, intravenous infusion of cells requires intact homing mechanisms, which significantly dissipate in the case of chronic infarction, for example. Further, this approach is hampered by low cardiac cell retention due to reticuloendothelial egress through a pulmonary first-pass effect [26,27]. For these reasons, intravenous infusion for cell-based therapies has largely been abandoned for methods that are more direct.

Bone marrow stem cell cytokine mobilization

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth factor that can mobilize cells from the bone marrow to the peripheral blood. It has been previously suggested that G-CSF mobilized bone marrow stem cells regenerate and repair myocardial tissue [28]; however, subsequent, pre-clinical animal models have shown mixed results in acute myocardial infarction animal models [28–30] and small, randomized human trials. For example, G-CSF as an adjunctive therapy post-acute infarction was not associated with in improved left ventricular function in human trials [31–35].

NEXT GENERATION DELIVERY APPROACHES

Irrespective of all available clinical delivery methods, cell retention has been poor with fewer than 10% of the injected cells detectable after 24 hours [7,36,37]. Many strategies have been tested to promote engraftment and survival of stem cells following transplantation, including cell preconditioning and encapsulation, genetic modification of donor cells, and myocardial tissue engineering [4,37].

Myocardial tissue engineering

Injectable bioactive hydrogels

One approach to improve cell survival and retention is to deliver bioengineered patches or injectable biomaterials that contain the cells of interest. Both natural or synthetic biomaterials have been explored to aid in cell engraftment. Bioactive hydrogels have shown efficacy in animal models, using a variety of cell types [9,38–45]. These hydrogels are thought to replenish locally damaged extracellular matrix while establishing a more hospitable environment for transplanted cells and myocardial regeneration. For example, Schmuck *et al.* previously showed that human cadaveric cardiac fibroblast derived matrix scaffolds (cECM) express abundant fibronectin. This biomaterial can be lyophilized and milled to powder form and combined with therapeutic cells to improve cell retention [9,46].

Bioengineered myocardial patch

Isolated cell transplantation may be insufficient for treatment of large areas of tissue injury. Bioengineered myocardial patch-like constructs may serve as an alternative to injectable strategies with goal of providing a viable and autologous tissue for repair and remodeling. A variety of patch-like constructs have been used, including extracellular matrix derived natural polymers and synthetic polyesters [47–52]. For example, human cadaveric cardiac fibroblasts derived extracellular matrix patches improve cell retention and migration in mouse and pig MI models [46]. Others have used 3D bioprinters [53,54], stacking of cell monolayers [55], and micro-fabricated systems [56] among many other approaches to assemble cardiac patch-like constructs. More recently, a human embryonic stem cell derived cardiovascular progenitors embedded in a fibrin patch were epicardially delivered during a coronary artery bypass procedure in humans [57].

Direct *in vivo* reprogramming

Circumventing the issues associated with cell delivery, others have tried to directly reprogram in-situ native non-cardiomyocyte cells into progenitor-like cells for cardiac regeneration [58]. Cardiac fibroblasts are abundant in the native myocardium and have recently been reprogrammed *in vivo* using retro and adenoviruses overexpressing specific transcription factors and micro-RNAs with impressive recovery of cardiac function in animal models [58–60]. Challenges including low reprogramming efficiency, potential toxicity of retrovirus and lentivirus vectors for gene transfer, and concern for immune response remain.

TRANSLATION INSIGHTS

Characteristics of an optimal delivery method for cell-based therapies for cardiac repair include being minimally invasive and easily accessible with a low cost. Such a delivery system should have minimal or no risk of adverse complications such as microembolization, arrhythmogenicity, and tissue injury. The system should address the critical problem of low cell retention, which is likely related to rapid egress from the tissue via lymphatics and veins in the injured myocardial environment [61,62]. Progress has been made using tissue bioengineered constructs to deliver cells. Engineered cardiac tissue scaffolds results in a 10-fold higher cell engraftment rate as compared with the direct myocardial injection of cells [62]. However, lethal arrhythmias due to the lack of electro-mechanical integration between the host-patch interface is a major problem. Implanting large patches also requires surgical access to the heart. A practical concession includes locally injectable bioengineered hydrogels to create a more hospitable microenvironment for transplanted cells to improve cell retention while circumventing the need for open chest surgery. Use of road map technologies such as electro-anatomic mapping or CT/MRI co-registration imaging may enable accurate targeted delivery of injectable biomaterials plus cells in the future.

CLINICAL TRIAL STRATEGIES

The advent of cell-based therapy trials has resulted unique approaches to ensure robust and informative clinical trial designs and strategies. Hypotheses, sample size, screening, randomization, blinding, control and treatment groups, endpoints, data monitoring, and statistical analysis are consistent elements of any human trial. However, in the context of cardio-regenerative medicine, adaptive trial design models have emerged, enabling a prospectively defined scheme to use accumulating data to modify the course of trial while it is ongoing [10,63,64]. While adaptive approaches are commonly used in cancer therapy trials, it is still uncommon for cardiovascular disease trials. For

DOI: 10.18609/cgti.2020.065

example, an adaptive trial design approach has been embraced with Phase 3 DREAM-HF clinical trials [65,66]. Adaptive strategies promise to facilitate a faster, cost effective pathways to clinical research objectives without compromising trial statistical integrity or ethics [65].

Furthermore, clinical trial design for autologous cell therapies where cells are harvested from the patient, and then re-administered to the same patient are viewed as an overall treatment strategy, where the risk implications of the harvest procedure itself are factored into the safety analyses. Double-blinding for autologous cell therapy trials usually requires two separate teams:

1. Unblinded harvest and treatment team;
2. Blinded follow-up team, which adds logistical challenges and cost [10].

The appropriate control group to use to test autologous cell therapy has also been debated. One approach is to compare the autologous cell treatment to standard of care, as is the case for the Phase 3 Bone marrow in Acute Myocardial Infarction (BAMI) trial [67]. This trial is comparing intracoronary infusion of bone marrow-derived mononuclear cells to standard of care. In contrast, the Phase 3 RENEW [68] which tested autologous $CD34^+$ cells required placebo injections in the control group. The ongoing Phase 3 DREAM Heart Failure and pivotal CardiAMP Heart Failure trials use sham procedures, where arterial access is obtained, but no transendocardial catheters are inserted in the blinded control group [65,66,69]. Sham or placebo injection procedures may introduce a 'placebo effect' phenomenon which has been observed to improve symptoms in studies, particularly when invasive procedures are performed [70].

TABLE 1

Randomized clinical trials with sample size ≥100 in the experimental arm (2010–2020).

Trial	Cells	Sample size (experimental/control)	Model	Route of cell administration	Primary efficacy endpoint	Outcome
SWISS-AMI [73]	BMMNC	133/67	ACS	Intracoronary	D in LVEF by quantitative MRI at 4 months	Negative
BOOST-2 [74]	BMMNC	127/26	ACS	Intracoronary	D in LVEF by quantitative MRI at 6 months	Negative
ACT34-CMI [75]	BMMNC-$CD34^+$	112/56	Refractory angina	Transendocardial	Frequency of angina episodes at 6 months	Positive
CHART-1 [76]	BMMNC-CSC	120/151	ICM	Transendocardial	FS hierarchical composite (all-cause mortality, worsening heart failure, MLFHQ, 6-min walk distance, LVESV, and ejection fraction) at 39 weeks	Negative
DREAM-HF [65]	BMMNC	566	ICM, DCM	Transendocardial	Time to recurrent HF-MACE prior to the first terminal cardiac event	Ongoing
Cardi-AMP-HF [66]	BMMNC	160/100	ICM	Transendocardial	Composite of 6-min walk distance (6MWD), death, or major adverse events that precludes assessment of 6MWD	Ongoing

ACS: Acute coronary syndrome; BMMNC: Bone marrow mononuclear cells; CSC: Cardiopoietic stem cell; DCM: Dilated cardiomyopathy; FS: Finkelstein–Schoenfeld; HF-MACE: Non-fatal decompensated heart failure major adverse cardiac event; ICM: Ischemic cardiomyopathy; LVEF: Left ventricular ejection fraction; LVESV: Left ventricular end systolic volume; MLFHQ: Minnesota Living with Heart Failure questionnaire.
Sources of data: PubMed, ClinicalTrials.gov, Cochrane Library.
Search Criteria: Randomized clinical trials with sample size ≥100 in the experimental arm, dates 2010–2020.

Finally, defining a primary endpoint in clinical trial is critical [71]. Several clinical trials are transitioning to composite endpoints to show therapeutic benefit with a more manageable sample size. Recent trials, such as the DREAM Heart Failure trial, are combining the composite endpoint analyses in an adaptive trial design. Composite endpoint scores should ideally have objective, clinically meaningful event categories that are interrelated and directionally concordant [10]. For instance, the CardiAMP Heart Failure trial has 6-minute walk distance as the primary endpoint but allocates death, hospitalization, and quality of life scores in a stepwise hierarchical fashion, which allows the most significant clinical outcome to supersede less clinically important outcomes [10,66]. Another approach, proposed by Finkelstein and Schoenfeld, utilizes simple non-parametric test which assigns a score of 1 (better), 0 (same), and – 1(worse) to the experimental patient group in comparison to the control arm for clinically meaningful events that are ordered in a hierarchy of clinical importance and at a pre-specified follow-up time net scores are compared [10,72]. Table 1 provides a list of randomized clinical trials with sample size ≥100 in the experimental arm from the years 2010–2020.

CONCLUSION

The optimal cell population and delivery method to repair the heart is unknown, but there is a flurry of effort worldwide to elucidate an answer. Nearly all cell delivery methods are plagued by low cell retention, although transendocardial injection and intracoronary infusion have prevailed as the most used methods of delivery in recent human trials. Advances in tissue bioengineering have led to variety of natural and synthetic tissue constructs that may overcome the problem of low cell retention. Injectable hydrogels may offer a pathway toward minimally invasive cell delivery with boosted cell retention, using transendocardial catheter injection as an example. Direct *in vivo* reprogramming has shown early promise; however, numerous practical hurdles prevent straightforward translation into human trials. Careful clinical trial design is critical for achieving an accurate estimate of the safety and efficacy of the therapeutic potential of cell-based therapies. Overall, despite the challenges that remain, cell therapy continues to hold great promise for patients afflicted with heart failure and other advanced cardiovascular diseases.

REFERENCES

1. Savarese G, Lund LH. Global Public Health Burden of Heart Failure. *Card Fail. Rev.* 2017; 3(1): 7–11.
2. Virani SS, Alonso A, Benjamin EJ *et al.* Heart Disease and Stroke Statistics-2020 Update: A Report From the American Heart Association. *Circulation* 2020; 141(9): e139–e596.
3. Timmis A, Townsend N, Gale C *et al.* European Society of Cardiology: Cardiovascular Disease Statistics 2017. *Eur. Heart J.* 2018; 39(7): 508–79.
4. Wu KH, Han ZC, Mo XM, Zhou B. Cell delivery in cardiac regenerative therapy. *Ageing Res. Rev.* 2012; 11(1): 32–40.
5. D'Amario D, Leone AM, Borovac JA *et al.* Granulocyte colony-stimulating factor for the treatment of cardiovascular diseases: An update with a critical appraisal. *Pharmacol. Res.* 2018; 127: 67–76.
6. Hare JM, Traverse JH, Henry TD *et al.* A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J. Am. Coll. Cardiol.* 2009; 54(24): 2277–86.
7. Sherman W, Martens TP, Viles-Gonzalez JF, Siminiak T. Catheter-based delivery of cells to the heart. *Nat. Clin. Pract. Cardiovasc. Med.* 2006; 3 Suppl. 1: S57–64.
8. Spinali KL, Schmuck EG. Natural Sources of Extracellular Matrix for Cardiac Repair. *Adv. Exp. Med. Biol.* 2018; 1098: 115–30.
9. Spang MT, Christman KL. Extracellular matrix hydrogel therapies: *in vivo* applications and development. *Acta Biomaterialia* 2018; 68: 1–14.
10. Raval AN. Clinical Trial Design for Investigational Cardio-Regenerative

DOI: 10.18609/cgti.2020.065

Therapy. *Adv. Exp. Med. Biol.* 2018; 1098: 199–211.

11. Keith MC, Tokita Y, Tang XL *et al.* Effect of the stop-flow technique on cardiac retention of c-kit positive human cardiac stem cells after intracoronary infusion in a porcine model of chronic ischemic cardiomyopathy. *Basic Res. Cardiol.* 2015; 110(5): 503.

12. Reinhardt M, Bader A, Giri S. Devices for stem cell isolation and delivery: current need for drug discovery and cell therapy. *Expert Rev. Med. Devices* 2015; 12(3): 353–64.

13. Vicario J, Piva J, Pierini A *et al.* Transcoronary sinus delivery of autologous bone marrow and angiogenesis in pig models with myocardial injury. *Cardiovasc. Radiat. Med.* 2002; 3(2): 91–4.

14. Gathier WA, van Ginkel DJ, van der Naald M, van Slochteren FJ, Doevendans PA, Chamuleau SAJ. Retrograde Coronary Venous Infusion as a Delivery Strategy in Regenerative Cardiac Therapy: an Overview of Preclinical and Clinical Data. *J. Cardiovasc. Transl. Res.* 2018; 11(3): 173–81.

15. Marshall DJ, Palasis M, Lepore JJ, Leiden JM. Biocompatibility of cardiovascular gene delivery catheters with adenovirus vectors: an important determinant of the efficiency of cardiovascular gene transfer. *Mol. Ther.* 2000; 1(5 Pt 1): 423–9.

16. Smits PC, van Langenhove G, Schaar M *et al.* Efficacy of percutaneous intramyocardial injections using a nonfluoroscopic 3-D mapping based catheter system. *Cardiovasc. Drugs Ther.* 2002; 16(6): 527–33.

17. Hamano K, Nishida M, Hirata K *et al.* Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. *Jpn Circ. J.* 2001; 65(9): 845–7.

18. Steinhoff G, Nesteruk J, Wolfien M *et al.* Cardiac Function Improvement and Bone Marrow Response -: Outcome Analysis of the Randomized PERFECT Phase III Clinical Trial of Intramyocardial CD133. *EBioMedicine* 2017; 22: 208–24.

19. Menasché P, Alfieri O, Janssens S *et al.* The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation* 2008; 117(9): 1189–200.

20. Hou D, Youssef EA-S, Brinton TJ *et al.* Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation* 2005; 112(9 Suppl.): I150–I156.

21. Narita T, Suzuki K. Bone marrow-derived mesenchymal stem cells for the treatment of heart failure. *Heart Fail. Rev.* 2015; 20(1): 53–68.

22. Kang SK, Shin IS, Ko MS, Jo JY, Ra JC. Journey of mesenchymal stem cells for homing: strategies to enhance efficacy and safety of stem cell therapy. *Stem Cells Int.* 2012; 2012: 342968.

23. Wysoczynski M, Khan A, Bolli R. New Paradigms in Cell Therapy: Repeated Dosing, Intravenous Delivery, Immunomodulatory Actions, and New Cell Types. *Circ. Res.* 2018; 123(2): 138–58.

24. Butler J, Epstein SE, Greene SJ *et al.* Intravenous Allogeneic Mesenchymal Stem Cells for Nonischemic Cardiomyopathy: Safety and Efficacy Results of a Phase II-A Randomized Trial. *Circ. Res.* 2017; 120(2): 332–40.

25. Bartolucci J, Verdugo FJ, González PL *et al.* Safety and Efficacy of the Intravenous Infusion of Umbilical Cord Mesenchymal Stem Cells in Patients With Heart Failure: A Phase 1/2 Randomized Controlled Trial (RIMECARD Trial [Randomized Clinical Trial of Intravenous Infusion Umbilical Cord Mesenchymal Stem Cells on Cardiopathy]). *Circ. Res.* 2017; 121(10): 1192–204.

26. Barbash IM, Chouraqui P, Baron J *et al.* Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation* 2003; 108(7): 863–8.

27. Fischer UM, Harting MT, Jimenez F *et al.* Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. *Stem Cells Dev.* 2009; 18(5): 683–92.

28. Orlic D, Kajstura J, Chimenti S *et al.* Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc. Natl Acad. Sci. USA* 2001; 98(18): 10344–9.

29. Misao Y, Takemura G, Arai M *et al.* Importance of recruitment of bone marrow-derived CXCR4+ cells in post-infarct cardiac repair mediated by G-CSF. *Cardiovasc. Res.* 2006; 71(3): 455–65.

30. Sato D, Otani H, Fujita M *et al.* Granulocyte colony-stimulating factor does not enhance recruitment of bone marrow-derived cells in rats with acute myocardial infarction. *Exp. Clin. Cardiol.* 2012; 17(3): 83–8.

31. Leone AM, Galiuto L, Garramone B *et al.* Usefulness of granulocyte colony-stimulating factor in patients with a large anterior wall acute myocardial infarction to prevent left ventricular remodeling (the rigenera study). *Am. J. Cardiol.* 2007; 100(3): 397–403.

32. Fan L, Chen L, Chen X, Fu F. A meta-analysis of stem cell mobilization by granulocyte colony-stimulating factor

in the treatment of acute myocardial infarction. *Cardiovasc. Drugs Ther*. 2008; 22(1): 45–54.

33. Ince H, Valgimigli M, Petzsch M *et al.* Cardiovascular events and re-stenosis following administration of G-CSF in acute myocardial infarction: systematic review and meta-analysis. *Heart* 2008; 94(5): 610–6.

34. Moazzami K, Roohi A, Moazzami B. Granulocyte colony stimulating factor therapy for acute myocardial infarction. *Cochrane Database Syst. Rev.* 2013(5): CD008844.

35. Kwon SG, Park I, Kwon YW, Lee TW, Park GT, Kim JH. Role of stem cell mobilization in the treatment of ischemic diseases. *Arch. Pharm. Res.* 2019; 42(3): 224–31.

36. Menasché P. Cell therapy trials for heart regeneration - lessons learned and future directions. *Nat. Rev. Cardiol.* 2018; 15(11): 659–71.

37. Kanda P, Davis DR. Cellular mechanisms underlying cardiac engraftment of stem cells. *Expert Opin. Biol. Ther.* 2017; 17(9): 1127–43.

38. Kofidis T, Lebl DR, Martinez EC, Hoyt G, Tanaka M, Robbins RC. Novel injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart after myocardial injury. *Circulation* 2005; 112(9 Suppl.): I173–I177.

39. Kutschka I, Chen IY, Kofidis T *et al.* Collagen matrices enhance survival of transplanted cardiomyoblasts and contribute to functional improvement of ischemic rat hearts. *Circulation* 2006; 114(1 Suppl.): I167–73.

40. Kraehenbuehl TP, Ferreira LS, Hayward AM *et al.* Human embryonic stem cell-derived microvascular grafts for cardiac tissue preservation after myocardial infarction. *Biomaterials* 2011; 32(4): 1102–9.

41. Kobayashi K, Ichihara Y, Tano N *et al.* Fibrin Glue-aided, Instant Epicardial Placement Enhances the Efficacy of Mesenchymal Stromal Cell-Based Therapy for Heart Failure. *Sci. Rep.* 2018; 8(1): 9448.

42. Chang MY, Huang TT, Chen CH, Cheng B, Hwang SM, Hsieh PC. Injection of Human Cord Blood Cells With Hyaluronan Improves Postinfarction Cardiac Repair in Pigs. *Stem Cells Transl. Med.* 2016; 5(1): 56–66.

43. Ichihara Y, Kaneko M, Yamahara K *et al.* Self-assembling peptide hydrogel enables instant epicardial coating of the heart with mesenchymal stromal cells for the treatment of heart failure. *Biomaterials* 2018; 154: 12–23.

44. Gaffey AC, Chen MH, Venkataraman CM *et al.* Injectable shear-thinning hydrogels used to deliver endothelial progenitor cells, enhance cell engraftment, and improve ischemic myocardium. *J. Thorac. Cardiovasc. Surg.* 2015; 150(5): 1268–76.

45. Alagarsamy KN, Yan W, Srivastava A, Desiderio V, Dhingra S. Application of injectable hydrogels for cardiac stem cell therapy and tissue engineering. *Rev. Cardiovasc. Med.* 2019; 20(4): 221–30.

46. Schmuck EG, Mulligan JD, Ertel RL *et al.* Cardiac fibroblast-derived 3D extracellular matrix seeded with mesenchymal stem cells as a novel device to transfer cells to the ischemic myocardium. *Cardiovasc. Eng. Technol.* 2014; 5(1): 119–31.

47. Huyer LD, Montgomery M, Zhao Y *et al.* Biomaterial based cardiac tissue engineering and its applications. *Biomed. Mater.* 2015; 10(3): 034004.

48. Wang B, Borazjani A, Tahai M *et al.* Fabrication of cardiac patch with decellularized porcine myocardial scaffold and bone marrow mononuclear cells. *J. Biomed. Mater. Res. A* 2010; 94(4): 1100–10.

49. Oberwallner B, Brodarac A, Choi YH *et al.* Preparation of cardiac extracellular matrix scaffolds by decellularization of human myocardium. *J. Biomed. Mater. Res. A.* 2014; 102(9): 3263–72.

50. Maureira P, Marie PY, Yu F *et al.* Repairing chronic myocardial infarction with autologous mesenchymal stem cells engineered tissue in rat promotes angiogenesis and limits ventricular remodeling. *J. Biomed. Sci.* 2012; 19: 93.

51. Chi NH, Yang MC, Chung TW, Chen JY, Chou NK, Wang SS. Cardiac repair achieved by bone marrow mesenchymal stem cells/silk fibroin/hyaluronic acid patches in a rat of myocardial infarction model. *Biomaterials* 2012; 33(22): 5541–51.

52. Ceccaldi C, Bushkalova R, Alfarano C *et al.* Evaluation of polyelectrolyte complex-based scaffolds for mesenchymal stem cell therapy in cardiac ischemia treatment. *Acta Biomater.* 2014; 10(2): 901–11.

53. Ong CS, Fukunishi T, Zhang H *et al.* Biomaterial-Free Three-Dimensional Bioprinting of Cardiac Tissue using Human Induced Pluripotent Stem Cell Derived Cardiomyocytes. *Sci. Rep.* 2017; 7(1): 4566.

54. Zhang YS, Arneri A, Bersini S *et al.* Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip. *Biomaterials* 2016; 110: 45–59.

55. Shimizu T, Yamato M, Isoi Y *et al.* Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and

DOI: 10.18609/cgti.2020.065

temperature-responsive cell culture surfaces. *Circ. Res.* 2002; 90(3): e40.

56. Engelmayr GC, Cheng M, Bettinger CJ, Borenstein JT, Langer R, Freed LE. Accordion-like honeycombs for tissue engineering of cardiac anisotropy. *Nat. Mater.* 2008; 7(12): 1003–10.

57. Menasché P, Vanneaux V, Hagège A *et al.* Transplantation of Human Embryonic Stem Cell-Derived Cardiovascular Progenitors for Severe Ischemic Left Ventricular Dysfunction. *J. Am. Coll. Cardiol.* 2018; 71(4): 429–38.

58. Kojima H, Ieda M. Discovery and progress of direct cardiac reprogramming. *Cell. Mol. Life Sci. CMLS* 2017; 74(12): 2203–15.

59. Qian L, Huang Y, Spencer CI *et al. In vivo* reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature* 2012; 485(7400): 593–8.

60. Song K, Nam YJ, Luo X *et al.* Heart repair by reprogramming non-myocytes with cardiac transcription factors. *Nature* 2012; 485(7400): 599–604.

61. Pavo N, Charwat S, Nyolczas N *et al.* Cell therapy for human ischemic heart diseases: critical review and summary of the clinical experiences. *J. Mol. Cell Cardiol.* 2014; 75: 12–24.

62. Zhang J, Zhu W, Radisic M, Vunjak-Novakovic G. Can We Engineer a Human Cardiac Patch for Therapy? *Circ. Res.* 2018; 123(2): 244–65.

63. Collins SP, Lindsell CJ, Pang PS *et al.* Bayesian adaptive trial design in acute heart failure syndromes: moving beyond the mega trial. *Am. Heart J.* 2012; 164(2): 138–45.

64. Lai TL, Lavori PW, Tsang KW. Adaptive design of confirmatory trials: Advances and challenges. *Contemp. Clin Trials* 2015; 45(Pt A): 93–102.

65. Borow KM, Yaroshinsky A, Greenberg B, Perin EC. Phase 3 DREAM-HF Trial of Mesenchymal Precursor Cells in Chronic Heart Failure. *Circ. Res.* 2019; 125(3): 265–81.

66. Raval AN, Cook TD, Duckers HJ *et al.* The CardiAMP Heart Failure trial: A randomized controlled pivotal trial of high-dose autologous bone marrow mononuclear cells using the CardiAMP cell therapy system in patients with post-myocardial infarction heart failure: Trial rationale and study design. *Am. Heart J.* 2018; 201: 141–8.

67. Mathur A, Arnold R, Assmus B *et al.* The effect of intracoronary infusion of bone marrow-derived mononuclear cells on all-cause mortality in acute myocardial infarction: rationale and design of the BAMI trial. *Eur. J. Heart Fail.* 2017; 19(11): 1545–50.

68. Povsic TJ, Junge C, Nada A *et al.* A phase 3, randomized, double-blinded, active-controlled, unblinded standard of care study assessing the efficacy and safety of intramyocardial autologous $CD34^+$ cell administration in patients with refractory angina: design of the RENEW study. *Am. Heart J.* 2013; 165(6): 854–861.e852.

69. Johnston PV, Duckers HJ, Raval AN, Cook TD, Pepine CJ. Not All Stem Cells Are Created Equal. *Circ. Res.* 2018; 123(8): 944–6.

70. Sheldon R, Opie-Moran M. The Placebo Effect in Cardiology: Understanding and Using It. *Can. J. Cardiol.* 2017; 33(12): 1535–42.

71. Banovic M, Loncar Z, Behfar A *et al.* Endpoints in stem cell trials in ischemic heart failure. *Stem Cell Res. Ther.* 2015; 6: 159.

72. Alexanian RA, Mahapatra K, Lang D *et al.* Induced cardiac progenitor cells repopulate decellularized mouse heart scaffolds and differentiate to generate cardiac tissue. *Biochim. Biophys. Acta Mol. Cell Res.* 2020; 1867(3): 118559.

73. Sürder D, Manka R, Lo Cicero V *et al.* Intracoronary injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: effects on global left ventricular function. *Circulation* 2013; 127(19): 1968–79.

74. Wollert KC, Meyer GP, Müller-Ehmsen J *et al.* Intracoronary autologous bone marrow cell transfer after myocardial infarction: the BOOST-2 randomised placebo-controlled clinical trial. *Eur. Heart J.* 2017; 38(39): 2936–43.

