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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:

Cellular immuno-oncology – overcoming manufacturing and development obstacles to commercial success

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SPOTLIGHT

COMMENTARY/OPINION

Towards the rational design of a next-generation dendritic cell vaccine for cancer immunotherapy

Marcelo Bravo, Timothy J Davies & Paul J Fairchild

As professional antigen presenting cells (APCs) capable of eliciting primary immune responses among naïve T cells, dendritic cells (DCs) offer an attractive target for immune intervention. While some strategies for vaccination have sought to deliver antigens direct to DCs *in vivo*, others have pulsed DCs with target antigens *ex vivo* prior to administration. Indeed, numerous clinical studies of cancer immunotherapy have been conducted over the past two decades based on this approach, most of them benefitting from the ease with which DCs may be differentiated *in vitro* from the peripheral blood monocytes of individual patients. Nevertheless, while therapies exploiting monocyte-derived DCs (moDCs) have been shown to be safe, clinical outcomes have been disappointing, efficacy having been limited by factors including the type of DCs used and the source of tumor antigens. Here we review recent developments in identifying DC subsets with more favorable properties for use in cancer vaccination, with particular emphasis on CD141^{+} DCs capable of antigen cross-presentation and discuss alternative sources, such as induced pluripotent stem cells (iPSCs), amenable to manufacture at scale. Furthermore, we assess how different sources of tumor antigens may complement this approach for the design of next generation DC vaccines.

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INTRODUCTION

Dendritic cells (DCs) are the most efficient antigen-presenting cells (APCs) for the activation of naïve T cells and play a critical role in initiating and regulating both innate and adaptive immune responses. Commonly referred to as 'nature's adjuvant', DCs have been considered attractive candidates for immunotherapy and have been used extensively for the treatment of a range of cancers [1], indeed, DC vaccines have been deployed against various malignancies in over 200 clinical trials, the four most targeted cancer types being melanoma (>1000 patients), prostate cancer (>750 patients), glioblastoma (GBM; >500 patients), and renal cell carcinoma (RCC; >250 patients) [2]. The extensive body of evidence obtained from these trials has shown that DC-based immunotherapy is safe and can induce anti-tumor immunity, both in patients with minimal residual disease following tumor resection and those at advanced stages of disease progression. Nevertheless, clinical responses have been disappointing, with objective response rates (ORRs) rarely exceeding 15% [3]. Furthermore, on the basis of a 4.1 month survival advantage and despite less than 5% of patients achieving an objective response, Sipuleucel-T (Provenge®) was approved by the US Food and Drug Administration in 2010 but was subsequently withdrawn from the market [4].

As other emerging immunotherapies such as immune checkpoint inhibitors and chimeric antigen receptor (CAR)-T cells have started delivering encouraging results, the interest in DC therapies has waned in recent years. At present, there is only a small number of Phase 3 trials underway in patients with advanced melanoma, glioma, and renal cell carcinoma which use overall survival as the primary endpoint [3]. Nevertheless, new clinical data and a reappraisal of existing evidence, have begun to shed new insights that are putting DC vaccines back in the spotlight.

THE RE-EMERGENCE OF DC VACCINES FOR CANCER TREATMENT

Anguille and colleagues have proposed that the assessment criteria typically used as the primary endpoint in most early trials of DC vaccines are suboptimal [3]. Typically, the primary endpoint used has been the classic response assessment criteria, such as the Response Evaluation Criteria in Solid Tumors (RECIST), which are based on a measure of tumor burden. However, Anguille *et al.* demonstrated that an increasing number of trials that had secondary endpoints for survival confirmed that DC therapy could confer a survival benefit. Specifically, an increase in median overall survival (OS) of at least 20% has been documented in most studies that had a secondary survival endpoint. Although many of these trials were early phase and not designed primarily to measure survival, the results obtained are nevertheless promising given that the bar for establishment of a clinically-meaningful improvement in median OS is generally set at 20% [3]. Interestingly, evidence is also accumulating that Sipuleucel-T may have had more efficacy in earlier stages of prostate cancer than previously appreciated [5]. Given that, in spite of the varying degree of success of chemotherapy, checkpoint inhibitors and cell-based therapies, a large fraction of patients remain unresponsive to intervention or are prone to relapse, there is renewed interest in exploring DC vaccination either alone or in combination with other forms of immune intervention, such as immune checkpoint inhibition [6].

