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SPOTLIGHT ON:

Clinical development strategy, tools and trial designs

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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.

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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

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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.

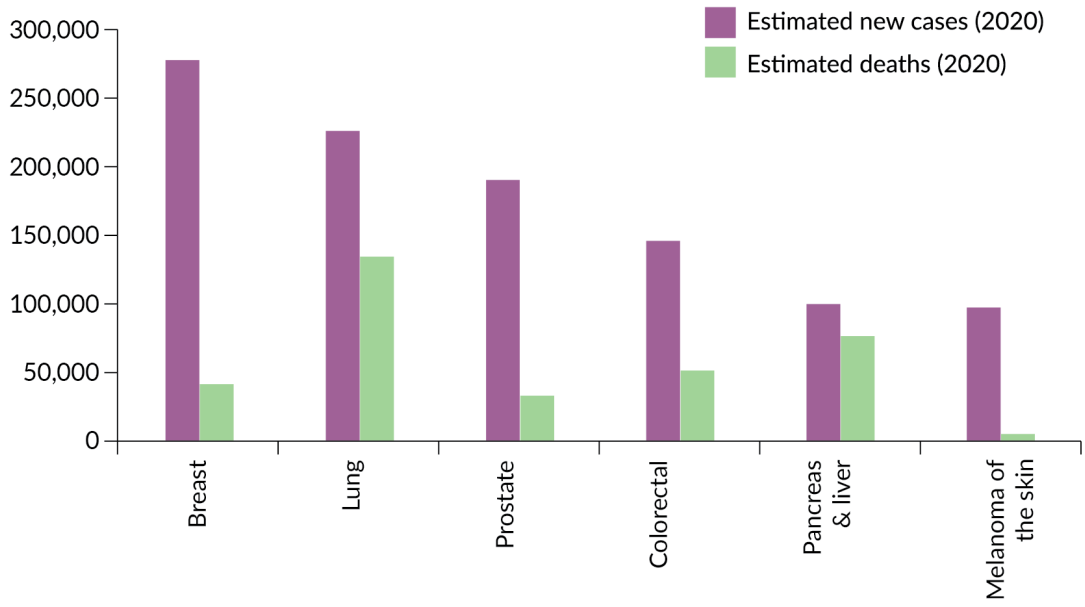

TABLE 1

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

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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

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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.

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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

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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.

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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

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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

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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.

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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

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AUTHORSHIP & CONFLICT OF INTEREST

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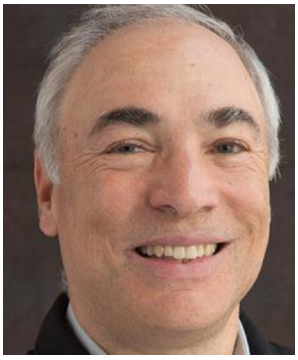
INTERVIEW

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.

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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

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"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,

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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

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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.

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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

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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

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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.

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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

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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.

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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.

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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].

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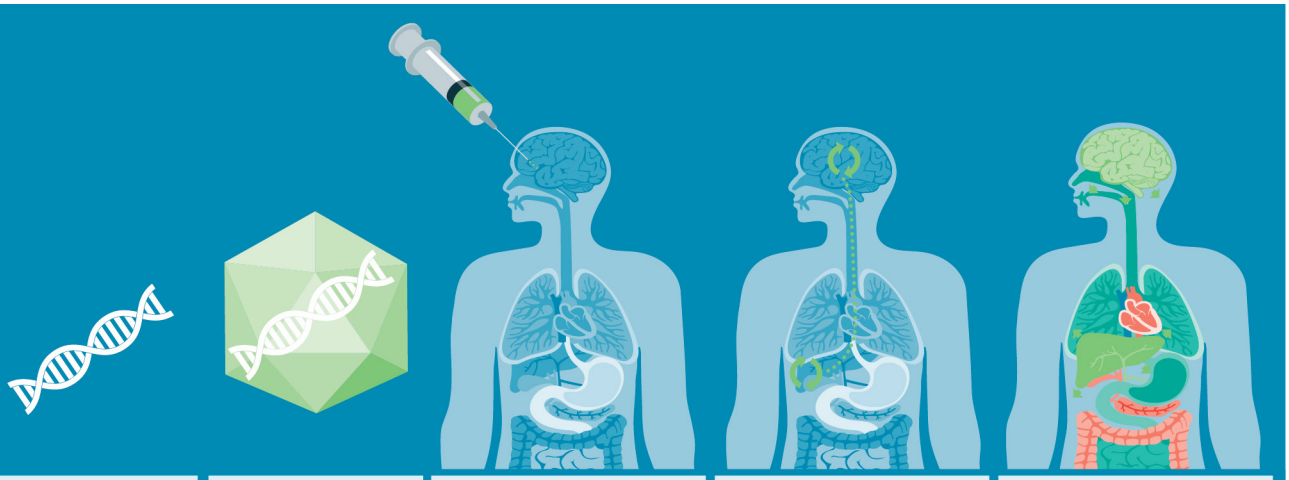
FIGURE 1

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.

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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.

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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

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CELL & GENE THERAPY INSIGHTS

CLINICAL DEVELOPMENT STRATEGY, TOOLS & TRIAL DESIGNS

SPOTLIGHT

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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.

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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

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> "...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

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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

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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.

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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

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"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?

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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

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AUTHORSHIP & CONFLICT OF INTEREST

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