75. Losordo DW, Henry TD, Davidson C *et al.* Intramyocardial, autologous $CD34^+$ cell therapy for refractory angina. *Circ. Res.* 2011; 109(4): 428–36.

76. Bartunek J, Terzic A, Davison BA *et al.* Cardiopoietic cell therapy for advanced ischaemic heart failure: results at 39 weeks of the prospective, randomized, double blind, sham-controlled CHART-1 clinical trial. *Eur. Heart J.* 2017; 38(9): 648–60.

AFFILIATIONS

Ruben A Alexanian
Division of Cardiovascular Medicine, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, 53572, USA

Amish N Raval
Author for correspondence:
Division of Cardiovascular Medicine, Department of Medicine and Department of Biomedical Engineering, University of Wisconsin School of Medicine and Public Health, Madison, WI, 53572, USA
anr@medicine.wisc.edu

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Raval reports grants from Biocardia Inc., Siemens Healthineers, Fujifilm Cellular Dynamics, Cellular Logistics and NIH/NHLBI 1U01HL148690-01 during the study.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Mar 3 2020;* ***Revised manuscript received:*** *Apr 14 2020;* ***Publication date:*** *May 6 2020.*

DOI: 10.18609/cgti.2020.065

CELL & GENE THERAPY INSIGHTS

CLINICAL DEVELOPMENT STRATEGY, TOOLS & TRIAL DESIGNS

SPOTLIGHT

EXPERT INSIGHT

Clinical trial design in gene therapy for neurodegenerative diseases: Sanfilippo A syndrome

Adelaida Morte, Esther Ortiz, Mariano Sust, Anna Vaque, Neus Gascon, & Carlos Plata-Salaman

Gene therapy (GT) represents a new therapeutic modality particularly suited for untreatable monogenic inherited genetic diseases. An important aspect in GT clinical trial design is the holistic view of the patient and disease. New regulatory guidances provide a framework for continuously evolving clinical trial design in GT and the nature of an intended therapeutic effect often requires unique designs. We present an example of an integrated clinical trial design for a GT (genetically modified AAV-9 containing the cDNA of the human sulfamidase gene) targeting Sanfilippo A syndrome (SFAS), a devastating neurodegenerative disease. With optimized delivery of the GT to the main target organ of SFAS, i.e., the brain (by using the intracerebroventricular administration), the trial design with multiple types of pre-defined complementary measures allows for an integrated assessment of safety/tolerability, pharmacodynamics/biomarkers and efficacy overtime, with the ultimate goal of a comprehensive view of an individual patient's response characterization.

Cell & Gene Therapy Insights 2020; 6(5), 387–396

DOI: 10.18609/cgti.2020.048

Gene therapy (GT) is evolving into a robust therapeutic platform that has the potential for the treatment of disease conditions currently categorized as untreatable, incurable

and catastrophic (i.e., early mortality associated with significant progressive deleterious impact on quality of life and the caregiver burnout syndrome). Examples include (in addition to SFAS) diseases such as Pompe disease (a glycogen storage disease) and Crigler Najjar syndrome (hereditary unconjugated hyperbilirubinemia).

Progress in GT is the result of new scientific, genetic, molecular pathophysiological and clinical knowledge [1,2]. GT clinical trials have been performed in multiple therapeutic areas including oncology, hematology, neurology, ophthalmology, metabolism, cardiovascular, infectious and immunological disorders. These studies included diverse patient populations who have been treated by different routes of administration. The results obtained from them have provided proof-of-mechanism and proof-of-concept evidence achieving demonstration of preclinical-to-clinical translation [1–3].

There are various types of GT strategies with different mechanisms of action. One strategy comprises the transfer of genetic material with the objective to enhance the expression of the transferred gene at levels high enough to be therapeutic. Another strategy encompasses the control of gene expression – for example, by antisense oligonucleotides or short interfering RNAs – that down regulate production of a disease-associated protein.

Regarding the first strategy, it can be considered as particularly suited to monogenic inherited genetic diseases and there is the potential that a single treatment may result in a life-time cure of a disease [1–3]. The transferring of genetic material can involve *in vivo* gene delivery to target cells via genetically engineered vectors, or *ex vivo* gene delivery to autologous cells (e.g., lymphocytes, hematopoietic) which are transferred back to a patient.

Whatever the approach, there are a number of GTs which have been successful in obtaining regulatory approval by Health Authorities. Various examples are included in Table 1 [4–17].

These and other product approvals have brought GT approaches into the reality of a new therapeutic modality. GT is also providing a new therapeutic strategy for targets and conditions that may not be suitable for standard pharmaceutical modalities. At the same time, it is also important to note that many clinical challenges remain. These include:

- Manufacturing and scale-up challenges: complex processes which are difficult to scale-up and, at the same time, have to comply with the strict Good Manufacturing Practice (GMP) regulatory framework;
- Technology challenges: optimization of GT vectors; need for more automated processes that do not impact cell quality and maximize reproducibility between lots as well as development of new techniques such as gene editing tools (e.g., Zinc Finger Nuclease and Clusters of Regularly Interspaced Short Palindromic Repeats, or CRISPR);
- Development of more nonclinical models with high translatability to human diseases;
- Clinical challenges: among others, the better understanding of humoral and cellular responses to achieve reduction of immunoregulatory responses to vector components and transgene and to gene-corrected cells. Also, as genetic diseases mostly occur in childhood, it is of high relevance to gather the complete knowledge of the mechanisms that would allow for long-term, high efficiency gene expression, and also the mechanisms that would allow the GT to reach all intended target cells (e.g., when enzymes are not secretable). Moreover, a more in-depth understanding of the possibilities of integration and malignancy, infection or other toxicities is also required.

These challenges are being systemically addressed through the cumulative knowledge generated by GT research including the design of GT clinical trials.

DOI: 10.18609/cgti.2020.048

TABLE 1

Examples of regulatory approved gene therapy products.

Control of gene expression	
Antisense oligonucleotides	**Fomivirsen** [4] **Mipomersen** [5] **Nusinersen** [6] **Eteplirsen** [7]
RNA oligonucleotides	**Pegaptanib aptamer** [8]
Interfering RNA	**Patisiran** [9]
Enhancement of gene expression	
In vivo: AAV vector	**Alipogene tiparvovec** (AAV-1 genetically modified to express the human lipoprotein lipase gene) [10] **Voretigene neparvovec-rzyl** (AAV-2 genetically modified to express the human retinal pigment epithelium 65 gene) [11] **Onasemnogene abeparvovec-xioi** (AAV-9 genetically modified to express the human survival motor neuron gene) [12]
In vivo: other viral vectors	**Talimogene laherparepvec** (live, attenuated herpes simplex virus type-1 genetically modified to express human GM-CSF) [13]
Ex vivo	**Strimvelis®** (autologous $CD34^+$ cells transduced with retroviral vector that encodes for the human adenosine deaminase cDNA) [14] **Zalmoxis®** (allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor and the herpes simplex 1 virus thymidine kinase) [15] **Tisagenlecleucel** (CD19-directed genetically modified autologous T cell immunotherapy comprised of autologous T cells that are genetically modified using a lentiviral vector to encode an anti-CD19 chimeric antigen receptor [CAR]) [16] **Axicabtagene ciloleucel** (CD19-directed genetically modified via retroviral transduction to express a CAR autologous T cell immunotherapy) [17]

Key characteristics of GT clinical trial designs such as patient selection, endpoints, and biomarker inclusion for both efficacy and safety, are playing a key role in the development of GT. Biomarkers, as objective measures of biological processes, have shown utility in the evaluation of therapeutic responses in GT clinical trials (e.g., levels of blood Factor IX in hemophilia B treated-patients or muscle function assessed by 6- or 10-meter walk test in Pompe disease-treated patients). An important aspect in the design of a clinical trial is the holistic view of the patient and disease, and how the design is 'tailored' to obtain the maximum amount of information. Since endpoints can be very diverse and may include clinical, physiological, hematological, biochemical, developmental, morpho-pathological, genetic and/or molecular measures for efficacy determination and safety monitoring, the clinical trial design can often be instrumental as a scientific tool in generating new information regarding efficacy and safety, preclinical-to-clinical translatability, and benefit–risk assessment.

The current scope of GT clinical trials reflects the importance of this new therapeutic tool and its overarching therapeutic reach. For instance, a search in www.clinicaltrials.gov (20 March 2020) using the term "gene therapy" yielded 1,574 clinical studies with the status completed and 1,028 recruiting patients, whilst a search in the EU Clinical Trials Register yielded 1,079 trials.

In parallel to these clinical activities, new regulatory guidance has been published, including guidance on: clinical trial design issues for all phases of a clinical development program for human GT products for the treatment of rare diseases [18]; design of long-term follow-up observational studies following administration of a GT product [19]; and structure and data requirements for a clinical trial application for exploratory (including first-in-human) and confirmatory trials [20].

Progress in specific therapeutic areas has also generated new guidance in 2020, such as the GT guidance for hemophilia [21], retinal disorders [22], and for mucopolysaccharidosis type III (Sanfilippo syndrome) [23].

The evolution of the scientific and medical knowledge and regulatory framework reflect the importance of GT for future medical treatments and also the uniqueness of each approach. The guidance provides a framework for continuously evolving clinical trial design in GT, and at the same time, the nature of an intended therapeutic effect often requires unique designs.

In this context, we present an example of a tailored and integrated clinical trial design for a GT targeting an inherited monogenic pathology, Sanfilippo A syndrome (SFAS), a devastating neurodegenerative disease. SFAS or mucopolysaccharidosis type IIIA (MPSIIIA) is characterized by the accumulation of the glycosaminoglycan (GAG) heparan sulfate (HS) due to the deficiency of an enzyme involved in the lysosomal degradation of HS: heparan N-sulfatase or sulfamidase.

SFAS patients appear to be normal at birth, and the earliest symptoms are usually recognized between 2 and 6 years of age. Then the disease progresses in three phases. The first phase typically presents with a slower or halted cognitive development, with speech deterioration or deficiency as the most severe sign. Intense sleeping disturbances, hyperactivity and extreme behavioral problems (e.g., impulsivity and aggressiveness) dominate the second phase of the disease that usually starts at the age of 3 to 4 years. The third phase is marked by progressive loss of motor skills and progressive dementia. When patients are around 10 years old, they present severe dementia, seizures, spasticity and dysphagia, with these symptoms and signs progressively worsening the patient's condition, eventually leaving him/her in a vegetative state; ultimately, patients usually die in their mid-late teenage years [24–28].

Somatic disease is relatively mild in SFAS patients and consists typically of frequent ear-nose-throat infections, episodic diarrhea, hepatomegaly and, more rarely, splenomegaly, skeletal abnormalities that usually appear later in the course of the disease (scoliosis, kyphosis, lumbar lordosis, hip dysplasia, and carpal tunnel syndrome), hirsutism, and mild facial dysmorphology (coarse facies).

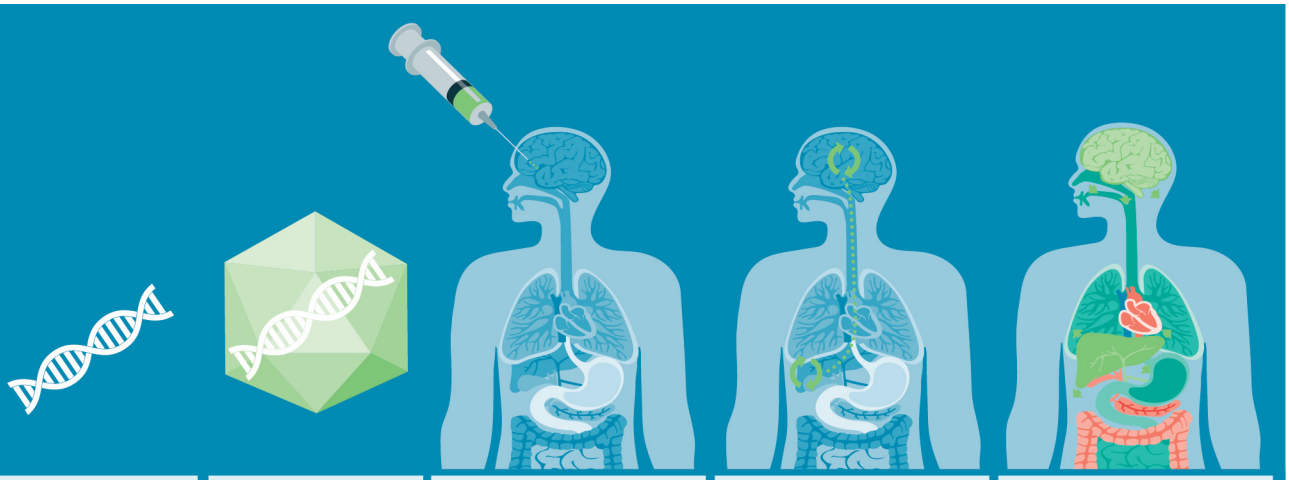
There is no specific therapy for SFAS and the nature of the disease is consistent with a therapy that can be designed to treat its precise biochemical deficiency and underlying pathophysiology. The medicinal product we are currently investigating in clinical Phase 1–2 testing is based on a non-replicating, non-pathogenic genetically modified AAV-9 containing the cDNA of the human sulfamidase gene with codon optimization, in order to maximize its efficiency in expression and translation of the human sulfamidase protein. This approach takes advantage of the intrinsic AAV-9 ability to achieve highly efficient transduction, including in non-dividing central nervous system (CNS) cells resulting in high levels of gene expression.

There are other GT clinical trials ongoing in SFAS patients: one consists on the intracerebral administration of a GT product (using a highly invasive procedure) [29] and another involves the administration by the intravenous route (not targeting directly to the main affected organ, the CNS) [30,31]. Other treatments for SFAS that have been tested in clinical trials have essentially focused on Enzyme Replacement Therapy (ERT) and Substrate Reduction Therapy (SRT). In relation to ERT, the results of the clinical trial testing the direct administration into the cerebrospinal fluid (CSF) of recombinant sulfamidase protein by an Intrathecal Delivery Device demonstrated good safety, but the treatment failed to slow cognitive decline. Regarding SRT, results from a Phase 3 clinical trial evaluating the use of high-dose genistein (molecule that down-regulates the expression of genes coding for enzymes involved in GAG synthesis) in children with Sanfilippo syndrome did not provide meaningful clinical benefit [32].

DOI: 10.18609/cgti.2020.048

FIGURE 1

Safety, Tolerability, Pharmacodynamic and Efficacy Endpoints (Study ESTEVE-SANF-201).



Therapeutic gene	**Vehicle**	**Administration**	**Biodistribution**	**Enzyme production**
Selection of the normal functional gene: human sulfamidase	Introduction of the therapeutic gene in a non-pathogenic AAV vector	Vector administration in the CSF and entry into the nucleus of brain cells. Beginning of production of the enzyme sulfamidase and distribution in the CNS	Diffusion of the gene therapy product from CSF into the blood-stream, reaching the liver. Hepatocytes produce and secrete the enzyme, which is spread throughout the body	Generalized restoration of enzyme activity, disappearance of glycosaminoglyycan accumulation, and correction of pathological alterations

Study ESTEVE-SANF-201 Assessments

SAFETY AND TOLERABILITY
- **Adverse events**
- **General laboratory: clinical biochemistry, hematology, urinalysis**
- **Specific laboratory:**
  - **Antigen-specific T-cell response to the vector and to the transgen:** ELISPOT, intracellular cytokine staining, lymphocyte proliferation with CFSE
  - **Humoral response monitorization:** ELISA to vector and to the transgen, neutralizing antibodies (NAb) to the vector in CSF and serum
  - **Shedding by qPCR (serum, saliva, urine, stool)**
  - **Inflammatory response (IL-1, IL-6, TNF-α, IFN-α, IFN-β)**
- **Vital signs and electrocardiogram**
- **Hepatic ultrasound**
- **Magnetic resonance imaging**

PHARMACODYNAMICS/ BIOMARKERS
- **Heparan sulfate:** plasma, urine, CSF
- **Heparan-N-sulfatase (sulfamidase):** leukocytes, CSF, fibroblasts

EFFICACY
- **Physical and neurological exam**
- **Neurobehavioral and cognitive scales:**
  - **Vineland III Adaptive Behavior Scale**
  - **Bayley III Scales: Cognitive, Language, Social-emotional, Motor and Adaptive Development**
  - **Sanfilippo Behavior Rating Scale**
- **Quality of Life by PedsQL Scales**
- **Sleep Disturbance Scale for Children**
- **Polysomnography**
- **Brain auditory evoked potentials**
- **Real-time ultrasound elastography of the Achilles tendon**
- **Hepatic ultrasound and magnetic resonance imaging (volume and structure)**

Time points: at screening, hospital admission, discharge, and at multiple times throughout 5 years post-administration (Table 1)

Safety, Tolerability, Pharmacodynamic and Efficacy Endpoints (Study ESTEVE-SANF-201). Top Panel: Intracerebroventricular administration and target profile of production and biodistribution of the gene therapy product. Bottom Panel: Summary of study ESTEVE-SANF-201 assessments

The trial design we present here takes advantage of optimized delivery to the main target organ of SFAS, i.e., the brain by using the intracerebroventricular (ICV) administration into the lateral cerebral ventricle (LCV), a routine procedure in neurosurgery operating

TABLE 2

Information on clinical trial of adeno-associated viral vector serotype 9 containing human sulfamidase gene.

Study ID	ESTEVE-SANF-201
EudraCT number	2015-000359-26: https://www.clinicaltrialsregister.eu/ctr-search/trial/2015-000359-26/ES
Clinical trial status	Ongoing
Study title	Phase 1/2 safety, tolerability and initial efficacy study of adeno-associated viral vector serotype 9 containing human sulfamidase gene after ICV administration
Orphan drug designation no.	EU/3/11/877
Product name	Adeno-associated viral vector serotype 9 containing human sulfamidase gene
Product code	AAV-9-CAG-coh-SGSH
Main objective of the trial	To determine the safety and tolerability, including the immune response, after ICV administration of a single dose of AAV-9-CAG-coh-SGSH in patients with MPSIIIA.
Secondary objectives of the trial	To assess the pharmacodynamic profile and the initial efficacy after ICV administration of a single dose of AAV-9-CAG-coh-SGSH in patients with MPSIIIA to estimate the dose required to significantly ameliorate the phenotype. To evaluate the correlation between the pharmacodynamic assessments and the clinical evolution, in order to establish the optimal biomarker to assess the evolution/amelioration of the disease. To collect data regarding potential tests that can be evaluation criteria for the subsequent pivotal study. To assess viral shedding.
SFAS pediatric patient population	Patients over two years of age with confirmed MPSIIIA (by genotype), with underlying missense mutation at least in one of the alleles for the disease and documented deficiency in sulfamidase enzyme activity of less than or equal to 10%.
Main inclusion criteria	Male and female patients aged 2 years or older. Patients with confirmed MPSIIIA by genotype (as described above). Onset of clinical manifestations related to MPSIIIA during the first 6 years of life. Patients with an adaptive behaviour score between 40 and 90 as evaluated by the Vineland Adaptive Behaviour Scale (Vineland-III). Patients not dependent on a wheelchair. Patients without severe sensory deficit (blindness, deafness that requires headset). Patients with stable symptomatic treatment (depending on weight) within the last 3 months, with no anticipated changes in medication regimen. Patients with no contraindication for surgical procedure and/or anesthesia. Patients taking non-steroidal anti-inflammatory drugs (NSAIDs) should discontinue their use. Patients medically stable to accommodate the protocol requirements, including travelling and assessments. Signed informed consent.
Main exclusion criteria	Patient deterioration that may compromise the interpretation of the study results. Patients with neutralizing antibodies (NAb) against AAV-9 in cerebrospinal fluid. Epilepsy resistant to treatment. Patients with significant co-morbid conditions. Any contraindication for anesthesia and product administration procedure, including major risk factors for hemorrhage. Any vaccination 30 days before investigational AAV-9-CAG-coh-SGSH administration. Patients who have received any medication with the objective of modifying the natural course of the disease, i.e. gene transfer agents or enzyme replacement therapy.
Primary endpoint(s)	**Safety and tolerability.** All safety and tolerability parameters (as summarized in Figure 1) will be evaluated at regular time points after AAV-9-CAG-coh-SGSH product administration and will be assessed by comparison to screening / baseline evaluations. **Pharmacodynamics and efficacy.** All pharmacodynamic and efficacy parameters (as summarized in Figure 1) will be evaluated at regular time points after AAV-9-CAG-coh-SGSH product administration and will be assessed by comparison to screening/baseline evaluations
Time point(s) of evaluation of endpoints	Depending on endpoint, times of evaluation may include: at screening; Day-1; Day-0; D1-discharge; weeks 2 and 4; month 2, 2.5, 3, 6, 9, 12, and 18; years 2, 3, 4, and 5.
Dosing regimen	First cohort (n=3): single dose administration of AAV-9-CAG-coh-SGSH 6.8 x10^{13} vg/patient. Second cohort (n=3): single dose administration of AAV-9-CAG-coh-SGSH 1.4 x 10^{14} vg/patient. Protocol amended to administer a higher single dose in a Third cohort.
Route of admin.	Intracerebroventricular (ICV) into the lateral cerebral ventricle (LCV).
Study duration	Follow-up period of 5 years post-administration.

DOI: 10.18609/cgti.2020.048

rooms. Due to the nature of the CSF circulation dynamics, administration into the LCV allows exposure of the AAV-9 containing the cDNA of the human sulfamidase to the complete brain ventricular system as well as the subarachnoid space (thereby allowing diffusion into the brain parenchyma via the ependymal lining of the ventricular system, as well as via the piamater surrounding the brain). At the same time, this route of administration reduces the potential for cellular immune responses (vector and transgene) and formation of neutralizing antibodies to the vector when compared to the intravenous route. Moreover, this optimized route of delivery allows the administration of the GT product with no need for concomitant immunosuppressants, minimizing confusing effects. The target profile of production and biodistribution of the GT product following the ICV administration is summarized in Figure 1. Since the CSF is ultimately absorbed into the venous vascular system, an amount of the ICV administered GT product also passes from the CSF into the bloodstream, reaching the liver that can also produce and secrete the sulfamidase which reaches peripheral target organs. This pathway was validated in mice and dogs; following administration of AAV-9 encoding sulfamidase into the CSF, sulfamidase activity increased throughout the brain and in blood in response to the transgenic expression throughout the CNS and liver [33].

This AAV-9 containing the cDNA of the sulfamidase gene with codon optimization was tested in preclinical efficacy studies with robust results [33,34]. The intracerebrospinal fluid administration of AAV-9 encoding sulfamidase corrected both CNS and somatic pathology, with prolonged survival in MPSIIIA mice [33]. This approach was also tested in a large animal species (dogs) using the intracisternal or ICV delivery of the AAV-9 encoding sulfamidase, resulting in transgenic expression throughout the CNS and increased sulfamidase activity in the CSF [33,34]. This expression is long-term: a single intra-CSF administration of AAV-9 encoding sulfamidase to dogs, at a clinically relevant dose, resulted in long-term stable increase in sulfamidase activity in the CSF throughout a period of study of ~7 years [35].

Based on the consistent and robust preclinical data in dogs and MPSIIIA mice model of SFAS that mimics the human biochemistry, pathology and clinical profile, and since the AAV-9 encoding sulfamidase was associated with long-term expression and was also safe in regulatory toxicology studies, we proceeded to clinical studies.

Because each GT may be unique, product-specific approaches in clinical trial design need careful consideration. In this context, several key aspects were considered when designing the ongoing Phase 1–2 study ESTEVE-SANF-201 (EudraCT 2015-000359-26) (Table 2 & Figure 1). These include the requirement of diagnosis confirmation of the deficiency in each patient by molecular genetics and biochemical testing. This first clinical trial includes patients over 2 years old as the standardization of the brain volume over this age maximizes the safety of product administration. The baseline clinical stage of the disease progression must be of mild or moderate impairment (Vineland-3 test score between 40 and 90), as this status gives room for clinical effects facilitating the interpretation of the study results. Baseline immune status must also be adequate, i.e., neither humoral nor cellular relevant immune response against the vector or the transgene has to be present. Patients must also have at least one missense mutation to minimize the specific immune reaction against the transgene. The study has been designed to include patients as homogeneous as possible, maximizing the safety and the interpretability of the overall results.

After treatment, a thorough follow-up is performed, including close monitoring of immune status changes both to the vector and to the transgene to obtain information of potential off-target effects, and close monitoring of short- and long-term safety by a complete battery of evaluations (Figure 1). For the assessment of pharmacodynamics and efficacy, multiple complementary endpoints using validated, feasible and sensitive-to-change scales

were also included (Table 2 & Figure 1) and will be assessed by comparison to screening/baseline evaluations. Concerning biomarkers, a complete evaluation in CSF, blood and urine is proposed, with the results being studied in the context of clinical improvement in the symptoms of the disease and in the cognitive and behavioral scales. These scales (Figure 1) have been selected in agreement with what is recommended by an expert panel in the field [36]. An Independent Data Monitoring Committee (IDMC) was established to periodically assess the accumulated study data for patients' safety and when appropriate, efficacy, as well as for the evaluation of the study conduct and progress, and for making recommendations concerning the continuation, modification, or termination of the trial. The study design also included the development and validation of all the associated specific analytical methods.

This clinical trial design is one among several examples of ongoing GT trials that allow:

1. An integrated assessment of safety, tolerability, pharmacodynamics and efficacy over time. This assessment is done using simultaneous and complementary evaluations that will be assessed by comparison to screening/baseline evaluations. The global analysis will indicate which of the assessments are more efficient in evaluating the therapeutic effect;

2. Analyses of temporal relationships of different types of measures, e.g., enzyme production with effects on HS in different compartments and their correlation, safety and tolerability in the presence or absence of immune responses, and biomarkers concordant with clinical improvement;

3. Short- and long-term monitoring of responses, including sustainability of the desired effect (e.g., reduction of HS in various compartments) as evidence of direct continuous expression of the transgene;

4. Strengthening of the interpretability of the clinical results to make a more robust composite of the benefit-risk of the GT medicinal product.

The ESTEVE-SANF-201 clinical study is being complemented by an Observational Natural History Study (Study EST–SFA–2013–01). The clinical information recorded in the medical chart of Pediatric Patients diagnosed with SFAS that have not received other than symptomatic treatment is retrospectively collected. This study is being conducted in 11 Medical Centers and will provide important information on disease progression and patient features that can support the development of the AAV-9 encoding sulfamidase product being tested in Study ESTEVE-SANF-201.