As of April 2019, there were 20 ongoing clinical trials evaluating personalized DC-based vaccines, 11 of which used tumor lysates as a source of antigen [1]. Among these, there are several promising Phase 3 trials including one testing an autologous monocyte-derived DC (moDC) vaccine loaded with autologous tumor lysate (DCVaxL) in patients with newly diagnosed glioblastoma [7], another study evaluating the efficacy of adjuvant vaccination

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using an autologous moDC vaccine loaded with autologous tumor RNA in patients with uveal melanoma [8] and a trial evaluating active immunization in adjuvant therapy of patients with stage 3 melanoma using natural CD141$^+$ DCs pulsed with appropriate peptides [9]. Although most of the current trials are based on autologous DCs differentiated *ex vivo* from peripheral blood monocytes and loaded with tumor cell lysate as a source of antigen, these Phase 3 trials highlight the breadth of 'design' modifications that are being explored to overcome the current limitations of standard moDC vaccines.

CONSIDERATIONS FOR VACCINE DESIGN: DC SOURCE

The two design elements that have most impact on the potential efficacy of a DC-based cancer vaccine are the source of DCs and the approach used to 'arm' the vaccine with an appropriate tumor-associated antigen (TAA). The reduced success of clinical trials has been variously attributed to the limited ability of administered DCs to directly prime T cells *in vivo* where they serve not only as APCs but as a source of antigen for processing and presentation by endogenous DCs [10–12]. Other confounding factors may include the late stage of disease progression of the patients recruited [13] and the suppressive tumor microenvironment [14]. Nevertheless, it has become evident over recent years that there are also significant limitations inherent in moDCs which have inspired efforts to identify alternative sources of DCs with properties more amenable to the induction of potent cell-mediated immunity.

The need for alternatives to moDCs

In order to achieve tumor eradication, cancer vaccines must elicit potent CD8$^+$ cytotoxic T lymphocyte (CTL) responses as well as the activation of CD4$^+$ Th1 cells required for optimal priming of CTLs and expansion of memory T cells [15]. Although all DCs function as efficient APCs, specific subsets are tasked with activating either CD8$^+$ and/or CD4$^+$ T cells [14]. Conventional DCs (cDCs) are broadly divided into two subsets, namely CD141$^+$ DCs (the so-called cDC1 subset) and CD1c$^+$ DCs, referred to as cDC2. The CD1c$^+$ population consists of highly-migratory cells which primarily stimulate CD4$^+$ T cells as a prelude to eliciting humoral immunity. In contrast, CD141$^+$ DCs are resident predominantly in secondary lymphoid tissues and have enhanced capacity to cross-present antigen to CD8$^+$ T cells [14,16,17] while the equivalent population in the mouse has also been demonstrated to stimulate the necessary CD4$^+$ T cell help to achieve optimal CTL priming [18]. Although the specific deletion of the cDC1 subset in mice has been shown to abrogate anti-tumor immunity, highlighting the importance of antigen cross-presentation [13,19], *in vitro* studies with human DC subsets have been rather more controversial. However, on the question of the ability of moDCs to induce antigen-specific CTL responses, a comprehensive study has been conducted by DanDrit Biotech, who undertook several clinical trials with their discontinued moDC-based vaccine, MelCancerVac. Attempts to generate TAA-specific T cell clones resulted primarily in CD4$^+$ clones, suggesting that T cell responses mounted against lysate-loaded moDCs were directed predominantly towards MHC class II-restricted epitopes consistent with the limited ability of these cells to cross-present exogenous antigen. Consequently, although it is relatively straightforward to differentiate sufficient numbers of moDCs from the peripheral blood monocytes of patients for subsequent vaccination, these cells fail to emulate the efficient cross-presenting capacity of CD141$^+$ DCs, highlighting the need to identify alternative sources with more appropriate credentials (Table 1).

Human blood dendritic cells

Accumulating evidence suggests that DC-based vaccines, consisting of naturally-

TABLE 1

Comparison of the advantages and disadvantages of different sources of DCs for cancer immunotherapy.

Source	Advantages	Disadvantages
Peripheral blood monocytes	▶ Autologous ▶ Readily accessible ▶ Well characterized ▶ Good safety profile	▶ Donor-to-donor variation ▶ Adversely affected by chemotherapy ▶ Poor capacity for antigen ▶ Cross-presentation ▶ Genome editing difficult
Circulating DCs	▶ Autologous ▶ Readily accessible ▶ Provides access to distinct DC subsets	▶ Cell numbers limited ▶ Adversely affected by chemotherapy ▶ Genome editing difficult
$CD34^+$ HSCs	▶ Good cellular yield ▶ Amenable to scale-up ▶ Provides access to distinct DC subsets	▶ Access is compromised ▶ Protracted timescale for differentiation ▶ Genome editing difficult
iPSCs	▶ Autologous or allogeneic sources available ▶ Amenable to scale-up ▶ Provides access to rare DC subsets ▶ Tractable for genome editing ▶ Refractory to chemotherapy	▶ Protracted timescale for differentiation ▶ Risks of tumorigenesis