TRANSLATION INSIGHT

Overall, we consider that this holistic design-oriented approach with multiple types of pre-defined complementary safety/tolerability, pharmacodynamic/biomarkers and efficacy domains (neurological, behavioral, social-emotional, cognitive, language and speech, motor, sensory (hearing), sleep, quality-of-life) assessments at different times is a compelling platform with the ultimate goal of an integrated view of an individual patient's response characterization to the GT therapy under investigation. The design also allows determination of what specific assessments would be most relevant for subsequent clinical studies to validate overall benefits, and to incorporate novel schemes such as adaptive designs and output analyses aggregating multiple data sets including those generated from clinical trials as well as observational studies. In conclusion, the integrated clinical trial design presented opens multiple options for future innovative designs to generate new medical knowledge and strengthen the interface between nonclinical and clinical science [37], and in research-to-patient translatability to advance the development of new GTs.

DOI: 10.18609/cgti.2020.048

REFERENCES

1. Shahryari A, Saghaeian JM, Mohammadi S *et al*. Development and clinical translation of approved gene therapy products for genetic disorders. *Front. Genet*. 2019; 10(868):1–25.
2. Deverman BE, Ravina BM, Bankiewicz KS, Paul SM, Sah DWY. Gene therapy for neurological disorders: progress and prospects. *Nat. Rev. Drug Discov*. 2018; 17: 641–59.
3. Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR. Gene therapy clinical trials worldwide to 2017: An update. *J. Gene Med.* 2018; 20(5): e3015: 1–16.
4. Fomivirsen: https://www.accessdata.fda.gov/drugsatfda_docs/nda/98/20961_Vitravene_prntlbl.pdf
5. Mipomersen: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/203568s000lbl.pdf
6. Nusinersen: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/209531lbl.pdf
7. Eteplirsen: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/206488lbl.pdf
8. Pegaptanib: https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021756s018lbl.pdf
9. Patisiran: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210922s000lbl.pdf
10. Alipogene tiparvovec: https://www.ema.europa.eu/en/documents/product-information/glybera-epar-product-information_en.pdf
11. Voretigene neparvovec-rzyl: https://www.fda.gov/media/109906/download
12. Onasemnogene abeparvovec-xioi:. https://www.fda.gov/media/126109/download
13. Talimogene laherparepvec: https://www.fda.gov/media/94129/download
14. Strimvelis: https://www.ema.europa.eu/en/documents/product-information/strimvelis-epar-product-information_en.pdf
15. Zalmoxis: https://www.ema.europa.eu/en/documents/product-information/zalmoxis-epar-product-information_en.pdf
16. Tisagenlecleucel: https://www.fda.gov/files/vaccines%2C%20blood%20%26%20biologics/published/Package-Insert---KYMRIAH.pdf
17. Axicabtagene ciloleucel: https://www.fda.gov/files/vaccines%2C%20blood%20%26%20biologics/published/Package-Insert---YESCARTA.pdf
18. Guidance for Industry. Human Gene Therapy for Rare Diseases. U.S. DHHS, FDA, CBER, January 2020: https://www.fda.gov/media/113807/download
19. Guidance for Industry. Long Term Follow-Up After Administration of Human Gene Therapy Products. U.S. DHHS, FDA, CBER, January 2020: https://www.fda.gov/media/113768/download
20. Draft. Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials. EMA/CAT/852602/2018. 31 January 2019: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-non-clinical-clinical-requirements-investigational-advanced-therapy_en.pdf
21. Guidance for Industry. Human Gene Therapy for Hemophilia. U.S. DHHS, FDA, CBER, January 2020: https://www.fda.gov/media/113799/download
22. Guidance for Industry. Human Gene Therapy for Retinal Disorders. U.S. DHHS, FDA, CBER, January 2020: https://www.fda.gov/media/124641/download
23. Draft Guidance for Industry. Mucopolysaccharidosis Type III (Sanfilippo Syndrome): Developing Drugs for Treatment. U.S. DHHS, FDA, CBER, February 2020: https://www.fda.gov/media/134893/download
24. Wagner VF, Northrup H. Mucopolysaccharidosis Type III. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A (Eds). *SourceGeneReviews*® University of Washington, Seattle; WA, USA; 2019.
25. Valstar MJ, Ruijter GJG, van Diggelen OP, Poorthuis BJ, Wijburg FA. Sanfilippo syndrome: A mini-review. *J. Inherited Metab. Dis.* 2008; 31(2): 240–252.
26. Meyer A, Kossow K, Gal A, Mühlhausen C, Ullrich K, Braulke T, Muschol N. Scoring evaluation of the natural course of mucopolysaccharidosis type IIIA (Sanfilippo syndrome type A). *Pediatrics* 2007; 120(5): e1255–e1261.
27. Jakobkiewicz-Banecka J, Gabig-Ciminska M, Kloska A, Malinowska M, Piotrowska E, Banecka-Majkutewicz Z, Banecki B, Wegrzyn A, Wegrzyn G. Glycosaminoglycans and mucopolysaccharidosis type III. Front Biosci. 2016;21:1393-1409. https://www.ncbi.nlm.nih.gov/pubmed/27100513
28. Heon-Roberts R, Nguyen ALA, Pshezhetsky AV. Molecular bases of neurodegeneration and cognitive decline, the major burden of Sanfilippo disease. *J. Clin Med.* 2020; 9(2): 344.

29. NCT03612869: https://clinicaltrials.gov/ct2/show/NCT03612869?term=lysogene&draw=2&rank=2

30. NCT02716246: https://clinicaltrials.gov/ct2/show/NCT02716246?term=abeona&draw=2&rank=4

31. NCT04088734: https://clinicaltrials.gov/ct2/show/NCT04088734?term=abeona&draw=2&rank=2

32. Gaffke L, Pierzynowska K, Piotrowska E, Węgrzyn G. How close are we to therapies for Sanfilippo disease? *Metab. Brain Dis*. 2018; 33(1): 1–10.

33. Haurigot V, Marcó S, Ribera A *et al*. Whole body correction of mucopolysaccharidosis IIIA by intracerebrospinal fluid gene therapy. *J. Clin. Invest*. 2013; 123(8): 3254–71.

34. Marcó S, Haurigot V, Bosch F. In vivo gene therapy for mucopolysaccharidosis type III (Sanfilippo syndrome): A new treatment horizon. *Hum. Gene Ther.* 2019; 30(10): 1211–21.

35. Marcó S, Haurigot V, Jaén ML *et al*. Safe and sustained elevation of sulfamidase after administration of AAV9-sulfamidase to the CSF of dogs: Seven year-follow up. P251. *Hum. Gene Ther.* 2019; 30(11): A1–A221.

36. van der Lee JH, Morton J, Adams HR *et al*. Cognitive endpoints for therapy development for neuronopathic mucopolysaccharidoses: Results of a consensus procedure. *Mol. Genet. Metab.* 2017; 121(2): 70–9.

37. Plata-Salamán CR. Editorial: Preclinical requirements for cell & gene therapies. *Cell Gene Ther. Ins*. 2019; 5(7): 477–82.

AFFILIATIONS

Adelaida Morte
Esteve Pharmaceuticals, S.A.

Esther Ortiz
Esteve Pharmaceuticals, S.A.

Mariano Sust
Esteve Pharmaceuticals, S.A.

Anna Vaque
Esteve Pharmaceuticals, S.A.

Neus Gascon
Esteve Pharmaceuticals, S.A.

Carlos Plata-Salaman
Esteve Pharmaceuticals, S.A.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors are full-time employees of Esteve Pharmaceuticals, S.A., a company with projects in gene therapy.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Feb 18 2020*; ***Revised manuscript received:*** *Mar 23 2020*; ***Publication date:*** *May 6 2020.*

DOI: 10.18609/cgti.2020.048

CELL & GENE THERAPY INSIGHTS

CLINICAL DEVELOPMENT STRATEGY, TOOLS & TRIAL DESIGNS

SPOTLIGHT

INTERVIEW

A streamlined approach to biomarker development in cellular immunotherapy

MAJID GHODDUSI has over 15 years of experience in some of the most challenging areas of oncology with focus on drug discovery and clinical development. Dr Ghoddusi has broad and overarching insights into unmet therapeutic areas with expertise in translational sciences and clinical biomarker development which allows him to provide unique perspective on how to propel therapeutic projects from discovery to approval. Trained as a translational pathologist he has held numerous positions at large pharmaceutical and small biotech companies including Novartis, Celgene and Juno Therapeutics. His current focus at Poseida Therapeutic is Gene and Cellular Therapy.

Cell & Gene Therapy Insights 2020; 6(5), 635–639

DOI: 10.18609/cgti.2020.075

Q What are you working on at the moment?

MG: Our lead product at Poseida is an anti-BCMA CAR T product candidate for patients with refractory relapsed multiple myeloma, which is currently in in the clinic. We also have an anti-PSMA CAR T product candidate in metastatic castration resistant prostate cancer for which we expect to begin dosing patients in the Phase 1 clinical trial this spring. It's our first CAR T product in solid tumors, so we are very excited to start working on this.

Q The Poseida Therapeutics R&D pipeline is very varied in terms of both technological approaches and indications. What approach or philosophy underpins it all?

MG: Poseida Therapeutics defines itself as clinical-stage biopharmaceutical company dedicated to utilizing proprietary gene engineering platform technologies to create next generation cell and gene therapeutics with the capacity to cure. We are developing a broad portfolio of product candidates in a variety of indications based on these core platforms, primarily including our non-viral piggyBac DNA Modification System and our Cas-CLOVER site-specific gene editing system

Q How do you seek to streamline biomarker discovery and development activities across such a broad portfolio?

MG: As you mentioned, the pipeline is varied, and the indications are quite distinct from each other. This means the modes of interaction with targets in each program is quite different. Therefore, the approach to biomarker discovery is going to be dependent on the biology of the disease, target engagement and also the characteristics of each product candidate.

Our biomarker activities start at the very early stages, alongside characterization of the product candidate itself. We look at markers that would define the manufacturing process and the final product candidate, as well as the clinical outcomes. Parameters that fall within these two processes are defined as potential biomarkers that could indicate possible utility in predict success, in terms of both manufacturing and clinical response.

Within this scope, we leave no stone unturned. We look for opportunities to potentially intervene to enhance the effectiveness of the product candidates, whether it's the manufacturing process, or to enhance safety profile and clinical efficacy.

Q Can you go into more depth on the need for and development of clinical biomarkers and assays at Poseida Tx to predict/guide treatment and correlate with outcomes?

MG: In the CAR T cell therapy area, although there has been phenomenal success in terms of overall response rate and the duration of response, yet the field is still searching for really profound biomarkers that allow us to potentially enrich the population of patients that respond better than the rest.

In multiple myeloma, patients still sometimes relapse, or those who are refractory sometimes produce no response once the final material is infused. Many companies are working on this with BCMA as the main target, but overall response indicate that experimental products are not yet entirely curative at this stage. This can be, to some degree, contrasted with

DOI: 10.18609/cgti.2020.075

> "...delivering a high percentage of TSCM cells will drive more gradual tumor killing, thereby inducing less inflammatory cytokine response and improving the tolerability profile of our CAR-T product candidates relative to those of existing CAR-T therapies. This allows engineered T cells to persist and be able to proliferate within the blood circulation for much longer."

non-Hodgkin's lymphoma, where companies and groups that target CD19 have seen better clinical outcomes, in terms of best overall response, duration of response, and persistence of the product delivered.

Our main mission is to find biomarkers that could allow us to predict which patient is going to benefit most and separate them from those who are less likely to respond, or respond only briefly. Finding these elusive markers will allow us to identify our tools for the long term, and improve our product candidates, and also to potentially assign patients into different categories, and to find the best therapeutic approaches for each group.

Q How do you define the next steps to be taken in the cellular cancer immunotherapy field?

MG: Within cell therapy, we believe our technologies allow us to create product candidates with engineered cells that engraft in the patient's body and drive lasting durable responses that may have the capacity to result in single treatment cures.

Solid tumors are an area where success has been very elusive when it comes to cellular therapies. It's not only an issue of honing the product and getting the CAR T cells to the tumor area, but also of overcoming the second, and perhaps more important, barrier of achieving infiltration of the CAR T cells into tumor microenvironment itself.

As we all know, the solid tumor microenvironment can be very hostile to T cells, and infiltrating lymphocytes in general. There are many pathways that essentially exhaust the cells and either neutralize or deactivate them, and these pathways are key reason why cellular therapy has not been as effective as they have been in hematological malignancies.

We seek to address barriers that impede honing of the CAR T cells to where they need to go, and then enable them to overcome the hostile environment so that they are able to effectively kill tumor cells. These are very tall orders, but various approaches are being tried to overcome these challenges – for example, putting several CAR molecules in one cell, something we can do with the larger cargo capacity of our non-viral piggyBac DNA Modification System.

Q What tools of the trade do you currently employ, and in what areas would you like to see innovation?

MG: In our field, genomics analysis sequencing has been an effective tool to characterize the genomic and transcriptomic characteristics of our products. Most importantly, sequencing analysis of single cells has allowed the field to look in detail at the different phenotypic populations of T cells and the composition of them, and see what percentage of them are actually stem cell-like memory T cells, central memory cells, or effector cells.

Knowing the composition of these cell populations allows us to better predict what the likely outcome is going to be. The more you move towards creating a product with abundance of stem cells, the more ability you have for self-renewal, and longer persistence within the blood circulation. Effector cells might be quite effective at the beginning in killing the tumor cells, but they often get exhausted much faster than stem cells. Poseida's proprietary tools allow for non-viral transposition of the construct into T cell's genome, essentially transduces stem cells, thus allowing us to come up with a product that is distinct from others in terms of very high composition of stem cell memory T cells, or TSCM. TSCM cells are a stem cell form of T cells that engraft, self-renew and mature into every T cell subtype, including the effector T, or TEFF, cells, which are tumor killing cells. We believe delivering a high percentage of TSCM cells will drive more gradual tumor killing, thereby inducing less inflammatory cytokine response and improving the tolerability profile of our CAR-T product candidates relative to those of existing CAR-T therapies. This allows engineered T cells to persist and be able to proliferate within the blood circulation for much longer.

Knowing the composition of the product and various sub-populations of the cells, both pre- and post-infusion, is of immense importance to us. Therefore, single cell analysis is something that we are working on to better characterize our product.

Q What are your chief goals and priorities for yourself, and Poseida as a whole, over the next 2 years?

MG: For Poseida, our focus is to take our current product candidates forward as efficiently as possible for patients who are in need of a tolerable and effective treatment, especially in multiple myeloma setting where there is currently no curative product available for patients.

"...we would like to see significant improvement in therapy of solid tumors, starting with prostate cancer..."

Our next product candidate, which we are very excited about, is an off-the-shelf allogeneic CAR T also for patients with multiple myeloma, with the same protein receptor target; anti-BCMA. We intend to utilize everything we have learned from the autologous program to inform the development of our

DOI: 10.18609/cgti.2020.075

allogeneic product candidate. Manufacturing autologous CAR T is less efficient than that of off-the-shelf allogeneic CAR T, so we believe there are benefits to the patient in terms of availability and potential systemic costs as well.

Looking further into the future, we would like to see significant improvement in therapy of solid tumors, starting with prostate cancer, and really crack the code for solid tumors in the CAR T space in general.

My personal goal in the near future is to find biomarkers that allow us to predict the clinical safety and efficacy of the product beforehand – or at least be able to predict what the reaction of the body of the patient could be, so that the product is safer and more effective within the clinic.

AFFILIATION

Majid Ghoddusi
Senior Director, Clinical Biomarkers, Poseida

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Ghoddusi currently holds stock options in Poseida Therapeutics.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

Interview conducted: *Mar 6 2020*; ***Publication date:*** *Jun 5 2020.*

CELL & GENE THERAPY INSIGHTS

CLINICAL DEVELOPMENT STRATEGY, TOOLS & TRIAL DESIGNS

SPOTLIGHT

INTERVIEW

Targeting lung damage in COVID-19 patients with CD34+ cell therapy

DOUG LOSORDO is Executive Vice President, Global Head of Research and Development and Chief Medical Officer of Caladrius Biosciences. Dr Losordo's career has been dedicated to the development of novel therapeutics aimed at the reversal of chronic conditions such as refractory angina, critical limb ischemia, coronary microvascular dysfunction, and heart failure. His guiding principle has been that the restoration of health should be our goal, not the management of ongoing disease. He has developed clinical programs in gene therapy and cell-based tissue repair targeting myocardial ischemia, diabetic neuropathy, refractory angina, critical limb ischemia, severe claudication, coronary microvascular dysfunction and most recently COVID-19 lung damage.

Cell & Gene Therapy Insights 2020; 6(5), 747–753

DOI: 10.18609/cgti.2020.087

Q What are you working on right now?

DL: I'm working on developing therapies designed to repair damaged tissue. That's really been the overarching theme of my career as a researcher and a therapeutic developer – the idea that biological tools can enable us to reverse damage that has occurred in various organs due to diseases, or other types of injury.

Q Can you give us some more background on the Caladrius CD34$^+$ cell platform?

DL: The idea behind this platform came out of a very deliberate search by a smart, very creative post-doctoral fellow who worked in the lab many years ago. He hypothesized that there must be a stem cell in our body that was designed and assigned to repair, replace, and maintain the vasculature.

That was 25 years ago now. While it was quite an innovative thought at the time, today it's almost second nature. We realize that all of the tissues in the body repair and replace themselves on an ongoing basis. Some do so more frequently than others, but none of the tissues in our bodies we have when we are kids are the same as those we have when we reach adulthood and beyond.

This researcher thought there must be a stem cell that was capable of replacing the endothelial cells, which are one of the key components of the vasculature. That was how he came up with the discovery that the CD34 cell, which was already pretty well known as a hematopoietic stem cell capable of replenishing the entire circulating blood system, also had this capability to stimulate the growth, repair or replacement of blood vessels, and in particular, the endothelial cells that line them.

Q Can you go deeper on the rationale underpinning the platform's latest clinical application in the fight against COVID-19?

DL: All cardiologists and vascular biologists have a somewhat vascular-centric view of the universe. We think everything revolves around the blood vessels. And to a certain extent, it really does. If you look at embryology, for instance, it's very typical for the vasculature to be the first thing that develops in an organ, and then the rest of the organ develops around the vasculature.

Our thinking was that we might be able to recreate that same scenario in a tissue repair setting. This seemed particularly rational in the setting of cardiovascular disease, where of course, one of the big problems is the loss of blood supply.

When people think about the loss of blood supply, they tend to think of a major blockage: a big blood vessel that gets clogged and causes a heart attack, or a stroke, or lower extremity ischemia. While that's all absolutely true, a few people recognized many years ago that hand-in-hand with the loss or obstruction of large blood vessels comes the destruction or attrition of the microcirculation. In fact, in some cases, the loss of the microcirculation is an independent process.

> "...the severe affects of COVID-19 on lung tissue occur at least in part due to microvascular damage."

We know that across multiple cardiovascular diseases, there is very good pathological evidence that in patients who get sicker, the

 DOI: 10.18609/cgti.2020.087

"There is certainly preclinical evidence that if you can trigger the recovery of the microcirculation in the lung, then the recovery of overall lung function and the regeneration of lost lung tissue will occur. More specifically, CD34 cell therapy in various forms of lung injury has been shown to result in better outcomes in animal models."

underlying pathology is the ongoing loss of the microcirculation. So with the discovery of this naturally occurring microvascular repair cell, we thought there might be a way to leverage that natural biology and restore microcirculation in tissues where it's been damaged, even in very chronic settings.

Over the past two decades, I've personally conducted a large number of clinical trials in literally hundreds of patients with all kinds of cardiovascular diseases, plus all the preclinical model studies that one needs to conduct before embarking on a clinical trial. Through these studies, I have documented that these cells do replenish the lost microcirculation in multiple types of tissues in a variety of different preclinical models, and that in the clinic, these cells administered in double blind, placebo-controlled studies resulted in significant long-term benefits in terms of reduced symptoms, improved function, and reduced mortality. Furthermore, all these benefits tied directly to the ability of the cells to replenish the microcirculation in the various target tissues.

At least on the surface, one might ask why we are putting a microvascular carousel into people who have had a virus – what has one got to do with the other? But what's interesting is that if you look at the literature – both very recently with COVID-19, and going back to some of the previous SARS virus events that occurred and even more routine viral infections like influenza – in all cases there is very good pathological evidence that these viruses attack the endothelium in the lung, often destroying the function of the microcirculation. This at least circumstantially seems to trigger the cascade of events that either leads to the death of the patient, or to the disability that occurs after recovery in those who survive the acute infection. While the underlying pathology is going to vary in different patients, there is at least one line of reasoning that says the virus attacks the endothelium in the lung, and there is actually some evidence that SARS viruses specifically attack the CD34 cells resident in the lung, leading to destruction of circulation and long-term damage.

So that's one part of the rationale: the severe affects of COVID-19 on lung tissue occur at least in part due to microvascular damage. The other part is the evidence in heart muscle, in brain, in skeletal muscle, and in kidney that the administration of these CD34 cells can restore function in these various tissues that have suffered an ischemic insult – in other words, something that leads to loss of blood supply.

"We have another study that's approved and ready to go here in the US for another of our pipeline therapies. That one is a CD34 cell used to treat coronary microvascular dysfunction..."

Is there any evidence that this same rationale could apply in the lung? There is certainly preclinical evidence that if you can trigger the recovery of the microcirculation in the lung, then the recovery of overall lung function and the regeneration of lost lung tissue will occur. More specifically, CD34 cell therapy in various forms of lung injury has been shown to result in better outcomes in animal models.

There's a lot of activity out there in this area at the moment, quite appropriately – it's been very heartening to see how the entire universe of drug developers from industry and academia have pivoted to address this crisis. There's an awful lot of work being done on anti-virals, vaccines, etc. But one of the areas where we saw a need that didn't appear to be being addressed to the same extent was those patients who have come through the initial crisis. They've survived, been taken off the ventilator, and the virus is cleared from their system. But their lungs are severely damaged, and we know from prior literature on ARDS that a lot of these individuals never recover full lung function. That's where we think we can fill a gap in the current armamentarium against this virus.

Q What can you tell us about the trial design for this initial COVID-19 study?

DL: This is Caladrius's first foray into lung disease, and so we obviously want to make sure we're always looking at safety and tolerability. The urgency of the COVID-19 situation doesn't given you a license to do crazy stuff. However, given the fact that these cells have an extensive track record of safety, our desire is to treat as many patients as we can in the initial study.

In fact, when we first approached the FDA with this protocol, it was under an expanded access strategy – we already had open INDs for these cells in a variety of different indications. We informed the FDA of the large amount of safety data we have generated on these autologous, unmodified cells, and that there has never been an adverse safety event related to them, and therefore we would like to administer the cells in an expanded access setting whereby everyone would be treated.

So this first study will not be a blinded study: the patients who receive the cells will know they are getting them, and we will monitor their progress in turns of recovery following the administration.

Q What might further steps in the clinic look like?

DOI: 10.18609/cgti.2020.087

DL: Once we have collected some initial evidence of bioactivity in the first handful of patients, step two will be a blinded randomized study. However, it will be a crossover design.

We have our own protocol for freezing the cells from these patients – the CD34 cell has a long track record of being successfully frozen and used for hematopoietic stem cell transplantation, so we already know the cells work after really long periods in liquid nitrogen. This means we can do a blinded randomized study, but also tell the control patients not to worry – you're going to get your cells, but it might just be a few months after the initial group of patients receive their cells. Under the circumstances and personally as a physician, I would feel very uncomfortable if these poor people who have suffered and continue to suffer because of lost lung function were denied their therapies at a later date.

I feel that we should be able to collect sufficient, comparative evidence from the initially treated versus the initial control subjects participating in the blinded study to be able to say, "OK, we're now seeing differences between treatment and control in the blinded phase – let's take those cells out of the freezer and treat the volunteer patients who were initially assigned to the control group". That would be the next stage of development.

Looking beyond that, I think there's going to be a very active and interesting discussion with all the regulatory agencies as to what the pathway to approval would be.

Q Can you comment on the rest of the Caladrius pipeline – how has COVID-19 affected your ongoing clinical development plans, and how are you seeking to minimize the impact of the inevitable disruption?

DL: I can tell you that we were coming down the home stretch of a pivotal clinical trial in Japan for our CD34 cell therapy in patients with critical limb ischemia – unfortunately, that's been slowed down somewhat. We have patients anxiously waiting in the wings – more than enough to complete the study – but we can't get them into the clinics, so that they can be screened and then enrolled.

There's no steering around that. The nature of the therapy means patients have to go into the clinics to be evaluated and undergo the study procedures. We really just have to wait until the current situation is behind us in Japan before we can finish that study.

We have another study that's approved and ready to go here in the US for another of our pipeline therapies. That one is a CD34 cell used to treat coronary microvascular dysfunction (CMD), which is a condition I alluded to earlier: despite the absence of blocked major blood vessels, patients with coronary microvascular dysfunction have destruction

> "...given the fact that these cells have an extensive track record of safety, our desire is to treat as many patients as we can in the initial study."

of the microcirculation that leads to the same symptoms that people have with blocked arteries – chest pain, heart attacks, heart failure, etc. Before our approach, there have really been no targeted therapies for this condition. We did a Phase 1 study recently that showed really remarkable improvement in the microvascular function in these patients after a single dose of cells. We were very actively planning the Phase 2 study and were more or less ready to go when COVID-19 came along. We've pivoted to COVID-19 to try to help those patients but are still planning to launch that Phase 2 study – it's just been pushed down the road a bit. We hope that we will be enrolling towards the end of this 2020, but of course that remains to be seen.

Q Finally, can you sum up your and Caladrius's near- and mid-term goals and priorities?

DL: The platform has a couple of pivotal programs. The pivotal program in Japan will finish as soon as things open up there. I think we will finish enrolment within a few months, and we should have a readout less than a year after we complete enrolment, because we already know some of the data looks very good. We also have a pivotal program in the United States that we can launch as soon as we have sufficient funding to finish it. That one will be a roughly 400-patient clinical trial.

Then of course we have the COVID-19 project, which is obviously the major priority for us right now. Launching the Phase 2 CMD program is probably the next thing after that. The data readout from COVID-19 should occur less than a year from the time we launch that clinical study, I would anticipate, because I think we'll have a 6-month endpoint – we should be able to see evidence of bioactivity within six months of administration. And I don't think it will take us very long to enroll patients, because there are so many of these poor people who have survived, but who are still suffering.