occurring blood-borne DCs loaded with TAA-derived peptides, display promising efficacy in melanoma patients [2]. Tel and colleagues reported on 15 patients with metastatic melanoma that received intranodal injections of plasmacytoid dendritic cells (pDC) loaded *ex vivo* with TAA peptides. *In vivo* imaging showed that administered pDCs were capable of migrating to multiple lymph nodes. Several patients mounted anti-vaccine $CD4^+$ and $CD8^+$ T-cell responses indicating that vaccination with naturally-occurring pDC is not only feasible with minimal toxicity but induces favorable immune responses in patients with metastatic melanoma [20]. Promising results using naturally-circulating DCs have subsequently been reported in Phase 1 trials of prostrate carcinoma [21] as well as acute leukemia [22].

Nevertheless, although peripheral blood DCs may provide an obvious alternative to moDC, this approach must overcome multiple hurdles. Circulating DCs constitute less than 1% of leukocytes in peripheral blood which may be further reduced by the impact of chemotherapy. In a study by Almand and colleagues, the number of DCs in the peripheral blood of cancer patients was dramatically reduced but was accompanied by the accumulation of cells lacking markers of mature hematopoietic cells, the appearance of which closely correlated with the stage and duration of the disease [23]. Consequently, isolating sufficient DCs may be challenging, especially given that multiple vaccinations may be required [1]. Another major limitation is that several studies have shown that DCs isolated from peripheral blood and lymph nodes of cancer patients are functionally compromised, displaying decreased expression of MHC class II and co-stimulatory molecules, and impaired T cell stimulatory capacity. Three studies, including one of breast cancer patients, have correlated DC phenotype and function with the stage of cancer, reporting that both functionality and expression of maturation markers decreases with advancing stages of cancer [24]. Furthermore,

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Almand and colleagues investigated 93 patients with breast, head and neck, or lung cancer and observed that the function of peripheral blood and tumor-draining lymph node DCs was equally impaired, consistent with a systemic rather than a local effect on DC function [23].

Given these limitations, methods for expanding DC subsets *in vivo* are of significant interest. One such approach uses Flt3L, a key cytokine involved in commitment of progenitors to the DC lineage, to expand DC numbers *in vivo*, even in patients with advanced cancer [25]. This approach may facilitate the isolation of different DC subsets in sufficient quantities to enable multiple rounds of vaccination. Balan and colleagues have reported trials of Flt3L administration in combination with poly-I:C:LC in melanoma and B cell lymphoma demonstrating safety and immunogenicity [13]. Furthermore, a recent study by the same group has demonstrated the capacity of Flt3L to augment all subsets of DCs when administered to high-risk melanoma patients, leading to responses to the TAA NY-ESO-1 when administered as a fusion protein with anti-Dec-205 monoclonal antibodies as a way of targeting the antigen to the DC compartment [26]. Nevertheless, there have so far been no vaccine trials using peripheral blood DCs expanded *in vivo* through administration of Flt3L which might serve as a source for purification and antigen loading *ex vivo* prior to reinfusion.

DCs differentiated from CD34$^+$ hematopoietic stem cells

Early studies of DC vaccination included several clinical trials in which DCs were differentiated from CD34$^+$ hematopoietic stem cells (HSCs). For example, Mackensen and colleagues reported promising results from a Phase 1 trial in melanoma patients of a vaccine consisting of peptide-pulsed DCs generated *in vitro* from CD34$^+$ HSCs [27]. Furthermore, Banchereau *et al.* reported immune and clinical responses in patients with metastatic melanoma who received a HSC-derived DC vaccine, also known to contain Langerhans cells (LCs) [28]. Syme and colleagues subsequently performed the first and only study in which a direct comparison was made between moDCs and DCs derived from CD34$^+$ HSCs in a group of cancer patients [29]. They concluded that DCs differentiated from HSCs may prove a more attractive source for clinical vaccination protocols, since cellular yield was superior and differences in patterns of costimulatory molecule expression did not appear to create a functional impediment. Based on these early studies, there has been renewed interest in this source of DCs and several groups are currently developing platforms exploiting CD34$^+$ HSCs for the large scale production of specific DC subsets, such as CD141$^+$ DCs, pDCs, LCs and CD1d$^+$ DCs [30]. Nevertheless, given that CD34$^+$ HSCs are found in trace numbers in peripheral blood making access difficult, and the timescale for their differentiation *in vitro* is protracted, moDCs have prevailed as the most common source of DCs currently employed in clinical trials [31].