AFFILIATION

Doug Losordo
Executive Vice President, Global Head of Research and Development and Chief Medical Officer, Caladrius Biosciences

DOI: 10.18609/cgti.2020.087

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Losordo reports personal fees from Caladrius Bioscience*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *May 7 2020;* ***Publication date:*** *Jun 17 2020.*

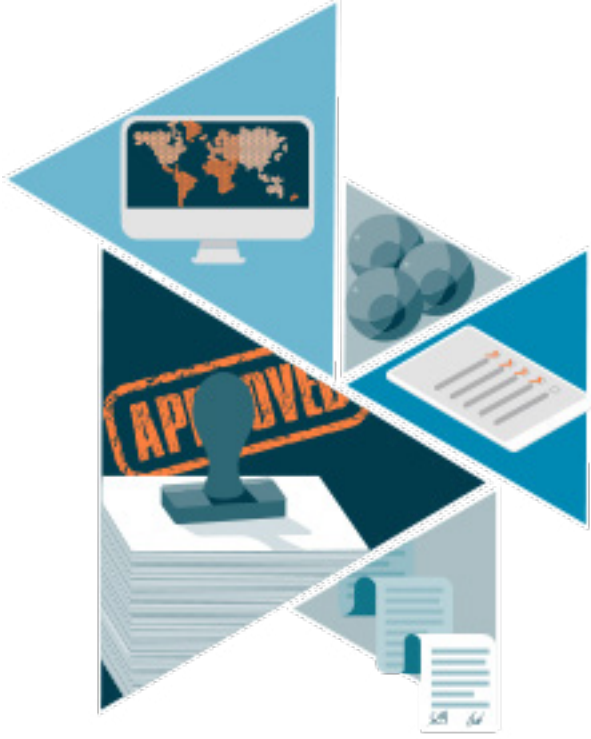
CELL & GENE THERAPY INSIGHTS

June 2020

Regulatory Insights

CELL & GENE THERAPY INSIGHTS

Regulatory Trends

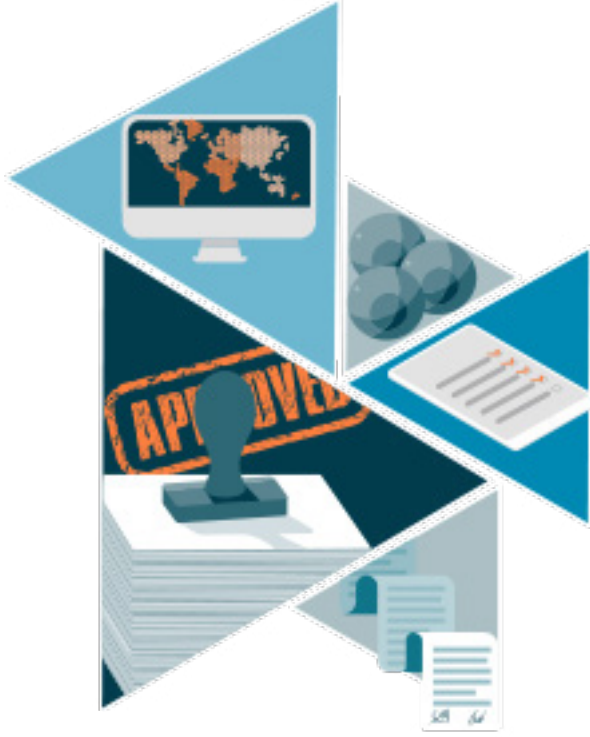


June 2020
Volume 6, Issue 5

REGULATORY PERSPECTIVE

INTERVIEW

CELL & GENE THERAPY INSIGHTS



Regulatory Insights

REGULATORY PERSPECTIVE

Regulatory considerations in the development of gene therapies for neurological disorders in the EU region: an industry perspective

Simon Bennett, Lauren Oliva, Stuart Beattie & Daniela Drago

Gene therapy medicinal products (GTMPs) offer hope to patients across a broad range of diseases, including neurological disorders. In the European Union (EU), the development of advanced therapies, including GTMPs, is governed by a comprehensive medicines regulatory framework, which has evolved over the last decade. The complexity of this regulatory framework presents some challenges to GTMP developers irrespective of the disease area, where some of the clinical and manufacturing aspects require careful consideration. As science and technology continues to advance there are opportunities for the EU GTMP regulatory framework to develop further in order to keep pace with innovation. The development potential for GTMPs in neurological disorders is an emerging area that shows promise and reflects advances in scientific understanding. However, there are some specific considerations for the development of GTMPs in neurological diseases that must be addressed in order to successfully deliver these promising treatments to patients.

Cell & Gene Therapy Insights 2020; 6(5), 877–885

DOI: 10.18609/cgti.2020.100

INTRODUCTION

Innovations in science and technology stand to transform the future of health care through the development of medicines for previously untreatable conditions [1–5] such as in type I spinal muscular atrophy (SMA) where, prior to the regulatory approval of new treatments, patients would not previously have been expected to reach their second birthday. Whilst this progress may impact the treatment of all diseases, it is particularly relevant in neuroscience where advances in neuroimaging and genomics [6] are unlocking new insights into brain activity and the genetic causes of complex neurological and neuropsychiatric diseases [7,8].

As our scientific understanding of disease pathology and therapeutic targets improves, medicine developers are increasingly turning to advanced therapy medicinal products (ATMPs) to overcome the limitations of small molecules and biologic products, which often require repeated administration over a lengthy time period for patients with chronic conditions and only treat the symptoms rather than the genetic cause of the disease. Among the most promising ATMPs are gene therapy medicinal products (GTMPs), which act to replace, suppress, or modify the action of a particular gene to improve clinical outcomes, either through delivery by a suitable viral vector, non-viral vector, or through a cell-based approach (via *ex vivo* genetic modification). This growth in interest is reflected in the number of ongoing clinical studies which include GTMPs, with 1,066 clinical trials underway globally at the end of 2019, of which 94 are Phase 3 trials [9]. These studies cover a range of disease areas including neurology, where a GTMP (Zolgensma®, onasemnogene abeparvovec) based on an adeno-associated virus (AAV) vector, has been approved for SMA. GTMPs are also in clinical development for several other conditions including Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease [10] and neuropathic pain.

In this article, we will outline the key regulatory considerations for the development of GTMPs in Europe from our perspective as a medicines developer and reflect how these may evolve in the future. Whilst many of these regulatory considerations apply to all GTMPs, we will primarily focus on therapies for neurological disorders.

DEVELOPING GTMPS IN EUROPE

The European regulatory framework

In the European Union (EU), ATMPs is a class of complex, innovative therapies which includes not only GTMPs but also somatic cell therapy medicinal products, tissue-engineered products, and combined ATMPs. The latter consist of one of the first three categories combined with one or more medical devices as an integral part [11].

The European Commission (EC) established the legal and regulatory framework for ATMPs in 2007, and Regulation 1394/2007/EC, also known as the "ATMP Regulation", came into force in 2008 [12]. Since then, the EU regulatory framework for advanced therapies has continued to evolve along with an increased use of these modalities. In the past 2 years, EU draft guidance covering the quality, non-clinical, and clinical requirements for investigational ATMPs in clinical trials has been published, in addition to guidance focused specifically on GTMPs [13,14]. The EC's growing experience with advanced therapies has also impacted the broader EU pharmaceutical legislation. For instance, the implementation of Commission Regulation 2018/781 in May 2018, which amended Regulation (EC) No 847/2000, altered the definition of the concept 'similar medicinal product' to take into consideration aspects related to ATMPs.

Development challenges in the current framework

A complex network of European regulations governs the quality, pre-clinical, and clinical

DOI: 10.18609/cgti.2020.100

aspects of GTMP development irrespective of therapeutic target. This complexity may be a contributing factor to the lower number of ATMP trials being conducted in EU Member States compared to other major jurisdictions, such as the US and China [15]. Globally, the number of new clinical trials with ATMPs increased by 32% during the 2014–2018 period. However, whilst there was an overall marked increase in North America (+36%) and Asia (+28%), this was not the case in Europe where the number of ATMP trials grew by less than 2% [16].

From a manufacturing perspective, the availability of investigational (i)GTMP that is compliant with Good Manufacturing Practice (GMP) early in clinical development is crucially important in order to deliver an efficient development plan. Unlike the traditional paradigm, where medicine developers often use material that is developed close to GMP-standard in non-clinical studies and then refine the manufacturing process during the clinical phase, regulatory review for GTMPs can be streamlined by ensuring GMP-compliant materials are used in non-clinical studies. This approach minimizes the subsequent need for comparability assessments and bridging studies. The investment required to ensure earlier availability of GMP-compliant iGTMP may be offset by the opportunity to implement an abbreviated clinical development program where proof-of-concept studies may serve as pivotal data to support a marketing application. This is most common for rare disease indications and may also apply to programs eligible for expedited development pathways, including the European Medicines Agency's (EMA's) Priority Medicine Evaluation (PRIME) scheme.

Potency assays are another major hurdle for many GTMP development programs. A prerequisite for GTMP approval is a validated analytical assessment that demonstrates the product's potency, or the ability of a product, as indicated by a laboratory test, to effect a given result. As per the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidance Q6B [17], a relevant, validated potency assay should be part of the specifications for a gene therapy drug substance and drug product. However, prior to commercial use, an *in vitro* functional potency assay may be challenging to develop for several different reasons. These reasons include lack of clarity regarding the mechanisms of actions and therefore the critical quality attributes (CQAs) which relate to efficacy and identification of suitable tests to assess those CQAs; reduced pre-clinical and early-stage clinical data that can be used to inform the method's development and validation; and variability in the CQAs due to unavoidable variability in the starting material and/or the challenges of the manufacturing process. Implementing wide acceptance criteria may initially appear to address this issue, at least in the short-term, but can cause problems later in development since such an approach may lead to variation in clinical results. Alternatively, a matrix of surrogate assays demonstrating infectivity and transduction *in vitro*, including *in vivo* potency may also be a feasible approach up to the submission of a marketing application.

Divergence in country-level interpretation and implementation of EU legislation further complicates the EU regulatory framework. A widely cited example is the requirement to comply with the individual EU Member State Genetically Modified Organism (GMO) legislation, which varies in definitions of contained use (Directive 2009/41/EC) or deliberate release (Directive 2001/18/EC) and is independent of the EU medicine legislation. This divergence is further exacerbated by varying expertise and application of differing review timelines for clinical trial authorizations (CTA) and GMO approval at a national EU Member State level. The impact of these conflicting policies can be lengthy lead times for investigator site initiation prior to patient recruitment. It is also sometimes the case that GMO approval takes longer than CTA approval, thereby delaying access to investigational studies for European patients. This may be another factor that is contributing to

the lower number of ATMP clinical trials recently seen in EU Member States.

Opportunities to improve GTMP regulation

The EMA's Regulatory Science Strategy 2025 (RSS2025) [18], published in March 2020 after 2 years of consultation and stakeholder engagement, outlines the priorities for the European medicines regulatory network to keep pace with innovation, and includes a proposal to encourage the translation of ATMPs into patient treatments. A range of potential actions are proposed within RSS2025 including provision by the EMA of sponsor assistance during development through early planning, method development, and clinical evaluation; enhancing evidence generation for broader stakeholders including Health Technology Assessment (HTA) bodies; and addressing challenges inherent to decentralized ATMP delivery. Furthermore, the EC published a Roadmap for the Pharmaceutical Strategy on the Timely Patient Access to Affordable Medicines in June 2020, which noted the importance of gene therapies and personalized medicine. Together these initiatives envision a regulatory environment that fosters the advancement of the EU and their success will depend on the support of all stakeholders in the GTMP ecosystem to share feedback and hold each other accountable to delivering tangible outcomes.

One important concept referenced in the EMA's RSS2025 which would facilitate GTMP development in the future is the introduction of a more flexible, iterative approach to scientific advice. This foresees the opportunity for ongoing dialogue between GTMP developers and regulators, including consolidated input from the EMA's scientific committees, which would start early and continue through development as issues arise for a program. Whilst several forums already exist for interaction between medicine developers and regulators e.g. through scientific advice meetings with Committee for Advanced Therapies (CAT) members or Innovation Task Force (ITF) discussions, a more iterative advice framework, where regulators are able to maintain a consistent view of the GTMP product across the lifecycle from early development to post-marketing could help to strengthen development support.

Concerning the challenge of national-level GMO compliance, some improvements have recently been made and medicine developers seeking resources about requirements in the EU can now access some useful new tools. Specifically, the EC recently introduced a web page dedicated to GMO aspects for investigational (i)GTMPs [19]. This resource outlines the specific GMO requirements at an EU Member State and individual Regulatory Authority level, and provides information on the use of a common application form that can be used across different EU Member States for viral vectors. Moreover, the EC issued a Frequently Asked Questions (FAQ) document on "Medicinal Products for Human Use Containing or Consisting of GMOs: Interplay Between the EU Legislation on Medicinal Products and GMOs," which – together with the draft "Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells" – provides further useful advice to GTMP developers navigating GMO requirements in the EU. Furthermore, the Alliance for Regenerative Medicines, an international group of industry, non-profit and patient organizations, has shared solutions to harmonize and simplify the GMO registration process for ATMP clinical trials [16]. One of their key proposals is to integrate the environmental and biosafety review into the CTA process, eliminating separate country-level reviews, which would improve timelines and documentation requirements. While this would necessitate access to the yet-to-be implemented clinical trial portal (the clinical trial information system) mandated by the EU Clinical Trial Regulation [20], if adopted this would be a positive step forward for efficient GTMP development. Across the ATMP trade associations, there are calls to

DOI: 10.18609/cgti.2020.100

request a derogation from EU GMO regulation, to prevent commencement of clinical trials being delayed by national GMO competent authorities. In June 2020, the EC published the proposed derogation to relax GMO requirements for vaccines under development for SARS-CoV-2, the virus responsible for the novel coronavirus pandemic.

GTMP DEVELOPMENT IN NEUROSCIENCE

As outlined in the introduction, gene therapy is an expanding area of interest in neuroscience medicine development. The first GTMP for the treatment of infants and children with SMA was approved in the European Union (and the UK) in May 2020. At the same time there are several GTMP candidates in development for diseases associated with the nervous system including Parkinson's disease, ALS, Huntingdon's disease, and Rett Syndrome. If this list is further broadened to include conditions related to neurosensory organs, including retinal degeneration and sensorineural hearing loss, the number of products in development becomes significant [21].

Treating monogenic versus multigenic diseases

Whilst some neurological conditions, such as SMA and X-linked retinitis pigmentosa (XLRP) are monogenic, other neurodegenerative conditions such as Alzheimer's and Parkinson's Disease are believed to be multigenic increasing the complexity of identifying gene therapy targets. Indeed, a recent study identified 67 autosomal genes (forming 9 gene clusters) as strongly associated with late onset Alzheimer's disease [22].

Early diagnosis in chronic diseases

The presence of long prodromal periods, where disease pathology may occur years in advance of clinical symptoms, and the lack of clinically accepted genetic markers presents difficulties for identification and recruitment of patients into clinical trials [23]. However, in Alzheimer's disease, an improved diagnostic framework, combined with the ability to confirm underlying disease pathology by use of cerebrospinal fluid (CSF) biomarkers or amyloid positron emission tomography (PET), has enabled more accurate diagnosis of Alzheimer's disease in pre-dementia subjects. This has provided the opportunity for earlier detection of disease and the measurement of pharmacodynamic effects, which has in turn created avenues to explore GTMPs in these areas.

Manufacturing at scale

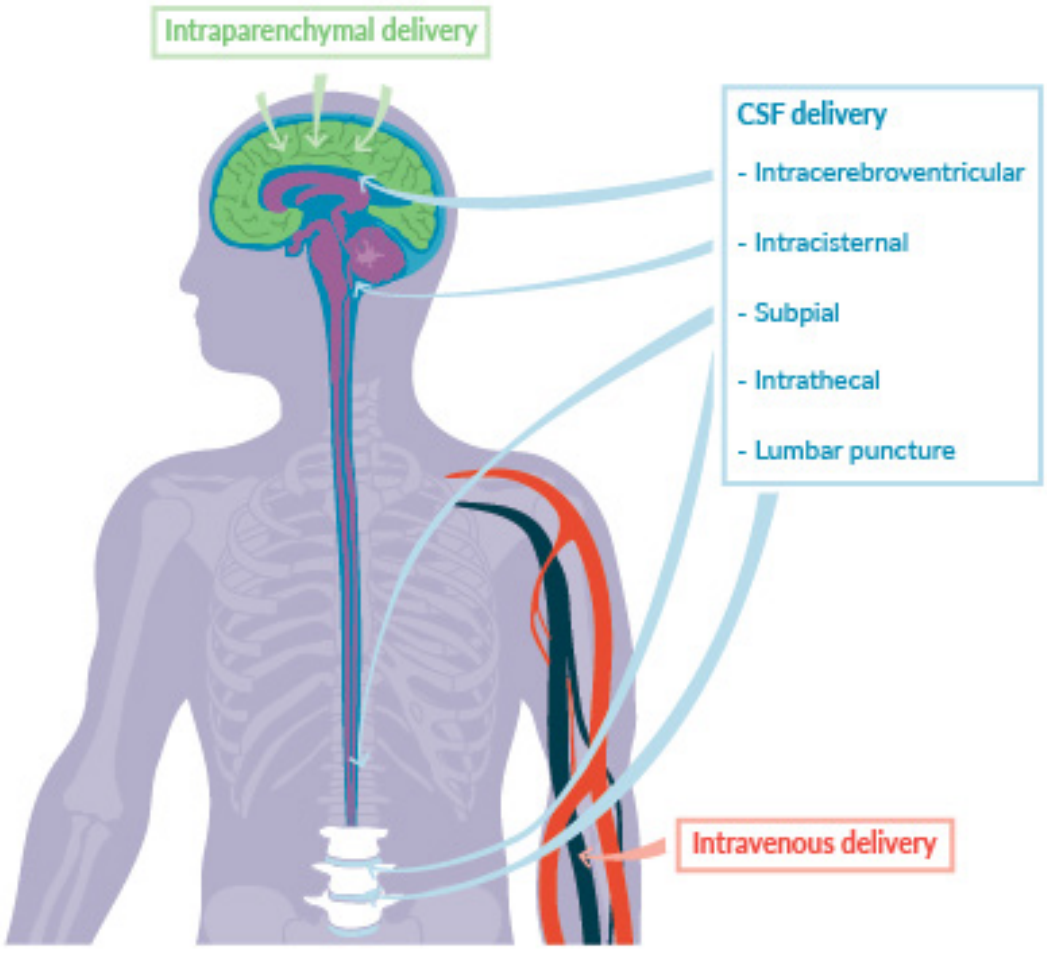
Whilst early advances and regulatory approvals of GTMPs in neuroscience have been in rare disease indications or for single patient use e.g. SMA, the potential to manufacture gene therapies with consistent quality for a large patient population is a challenge that has not yet been faced. The degree of up-scale of rAAV manufacture that might be needed in such circumstances would also depend on the dose, route of administration and target cell types, in addition to the phase of clinical trial, and potential commercial supply requirements. In order to achieve this, manufacturing may need to move away from the current typical approach involving the transient transfection of adherent cells in plastic vessels. The use of producer mammalian cell lines in suspension culture and of insect cells and recombinant baculoviruses may be needed to meet the challenge of larger-scale requirements [24].

Route of administration

Identifying the optimal delivery route for GTMPs targeting the central nervous system (CNS) is exceedingly challenging due to the limited compartment volume and potential risks of invasive procedures, such as direct

intraparenchymal injection. While the most experience has been gained through local injection into the eye to target ophthalmic disorders, where outcome can be monitored non-invasively [25], many neurological diseases may necessitate investigative alternatives to achieve transduction efficiency of the desired cell types. The potential risk, which varies depending on the route of administration and in some cases is yet to be fully defined (i.e. subpial), should be considered when assessing the overall risk benefit for the development programme. Therapeutic levels of gene expression in the CNS has been achieved through several different routes [10] including intravenous (iv) (Zolgensma® marketed in the EU), intraparenchymal, intrathecal (lumbar puncture), intercerebroventricular, intra-cisterna magna (all in clinical development) and subpial administration (in pre-clinical development). Some naturally occurring serotypes of AAV, such as AAV9, have strong neuronal tropism [26] which has been further enhanced through engineering AAV capsid variants developed through directed evolution of shuffled genome libraries. In mice, these have demonstrated the potential to preferentially transduce specific cell types in the CNS, including astrocytes, neural stem cells and cells within the retina [27]. In 2019, a variant of AAV9, AAV-PHP.B, was shown to efficiently deliver transgenes across the blood brain barrier in C57BL/6J mice after iv administration [28]. Similarly, injection of different serotypes via the round window membrane of the neonatal mammalian inner ear demonstrated successful transduction of several cell types (Figure 1) [29].

FIGURE 1



DOI: 10.18609/cgti.2020.100

Safety & risk–benefit considerations

Whilst AAV GTMPs are generally well tolerated, the inability to regulate transgene expression remains a concern. For example, promotors that induce strong expression are more prone to inactivation or gene silencing and unintended toxicity may occur if a transgene is overexpressed leading to off-target effects [10].

Immunogenicity is a thematic concern for GTMPs as a patient may develop neutralizing antibodies through prior exposure to components of a GTMP. In the nervous system the occurrence of an inflammatory responses to GTMPs may be reduced due to the immune privileged nature of the brain and other compartments of the nervous system.

Beyond these immediate safety challenges, the chronic nature of neurological disease like Alzheimer's and Parkinson's disease means that patients will require follow-up for considerable time after treatment, and the true clinical impact of GTMPs in these conditions, both in terms of safety and efficacy, may not be fully known for many years after treatment. This raises the question of whether GTMPs for these chronic neurological conditions may be approved based on their effect on a biomarker alone, or whether evidence of clinical benefit will always be expected. Although rAAV vectors demonstrate a very low integration frequency into chromosomes, with a largely random distribution without preference for risky integration hotspots [30], adult CNS neurons are typically described as permanently postmitotic. This potentially allows for long-term expression of the transgene in the CNS, without the requirement for significant integration of the viral vector-delivered therapeutic sequence [31].

Potency assays

Potency assays must demonstrate that a vector can transfer a gene into a cell and show that the transferred gene has the desired biological effect in the transduced cells. Whilst some of the challenges associated with developing potency assays were mentioned previously, the development of validated assays for gene therapies for neurological conditions is especially challenging. This is due to the complex mechanism of actions within the brain and CNS and the limited points of access to determine the *in vivo* product outcomes, such as migration from the site of administration.

Gene editing technologies

An emerging gene therapy technique with great potential is gene editing. The most widely known approaches in this area currently include zinc finger nucleases (ZFN), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease Cas9 (CRISPR/Cas9). These technologies are of great interest due to their ability to very precisely target specific gene sequences and offer potential for investigation in a wide range of conditions, including neurological disorders.

While there is limited regulatory guidance specific to gene editing technologies, a number of the challenges that surround their translation into viable treatments were discussed at an Expert Workshop convened by the EMA in late 2017 [32], including monitoring for off-target toxicity and aspects of manufacturing. It is a topic where regulatory guidance in these emerging areas would be expected to develop over the next few years as more products enter clinical development and experience grows. The approach of the EMA to organize expert workshops to discuss such developments is helpful in advancing the use of these technologies in medicine development and further engagement in the future should be encouraged.

CONCLUSION

The development of therapies for neurological disorders – long considered too risky, too hard, and too uncertain – is starting to

be addressed successfully. Increased research focusing on unravelling how the most mysterious organ of the body works is accelerating the pace of innovation. Notably, a remarkable increase in the effort to explore AAV gene therapy in the CNS has recently emerged. At the same time, several steps have been taken to improve regulatory frameworks for GTMPs. Some regulatory challenges still exist, and enhancements can be made to overcome potential regulatory barriers to bringing innovative complex new therapies to patients in need. As advances in optimizing AAV capsids, vector delivery, and transgene design continue – with scientific rigor, regulatory flexibility, and clinical innovation – GTMPs in neuroscience will need to advance. This is a challenge for medicine developers and regulators - and a glimmer of hope for patients suffering from these debilitating diseases.

REFERENCES

1. Rousseau CF, Mačiulaitis R, Śladowski D, Narayanan G. Cell and gene therapies: European view on challenges in translation and how to address them. *Front. Med*. 2018; 5: 158.
2. Burns LR (Ed.). *The Business of Healthcare Innovation*. Cambridge University Press, 2012.
3. Cuende N, Izeta A. Clinical translation of stem cell therapies: a bridgeable gap. *Cell Stem Cell* 2010; 6(6): 508–12.
4. Gardner J, Webster A. The social management of biomedical novelty: facilitating translation in regenerative medicine. *Soc. Sci. Med.* 2016; 156: 90–9.
5. Omachonu K, Vincent K, Norman G. Einspruch. Innovation in healthcare delivery systems: a conceptual framework. *Innov. J.* 2010; 1–20.
6. Williams N, Henson R. Recent Advances in Functional Neuroimaging Analysis for Cognitive Neuroscience. *Brain Neurosci. Adv.* 2018; 2: 1–4.
7. Bob P. *Brain, Mind and Consciousness: Advances in Neuroscience Research*. Springer Science & Business Media, 2011.
8. Ehlers M. Neuroscience is the next oncology. Innov. Clin. Neurosci. 2018; 15(3–4): 15–6.
9. Lambert JL. State of the Industry: The Financial, Clinical, and Scientific Landscape for Cell and Gene Therapies. *Cell Gene Ther. Ins*. 2020; 6(1): 47–54.
10. Deverman BE, Ravina BM, Bankiewicz KS, Paul SM, Sah DWY. Gene therapy for neurological disorders: progress and prospects. *Nat. Rev. Drug Discov*. 2018; 17: 641–59.
11. Drago D, Houn F, McBlane J. Regulatory Framework for Advanced Therapy Medicinal Products. Fundamentals of EU Regulatory Affairs. *RAPS* 2020; 419-4338.
12. Regulation (EC) No 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 (2007). Off J L 324:121–137.
13. EMA Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials [draft] 2019: EMA/CAT/852602/2018.
14. EMA Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products 2018: EMA/CAT/80183/2014.
15. Data on file.
16. Alliance for Regenerative Medicine. Possible solutions to improve the European regulatory procedures for clinical trials with Advanced Therapy Medicinal Products consisting of or containing Genetically Modified Organisms. 2017; report.
17. ICH Topic Q 6 B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products. Note for guidance on specifications: Test procedures and acceptance criteria for biotechnological/biological products. CPMP/ICH/365/96.
18. EMA Regulatory Science to 2025 – Strategic Reflection.
19. European Commission: https://ec.europa.eu/health/human-use/advanced-therapies/gmo_investiganional_en
20. Regulation (EU) No 536/2014 of the European Parliament and the Council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC
21. Data on file.
22. Ki Kwok M, Lin Lin S, Schooling CM: Re-thinking Alzheimer's disease therapeutic targets using gene-based tests. *EBioMedicine* 2018; 37: 461–70.

DOI: 10.18609/cgti.2020.100

23. EMA Guideline on the clinical investigation of medicines for the treatment of Alzheimer's disease. 2018; CPMP/EWP/553/95 Rev.2

24. Kotin RM, Snyder RO. Manufacturing Clinical Grade Recombinant Adeno-Associated Virus Using Invertebrate Cell Lines. *Hum. Gene Ther.* 2017; 28(4): 350–60.

25. Rodrigues GA, Shalaev E, Karami TK, Cunningham J, Slater NKH, Rivers HM. Pharmaceutical Development of AAV-Based Gene Therapy Products for the Eye. *Pharm. Res*. 2018; 36(2): 29.

26. Hudry E, Vandenberghe LH. Therapeutic AAV Gene Transfer to the Nervous System: A Clinical Reality. *Neuron* 2019; 839–62.

27. Castle MJ, Turunen HT, Vandenberghe LH, Wolfe JH. Controlling AAV Tropism in the Nervous System with Natural and Engineered Capsids. *Methods Mol. Biol*. 2016; 1382: 133–49

28. Hordeaux J, Yuan Y, Clark PM *et al*. The GPI-Linked Protein LY6A Drives AAV-PHP.B Transport across the Blood-Brain Barrier. *Am. Soc. Gene Cell Ther*. 2019; 912–21.

29. Kim MA, Ryu N, Kim HM *et al*. Targeted Gene Delivery into the Mammalian Inner Ear Using Synthetic Serotypes of Adeno-Associated Virus Vectors. *Am. Soc. Gene Cell Ther*. 2019; 197–204.

30. Kaeppel C, Beattie SG, Fronza R et al. A largely random AAV integration profile after LPLD gene therapy. *Nat. Med.* 2013; 19(7): 889–91.

31. Anda F, Madabhushi R, Rei D *et al*. Cortical neurons gradually attain a post-mitotic state. *Cell Res*. 2016; 26: 1033–47.

32. Report of the EMA expert meeting on genome editing technologies used in medicinal product development. 12 September 2018; EMA/47066/2018.

AFFILIATIONS

Simon Bennett
Author for correspondence
Biogen Limited, Innovation House, 70 Norden Road, Maidenhead, Berkshire, SL6 4AY, UK
simon.bennett@biogen.com

Lauren Oliva
Biogen Inc, 300 Binney Street, Cambridge, MA 02142, UK

Stuart Beattie
Biogen Limited, Innovation House, 70 Norden Road, Maidenhead, Berkshire, SL6 4AY, UK

Daniela Drago
Biogen Inc, 300 Binney Street, Cambridge, MA 02142, USA
and
George Washington University's School of Medicine and Health Sciences, 2600 Virginia Avenue, Suite T100, Washington, DC 20037, USA

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Bennett, Dr Oliva, Dr Beattie and Dr Drago are all employees of Biogen Limited.*

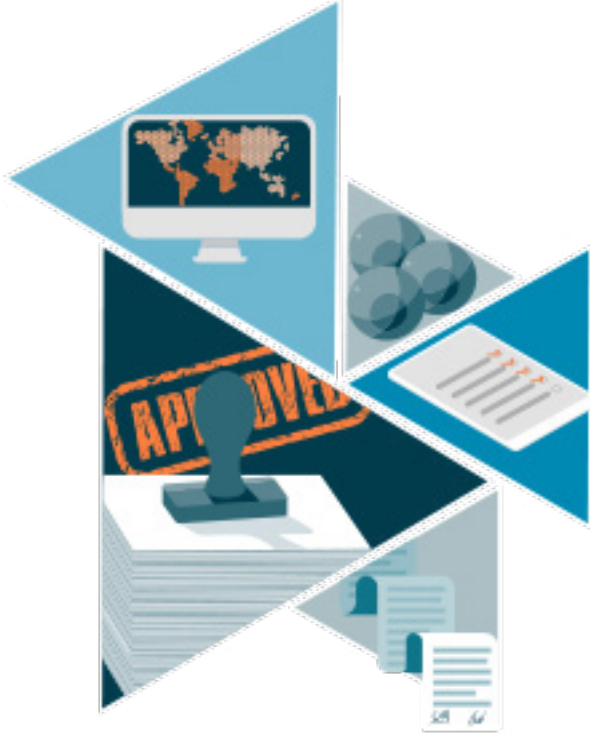
Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Jul 1 2020*; ***Revised manuscript received:*** *Jul 22 2020*; ***Publication date:*** *Jul 30 2020.*

CELL & GENE THERAPY INSIGHTS



Regulatory Insights

INTERVIEW

Regulatory affairs in regen med: building trust, expediting development and navigating the impact of COVID-19

MANAL MORSY's extensive experience in drug development with particular focus on strategic regulatory aspects taking into account early research, clinical, commercial, and post marketing development have made her a global leader in pharmaceutical regulatory affairs. Her 30 years of successful experience in technically and managerially leading teams to successful biologics' (vaccines and cell therapy) and new chemical entities' key regulatory designations (RMAT, SPA, Fast Track, Priority review, Orphan) as well as multiple global marketing traditional and conditional authorization approvals have made her an asset to Athersys Inc., where she currently serves as the Senior Vice President and Head of Global Regulatory Affairs.

Cell & Gene Therapy Insights 2020; 6(5), 799–805

DOI: 10.18609/cgti.2020.092

Q Can you tell us what you're working on right now?

MM: We are currently working on several high priority programs using our stem cell product, MultiStem®. Our current clinical development includes our MASTERS-2 study for treatment of ischemic stroke, which is being conducted under a US FDA protocol assessment agreement for registration studies. We are also working on our MACOVIA study, which is specifically for COVID-19 induced acute respiratory distress syndrome (ARDS), and a Phase 1/2 trauma study.

We have Fast Track designation for the ischemic stroke and ARDS programs, and we also have a Regenerative Medicine Advanced Therapy or RMAT designation for the stroke program. In addition, we are supporting the development of regulatory activities associated with ischemic stroke and ARDS studies being conducted by Helios, our partner in Japan.

Q Can you tell us more about the specific utility of the MultiStem® platform for application in COVID-19?

MM: We observed in our prior preclinical and clinical work that MultiStem® can modulate the immune system response, especially in severe inflammation associated with indications such as trauma, ischemic stroke events, and acute inflammatory responses in general.

Our MUST-ARDS clinical study suggests the potential of MultiStem® to treat ARDS patients. Our MACOVIA study builds on this experience, and it's intended to evaluate MultiStem®'s potential in treating patients who develop ARDS as a result of COVID-19 infection and pneumonia.

Beyond COVID-19, there are many patients who develop ARDS for a variety of reasons and who face significant mortality risks and difficult long-term recovery with limited treatment options. We believe that MultiStem® could also be efficacious in these types of cases.

Q What does the regulatory pathway look like for this particular application, in light of the unusual, urgent scenario we are facing with COVID-19? What role for compassionate use, for example?

"Beyond COVID-19, there are many patients who develop ARDS for a variety of reasons and who face significant mortality risks..."

MM: The FDA has worked with exceptional speed and flexibility and has been extremely responsive. Especially for COVID-19 treatment development, they have worked to enable more rapid development of technologies that may help in treating COVID-19 patients and also in designing and starting studies.

DOI: 10.18609/cgti.2020.092

"...although we are interested in speed, we of course want to conduct well designed, placebo-controlled studies that robustly determine the potential for MultiStem® treatment to help COVID-19 patients who develop ARDS. The FDA worked with us to enable this. Our aim is to complete a successful MACOVIA study as quickly and efficiently as possible..."

In our case, for the MACOVIA study activities, we have benefited from rapid reviews and discussions with the FDA regarding protocol finalization allowing for rapid site initiation. This was significant and enabled us to get our first couple of patients in less than 6 weeks from the beginning of putting a study design together until enrollment – the speed with which we have been able to move is really incredible.

But although we are interested in speed, we of course want to conduct well designed, placebo-controlled studies that robustly determine the potential for MultiStem® treatment to help COVID-19 patients who develop ARDS. The FDA worked with us to enable this. Our aim is to complete a successful MACOVIA study as quickly and efficiently as possible, helping make this product available as soon as possible to as many patients as may need it.

In terms of compassionate use, it's a requirement for each company to post their compassionate use policy on their website. When the right-to-try legislation was passed, our company had numerous in-depth discussions before we reached the conclusion that it is not feasible for us to provide extended access to our investigational drugs or biologics outside of our clinical trials at this time. This policy is on our website and can of course be reviewed. The reason, which we deliberated so much about and reached the decision we did, was to ensure the long-term availability of our therapies. This can best be achieved by demonstrating safety, tolerability and effectiveness through the context of properly designed and authorized controlled clinical trials, which are established and conducted to address the requirements of regulatory agencies for approval.

For us, it was more important that we make sure we are conducting these studies to reach as many patients as possible rather than offering extended access outside of controlled clinical studies, which may unintentionally prevent or delay access for a much broader patient population in a period of great need.

Q Turning to the lead neurological indication of MultiStem®, ischemic stroke, can you frame for us the challenges you encounter in your role in coordinating regulatory approaches in Japan, Europe, and the USA?

MM: Making sure that our regulatory strategy takes into account the global perspective has always been an objective both for me and the company as a whole and has been at the forefront of how we manage our programs. Although there are sometimes unique requirements for Japan vs EMA vs FDA, continuous communication and transparency with regulators plays a significant role in ironing out many of these challenges and building strategies that are satisfactory for all.

I've always maintained that it is critical to obtain agreement upfront with regulatory agencies, especially on key strategic decisions. One common theme I have observed over the years is how very helpful and collaborative regulators are, especially when relationships with them are established based upon transparent trust and collaborative grounds.

In terms of the differing regulatory expedited pathways that we have, we firstly obtained Fast Track and RMAT designation for the stroke program, and FDA agreement for the MASTERS-2 clinical study. Then we obtained a positive Scientific Advice opinion on the pivotal MASTERS study design and registration plan from the EMA. We were very transparent with the EMA: we told them that we had already received FDA Special Protocol Assessment (SPA) and Fast Track designation, and what that meant, and explained our intent to accelerate development. Our questions were very clear relating to the fact we are looking for alignment. It was a very positive interaction. At the same time, the same program in Japan – represented by the TREASURE study conducted by our partners, Healios – received Sakigake designation.

Therefore, we have managed a global expedited pathway for MultiStem® therapy in stroke, even though there are differences in the various regions. Across these regions, we have successfully pursued a common approach for enhancing agency communication, which will hopefully accelerate our goal of facilitating a rapid path to approval.

Q Regarding RMAT designation specifically, what are your reflections upon your experience of it to date? What have been the key learnings?

MM: RMAT designation is the breakthrough designation for regenerative medicine, with the added advantage that it also supports an accelerated pathway including early interaction with FDA to discuss any potential surrogate or intermediate endpoint, and potential ways to satisfy post-accelerated approval requirements. All the privileges of breakthrough designation are available through RMAT, meaning intensive guidance on efficient drug development beginning as early as Phase 1, organizational commitment involving senior managers on the FDA side, all Fast Track designation features and actions to expedite development and review, and eligibility for

"I've always maintained that it is critical to obtain agreement upfront with regulatory agencies, especially on key strategic decisions."

DOI: 10.18609/cgti.2020.092

rolling submission review. The interactions with the FDA have been exceptional with very rapid turnaround in communications.

In terms of learnings, I believe that as a regulatory professional, to advance excellent candidate drug development and benefit patients with high unmet medical needs, every possible regulatory pathway made available to facilitate, support, and expedite development should be considered. There are significant advantages to early and transparent collaborative communication between industry and regulators, and I believe it's crucial to identify obstacles and tackle them together early on in order for a sponsor to be enabled to continue the development pathway for critical programs in an efficient and expedited manner.

> "I've been very encouraged by the readiness of regulators to engage with industry and revise guidelines and regulations..."

Q Looking back on your own extensive career in biopharma regulatory affairs, what best practices have you picked up along that journey, particularly in relation to operating in the regenerative medicine field?

MM: As I mentioned earlier, one of the key learnings that I owe to my tenure at Merck and J&J is the importance of building a trusting and transparent relationship between myself as a regulatory professional, my company, and the regulators. It's of the utmost importance to establish that relationship very early on.

One of the importance practices I've learned in the biotechnology environment is the need to rapidly tackle issues associated with novel therapies in collaboration with regulators, which again is built on this foundation of trust and transparency. For example, early on in my regulatory career at Merck, I worked on a program developing an HIV vaccine. Later on, at J&J, I worked on a high unmet medical need orphan drug program for multi-drug resistant TB. Subsequently, I was involved with a novel Duchenne muscular dystrophy (DMD) orphan indication program at PTC Therapeutics.

All three of these programs required innovative paving of uncharted development pathways: there was no other HIV vaccine development or approved treatments at the time of the first project; TB had a drug that was 30 years old so there was nothing to follow in terms of endpoints or study design; and Duchenne muscular dystrophy was an orphan indication with no development activities at the time, DMD patients were mainly getting corticosteroids – there was no DMD specific drug option. All three programs required very intense collaboration with regulators. Each of them presented unique challenges, requiring collaborative problem-solving with the FDA and other regulators. Working very closely with the regulators to carve an uncharted path, such as selecting meaningful endpoints and agreeing on relevant study designs with limited information available to build on, was both challenging and energizing at the same time.

These experiences and key practices, such as building transparent and trust-based regulatory relationships and collaborative problem solving, are very relevant to and effective in the new, innovative and unprecedented regenerative medicine field.

Q Looking to the future, where would you like to see evolution to further expedite development and commercialization of regenerative medicine products?

MM: I like what I'm seeing in terms of openness from regulators to interact more frequently and effectively with industry. So much has changed from when I started 30 years ago and is continuing to change.

The evolving process includes closer communication between industry and regulators, and better understanding of the true obstacles in manufacturing, clinical trial design, and even conducting those trials, especially under the circumstances we're in right now. I've been very encouraged by the readiness of regulators to engage with industry and revise guidelines and regulations in a much more effective and expedited fashion than has ever been seen in the past. I would really like to see this continue.

Q Can you summarize for us both your own and Athersys' key priorities for the foreseeable future?

MM: At Athersys, we are focused on advancing and completing clinical development as thoughtfully and efficiently as possible to enable us to bring an important, novel therapy to patients in areas where there are many unmet needs.

Our ischemic stroke and ARDS programs are key priorities, as our preparations to supply the therapy following successful studies, and hopefully regulatory approval, to patients who need it most. Again, continuing the collaborative relationship with regulatory agencies will be important in achieving these objectives.

My personal goal is to get these drugs to the market. Especially when a drug has multiple high unmet need serious critical indications, I think it is very important to make it available as widely as possible to patients.

AFFILIATION

Manal Morsy
Senior Vice President & Head of Global Regulatory Affairs, Athersys Inc.

DOI: 10.18609/cgti.2020.092

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *This article addresses topics relevant to Dr Morsy's employer, Athersys, Inc., where she is a senior manager and a shareholder.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *May 27 2020;* ***Publication date:*** *Jul 10 2020*

CELL & GENE THERAPY INSIGHTS

April 2020

Commercial Insights

CELL & GENE THERAPY INSIGHTS

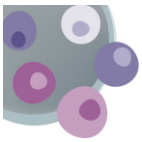
COMMERCIAL INSIGHT: APRIL 2020

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.

Cell & Gene Therapy Insights 2020; 6(5), 761–774

DOI: 10.18609/cgti.2020.089



CELL THERAPY – Mark Curtis. Director, Manufacturing Partnerships, AVROBIO

Interest in allogeneic approaches to T cell therapy continues to gain momentum as drug developers, both small and large, see value in bulk value manufactured drug product. In April we saw two notable deals that will advance allogeneic cell therapies towards the clinic. The first was a deal between Sangamo and Mogrify, where Sangamo will leverage Mogrify's computational platform to convert iPSCs to T cells. The second was a deal between J&J and Fate Therapeutics, where J&J will gain access to four targets for CAR-T and CAR-NK therapies. On the autologous front, Gilead/Kite partnered with Australia-based oNKo-innate, to leverage the company's genome-wide screening platform to develop NK cell therapies. Also, recently formed start-up, TScan, which has a novel genome-wide TCR/target discovery platform created at Harvard, partnered with Novartis to develop TCR therapies targeted to kidney cancer and other solid tumor types.

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

With news headlines dominated by the COVID-19 pandemic, it's great to see that the world of gene therapy can offer assistance to vaccine development. The announcement that researchers at two Harvard-affiliated hospitals are adapting a proven form of gene therapy to develop a coronavirus vaccine offers an alternative approach to traditional vaccine development, and one that will be watched closely by the scientific community. Elsewhere, there have been a couple of early clinical development updates, with the news that Orchard Therapeutics has started its first trial in MPS-IIIA, and publication of early clinical data from a research group in Germany in CNGA-linked achromatopsia.

Clinical Regulatory

COVID-19 VACCINE DEVELOPMENT LOOKS TO GENE THERAPY FOR INSPIRATION

With researchers around the globe working to find treatments for COVID-19, one team from Massachusetts Eye and Ear and Massachusetts General Hospital believe they have found a unique approach: adapting adeno-associated virus (AAV) vectors for use in a coronavirus vaccine. The vaccines candidates have only been tested in mice so far, but primate studies are due to begin shortly, and clinical studies are anticipated to start later this year.

Dr Luk H Vandenberghe, director of the Grousbeck Gene Therapy Center at Massachusetts Eye and Ear, explained in an interview that their angle has several advantages – including that AAV is already used in approved gene therapies and has an established safety profile. As AAV vectors are already widely manufactured, production could be more easily scaled up to meet demand should any of the trial vaccines prove successful – and biotechnology companies with existing AAV manufacturing facilities could potentially aid in making the required vector.

Dr Vandenberghe commented:

"We are leaning on an established industry. AAV as a class has had investments in the dozens of billions of dollars. There's capacity out there that can be leveraged if we are lucky and successful."

FDA AUTHORIZES IND FOR THE STUDY OF CALADRIUS' CLBS119 IN COVID-19 SURVIVORS

Caladrius Biosciences has announced that the FDA has authorized an IND application for the study of its cell therapy offering, CLBS119, in COVID-19 patients.

CLBS119 is a $CD34^+$ cell therapy that could potentially repair damage to the lungs caused by COVID-19; evidence suggests that COVID-19 survivors who required ventilator support during their illness continue to suffer from the effects of lung injury caused by the virus. In clinical and preclinical studies, $CD34^+$ cells have shown evidence of vascular repair in multiple organs, including in models of severe lung inflammation.

"COVID-19 appears to damage the vasculature of the lungs and repair of that vasculature will be necessary for patients to achieve a full recovery. Although many therapies are

DOI: 10.18609/cgti.2020.089

targeting the SARS-CoV-2 virus itself or the reduction of severe inflammation during the acute phase of the illness, no therapy has been shown to repair COVID-19 induced lung damage," commented Douglas W Losordo, Chief Medical Officer at Caladrius. "CLBS119 offers the potential to repair the lung damage caused by COVID-19 and to address a serious unmet need for patients," he added.

GENE EDITING APPROACH REVERSES DIABETES IN MICE

A recent study in *Science Translational Medicine* has documented a technique for reversing preexisting diabetes in a murine model using CRISPR-Cas9 gene editing. Diabetes is caused by the death or dysfunction of β cells within the pancreas and can be treated using insulin injection, but it is a lifelong condition which brings a risk of a variety of side effects and complications including damage to the eyes, kidneys and nervous system. Transplantation of allogeneic pancreatic islets containing β cells has previously seen success in treating diabetes, but comes with drawbacks in the form of low donor numbers and the need for an immunosuppressive regimen.

The study involved using cells from a patient with Wolfram syndrome 1, a variant of a rare genetic disorder resulting in childhood diabetes and other symptoms including vision loss. The researchers used CRISPR-Cas9 to correct a diabetes-causing pathogenic variant in induced pluripotent stem cells (iPSCs) derived from the patient in order to generate functional β cells. These cells were then transplanted into severely diabetic mice, where they successfully performed insulin secretion in response to glucose and rescued the diabetic phenotype, with blood glucose levels remaining lowered for the 6-month observation period. This is in contrast to previous methods for generating patient iPSC-derived β cells, where the cells did not show as much function *in vitro* and *in vivo*, and were unable to achieve normoglycemia. The authors are hopeful that the technique could lead to the development of autologous cell replacement therapy for various forms of diabetes.

EXCELLTHERA'S ECT-001 RECEIVES ORPHAN DESIGNATIONS FROM EMA AND FDA

Clinical-stage molecular medicine company ExCellThera has been granted orphan medicinal product designation from the EMA for its ECT-001 cell therapy, a treatment for use in hematopoietic stem cell transplantation. ECT-001 has also been granted Orphan Drug Designation for the prevention of graft-versus-host disease and Regenerative Medicine Advanced Therapy (RMAT) designation in the treatment of hematologic malignancies from the FDA.

ECT-001 combines the small molecule UM171 and an optimized culture system in order to reduce transplant-related mortality, chronic graph versus host disease and relapse in cord blood transplant therapies for blood cancers, thereby improving patient outcomes. Several clinical studies involving ECT-001 are currently underway, including in multiple myeloma and high-risk leukemia. ExCellThera intend to complete enrollment in Phase 2 trials in the next few months.

Dr Guy Sauvageau, CEO and founder of ExCellThera, commented:

"We are extremely pleased that our lead technology, ECT-001 Cell Therapy, has been granted orphan medicinal product designation from the EMA as treatment in hematopoietic stem cell transplantation. The clinical data generated to date emphasizes the potential of this cell therapy to change the transplantation treatment paradigm for patients, resulting in better recovery and overall health."

NANTKWEST AND IMMUNITYBIO TEAM UP TO TAKE ON COVID-19

Two immunotherapy companies under the NantWorks umbrella, NantKwest and ImmunityBio, have announced that they are combining resources in order to develop potential coronavirus therapeutics and vaccines. The companies are in active talks with the FDA and hope to combine ImmunityBio's experience in vaccine development, and its platform of immunomodulators for cancer and infectious diseases, with NantKwest's expertise in off-the-shelf cell therapies.

The aim is to treat patients at different phases of the disease, from moderate infection through to severe acute respiratory distress syndrome, through a number of different approaches. ImmunityBio's adenovirus vector-based Ad5 vaccine platform will be used as a potential avenue to protect against infection. In mild to moderate infection, the collaboration plans to trial natural killer and T cell stimulation using NantKwest's haNK – CD-16, off-the-shelf natural killer cells, given alone or combined with convalescent plasma – and ImmunityBio's interleukin 15 'superagonist' cytokine, N-803. For later-stage patients requiring ventilatory support, NantKwest's bone marrow-derived mesenchymal stem cells (MSCs) will be deployed.

N-803 is currently in clinical trials for other indications, and has received Breakthrough Therapy designation from the FDA for the treatment of BCG-unresponsive non-muscle invasive bladder carcinoma *in situ*, while the second generation of Immunitybio's Ad5 platform has reached safety endpoints in Phase 1 and 2 studies. For MSC production, NantKwest's proprietary isolation and expansion methods will be combined with ImmunityBio's automated, closed system to grow cells within 7–9 days.

Investigational New Drug (IND) applications are now pending for the planned trials.

Patrick Soon-Shiong, Chairman and CEO of both NantKwest and ImmunityBio, commented:

"While development of therapies is urgently needed in this crisis, as urgent is the need to develop a vaccine with long-lasting cell-mediated immunity. Developing vaccines in the time of pandemics requires novel approaches and the use of modernized genomics, molecular dynamics, and vectors that are proven to induce cell-mediated immunity, with mass scale production capabilities."

COLOR BLINDNESS THERAPY SEES SUCCESS

A first-in-human trial of a subretinal gene therapy to treat color blindness suggests that the treatment may be both safe and effective, paving the way for future trials. Total color blindness, or achromatopsia, is caused when the cone cells in the eye which provide bright

DOI: 10.18609/cgti.2020.089

light and color vision do no react appropriately to light. The condition can have a significant visual impact and is associated with problems including day blindness and involuntary eye movements, with no treatments currently available.

The authors of the study, published in *JAMA Ophthalmology*, recruited nine patients with achromatopsia linked to variations in CNGA3, one of six genes known to cause the majority of cases of total color blindness, to receive surgery and subretinal injection of the gene therapy vector AAV8.CNGA3 in one eye. During the 12 month follow up period, no substantial safety problems were observed, and all nine treated eyes showed improvement in cone function, including improved visual acuity.

Similar to Luxturna, an FDA-approved gene therapy for treating vision loss, the biggest benefits could be gained if patients are treated at a young age before structural loss of cone photoreceptors occurs – and the researchers are hopeful that a follow-up study in pediatric patients with a *CNGA3* mutation could be a promising next step.

Expert Pick

The publication of the first clinical trial of an AAV-based gene therapy treatment in 9 patients with CNGA-linked achromatopsia suggests the treatment is safe and may activate cone photoreceptors. Achromatopsia, also known as rod monochromacy, is present in about 1:30,000 births, with *CNGA*-linked achromatopsia making up around 25% of the overall population of patients. It is an autosomal-recessive genetic disease defined by loss of cone cell function in the retina, classically presenting with color blindness, photophobia, nystagmus, and decreased visual potential with visual acuity often less than 20/200. The new data from a group of ophthalmologist at the University Hospital Tübingen in Germany provides encouragement for this group of patients, but the real test will be whether significant functional improvements can be achieved in children with the condition. – Richard Philipson

IMPROVING GENE THERAPY FOR THE CNS WITH SHORT GENE PROMOTERS

Gene therapy is a promising approach for treating diseases of the central nervous system, but there are still limitations, such as the relatively small payload capacity of AAV vectors which can affect their ability to cause long-term transgene expression within the

CNS. Another issue is the relatively large gene promoters used which can be repressed after introduction to the CNS.

Esteban Engel, a researcher in viral neuroengineering at the Princeton Neuroscience Institute, and his team have developed a technology which could help solve the problem: short gene promoters which could save space and allow AAV vectors to carry larger or multiple genes. The promoters were designed by adopting attributes from the herpesvirus family, viruses which can remain in the body for years by creating chronic infection within the nervous system. In a study in *Molecular Therapy: Methods & Clinical Development*, the team demonstrate that the promoters can provide efficient and long-term transgene expression in the brain and spinal cord in a mouse model.

The use of such promoters could be especially useful in the treatment of diseases such as Parkinson's and Pompe disease, which require the delivery of relatively large genes to the CNS.

"These new promoters will allow us to deliver larger genes or multiple small genes, and the genes can remain active for as long as they are needed," commented Engel.

KITE PHARMA SETS SIGHTS ON A NEW GENERATION OF CAR T THERAPIES UTILIZING TENEOBIO TECHNOLOGY

Kite Pharma has struck a new deal with Teneobio to license a suit of antibodies that target B-cell maturation antigen (BCMA) in order to develop next generation CAR T therapies for multiple myeloma. The two companies will also make use of Teneobio's heavy-chain antibody platform to discover antibodies for up to four more targets to be used in multiple myeloma treatment. According to Peter Emtage, Kite's senior vice president of research, the deal is part of a strategy to replicate the success Kite has had with Yescarta in blood cancers that express CD19.