DC vaccines based on iPSC-derived CD141$^+$ DCs

A recent development has been to exploit the potential of induced pluripotent stem cells (iPSCs) whose unlimited self-renewal capacity and inherent pluripotency may give rise to specific cell types that would otherwise prove inaccessible in patients. Indeed, an unlimited number of DCs with little variability could be derived from iPSCs, reprogrammed from cells such as dermal fibroblasts that are least affected by long-term chemotherapy, an advantage for cancer patients displaying functional defects among moDCs [32]. Several groups have successfully derived DCs from iPSCs: Senju and colleagues first reported the generation of DCs from human iPSCs that exhibited the morphology of typical DCs and the capacity for efficient antigen

presentation and activation of naïve T-cells [33]. However, Silk *et al.* subsequently developed protocols for the directed differentiation of CD141$^+$ DCs from patient-specific iPSCs, displaying the additional capacity for cross-presentation of TAAs to CD8$^+$ T cells [34]. Given the proliferative capacity of iPSCs, this process therefore has the potential for mass production of otherwise inaccessible subsets of DCs required for vaccination purposes.

Turnis and Rooney have suggested that for optimal induction of tumor-specific T cells, an ideal DC vaccine should exhibit three essential qualities: the ability to migrate to lymph nodes where T cell activation first occurs; maintenance of a mature phenotype over time to activate and expand tumor-specific T cells; and the capacity to cross-present TAAs as a prelude to the activation of CTLs [35]. In addition, the DC vaccine should be amenable to scale-up of manufacturing to ensure the availability of cells at a scale necessary for repeated vaccination. It is in these four areas that iPSC-derived CD141$^+$ DCs show advantages compared to other sources of DCs since they share many characteristics of the rare lymph node-resident human cDC1 subset. Unlike moDCs, this novel population co-expresses the chemokine receptors CCR7 and XCR1 which guide migration towards secondary lymphoid tissues and CD8$^+$ T cells, respectively [36]. Indeed, XCR1 has been found to be selectively expressed among cDC1 cells and to confer on them the unique ability to migrate in response to its ligand XCL1 [37]. Accordingly, the selective expression of XCR1 by this novel source of DCs may promote their recruitment to sites of CTL activation in the lymph nodes [38] and to peripheral sites of inflammation where natural killer (NK) cells and CTLs may actively secrete XCL1 [39].

Primary cDC1 were initially identified as a unique subset based on their propensity for antigen cross-presentation when tested *in vitro* with soluble or cell-associated antigen [37,40–42]. In common with their *in vivo* counterparts, Silk and colleagues demonstrated that iPSC-derived CD141$^+$ DCs cross-present exogenous TAA directly to MHC class I restricted CTL clones as well as naïve primary T cells [34], properties which permit target antigens to be introduced either as recombinant proteins or whole tumor cell lysates from which appropriate MHC class I and class II-restricted epitopes may be selected during antigen processing.

Finally, the central role played by iPSCs in this source of DCs provides opportunities to apply genome engineering to the rational design of DC vaccines displaying additional functionality. Coupled with opportunities for the mass production of large numbers of high-quality cells, iPSC-derived CD141$^+$ DCs have multiple advantages that make them attractive candidates for the next generation of DC vaccines.

CONSIDERATIONS FOR VACCINE DESIGN: ANTIGEN SELECTION

The second critical factor in vaccine design for cancer immunotherapy is the choice of antigen or antigen cocktail with which to load DCs prior to administration.

Tumor-associated & tumor-specific antigens

Tumor-associated antigens (TAAs) include gene products that are involved in tissue differentiation that are preferentially over-expressed by cancer cells but may also have a wider distribution, being expressed at lower levels by some normal tissues. While over-expressed tumor antigens include HER2, TERT and anti-apoptotic proteins, such as BIRC5, tissue differentiation antigens include mammaglobin-A, PSA, Melan-A and PMEL [43]. Cancer testis antigens (CTAs) are a specialized subset of TAAs that are thought to provide higher tumor specificity, as they are not expressed in normal adult tissues with the exception of germline and trophoblastic cells, but are, nevertheless, highly expressed

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by numerous cancers. More than 60 genes encoding CTAs have been identified, the best studied of which are the MAGE family, SAGE1 and CTAG1A [44].

It is important to note, however, that in addition to lacking complete specificity for the tumor, TAAs are self-components and are, therefore, subject to some degree of central and peripheral tolerance. Breaking such immunological tolerance inevitably carries the risk of autoimmunity directed against those tissues expressing the relevant genes at low levels. Furthermore, those peripheral T cells specific for TAAs may have escaped normal tolerance mechanisms due to their moderate or low affinity for antigen: accordingly, vaccination against such antigens may lead to weak T cell responses with poor anti-tumor activity [45].

Tumor-specific antigens (TSAs) include proteins derived from oncogenic viruses associated with cancers such as cervical cancer, induced by human papillomavirus (HPV), hepatocellular carcinoma, secondary to hepatitis B virus infection, and human herpesvirus 8-associated Kaposi sarcoma [46]. As *bona fide* foreign antigens, these proteins play no part in central tolerance. Furthermore, being expressed solely by cancer cells they are highly specific for the tumor, making them ideal for use in cancer vaccines [44].

Defined antigen vaccines targeting a single TAA or TSA may, however, be ineffective due to immune escape via downregulation or mutation, these so-called escape mutants losing expression of key epitopes. Using multiple defined antigens mitigates against this risk and may be a crucial design component for achieving clinical benefit [47]. Another approach to mitigate this risk is to select TAAs that are essential for cell function and cannot, therefore, be downregulated by the tumor. An example is carcinoembryonic antigen (CEA), an adhesion molecule without which colorectal cancers could not metastasize. Another issue which may explain the limited clinical efficacy of earlier vaccines is that selection of appropriate antigens was based on their reported expression pattern in the relevant type of tumor; nevertheless, expression of these antigens by the tumor tissue of individual patients was rarely verified [44]. Consequently, where tumor biopsies are available, treatment eligibility criteria should be established based on confirmation of expression of the TAAs to be targeted [48].

Neoantigen vaccines

Recent years have witnessed a growing interest in the use of so-called neoantigens that arise from tumor-specific mutations, indeed, the high mutational rate of some tumors results in the expression of neoantigens that are exquisitely tumor specific and highly immunogenic due to the lack of central tolerance [49]. Although tumor neoantigens have long been conceptualized as ideal antigenic targets, their routine identification and evaluation has only recently become feasible with the advent of next generation sequencing and bioinformatics tools for detection of all coding mutations within tumors and algorithms to reliably predict those mutations capable of generating epitopes with high-affinity for the patient's MHC molecules [50]. Although targeting of neoantigens is a recent development, some groups have published promising results [45]. For example, Carreno and colleagues reported that a DC vaccine loaded with neoantigenic peptides elicited neoantigen-specific T cell responses as a result of which some patients showed stabilized or non-recurrent disease [51]. Furthermore, the use of RNA-vaccines that deliver patient-specific neoantigenic epitopes directly to DCs *in vivo*, has recently facilitated a personalized approach to cancer immunotherapy, leading to objective responses in two of five patients with metastatic melanoma [52].

Despite these successes, some tumors carry a higher mutational burden than others, creating a disparity between cancer types with respect to the likelihood of identifying appropriate neoantigens [51]. Furthermore, even in those so-called 'hot' tumors, which show enhanced responsiveness to treatments such as

immune checkpoint inhibitors, it is necessary to identify so-called 'trunk' mutations which, having contributed to the original transformation, are expressed ubiquitously throughout the tumor. Their identification must, however, be achieved against a background of high mutational burden creating numerous 'branch' mutations expressed as a patchwork throughout the tissue but representing inappropriate targets. This approach also requires the availability of fresh tumor material and is, therefore, applicable only to solid tumors that can be surgically resected. Consequently, by being inherently patient-specific, this approach may be limited by pragmatic issues of complexity, cost and challenging timelines between tumor resection and injection of the first vaccine, a delay of several months potentially proving a major challenge for uptake by patients.

Tumor lysates as a source of patient-specific antigens

For indications where surgery can be performed as part of treatment, a common approach to antigen loading has been the use of tumor lysates as a source of antigen [45]. Since these contain the full spectrum of relevant target antigens, both TAAs, TSAs and neoantigenic epitopes capable of activating both $CD4^+$ and $CD8^+$ tumor-specific T cells [53], their use may help reduce the chances of tumor escape. Accordingly, there have been several positive reports of the induction of a potent anti-tumor response using this approach. Notably, May and colleagues reported a significant OS advantage for renal cell carcinoma (RCC) patients treated with an autologous tumor lysate vaccine. Patients at an advanced tumor stage (pT3) revealed 5- and 10-year OS rates of 71.3% and 53.6%, respectively, among those treated compared to 65.4% and 36.2% in the control group. Significantly, patients in the vaccine group showed a significantly improved survival both across the whole treatment group and the subgroup with pT3 stage tumors [54]. Furthermore, a meta-analysis of approximately 1,800 patients showed that those who were immunized with whole tumor vaccines had a significantly higher ORR (8.1%) compared to patients vaccinated with defined tumor antigens (3.6%) [55], providing a strong rationale for using whole tumor cell lysate for cancer vaccination. Interestingly, these findings may be further enhanced in the future by the oxidation of tumor lysates which was found to augment the capacity of DCs to induce TSA-specific T cell responses both *in vitro* and *in vivo*. Indeed, of five patients with ovarian cancer treated with autologous DCs pulsed with oxidized tumor lysate, two experienced durable progression free survival of 24 months or more [56].