The advantage of heavy chain antibodies is their small size, which could potentially allow for fitting multiple chimeric antigen receptors within a vector. This could mean the creation of CARS that target more than one antigen, which could reduce relapse – a common issue in multiple myeloma patients who may relapse and exhaust the front line treatments available to them. Research has shown that relapse may occur because some of the patient's cancer cells don't carry the antigen the CAR T is targeted against – therefore adding an additional target could help prevent this.

One of the licensed antibodies is currently in use in a Phase 1 study at the National Cancer Institute against multiple myeloma, and Kite plan to file an Investigational New Drug application based on the resulting data.

"We have limited payload in lentiviral or retroviral platforms, so the ability to put smaller CARs means we will be able to marry those in the future—as we develop our multiple myeloma strategy—with other modulators, say, of the tumor microenvironment, or other enhancements of T-cell activity," commented Emtage.

FIRST SANFILIPPO TYPE A PATIENT DOSED IN TRIAL OF OTL-201

The first patient has been dosed in a clinical trial investigating Orchard Therapeutic's OTL-201 for treatment of Sanfilippo syndrome type A, the company has announced.

DOI: 10.18609/cgti.2020.089

The open-label, Phase 1/2 proof-of-concept study is recruiting 3–5 patients aged between 3 months and 2 years.

Sanfilippo syndrome type A is caused by mutations in the *SGSH* gene that cause a deficiency in the enzyme sulfamidase, which leads to the accumulation of long sugar molecules within cells, eventually resulting in neurodegeneration, developmental regression, and a severely curtailed lifespan. OTL-201 is a cell-based gene therapy which introduces a working copy of the *SGSH* gene using the patient's own stem cells.

The trial will use sulfmidase levels as a marker of the therapy's efficacy, and will include overall survival, cognition and behavioral changes and quality of life improvements as additional outcomes; participants will be followed for 3 years after receiving the therapy.

"I am very encouraged that we, together with our research and clinical collaborators in Manchester, could achieve this important milestone in our efforts to develop a gene therapy for MPS-IIIA despite the current, challenging global health circumstances," commented Orchard CEO Bobby Gaspar.

Ones to Watch

The first patient dosed in Orchard Therapeutics clinical trial of OTL-201 in Sanfilippo Type A (MPS-IIIA) marks an important milestone for the company, and accelerates its move away from ADA-SCID and towards metabolic diseases as an area of focus. In MPS-IIIA, a mutation in the N- SGSH gene results in the build-up of mucopolysaccharides in the brain and other tissues, leading to intellectual disability and loss of motor function. Life expectancy of children born with MPS-IIIA is estimated to be 10–25 years, and there is currently no approved treatment. The company uses a lentivirus-based, *ex vivo* transduction method for treatment, and patients therefore have to undergo bone marrow conditioning prior to re-infusion of transduced stem cells. The road ahead is long, with a 3 year follow-up period for treated patients, but this is an important first step. – Richard Philipson

Licensing agreements & collaborations

EVOTEC ANNOUNCES MOVE INTO GENE THERAPY ALONGSIDE A NEW PARTNERSHIP WITH TAKEDA

Evotec has announced a new alliance with Takeda in order to expand into gene therapy R&D – the company has established a 20 person gene therapy unit alongside Takeda's gene therapy center in Orth an der Donau, Austria. Further details of the multiyear partnership have not been released, but it features an upfront fee and other payments over time, and will see Evotec apply its gene therapy and drug discovery capabilities to Takeda's cancer, rare disease, neuroscience and gastroenterology programs.

Although a transfer of employees has not been referenced, Friedrich Scheiflinger, who until recently working in drug discovery for Takeda in Austria, will now be leading the new Evotec gene therapy unit – and as Evotec has commented that it's new team ""have worked together for many years", it is likely that other Takeda employees have also made the move.

Werner Lanthaler, Chief Executive Officer of Evotec, commented:

"We are delighted to initiate our new gene therapy platform and step into this field, which perfectly fits into our business strategy going forward. In recent years, precision medicines based on cell and gene therapies have emerged and are predicted to grow significantly. Gene therapy is a promising approach in the development of genetic medicines for patients, especially for inherited and rare diseases. Finding the best candidate agnostic of modality for any given disease biology will ultimately bring forward the best medicine for patients."

SANGAMO ALLY WITH MOGRIFY TO SECURE TREG SUPPLY

Startup Mogrify will generate regulatory T cells (Tregs) using its direct cell conversion technology for use in Sangamo Therapeutic's allogeneic CAR Treg therapies under the terms of a new deal.

Access to a reliable cell supply has emerged as a critical consideration for developing off-the-shelf cell therapies, as sources of T cells can be limited. Sangamo hopes that the license agreement with Mogrify will provide a

DOI: 10.18609/cgti.2020.089

scalable Treg supply to support the development of allogeneic CAR Treg therapies.

Under the terms of the agreement, Mogrify will work on the discovery and optimization of the cell conversion technology making it possible to convert induced pluripotent stem cells and embryonic stem cells into Tregs. Sangamo will then have the exclusive rights to the resulting process and will use its zinc finger protein gene engineering technology to develop novel cell therapies.

"This license agreement provides Sangamo with access to Mogrify's cell conversion technology, which will diversify our options as we develop off-the-shelf allogeneic CAR-Treg cell therapies," commented Jason Fontenot, Head of Cell Therapy at Sangamo. "We expect this collaboration to accelerate our development of scalable and accessible CAR-Treg cell therapies, so that we can potentially deliver treatments to patients with inflammatory and autoimmune diseases more rapidly."

Expert Pick

There are a variety of approaches to generating T cells for allogeneic cell therapies. Industry is moving away from donor sources, which are limiting in terms of expansion potential, and focusing on induced pluripotent stem cells as starting material. Several companies utilize directed differentiation techniques to produce T cells, which guide stem cells down specific developmental pathways through the implementation of exogenous transcription factors. Mogrify's technology is also based on transcription factors, however, is unique in that it takes a computational approach allowing for the identification of optimal combinations of transcription factors that drive the direct conversion of stem cells to T cells. One aspect of Mogrify's platform that is particularly powerful, is the potential to drive efficiency and markedly reduce cost-of-goods in T cell production through the identification of small molecule cocktails that target a group of pre-identified transcription factors to drive the conversation process. – Mark Curtis

BIOMARIN INVESTS IN GENE THERAPY STARTUP

BioMarin has recently announced its plans to back DiNAQOR, a Swiss gene therapy startup with a focus on heart failure. The preclinical collaboration and license agreement aims to develop novel gene therapies to treat rare cardiomyopathies – neither company has disclosed financial details of the deal but DiNAQOR will receive an upfront payment. It will also be eligible for tiered royalties on worldwide sales, along with development and commercial milestone payments.

BioMarin has licensed DiNAQOR's lead program DiNA-001, against myosin-binding protein-C hypertrophic cardiomyopathy, and will collaborate on several other programs in the pipeline, along with investing in the company.

Lon Cardon, Chief Scientific Strategy Officer and Senior Vice President at BioMarin, commented:

"We are thrilled to collaborate with the researchers at DiNAQOR to conduct this pioneering work on the development of gene therapies for inherited cardiomyopathies. We believe there is tremendous potential in combining our experience in gene therapy research and development with DiNAQOR's in-depth knowledge of genetic heart diseases."

GILEAD AND KITE BROKER KILLER NEW DEAL

Through its acquisition of Kite Pharma, Gilead Sciences has announced a new deal with Australian biotech oNKo-innate for a three year research collaboration focused on using natural killer (NK) cell technology for the development of new therapies.

oNKo-innate will use genome screening techniques to discover new immune cell targets that boost NK anti-tumor immunity, and to create NK cell therapies. For Gilead, oNKo-innate will find targets to feed into its immuno-oncology discovery programs, and for Kite, oNKo-innate will "create and evaluate NK constructs for Kite's development of next-generation cell therapies."

NK cell technology could bring several benefits to Kite and Gilead's cell therapy offerings – it is thought NK cells could solve some of the current issues hampering the success of CAR T therapy in treating blood cancers, and cut down on some of the adverse effects seen with CAR T such as cytokine release syndrome, which can cause a systemic inflammatory response.

Jai Rautela, co-founder and CEO of oNKo-innate, commented:

"With more than 20 years of collective academic expertise in NK cell biology, we have long believed in the potential for NK cells to play a role in cancer immunotherapy. We look forward to bringing this NK cell expertise and our unique screening techniques into a collaboration with Gilead and Kite to serve a common goal of discovering new treatments for patients."

ADICET BIO AND RESTORBIO ANNOUNCE REVERSE MERGER

Privately held Adicet Bio has come to an agreement to combine with resTORbio in order to go public and trade on the Nasdaq Global Market using resTORbio's Nasdaq listing, in a move known as a "reverse merger".

resTORbio has been seeking a purchase of the firm or its technology after its lead drug candidate failed a critical Ohase 3 trial last November and the company saw shares fall over 87%. Under the merger the new company will retain the Adicet Bio name and combine resources to advance Adicet's allogeneic cell therapy work, including a lead program targeting the cancer protein CD20 in patients with non-Hodgkin lymphoma – and will continue to pursue development of ResTORbio's failed drug for an undisclosed COVID-19 related application.

Chen Schor, resTORbio CEO, will be replacing Adicet CEO Anil Singhal as the head of the new company, with Singhal becoming a board advisor. If the transaction is greenlit by each company's shareholders, it is anticipated to close later this year – and as resTORbio's stock price saw a jump of 26% since the announcement, this could be a sign investors like the idea.

DOI: 10.18609/cgti.2020.089

Finance

TEAM BEHIND ZOLGENSMA LAUNCH NEW GENE THERAPY COMPANY

A group of former AveXis executives and investors have recently unveiled Taysha Gene Therapies, a company speeding on to the scene with 15 programs, $30 million in seed funding, a partnership with the University of Texas Southwestern Medical Center (UT Southwestern), and plans to be in the clinic by the end of 2020.

Taysha is working on adeno-associated vector gene therapies for monogenic diseases of the central nervous system – with a lead program targeting GM2 gangliosidosis, and further trials planned for Rett syndrome, SURF1 deficiency and *SLC6A1* genetic epilepsy. The company also has the option to choose four more prospects from UT Southwestern – the research center will handle discovery and preclinical work, with Taysha poised to pick up clinical development and handle regulatory strategy, manufacturing and commercialization.

"We essentially flew the plane and built it at the same time when we were developing AveXis ... We have people with the experience of being able to develop, manufacture and commercialize a gene therapy program and we're marrying that with a best-in-class academic research institution,"

Taysha CEO and co-founder, R. A. Session II, commented.

NOVARTIS AND TSCAN TEAM UP TO TACKLE SOLID TUMORS WITH TCR

In a deal worth $30 million, Novartis and Harvard University spinout TScan Therapeutics have united to develop new T cell receptor (TCR) therapies to treat solid tumors. TScan will identify targets and the TCRs that target them, and Novartis has the right to license up to three treatments, with the right of first refusal on any other targets and TCRs that result from the collaboration.

TScan's technology looks at both T cells themselves and at what they target in tumor cells – allowing target and therapeutic discovery to become part of the same process and providing immediate leads to take towards the clinic. This is the aspect that captured Novartis' attention, according to TScan CSO Gavin MacBeath. TCR treatment could also see success in solid tumors, as unlike CAR T

which recognizes surface antigens, TCRs target proteins within the cells.

"There hasn't been technology that enables you to, on a completely genome-wide basis, discover what the natural target of a T-cell receptor is. Previously there have been limited efforts at this, where people had a small collection of targets they could look at to see if a T-cell receptor is recognizing them. But there was no path forward to finding a natural target," added MacBeath.

TScan is also developing its own pipeline, including both a blood cancer program and the freedom to develop any targets and TCRs that Novartis doesn't pursue. A long-term goal of the company is to match targets in a given tumor with a TCR using a repository, allowing for quick identification of potential treatments.

Ones to Watch

TCR therapies have a key advantage over CAR-T therapies, which is that they are able to hit intracellular targets, though most companies developing TCR therapies have focused on a common group of targets. TScan's technology, developed at Harvard, takes a genome wide approach to discovering natural TCR targets giving TScan the ability to greatly expand the universe of TCR targets. While it is partnered with Novartis, TScan will retain its own portfolio of drug targets to bring forward to the clinic and will generate value through both licensing and its own clinical development efforts. – Mark Curtis

UK GENE THERAPY INNOVATION RECEIVES £16 MILLION BOOST

The UK Medical Research Council (MRC) and independent research charity LifeArc are offering grants for up to 5 years to UK-based research organizations in order to create a network of "Gene Therapy Innovation Hubs", to the tune of £16 million. The hubs will offer clinical grade vectors, along with translational and regulatory guidance, to academic-led gene therapy clinical trials in order to aid in progressing novel therapies.

Together, the hubs will form a centrally coordinated network to encourage sharing of knowledge and capabilities, and allow researchers to overcome hurdles to translation, such as a lack of vector production capacity and the complexities of navigating the clinical translation of a therapy.

Professor Fiona Watt, Executive Chair of the MRC, said:

"We are delighted to be working in partnership with LifeArc on this exciting initiative. Continued investment in translational research and in advanced therapies remains a major priority for the MRC and, through this partnership, we aim to support clinical development of the most exciting gene therapy projects from

DOI: 10.18609/cgti.2020.089

the UK's world-leading academic researchers. This investment will streamline and accelerate progress towards a new generation of genetic medicines for patients."

A FATED DEAL WITH J&J

Johnson and Johnson has penned a $50 million upfront collaboration with Fate Therapeutics to use Fate's induced pluripotent stem cell (iPSC) platform to develop cell therapies based on four targets. Similar to Sangamo's decision to collaborate with Mogrify, J&J is also looking for a renewable cell source to aid in the development of off-the-shelf allogeneic cell therapies.

With J&J choosing the targets and providing the funding, Fate will working to develop CAR NK and CAR T cell therapies up to the point of filing an IND – J&J will then have the option of obtaining exclusive rights to the prospective treatments. As they advance, J&J could pay up to $1.8 billion in development and regulatory milestones, and up to $1.2 billion in commercial milestones. Additionally, Fate will have the option to co-commercialize the drugs in the US, and will receive royalties on worldwide commercial sales.

Movers & shakers

CATALENT APPOINTS NEW PRESIDENT OF CELL & GENE THERAPY

Manja Boerman will take on the role of President of Cell & Gene Therapy for Catalent from June 1, 2020. Catalent's gene therapy activities have been led by Pete Buzy since 2019, and he will remain in an advisory role for the next 12 months to support the transition.

When joining Catalent in December last year, Dr Boerman brought over 20 years' experience in the biotechnology and

pharmaceutical spaces, both in start-up environments and late-stage clinical development of cell therapies. She most recently served as president of the UK-based CMO Aesica Pharmaceuticals. Prior to this, she held positions with Charles Rivers Laboratories where she was involved with opening a new facility in Boston, and served as CEO for a Netherlands-based biotechnology company. Her previous position as Catalent's Region President of Biologics, Europe, will be filled by Mario Gargiulo, who previously worked in various leadership positions at Bristol Myers Squibb.

– Written by Roisin McGuigan,
Cell and Gene Therapy Insights

DOI: 10.18609/cgti.2020.089

CELL & GENE THERAPY INSIGHTS

RESEARCH ARTICLE

The necessity of automated manufacture for cell-based immunotherapies: a cost-based analysis

Ryan McCoy, Jahid Hasan, Stephen Ward & Nicholas Gaddum

Automation adoption is a fundamental requirement to de-risk manufacturing processes and support sustainable commercial realization of cell and gene therapies. In this study, we examined cost and productivity sensitivities to increasing automation for the manufacture of cell-based immunotherapies. Firstly, we stratified automation adoption into four strategic levels (Manual, Bolt-together, Integrated and High-throughput) and adapted each to support the manufacture of an exemplar CAR-T immunotherapy. Then, using an internally developed modelling tool, we demonstrated automation adoption at the Bolt-together level reduced the Cost of Manufacture (23%) to Manual processing with limited further reductions seen as a function of increasing automation levels (max 30%). However, more significantly, we illustrated how automation adoption delivers increased throughputs (batches/yr) proportional to automation level in the example modelled, when maintaining facility footprint and constraints. This study highlights the value of employing modelling tools to strengthen early-stage development activities with respect to the assessment of automation adoption strategies to support commercial realization and confirms the requirement for automation if cell and gene therapies are going to realize their full potential at industrial scale.

Cell & Gene Therapy Insights 2020; 6(5), 673–690

DOI: 10.18609/cgti.2020.071

INTRODUCTION

Cell and gene therapies (CGTs) are showing immense promise to transform the management of chronic disease, from diffuse cancers to monogenetic disorders and eye disease. There are currently 14 licensed products in total being actively reimbursed within the US (9) and EU (9) respectively – with the ability to supply the market, a core component considered during licensure. The immune-oncology sector has dominated recent advances in the field, with gene modified Chimeric Antigen Receptor (CAR) T-cell immunotherapies demonstrating durable responses in up to 80% of complex liquid tumor patients [1–5]. FDA and EMA awarding of marketing authorization to Novartis's Kymriah® and Kite's Yescarta® in 2017 and 2018 respectively, swiftly followed by high profile acquisitions (Gilead's $12 billion for Kite and Celgene's $9 billion for Juno Therapeutics), signals commercial commitment to CGTs. Furthermore, the CGT industry attracted greater than $16 billion dollars in financing over the first 3 quarters of 2019 [6]. However, positive clinical data and the receipt of marketing authorization do not guarantee commercial success. Health payer reimbursement, clinical adoption, supply logistics and cost-effective robust manufacturing strategies have all been identified as key deterministic factors of commercial success [7–9]. Each of these present unique challenges, and analysis can employ different tools. Herein we apply an engineering approach to analyze the latter, where various manufacturing strategies of a CAR T-cell product are viewed considering the associated cost and capacity.

For this we need to examine the evolution of these therapies and how they move from discovery to clinical use. Novel therapies generally emerge from laboratory research environments. Early stage development benefits from flexible, manual processing strategies allowing agile exploration of new biological technologies. Ideally, therapies showing promise would then benefit from process and analytical industrialization through automation to increase potential for commercial success. However, at this stage of development funds are scarce, and industrialization investment has to compete with clinical data generation. In turn, this locks-in those flexible, manual processes which benefit discovery, with limited scalability for commercial roll-out.

CGT manufacturing process industrialization has become a specialism in itself seeding the growth of companies with appropriate capabilities. Commercial entities providing this function typically provide expert process diagnostics, analytical test development and access to automated commercial bioprocessing systems. When automating manufacturing systems, two models currently exist. Firstly, manual processing steps can be closed and automated on an individual unit operation basis. These systems can then be 'bolted' together. This daisy-chaining of systems support the generation of a closed end-to-end automated process, but often still requires manual intervention to transfer material between systems representative of different unit operations. Secondly, integrated solutions exist, whereby multiple unit operations are combined onto a single platform [10]. These reduce operator intervention and increases process consistency, however they are less flexible, and inherent process bottlenecks renders other unit operations unavailable. Finally, in-built analytical functionality is currently limited, meaning process monitoring still requires sampling for off-line analysis.

To meet the expected 'high-throughput' required for realizing commercial success of autologous immunotherapies, integrated processing may reflect automotive and pharma manufacture, allowing parallel processing of multiple patient therapies on a single platform. This shift from manual processing, to 'bolt-together' automation, to integrated solutions, and eventually to a futuristic 'high throughput' system, presents new commercial and quality challenges [11] that require addressing prior to health sector adoption [12]. Challenges already recognized include:

DOI: 10.18609/cgti.2020.071

development of enabling technologies to allow safe manufacturing stream parallelization; incorporation of process analytical technologies (PAT) for monitoring and control; shifting from scheduled (stepwise recipes) to adaptive (feedback control) processing. These developments will enable scalability, and are a prominent discussion point within the industry [13] when contemplating the vision for idealized 'high-throughput' systems of the future.

When considering the therapy reimbursement cost, investor-funded development costs need to be recovered as well as the ongoing manufacturing cost of goods (CoGs). These are then viewed in-line with the targeted disease prevalence (rare versus common) which means that a one-solution-fits-all strategy to automation adoption will not be appropriate. At this point CoGs can be affected through process industrialization, and therefore the art of CoGs modelling should direct automation planning.

Current modelling strategies for determining the manufacturing CoGs fall into two distinct categories. The first utilizes a goal seeking orientated approach, identifying the most cost-effective solution in an 'unconstrained' environment, by mass balancing different combinations of bioprocessing technology solutions [14–16]. The second, constrains the manufacturing facility design or the process toolchain and seeks to maximize/optimize a pre-defined measure of success, such as cost or throughput [17,18]. Technically, whilst both modelling strategies can provide valuable insight, gaps exist, especially around the oversight of the assumptions and constraints. This makes it challenging to compare model outputs from different authors to support comparative analysis. Furthermore, from a practical perspective, companies rarely have the capital to build facilities from the outset that are capable of meeting projected demands for different stages of the development or commercialization pathway. This coupled with the high risk of investment early in the development program, when supporting clinical data is yet to be realized, means this second strategy for CoGs modelling has the potential to be of greater value in understanding how to maximize utility of collaborations with CMOs, or of leased manufacturing space, where the facility infrastructure is already in place.

The aim of this study is to apply different automation strategies to CGT Catapult's exemplar CAR-T cell therapy process to interrogate their potential to optimize Cost of Manufacture (CoM) and manufacturing throughput. Manufacturing costs including suite layout, process equipment utilization, staff scheduling, and quality control/release aspects have all been considered in order to provide a comprehensive analysis with the assumptions and constraints clearly detailed. This study supports understanding of how the implementation of four different levels of automation (manual, 'bolt-together', integrated and a forward looking idealized 'high-throughput' system) may impact the scalability of CAR-T therapy manufacture.

MATERIALS & METHODS

The economics and resource utilization model applied in this study was developed using Microsoft Excel® (Microsoft Office 2016, Microsoft Corporation, WA, USA). This model was designed to evaluate the relationship between key manufacturing strategy considerations in terms of manufacturing suite footprint, throughput, labor utilization and CoM as a function of applied automation level. The model focused exclusively on what occurs within the manufacturing suite and quality control (QC) activities. As the model omits the parameters described below, cost projections as a function of automation level are described as CoM rather than CoGs.

- Pre- and post-manufacturing unit supply chain logistics and staffing thereof;
- Cold chain storage capacity and staffing thereof;
- Warehousing and staffing thereof;

- Quality management/assurance oversight (except for the QP);
- Commercial licenses associated with the use of reagents and equipment;
- Batch failure rates – it was assumed all batches were completed successfully;
- R&D costs during development.

Automation level definition

To stratify process automation into different categories for evaluation, an indicative structure describing four discrete levels was proposed as defined below and illustrated in Figure 1.

Manual

A process with a low level of automation. Most unit operations are performed utilizing conventional manual handling methods. This approach requires use of Biological Safety Cabinet (BSC) isolators to provide a Grade A processing environment for material manipulation in a Grade C background. Process material is cultured in laboratory incubators. For the exemplar CAR-T process modelled in this study, only the cell isolation processing step utilizes an automated unit operation (CliniMACs® Plus). All analytical techniques are performed off-line.

Bolt together

A process where automation is applied at the individual unit operation level. This approach still requires operator intervention to transfer material between unit operations but can be performed completely using closed processing technologies. As each automated device represents a single unit operation and is operated independently, it means that multiple devices within the same process stream can be utilized at the same time – supporting concurrent manufacture of different patient therapies (e.g., Patient A material may be processed by Device 1 whilst Patient B material is being processed by Device 2). All analytical techniques are performed off-line. Cell expansion has 'on-line' monitoring capability for some process parameters (pH, Dissolved Oxygen [DO]).

Integrated

A process where automation is applied across multiple unit operations in a unified manner utilizing closed processing technologies and disposable single use tubing/reactor sets. The only operator intervention during processing is to remove samples or exchange reagent reservoirs. Each integrated platform can only handle a single batch at any one time. All analytical techniques are performed off-line. Cell expansion has 'on-line' monitoring capability for some process parameters (pH, DO).

'High-throughput'

A hypothetical, automated system capable of processing parallel streams of patient material utilizing closed processing technologies. The system has integrated at-line and on-line analytics (minimizing the need for operator intervention). The system takes advantage of shared functionality across the parallelized streams.

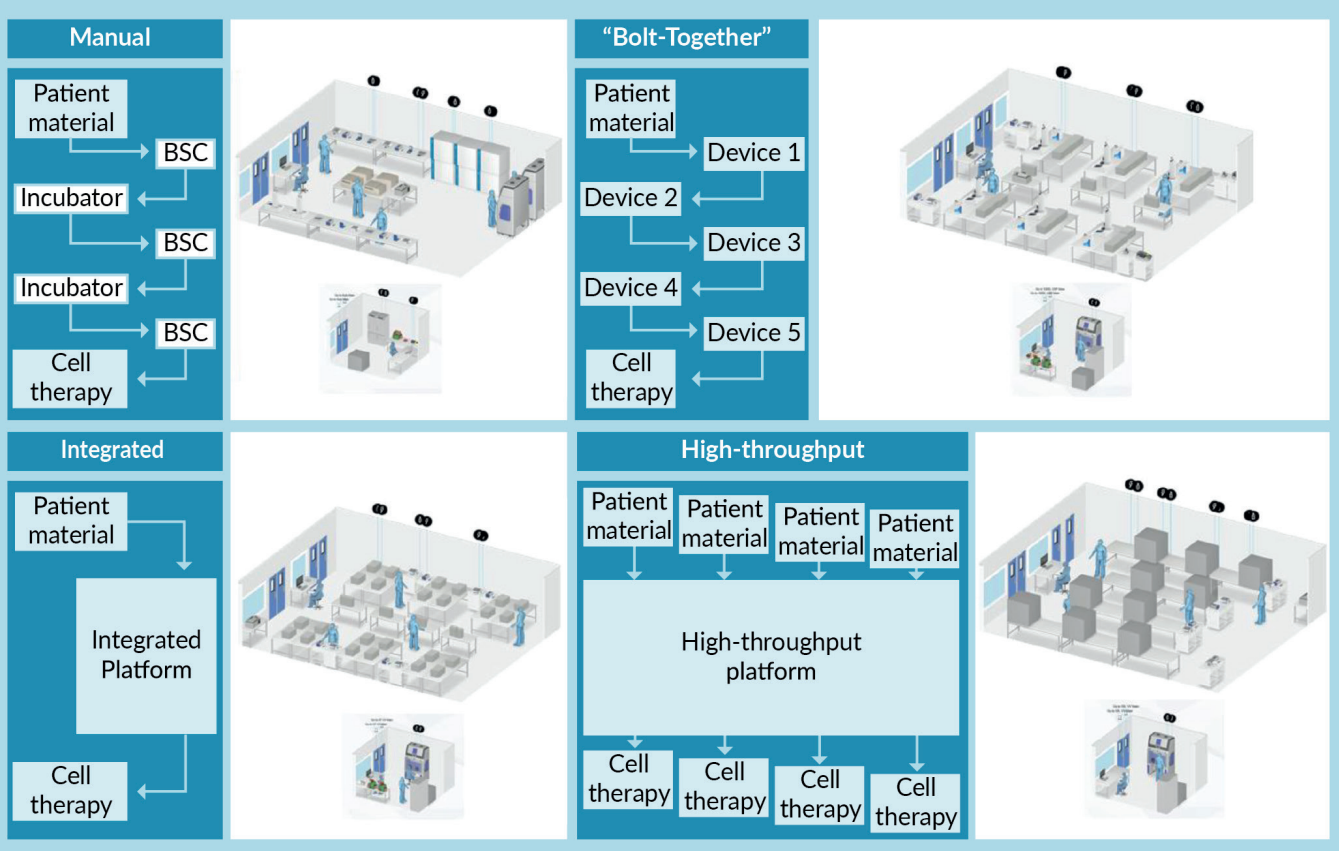
CAR-T cell process definition

A generic exemplar 7-day CAR-T immunotherapy process, capable of generating a single dose of 1 billion $CD3^+$ cells per patient, was defined for use in this study and then adapted to the automation levels described above (Figure 2). Technologies used (using generic names), key processing parameters and analytical testing regimes for each are captured in Table 1 and Table 2 respectively. Differences in the cell seeding strategy between manual and automated technologies to achieve the target dose in the proposed timeframe, was based on the authors practical experience of implementing processes within similar expansion technologies and operational conditions; with the point of commonality chosen as a seeding

DOI: 10.18609/cgti.2020.071

FIGURE 1

Indicative structure of operation the four levels of automation and translation these concepts into manufacturing designs for the exemplar CAR-T immunotherapy process studied.