Although promising, a significant limitation to the use of autologous cancer tissue as a source of antigens is the requirement for sufficient patient material, making it applicable only to solid tumors that can be surgically resected [45]. An alternative approach that merits consideration is, however, the use of tumor lysates of allogeneic origin. Allogeneic vaccines based on a cocktail of human tumor cell lines might enable large-scale production and standardization of quality and composition [45]. Possibly the best example is TRIMEL, a cell lysate derived from three allogeneic melanoma cell lines established from metastatic lymph nodes and used in TAPCells, a DC vaccine tested in more than 120 stage 3 and 4 melanoma patients and 20 castration-resistant prostate cancer patients in a series of Phase 1 and 1/2 clinical trials. The TAPCells vaccine was shown to induce T cell-mediated memory that correlated with increased survival of melanoma patients while in patients with prostate cancer, is was shown to prolong prostate-specific antigen (PSA) doubling time [57]. TRIMEL was, therefore, shown to include all the necessary elements to induce a vigorous immune response, promote the recognition and destruction of tumors *in vitro* and the stabilization of the disease *in vivo* in a proportion of treated patients [58,59].

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iPSCs as source of TAAs

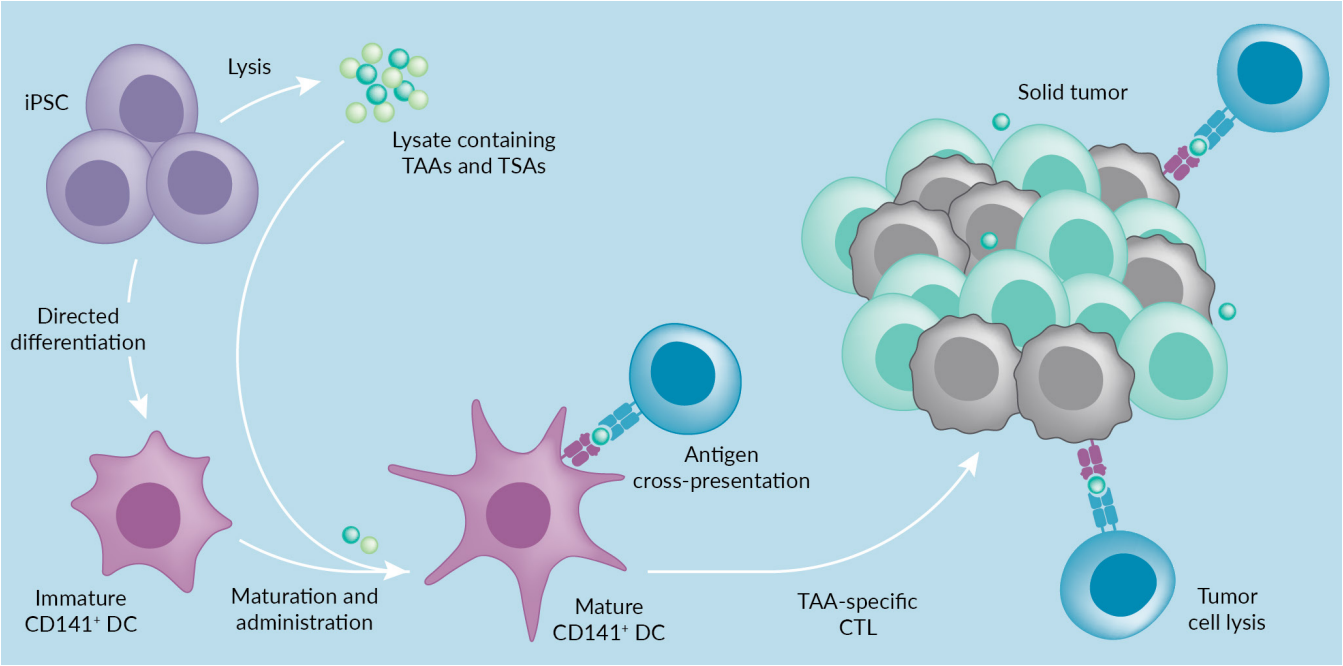
It has been known for over a century, that immunization with embryonic or fetal tissue could lead to the rejection of transplanted tumors in animal models [60]. More recently, studies identified antigens shared between tumors and embryonic cells which led to the hypothesis that embryonic stem cells (ESCs) might be used to induce anti-tumor immunity. Indeed, cancer cells and ESCs share many cellular and molecular features including a rapid proliferation rate, upregulation of telomerase, increased expression levels of oncogenes, and similar gene expression profiles, microRNA signature and epigenetic status. Similar to ESCs, iPSCs share genetic and transcriptomic signatures with cancer cells [61], as well as the ectopic expression of certain genes encoding 'developmental antigens'. These are strongly expressed in the pluripotent state but would normally be down-regulated early during ontogeny, being lost prior to development of the immune system and the induction of self-tolerance [62]. Upon reprograming somatic cells to pluripotency, these genes are strongly upregulated but may not be silenced upon subsequent differentiation *in vitro*, potentially prompting the rejection of tissues differentiated from them, even in syngeneic recipients [63]. Nevertheless, the same genes that are making the application of iPSCs challenging in regenerative medicine may be the key to their use as a source of antigen to drive anti-tumor responses as they are shared by many tumors. For example, CT46/HORMAD1 is a CTA which is strongly up-regulated by iPSCs but has also been shown to be expressed in 31% of carcinomas [64].