Each manufacturing suite consisted of 2 rooms, with the larger primary room dedicated to core processing activities and the smaller auxiliary room to in-process control analytics and reagent preparation activities (where appropriate).

density of one million $CD3^+$ cells/mL. Two databases were generated and populated with the unit costs for the reagents and consumables (Supplementary Table 1) or the equipment (Supplementary Table 2) used for each automation level. Where the consumable kits or physical equipment didn't exist (e.g., for the integrated and parallel processing solutions), prices were estimated based on benchmarked values against existing technologies. Processes for all automation levels were assumed to occur in a Grade C manufacturing environment.

The model: set-up & workflow

An overview of the four-step method developed for the model set-up and workflow is defined in Figure 3.

Step 1

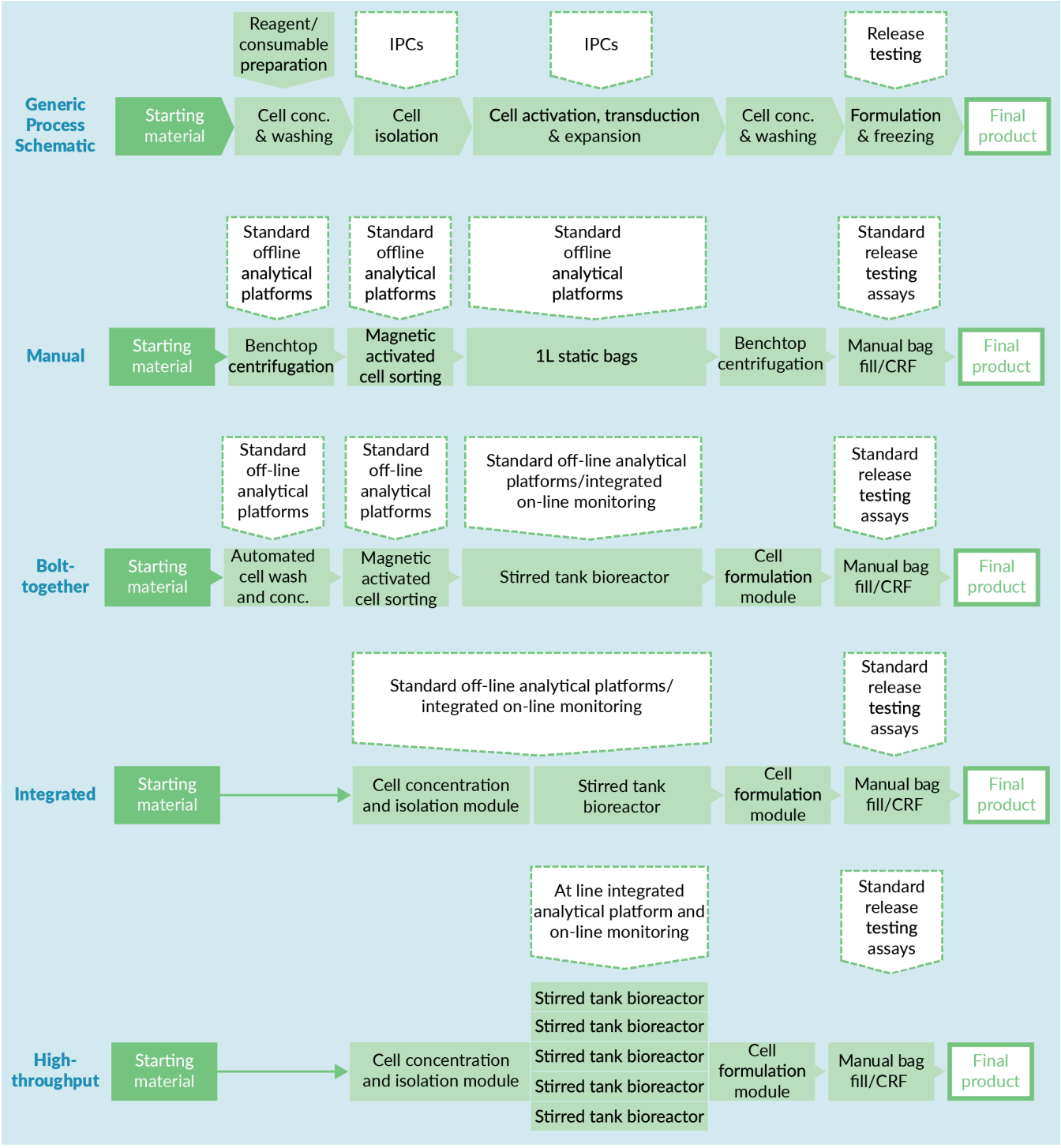
Generic model assumptions and constraints, independent of automation level, for the manufacturing suite (and facility) operation (Table 3) and regulatory or quality compliance (Table 4) were defined. Included here is a maximum number of operators working in the clean room at any time due to particle generation.

Step 2

As described above, discrete levels of automation were defined and translated into CAR-T manufacturing processes based on the exemplar CAR-T process. Specific assumptions as a function of each automation level were captured at this point (Table 1).

FIGURE 2

Overview of how CGT Catapult's exemplar CAR-T process was adapted to demonstrate the four different automation levels employed in this study, as previously detailed in Figure 1.



The 'Generic Process Schematic' reflects the unit operations/process steps that were considered as part of the exemplar process. The streams for each of the four automation levels modelled then illustrate the technologies used and the integrated vision of these unit operations for 'Integrated' and 'High-Throughput' automation levels.

DOI: 10.18609/cgti.2020.071

Step 3

Cleanroom suite equipment layout designs were established and refined in an iterative manner, by determining the number of parallel streams that could be simultaneously processed without exceeding resource or equipment constraints. In brief, we assumed operators could work in an independent fashion with key processing steps having to be verified by a secondary operator in accordance with accepted pharmaceutical good manufacturing practice. The maximum number of 'processing' operators required to run each batch per day, were defined to be different dependent on the level of automation. Therefore, one key difference applied in the model was the ratio of 'processing' operators to 'secondary sign-off' operators required for each automation level. Next, based on maximum number of operators available, the total number of man hours available per suite per day were calculated. We then determined the number of parallel batches possible within the available man hours, keeping in mind the equipment constraints for each option as determined by the current iteration of the suite layout. If a piece of equipment was determined to be a bottleneck, then the additional items would be included, and the above process would be repeated. In this study, only the manual process option was equipment limited, with isolator availability identified as the bottleneck. For the other automation levels, where equipment constraints were not limiting, the scheduling pattern providing the most balanced resource utilization was selected. The finalized suite layouts were visualized using HakoBio Software (OUAT! Live Sciences, Brussels, Belgium) (Figure 1).

Step 4

The model for each automation level created was then run. Each model could be based on constant footprint or constant facility throughputs assumption, to determine the impact on key measured responses (e.g., cost of manufacture, labor requirements etc.). Costs were then extracted from the model and represented as either facility costs (a function of cleanroom rates, equipment depreciation (based on 10-year lifetime) and renewal rates), labor costs (as a function of total staff numbers, including process operators, QC operators and QPs) or variable costs (e.g., raw materials, consumables, QC analysis reagents etc.). Variable costs were calculated on a per batch basis. The facility and labor costs were calculated annually and then divided by the throughput to determine the contribution per batch to the CoM.

The Cost of Manufacturing (CoM) per batch was determined by summing the annual suite (facility and labor) costs, dividing by the number of manufactured batches per suite, per year (throughput), and then adding the variable costs per batch.

RESULTS

Cost of manufacturing per batch (CoM) & annual throughput

Figure 4A illustrates the calculated CoM and breakdown into facility, labor and variable contributions for each automation level. Compared to Manual production, the introduction of Bolt-Together automation reduced CoM by 23% (£32,707 and £25,206, respectively) per batch. Interestingly, increasing automation level further did not substantially reduce the batch cost for Integrated (24% decrease, £24,717) and offered a modest improvement for High Throughput (30% decrease, £22,944). However, facility throughput was enhanced considerably when compared to Manual (320 batches p/a); 290% with Bolt Together (936 p/a), 400% with Integrated (1272 p/a) and 760% with High-Throughput (2436 p/a), see Figure 4B.

Further analysis of Figure 4A revealed that almost all the batch cost could be attributed to the variable costs for the Bolt-Together, Integrated and High-Through automation strategies (86, 88 and 94% of the total cost, respectively). This was a function of the fixed

TABLE 1

Assumptions and constraints associated with the exemplar CAR-T process modelled in the context of the four levels of automation.

Hypothetical CAR-T process assumptions		
Process step	**Automation level**	**Assumption**
Starting material	**All**	**Apheresis (fresh)**
Cell conc. and washing	Manual	Benchtop centrifugation using commercially available buffer
Cell conc. and washing	Bolt Together	Stand-alone closed and automated volume reduction and wash technology – cells washed in commercially available buffer
Cell conc. and washing	Integrated High-throughput	Volume reduction and wash technology within integrated platforms. Cells washed into commercially available buffer
Cell isolation	Manual	Stand-alone Magnetically Activated Cell Sorting Technology – (CD4/CD8 positive selection)
Cell isolation	Bolt Together	Stand-alone Magnetically Activated Cell Sorting Technology – (CD4/CD8 positive selection)
Cell isolation	Integrated High-throughput	Magnetically Activated (or equivalent) Cell Sorting Technology integrated into platform design – (CD4/CD8 positive selection)
Cell seeding	Manual	400 million $CD3^+$ cells at 1 million cells/mL in cell expansion bags
Cell seeding	Bolt-Together Integrated High-throughput	100 million $CD3^+$ cells at 1 million cells/mL in STRs
Activation	All	CD3/CD28 based Polymeric Nanomatrix activation agent
Transduction	All	Lentiviral vector added on day 1 at MOI of 1:1 to $CD3^+$ cells. (Total seeding volume of 'Manual' process is 4x other systems and therefore 4x amount of vector is required)
Expansion	All	Basal media + 5% human AB serum
Expansion	All	Cytokine addition on day 1, 3, 5 (IL-7 and IL-15) through closed processing techniques
Expansion	All	Scheduled feeding regime – medium addition on days 3 and 5 (equal to 40% media volume addition in system on each day, so that after two feeds system volume has doubled)
Expansion	All	Total process length – 7 days
Expansion	Manual	1 L Bags (maximum working volume 800 mL) – static culture in incubators (1 patient per shelf, 3 patients per incubator)
Expansion	Manual	Expected >2.5-fold cell fold expansion
Expansion	Bolt Together	Seven individually operated single stirred tank bioreactors operated on various/a single platform(s)
Expansion	Integrated	A single stirred tank bioreactor operated as part of integrated platform
Expansion	High-throughput	Seven individually operated single stirred tank bioreactors operated on single platform that have centralized common resources for media and cytokine addition
Expansion	Bolt Together Integrated High-throughput	Expected >10-fold cell fold expansion
Post-expansion cell conc., washing and pre-formulation	Manual	Benchtop centrifugation
Post-expansion cell conc., washing and pre-formulation	Bolt Together	Stand-alone closed and automated volume reduction and wash technology
Post-expansion cell conc., washing and pre-formulation	Integrated High-throughput	Volume reduction and wash technology in integrated platform
Formulation buffer	All	Cryostor10
Formulation	Manual/Bolt Together/Integrated	Manual volume addition of formulation reagents to attain required cell concentration in formulation buffer post-cell count analysis
Formulation	High-throughput	In line analytics determine cell count post-wash and initiates volume addition to achieve correct cell concentration in an automated fashion
Formulation	All	1 billion viable $CD3^+$ cell dose. (50 million cells/mL, single bag, 20mL working volume)
Freezing	All	Controlled Rate Freezing Technology – performed outside suite – considered non-rate limiting

facility costs being averaged across an increasing number of batches, reducing them to an appreciably small percentage of the total costs (3–7%).

To determine the number of equivalent manufacturing suites required for the manual, bolt-together and integrated automation levels to achieve the same throughput as the 'high-throughput' automation level, the model was switched from a 'constant footprint' to 'constant throughput' scenario, whereby the 'high-throughput' result (2436 batches per annum) was used as the target value. From this analysis the Manual, Bolt-Together and Integrated automation levels required 8, 3 and 2 suites respectively to achieve the same throughput (Figure 4B). Scaling out of the Manual, Bolt-Together and Integrated automation levels had a minimal impact on the total batch cost or the percentage distribution of facility, labor and variable costs (<1%) [Data Not Shown].

The model output in relation to facility staffing and productivity, as a function of automation level, is illustrated in Figure 5. To generate the staff utilization profiles, the total number of hours required to perform the scheduled operations on a given day was determined. This was then used to generate the number of operators required each day, factoring in the assumptions around primary and 'sign-off' operators (Table 4). The total number of staff required each day to perform operations was similar for Manual, Bolt-Together and Integrated automation levels leading to similar sized payrolls (22, 22, 24 total staff, respectively), whilst High-Throughput had a reduced payroll size (19 total staff), which was mainly attributed to the reduced QC staff numbers, as a function of the assumed in-built analytical capability of the system.

Interestingly, workforce composition between Operators and Quality Control (QC) changed as a function of automation level (Figure 5A). For a Manual automation level, the ratio of operators (18 Staff) to QC (4 staff) was 4.5:1 reflecting the highly labor-intensive nature of the process and low QC throughput as a result. Bolt-Together and Integrated automation levels had ratios of Operator to QC staff of 1.75:1 and 2:1, respectively, with total Operator numbers reducing in comparison to the Manual automation level (14 and 16 staff, respectively) and QC staff increasing (8 staff for both). This reflects the assumptions built into the model whereby the use of automation improves operator efficiencies, but not QC and thus, increased QC support is required to accommodate the increasing production capability. For the High-Throughput automation level we see Operator numbers stay constant compared to other automation levels (15 staff), but QC staff numbers reduce to four. This illustrates the value potential of the assumed in-built analytics which reduces the QC burden associated with running in-process controls.

When considered in the context of the overall annual throughput per staff member, productivity was significantly enhanced from 14.5 batches/staff member for Manual to 128.2 batches/staff member for High-Throughput, whilst Bolt-Together and Integrated automation showed similar figures at approximately 42.5 and 53.0 batches/staff member (Figure 5B).

Annual throughput per unit footprint also increased as a function of automation level, from a baseline of 3.2 batches/m^2 for a Manual, to 24.3 batches/m^2 for High-Throughput. This highlights that whilst staff numbers remain reasonably constant and footprint is fixed, the value of each staff member or each square meter becomes much greater with the implementation of automation.

Upfront capital investment

Whilst supporting higher throughputs and lower CoM, the potential drawback of automation lies in the significant upfront capital investment to realize these benefits. From solely a capital expenditure perspective, facility set-up for the Bolt-Together automation strategy required 2x the investment compared to Manual, whilst Integrated and

TABLE 2

Assumptions and constraints associated with the in-process controls for the exemplar CAR-T process modelled in the context of the four levels of automation.

In-process controls (IPC) and release testing (RT) assumptions		
Test	**Process**	**Definition**
IPC 1 – Post-wash/pre-cell isolation	Manual Bolt Together Integrated	Off-line viable $CD3^+$ cell count (flow cytometry)
IPC 1 – post-wash/pre-cell isolation	High-throughput	At line integrated viable $CD3^+$ cell count (flow cytometry)
IPC 2 – post-cell isolation	Manual Bolt Together Integrated	Off-line viable $CD3^+$ cell count (cytometry)
IPC 2 – post-cell isolation	High-throughput	At line integrated viable CD3+ cell count (cytometry)
IPC 3 – during formulation	Manual Bolt Together Integrated	Off-line viable $CD3^+$ cell count (cytometry)
IPC 3 – during formulation	High-throughput	At line integrated viable $CD3^+$ cell count (cytometry)
RT – sterility	All	Standard sterility testing of final product – assumed this QC test is outsourced as a standard cost per batch
RT – endotoxin	All	Standard endotoxin testing of final product – assumed this QC test is outsourced as a standard cost per batch
RT – mycoplasma	All	Standard mycoplasma testing of final product – assumed this QC test is outsourced as a standard cost per batch
RT – identity	All	Quantitative flow cytometry-based assay – up to eight identity markers including viability. (includes % transduction). Test is performed in-house by QC team
RT – potency assay	All	Cell based killing assay – test is performed in-house by QC team
RT – pH	All	pH testing of final product – assumed this QC test is outsourced as a standard cost per batch
RT – adventitious agent	All	Process assessed designed to be Xeno free so no adventitious agent testing required for murine, bovine, porcine viruses, etc. Human adventitious agent testing of final product assumed to not be required as it is autologous product and would have been tested as part of patient screening
RT – viral copy number per cell	All	Assumed QC test is outsourced as a standard cost per batch
RT – integration site analysis	All	Safety assay to address the risk deriving from insertional mutagenesis. Assumed QC test is outsourced as a standard cost per batch

IPC: In-process control; RT: Release testing.

High-Throughput solutions required 5–6x the capital investment (albeit they delivered 4–8x the productivity). The scalability of automation is a substantial advantage as it facilitates incremental expansion (or reduction) of the process, if required, much more efficiently and with better control (leading to lower failure rates). Furthermore, this capital expenditure is to realize the full throughput potential of each suite. In terms of business strategy, it is much more likely that company will experience a ramp up phase in production as they expand their reach within the respective market space, thus it may be feasible to stage this capital expenditure outlay.

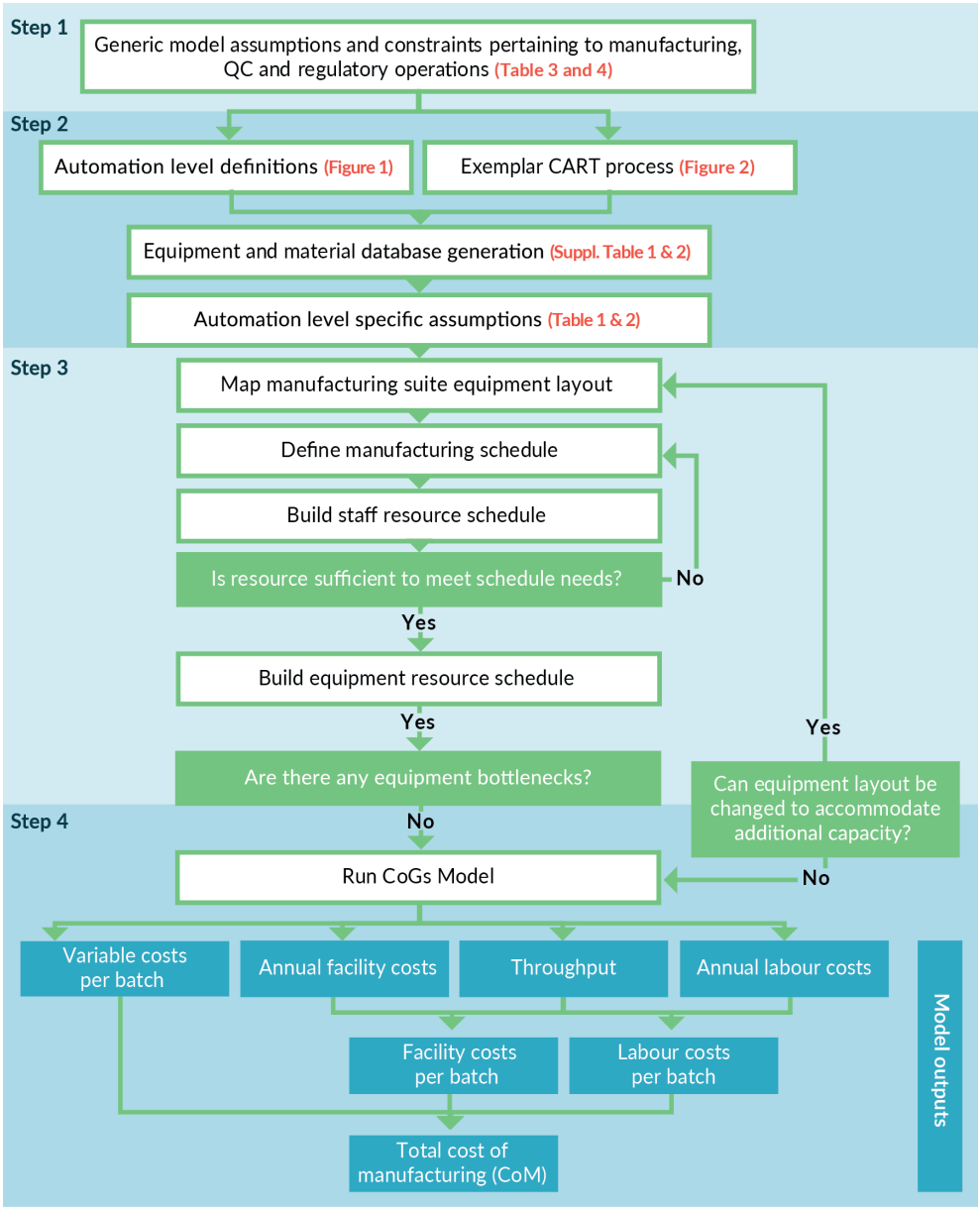
DISCUSSION

The work examines cost and productivity sensitivities to increasing automation of the manufacturing processes for cell-based immunotherapies. Manual, Bolt-Together and Integrated automation solutions were all

DOI: 10.18609/cgti.2020.071

FIGURE 3

Overview of the set-up structure and workflow for the model.



Steps reflect the different activities undertaken as described in the methods section.

modelled upon existing automation strategies currently available, wholly or in-part, to therapy developers. The High-Throughput automation level was a hypothetical vision; a system with integrated analytics (cell counting/flow cytometry to support cell selection and formulation) and parallelized expansion for multiple batches concurrently. Although the system has not been realized yet, the power of modelling tools can still demonstrate the value of such systems, thereby supporting the business case for their development. Critical to this analysis are the assumption provided in Tables 1–4, as well as the Supplementary Tables.

We demonstrated that the introduction of automation at the Bolt-Together level reduced the CoM by 23% compared to Manual processing, with Integrated automation showing a marginal improvement (24% decrease) and High-Throughput enabling additional cost reductions (30% decrease). More

TABLE 3

Assumptions associated with the operation of the manufacturing facility and cleanroom suite, which were utilized for constraining the model and determining the facility associated costs.

Manufacturing facility and cleanroom operational assumptions	
No. of cleanroom suites	1
Cleanroom grade	Grade C
Cleanroom footprint	100m^2
Maximum equipment footprint	65% of total area. This ensured integrity of required work-flows could be maintained (personal flow, waste streams, product streams etc.)
Max. no of operators and equipment load per suite	In a real-world scenario these factors are driven by particulate data and the ability to maintain particulate counts below required operating levels (Grade C in this model). It was assumed a combination of equipment and operator particulate generation would allow a maximum of 12 people in the cleanroom suite at one time, in addition to the required equipment for the process being modelled
Equipment location	It was assumed equipment could be positioned in a manner that would not compromise air handling/air-flow within the suite and would not impact on material, product or waste stream work-flows
Cryo- and warehouse facilities	Cryo- and warehouse facilities are segregated into another part of facility and are unconstrained in terms of operational/storage capacity
QC facilities	QC facilities are based on-site and are unconstrained in terms of operational capacity
QC staffing	Each assay required had a 'processing time' associated with it. Staffing requirements were calculated based on total time required to perform assays
Facility operational period	Maximum of 320 days per annum
Cleanroom rental cost	Fixed cost – includes environmental monitoring, gowning, central facility services (e.g., waste management) etc. Analysis of variations in these costs [Data not shown] as a function of the models employed were not considered significant enough to warrant individual inclusion and thus a fixed rate was applied for all models
Manufacturing shift length (and maximum working time per day)	14h (2 x 8h overlapping shift patterns) – during periods where shifts overlap, no more than 12 people would be allowed in the cleanroom at any given time (as per the 'Max. no of operators and equipment load per suite')
Operator/QC staff working days per annum	5/2 shifts (224 working days a year per operator, after allowance for 28 days of holiday and 8 bank holidays are accounted for)
Batch scheduling	It was assumed that the arrival of patient apheresis material at the manufacturing centre would be managed from a clinical and logistical scheduling perspective to support the proposed manufacturing schedule. Whereby incoming material would be stored for no longer that 24h

These were applied to all four levels of automation modelled.

significantly, automation adoption delivered much greater throughputs (batches/yr) (Manual 320, Bolt Together 936, Integrated 1272, High Throughput 2436, respectively). Thus, the reduction in CoM observed could be primarily attributed to automation increasing throughput and diminishing facility costs proportionally (from 14% for manual to 3–7% for other automation levels). Secondly, increasingly efficient use of the manufacturing team, as a function of automation adoption, yielded reduced labor costs (from 10% for Manual, to 5–6% for Bolt-Together/Integrated, and 2% for High-Throughput) per batch. Furthermore, adoption of automation creates a different set of operator skill requirements to that of manual, which could be argued are easier to recruit for and train-up on when establishing a manufacturing team (especially operators). This may be counter balanced by the need for greater numbers of QC staff (for bolt-together and integrated platforms), however, at a time when the forecast recruitment needs vastly outstrips supply [19], the ability to maximize the number of batches per staff member is greatly beneficial. The authors recognize that the upfront development of automation is costly and time consuming and developers often decide against it during the early stages of development. The impact unfortunately, is that from a regulatory perspective you get 'locked-in'

DOI: 10.18609/cgti.2020.071

to your process reasonably early within the development lifecycle. When funding and resources are then more amenable, it becomes significantly more challenging, but not impossible, to implement; not just through the cost of developing the new process, but the costs of showing comparability and through obtaining commercial licenses etc. Hence the automation need at commercial scale should be identified early in the development lifecycle, so it can be built-in to the development plan, with a view to minimize the cost (and risk) of subsequent requirements to change. The authors expect it to take in the region of 9–15 months for development teams to translate a process similar to the exemplar manual process herein, to something reflecting a bolt-together or integrated solution at an estimated cost in the region of £0.75–1.5 million (Note this can be highly depended on the size of the development team employed, the technologies being applied and doesn't include the capital expenditure required to set-up the development laboratory with the technologies being adopted, the cost associated with formal comparability studies (which may be significant if non-clinical work needs to be repeated or clinical bridging studies undertaken).

As we sought to maximize throughput during model set-up, the challenges associated with the scalability of the manual process became apparent. We set out an iterative decision tree to assist developers in planning manufacture to maximize capacity, see Figure 3. The Manual process required considerable isolator usage, which made it time inefficient and, in this study, became the primary bottleneck. When this was coupled with a manufacturing protocol requiring operator interventions across multiple process days, the result was an equipment unit operation with a very low throughput (mean of 0.5 batch/day in this study).

As this model assumes unimpeded supply of patient apheresis material, the process design is of maximum suite capacity. Therefore, labor and facility contributions to CoM were minimized. The reality is that apheresis supply is likely to be uneven, a result of many contributing factors including clinical slot availability, logistic considerations [20] and manufacturing/scheduling strategies. Implementation of starting material cryopreservation is one strategy being utilized by developers to help to smooth starting material supply into the facility and therefore maximize throughput. Our model (Figure 4) suggests that the automated strategies could accommodate significant reductions in throughput without a significant change in CoM as the facility contribution was a very low proportion of the total CoM (e.g., 3–7% with decreasing automation level). Variable

TABLE 4

Assumptions and constraints associated with regulatory and quality compliance for the modelled processes.

Regulatory and quality assumptions	
BMR and counter sign off	It was assumed state of the art electronic traceability system/batch manufacturing records (BMR) would be employed and the role of dual operator sign-off would be reduced as a function of automation level, thereby allowing greater levels of interdependent operator working within increased automation. Where dual sign-off would then only be required at critical process points. To model this the following 'primary' to 'sign-off' operator ratio was applied to each automation level: 2:1 for Manual 3:1 for Bolt Together 3:1 for Integrated 4:1 for High-Throughput
Multi-product segregation	It was assumed that the methods employed could be validated (e.g., through temporal and spatial separation approaches) to facilitate 'side-by-side' product manufacture within the same suite/on the same automated platform
QP sign-off	It was assumed that 1 x QP can sign-off up to 4 products per day

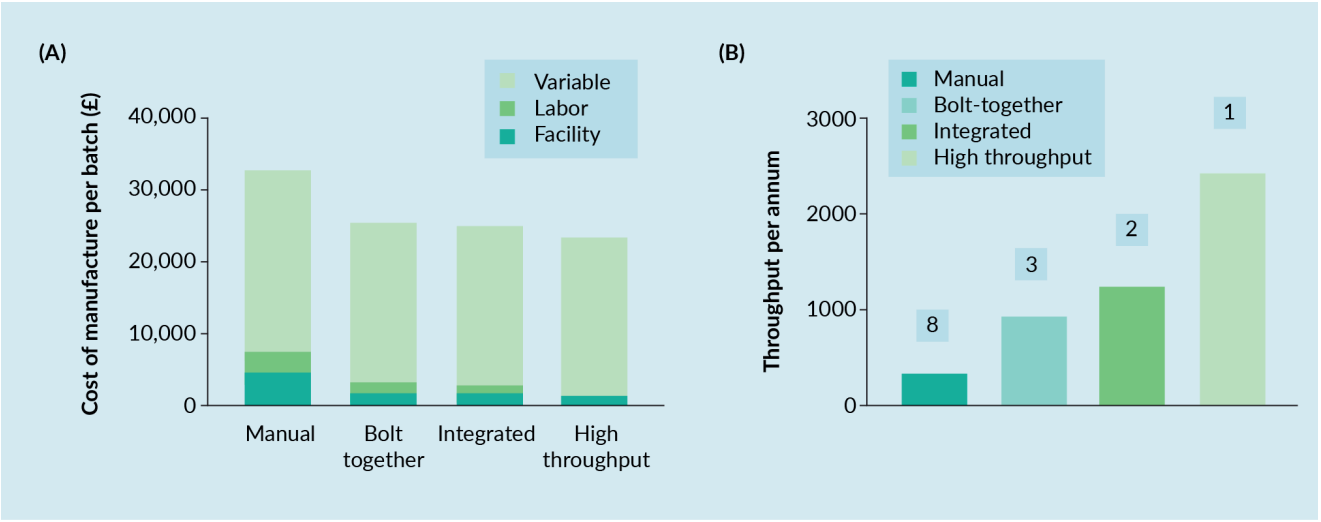
These were applied to all four levels of automation modelled.

costs therefore accounted for the majority of CoM for Bolt-Together, Integrated and High-Throughput (88, 89 and 94%, respectively), as process automation afforded higher throughput. Targeting key variable cost drivers is therefore paramount to achieving further cost reductions. Typically, factors such as gene delivery vector, selection antibodies, cytokines and single use disposable kits are high cost items, representing opportunities for savings. A significant proportion of this cost is driven by the scale of operations for a single batch and the efficiencies of individual unit operations. As therapeutic mechanisms of action understanding for immunotherapies improves, quality attributes deemed critical (CQAs) will be refined. It is expected then that the field will shift towards lower dose, but higher purity and potency products. This in turn, is projected to reduce physical scale and processing times for individual autologous batches. Coupled with the incorporation of emerging technologies, such as non-viral gene delivery systems, the future looks promising with respect to significantly reducing the variable cost proportion of autologous ATMP manufacture.

High variability in CoGs model structure exists between groups, particularly around assumptions and constraints applied, making it challenging to compare their outputs. However, a recent publication by James [21], which also utilized modelling approaches to examine the impact of automation adoption on CAR-T manufacture, showed a number of parallels to our own. It illustrates similar levels of cost reduction to this study through the application of automation for a "closed automated system with centralized incubation" (22% CoGs reduction compared to 25–25% in this study). When comparing both models, manual processes showed comparable annual throughput per unit area, and per staff member, (2.1 and 3.2 batches/yr.m^2, and 14.5 and 19 batches/yr.staff, respectively). Whilst James' "closed automated system with centralized incubation" strategy showed a significantly lower throughput per unit area (10 batches/yr.m^2) compared to our 'high-throughput' strategy (24.3 batches/yr.m^2), the staff productivity in his model was significantly higher (153 to 128 batches/yr.staff, respectively). By evaluating the assumptions behind these metrics, we can

FIGURE 4

(A) Cost of Manufacturing (CoM) per batch as a function of automation level. (B) Annual throughput per suite as a function of automation level.

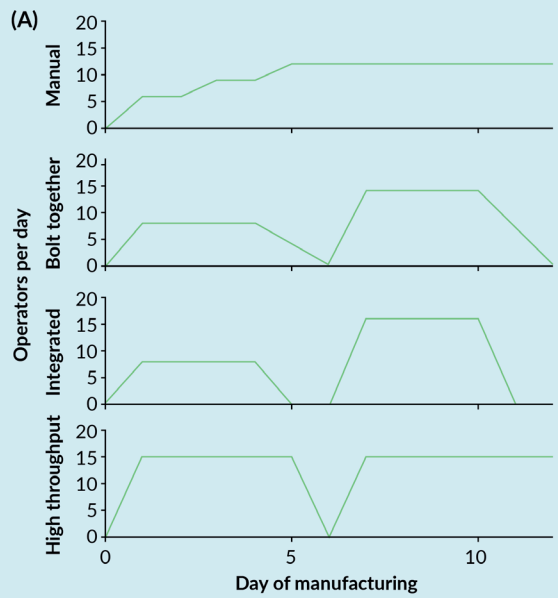


(A) Contributing costs are illustrated individually in terms of facility, labor and variable associated costs. **(B)** The 'high-throughput' automation level yielded the greatest annual production. To determine the number of manufacturing suites required for the manual, bolt-together and integrated automation levels to achieve the same throughput as the 'high-throughput' automation level, the model was switched from a 'constant footprint' to 'constant throughput' scenario. The number of suites are shown in boxes above each bar.

DOI: 10.18609/cgti.2020.071

FIGURE 5

Staff utilization and productivity.



(B)

Automation level	Total staff members (operators + QC)	Batches per annum (batches/yr)	Annual batches per staff member (batches/yr.staff)	Annual batches per footprint (batches/yr.m²)
Manual	x22 = x18 + x4	320	14.5	3.2
Bolt-together	x22 = x14 + x8	936	42.5	9.4
Integrated	x24 = x16 + x8	1272	53.0	12.7
High-throughput	x19 = x15 + x4	2436	128.2	24.3

: Total staff members; : Operator ; : QC

(A) Day-to-day variation in operator staffing levels for the four automation levels. To generate the staff utilisation profiles, the total number of hours required to perform the scheduled operations on a given day was determined. This was then used to generate the number of operators required each day, factoring in the assumptions around primary and "sign-off" operators (Table 4). For each automation level the total number of operators only, required to complete processing per day is shown by the solid line. After the initial ramp-up phase of production, a unique cyclic pattern in daily operator requirements for each automation level was derived, as illustrated within the dotted lines. (B) Throughput variation with respect to total staffing requirements and manufacturing suite footprint, for the four automation levels. Based on model constraints and assumptions, whereby employees work a 5/2 shift pattern, with each employee working 224 working days/year, the total payroll staffing size (Operators and QC staff) required to support operations are shown in the first column. Annual throughput per staff member (Batches/yr.staff) and per footprint (batches/yr.m²) for the manufacturing suite are shown in the other columns respectively.

hopefully start to understand what has led to the variations observed. For example, we hypothesized that the higher throughput per unit area in our study can be attributed to the use of STRs as the expansion system. These systems use heating jackets to maintain temperature, which in turn result in a zero impact on footprint in addition to the baseline consumables or equipment, rather than requiring additional incubator resources and associated footprint.

In addition to reducing CoGs, automation offers improved process robustness and the reduced risk of batch failure. This is a critical factor especially for autologous therapies where a repeat batch may not be possible for a patient that is progressing clinically. It is highly challenging to quantify such parameters as a function of automation level, but qualitatively however, it is possible to surmise at the potential impact. Automation naturally tends to adoption of closed processing technologies, reducing operator interventions/manipulations. This in turn, decreases risks associated with microbial contamination (during manufacture and due to decreased 'spray and wipe' load), or operator induced errors. Furthermore, automation opens the door for adaptive control strategies whereby in-built analytics measure, and feedback control directs process decisions to reach key target parameters, thus reducing batch failure rates. Importantly however, the high initial capital expenditure follows the law of diminishing returns, whereby the majority of risk reduction and removal of 'process hazards' can be achieved with semi-automated solutions (such as the Bolt-Together and Integrated automation in this study) [21]. Thus, investment in automation beyond this level, purely for risk reduction purposes, should be considered cautiously as the value is diminished.

From a more overarching perspective, as automation level increases, single batches

become increasingly tied into a single item of equipment, which in the case of high-throughput means you have multiple batches 'operational' on a single item of equipment at any given time. The consequence of this is you become more exposed from a risk perspective to failure of that automated system. Furthermore, increasing levels of automation, typically translates to increasing single supplier dependencies (especially for integrated and 'high-throughput' systems). Thus, as the manufacturer, you become increasingly exposed to the ability of that supplier to meet your daily operational needs, thus co-evolutionary collaborations will be critical in ensuring automation technology providers are capable of growing in line with individual therapy developers and the broader community.

The concept of High Throughput has not been coined in this paper, as this has been a point of discussions at the recent Phacilitate Special Interest Group automation meetings [22]. However, to the authors' knowledge this is the first publication analyzing cost or production implications of this concept, where patient material can be manufactured in parallel. This study demonstrates clear commercial benefits, but important engineering and regulatory challenges need to be addressed if High Throughput is to be realized for ATMP manufacture.

CONCLUSION

Immunotherapy development, specifically for CAR-T and TCR focused products, is a rapidly changing landscape. The processes of 5 years ago are considerably different to today and will be very different again in 5 years' time. Automation allows the development of de-risked manufacturing processes, enabling increased throughput and reduced CoM. It is therefore a critical aspect of scalable manufacture, in order to support commercial viability and that these novel medicines can be supplied to the populace. Each product and process is unique. Therefore, the degree of automation adoption should be tailored to the manufacturing needs and supply level demands. We have demonstrated the value to therapy developers of employing modelling tools to support early stage manufacturing development decision making in relation to assessing automation adoption strategies. Embracement of these tools can only make the development process stronger.

REFERENCES

1. Lee DW, Kochenderfer JN, Stetler-Stevenson M *et al.* T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *The Lancet* 2015; 385(9967): 517–28.
2. Maude SL, Frey N, Shaw PA *et al.* Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia. *N. Eng. J. Med.* 2014; 371(16): 1507–17.
3. Maus MV, Grupp SA, Porter DL *et al.* Antibody-modified T cells: CARs take the front seat for hematologic malignancies. *Blood* 2014; 123(17): 2625.
4. Schuster SJ, Bishop MR, MD, Tam CS *et al.* Primary Analysis of Juliet: A Global, Pivotal, Phase 2 Trial of CTL019 in Adult Patients with Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *Blood* 2017; 130(Suppl 1): 577.
5. Borchmann P, Jäger U, McGuirk JP *et al.* An updated analysis of JULIET, a global pivotal Phase 2 trial of tisagenlecleucel in adult patients with relapsed or refractory (r/r) diffuse large b-cell lymphoma (DLBCL). In: The 23rd Congress of EHA. 2018. Stockholm, Sweden.
6. Alliance Regenerative Medicine. Quarterly Regenerative Medicine Sector Report – Q3 2019: https://alliancerm.org/publication/q3-2019-data-report/
7. Jarosławski S, Toumi M. Sipuleucel-T (Provenge®) – Autopsy of an Innovative Paradigm Change in Cancer Treatment: Why a Single-Product Biotech Company Failed to Capitalize on its Breakthrough Invention. Vol. 29. 2015.

DOI: 10.18609/cgti.2020.071

8. uniQure, uniQure announces it will not seek marketing authorization renewal for Glybera in Europe. 2017.

9. Abou-El-Enein M, Elsanhoury A, Reinke P. Overcoming Challenges Facing Advanced Therapies in the EU Market. *Cell Stem Cell* 2016; 19(3): 293–7.

10. Granzin M, Soltenborn S, Müller S *et al.* Fully automated expansion and activation of clinical-grade natural killer cells for adoptive immunotherapy. *Cytotherapy* 2015; 17(5): 621–32.

11. Levine BL, Miskin J, Wonnacott K *et al.* Global Manufacturing of CAR T Cell Therapy. *Mol. Ther. Meth. Clin. Dev.* 2017 4(Suppl. C): 92–101.

12. Brindley DA, Smith JA, Reeve B *et al.* Automation of CAR-T Cell Adoptive Immunotherapy Bioprocessing: Technology Opportunities to Debottleneck Manufacturing. *BioProcess Int*. 2016; 14(4): 22–31.

13. McCall. Rapporteurs' Report. in Phacilitate Automation – Special Interest Group. 2017. Edinburgh.

14. Hassan S, Simaria AS, Varadaraju H *et al.* Allogeneic cell therapy bioprocess economics and optimization: downstream processing decisions. *Regen. Med.* 2015; 10(5): 591–609.

15. Jenkins MJ, Farid SS. Cost-effective bioprocess design for the manufacture of allogeneic CAR-T cell therapies using a decisional tool with multi-attribute decision-making analysis. *Biochem. Eng. J.* 2018; 137: 192–204.

16. Simaria AS, Hassan S, Varadaraju H *et al.* Allogeneic cell therapy bioprocess economics and optimization: single-use cell expansion technologies. *Biotechnol. Bioeng*. 2014l 111(1): 69–83.

17. Lopes AG, Sinclair A, Frohlich B. Cost Analysis of Cell Therapy Manufacture: Autologous Cell Therapies, Part 1. *Bioprocess Int.* 2018; 16(3): 3–9.

18. Lopes AG, Sinclair A, Frohlich B. Cost Analysis of Cell Therapy Manufacture Autologous Cell Therapies, Part 2. *Bioprocess Int.* 2018; 16(4): 12–9.

19. Cell and Gene Therapy Catapult. UK cell and gene therapy skills demand report 2019: https://ct.catapult.org.uk/sites/default/files/publication/Catapult_01_UK%20Skills%20Demand%20Report%202019_published_v2.pdf

20. Ellison S, McCoy R, Bell M *et al.* Logistics by design: a framework for advanced therapy developers to create optimal Logistics Platforms. *Cell Gene Ther. Ins.* 2018; 4(10): 1019–40.

21. James D. How short-term gain can lead to long-term pain. *Cell Gene Ther. Ins.* 2017; 3(4): 271–84.

22. Ball O, Robinson S, Bure K *et al.* Bioprocessing automation in cell therapy manufacturing: Outcomes of special interest group automation workshop. *Cytotherapy* 2018; 20(4): 592–9.

AFFILIATIONS

Ryan McCoy
Cell and Gene Therapy Catapult, London, UK

Jahid Hasan
Cell and Gene Therapy Catapult, London, UK

Stephen Ward
Cell and Gene Therapy Catapult Manufacturing Centre, Hertfordshire, UK

Nicholas Gaddum
Cell and Gene Therapy Catapult, London, UK

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

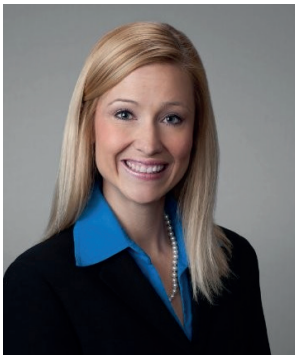
Submitted for peer review: *Feb 20 2020;* ***Revised manuscript received:*** *May 4 2020;* ***Publication date:*** *Jun 8 2020.*

DOI: 10.18609/cgti.2020.071

CELL & GENE THERAPY INSIGHTS

INTERVIEW

The power of patient advocacy in connecting industry, academia and patients: the story of SLC6A1



AMBER FREED and her husband Mark are the parents of adorable twins, Miss Riley James and Mr Maxwell Norman. Maxwell was 18 months old when the Freed family received his devastating diagnosis of SLC6A1. Ms Freed left her career in equity analysis the day Maxwell was diagnosed and dedicated her life to finding a cure. In 18 months, Amber has single-handedly driven multiple translational treatments forward and become a leader within the rare disease community. Ms Freed serves as the Founder and CEO of SLC6A1 Connect. SLC6A1 Connect's work has elevated awareness and created an ecosystem that can systematically help fund and consolidate research and treatment efforts. Her efforts have been highlighted in the Huffington Post, Buzzfeed, Bloomberg, CNBC and many more. Ms Freed was featured in the best-selling book, Shortcut to Prosperity, as an example of grit well before her skills were put to the ultimate test. Prior to Founding SLC6A1 Connect, Ms Freed served in a variety of equity and financial analysis roles, most recently in consumer equity research with Janus Henderson Investors. Prior to Janus, Ms Freed was a Vice President with Stout, Risius & Ross in Houston, Texas, focusing on private company and personal valuations. Ms Freed has also served in roles with RK Capital Management, Dividend Capital Trust, and KPMG LLP. Ms Freed attended the University of Denver for both undergraduate and graduate school, receiving degrees in Accounting on an academic scholarship. She was nominated for the Global Genes Rare Champion of Hope Award and sits on the Board of CombinedBrain. Amber can be reached at any hour of the day to advance science.

Cell & Gene Therapy Insights 2020; 6(5), 691–695

DOI: 10.18609/cgti.2020.081

Q How did you first learn about SLC6A1 and become an advocate?

AF: I spent my career in equity research and gave birth to twins in March of 2017 named Maxwell and Riley. They are the light of my life.

At 4 months, I noticed Maxwell wasn't progressing like his twin sister and he had bizarre symptoms; like the inability to use his hands. Every doctor dismissed my concerns but mother's intuition said differently as Maxwell missed every developmental milestone.

I remember the day Maxwell was diagnosed. My husband and I were called back to a cold, sterile room at the children's hospital full of doctors with sad faces. Genetic testing revealed that Maxwell had a rare neurological disease called SLC6A1. We were handed a five-page research article from Denmark and the doctors acknowledged our understanding of the disease would quickly surpass their own. We had no idea of what Maxwell's future would hold. All of the dreams we had for our baby slipped through our fingers as we tried to digest every parent's worst nightmare. It was the lowest moment of our lives and a sadness for which there is no words.

In that moment I decided to fight like a mother. If anyone was going to cure SLC6A1, it was going to be me. A determined mother does better work than any doctor or detective. I left my career the same day as Maxwell's diagnosis and have devoted 80 hours a week to curing this disease myself.

Q What is known about SLC6A1 so far, and what research still needs to be done?

AF: SLC6A1 encodes a GABA transporter, GAT-1, and mutations in the gene cause a progressive neurodevelopmental disease. It begins with a movement disorder, speech apraxia, intellectual disability, and develops into a debilitating form of epilepsy. There are currently no drugs that effectively treat SLC6A1. The patient organization is pursuing novel translational approaches and there is a large unmet need.

Q A lot of your advocacy work has focused on the development of a gene therapy approach to treat SLC6A1 – why is it considered a good candidate?

AF: It's a perfect candidate for a gene therapy approach. SLC6A1 a monogenic, haplo-insufficient, loss of function, and the required genetic material fits well into an adeno-associated viral vector that is already being used for spinal muscular atrophy and retinal diseases. SLC6A1 is also a candidate for an RNA approach such as an antisense oligonucleotide, or some micro-RNA approaches.

Q What stage is the potential gene therapy currently at?

DOI: 10.18609/cgti.2020.081

"One of my first observations about patient advocacy, industry, and academics, is that everyone is fragmented. I aim to bridge that gap. We provide the patients, advocacy, and research access to unite key stakeholders ... We originally thought this disease was incredibly rare. But once we got everyone in the same room to discuss this, we found out something amazing: it's not."

AF: We began preclinical work on a gene therapy approach in the fall of 2018 in conjunction with the University of Texas Southwestern. We are now finishing preclinical studies and progressing towards a clinical trial. The next steps will be toxicology, completing a natural history study, manufacturing and actually holding the clinical trial itself.

Q How do you connect with the right people in industry and academia?

AF: I believe that the value proposition and the authenticity of our patient organization has advanced our advocacy efforts by decades. Many scientists and companies may want to engage with patient organizations, and in many ways I think the onus is on the patient organizations to reach out. We know our disease better than anybody and we must educate academics and companies about us.

To this end, I've designed a one-page overview of SLC6A1 to help interested parties 'speed date' with SLC6A1. I pride myself on our rapid response times, accessible registry/natural history study and collaborative culture. We are the most enthusiastic patient population you will ever meet – we are putting excitement back into inhibitory neurotransmitters. SLC6A1 Connect hosts an annual symposium and over 100 scientists and biotechnology companies attended last year.

Q As a volunteer organization, how do you reconcile your own goals with those of researchers or for-profit businesses you work with?

AF: Coming from a background in capital markets, I have a sound understanding of how collaboration leads to greater success. One of my first observations about patient advocacy, industry, and academics, is that everyone is fragmented. I aim to bridge that gap. We provide the patients, advocacy, and research access to unite key stakeholders.

We host a monthly virtual lab meeting where every interested party is welcome and shares thoughts. The virtual lab meeting has been instrumental in building strong relationships and

advancing our mission to cure children. Some examples of shared efforts include our registry, natural history study, creating centers of excellence and academic/biotechnology partnerships.

We now have partnerships spanning the USA, Europe and Asia. We originally thought this disease was incredibly rare. But once we got everyone in the same room to discuss this, we found out something amazing: it's not.

SLC6A1 was added to genetic testing panels in 2017. Prior to 2017, SLC6A1 essentially didn't exist. We now know that our prevalence is actually 1 in 38,000. We quickly realized that SLC6A1 is a newly discovered disease and is actually not so rare. We are the tenth cause of autism, sixth cause of epilepsy, and play a major role in many psychiatric conditions. Our quest to save Maxwell quickly transcended our little family, and we held the ability to impact a multitude.

Q What advice do you have for anyone in academia or industry looking to engage with patient advocates?

AF: I think that the patient advocate role within organizations themselves is very important. And there are many patient organizations that get lost – they may not have the right professional background to know how to reach out and get in touch. The more information you can provide on your website the better – and providing a contact is incredibly helpful.

Another area to consider is companies that have developed drugs and then shelved them, for whatever reason. There is a potential market out there for rare diseases. I would advise companies to keep an open line of communication with academics that are keyed into non-profit organizations, sit on the board, or maybe make new connections and test drugs in animal models. There's so much more that can be done and a large opportunity for pharmaceutical companies.

Q What are your key priorities and goals for your advocacy work for the next 2 or 3 years?

AF: We will advance a gene therapy. It is not a question, it is a fact. My second goal is to advance an antisense oligonucleotide therapy. My third goal is to raise awareness for rare disease overall. Rare diseases are an extremely lonely place to be, and they are often forgotten. We are only able to rely on a couple of scientists, and there's no infrastructure for us.

However, rare diseases are not as rare as they might seem, and what can be a breakthrough for one can be a breakthrough for

"I would advise companies to keep an open line of communication with academics that are keyed into non-profit organizations..."

 DOI: 10.18609/cgti.2020.081

many. I really want to shine a spotlight on the opportunities available and the amazing things happening right now in rare diseases. If anybody had said that children with spinal muscular atrophy would be walking at the age of four 10 years ago, no doctor would have believed it. On my darkest days, I watch videos of children who have received Zolgensma®, to keep my spirits up and to remind myself that this will happen for my son too.

Ultimately, we're an organization of mums trying to cure our children. I have put my entire retirement accounts into this and I spend day and night fundraising. If anybody is reading this and has an interest in SLC6A1 or GAT-1, please reach out to Amber Freed. I return text messages at 3am. I am relentless, and I won't stop until there is a cure for every child.

AFFILIATION

Amber Freed
CEO, Founder & Mom; SLC6A1 Connect

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author declares that they have no conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited.*

Interview conducted: *May 8 2020;* ***Publication date:*** *Jun 10 2020.*