While it is undoubtedly early days in exploring the application of iPSCs as cancer vaccines, Li and colleagues evaluated the use of a human xenogeneic iPSC line as a cancer vaccine in a transplantable mouse model of colon cancer. They found that iPSCs were able to induce significant expansion of IFNγ- and IL-4-producing cells, although this did not result in tumor rejection [65]. More recently, however, Kooreman *et al.* reported proof of principle experiments using irradiated iPSCs as an autologous anti-tumor vaccine. Vaccination of mice was shown to protect against growth of tumors as distinct as mesothelioma, melanoma and breast cancer. Furthermore, adoptive transfer of T cells from vaccinated mice protected unvaccinated recipients from tumor growth, consistent with the induction of antigen-specific T cell responses. Interestingly, this study also used RNA sequencing to compare expression profiles between human iPSCs and cancer tissues and demonstrated the shared expression of numerous TAAs and TSAs [66]. Subsequent studies by the same group have further demonstrated how shared expression of cancer signature genes between iPSCs and pancreatic ductal adenocarcinomas (PDAC) enabled the generation of $CD8^+$ effector and memory T cells specific for tumor antigens in mice vaccinated with iPSCs, thereby preventing tumorigenesis in 75% of PDAC mice [67].

While these researchers have explored the use of iPSCs as whole-cell cancer vaccines, there is a significant opportunity to use iPSCs as the source of antigen in combination with a DC vaccine. This approach would ensure that tumor antigens are processed for presentation to CTLs, provided the DCs used in the vaccine have cross-presenting capacity. In this context, the recent optimization of protocols for the directed differentiation of the $CD141^+$ DCs from human iPSCs [34, 36] suggests a compelling scenario in which a signature iPSC line may not only provide a ready source of tumor antigens but an inexhaustible supply of cDC1 cells, capable of their cross-presentation to the patient's T cell repertoire (Figure 1). Although iPSCs could be produced in a patient-specific manner, benefit may also be derived from the use of a semi-allogeneic source, sharing with the patient one or more MHC class I loci to allow for cross-presentation [68]. A source of iPSCs derived under cGMP conditions from an HLA-A*0201^+ donor would, for example, be compatible with >20% of the US Caucasian population whilst providing an ongoing source of tumor antigens, an approach which would pave the way for the manufacture of

FIGURE 1

Scheme showing the potential use of iPSCs as a novel source of DC subsets, such as the CD141⁺ cDC1 subset capable of antigen cross-presentation to MHC class I-restricted CTLs.



The parent iPSC line may serve as a rich source of TAAs and TSAs with which to load the DCs prior to maturation and administration to recipients, thereby eliciting a TAA-specific CTL response capable of inducing tumor regression.

a readily available off-the-shelf product. The derivation of additional iPSC lines expressing the most prevalent MHC class I alleles could cater for a significant proportion of the population [68].

TRANSLATIONAL INSIGHT

Translation of novel sources of DCs to the clinic is likely to be challenging: for blood borne DCs and DCs differentiated *in vitro* from CD34⁺ HSCs, scale up and consistency of the cell therapy product poses significant issues, while the specter of tumorigenicity continues to cloud the use of iPSCs. Nevertheless, exploiting pluripotency as a means of accessing those rare subsets of DCs most suited to the induction of anti-tumor responses may avoid many of the anticipated issues likely to be encountered upon the use of iPSCs in the context of regenerative medicine. In particular, the success of immunotherapy does not depend on the long-term survival of administered DCs but rather the legacy they leave behind within the T cell repertoire: the eventual demise of the administered cells is not, therefore, an obstacle to be overcome, but rather a strategic advantage, ensuring the clearance of all material derived from iPSCs and greatly improving the safety profile of the cell therapy product. Consequently, although the promise of enlisting nature's adjuvant to elicit anti-tumor immunity has beguiled researchers for more than 20 years, recent developments that have diversified the sources of tumor antigens available while providing access to alternative populations of DCs, suggest that the field may now be ripe for a renaissance.

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CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNO-ONCOLOGY: OVERCOMING MANUFACTURING & DEVELOPMENT OBSTACLES TO COMMERCIAL SUCCESS

SPOTLIGHT

INNOVATOR INSIGHT

CMC obstacles in cell and gene therapy: four solutions to solve six challenges

Subbu Viswanathan & Marc Puich

Advanced therapy developers find themselves paying increasing attention to three letters — CMC. Short for "Chemistry, Manufacturing, and Controls," this portion of the regulatory approval package has taken on predominant importance in cell and gene therapies (CGTs), and represents significant risk to clinical trial and approval timelines. At least 14 products were delayed in 2020 due to CMC issues. Manufacturers must proactively address six major types of obstacles early in their clinical trial process to prevent significant, costly regulatory delays later. Four proven solutions can address these challenges, automate data management and compliance, and streamline the path to a robust CMC package. This article addresses these challenges and presents relevant solutions, in the interest of simplifying CMC activities for the benefit of patients and the entire advanced therapy sector.

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In the fast-changing world of cell and gene therapies (CGTs), advanced therapy manufacturers are increasingly finding that the regulatory approval progress comes down to three letters — CMC.

Chemistry, Manufacturing, and Controls (CMC) is an essential part of regulatory approval for any medicinal product. CMC applies to both the drug product itself (the product's manufacturing process, quality control release testing, and specifications and stability) and the manufacturing facility that creates the product (the facility's design, operating procedures, and maintenance).

The category of advanced therapies – treatments based on genes, tissues, and/or cells

– is broad and growing every day. Included in this category are cell therapies, gene therapies, gene-modified cell therapies, personalized cancer vaccines, and cell and gene therapy devices. Pausing for a moment, it's remarkable to consider that human cells and tissues (both autologous and allogeneic) can be used to create drug products. Then further consider that the variety of cells and technologies currently being used in advanced therapies may only be a start. CAR-T therapies receive a lot of press, but there are many other cell types and technologies being explored.

A sampling of the types of cells used includes T-cells, dendritic cells, tumor-infiltrating lymphocytes (TILs), induced pluripotent somatic cells (iPSCs), stem cells, natural killer cells (NK), red blood cells, and more. These cell-based products can either be autologous (a patient's own cells are used) or allogeneic (a donor other than the patient provides the cells). And while there is a great deal of excitement and research around 'off-the-shelf' allogeneic products, where one large batch of drug product can be used to dose many patients, the current reality is that some level of donor-to-patient matching is often still required to avoid graft-versus-host disease. Personalized cancer vaccines start with human cells, and can either simply use the analysis of the cells to determine the best vaccine formula for a patient, or may include inert cells in the vaccine itself.

When the variety of cell types and level of personalization is combined with the variety of technologies for cell modification, activation, and expansion, it becomes clear why the processes – and CMC – are so complex and constantly evolving.

These complex and novel therapies must be successful within the established clinical development framework, albeit appropriately adapted to suit the new therapies, and many companies find it challenging to produce a robust CMC section for Investigational New Drug (IND) and Biological Licence Applications (BLA) (Figure 1).

CMC challenges arise for advanced therapies because:

1. A key raw material is living human cells from a donor or patient; and
2. The often patient-specific, personalized nature of the products.

These two factors introduce enormous variability and complexity into the product and processes not seen with other drug products, with one result being an exponential amount of data. The CMC portion of filing packages is affected in particular because the purpose of the CMC section is to provide enough evidence and assurance of "product safety, identity, quality, purity, and strength (including potency) [1]", and in early submissions, "emphasis should be placed on control of the raw materials and the new drug substance [2]" to support the assertion that patients will receive a well-understood, consistently produced, safe, and efficacious product.

In order to provide this evidence, CMC sections have become the dominant part of CGT filings – and a significant hurdle in gaining approval for both INDs and BLAs. According to US Food and Drug Administration (FDA) leaders, as much as 80% of a regulatory submission in cell and gene therapies is CMC and product related, compared to 20% for a traditional medicinal product [3]. The typical Biologics License Application (BLA) filed with the US FDA or Marketing Authorization Application (MAA) filed with the EU EMA is already large, often in the range of several thousand pages. In contrast, an industry executive recently noted that a single gene therapy BLA consisted of about 60,000 pages [4], at least ten times a typical BLA submission (Figure 2).

One of the main drivers behind this huge increase in filing size is the enormous amount of data generated in the end-to-end process of advanced therapies and the need to connect the individual patient supply chain data with the individual patient clinical data and incorporate it in the overall analysis. For example, a typical BLA will have pooled data from three to five conformance lots for manufacturing, plus the development data from the GMP lots used in clinical trials.

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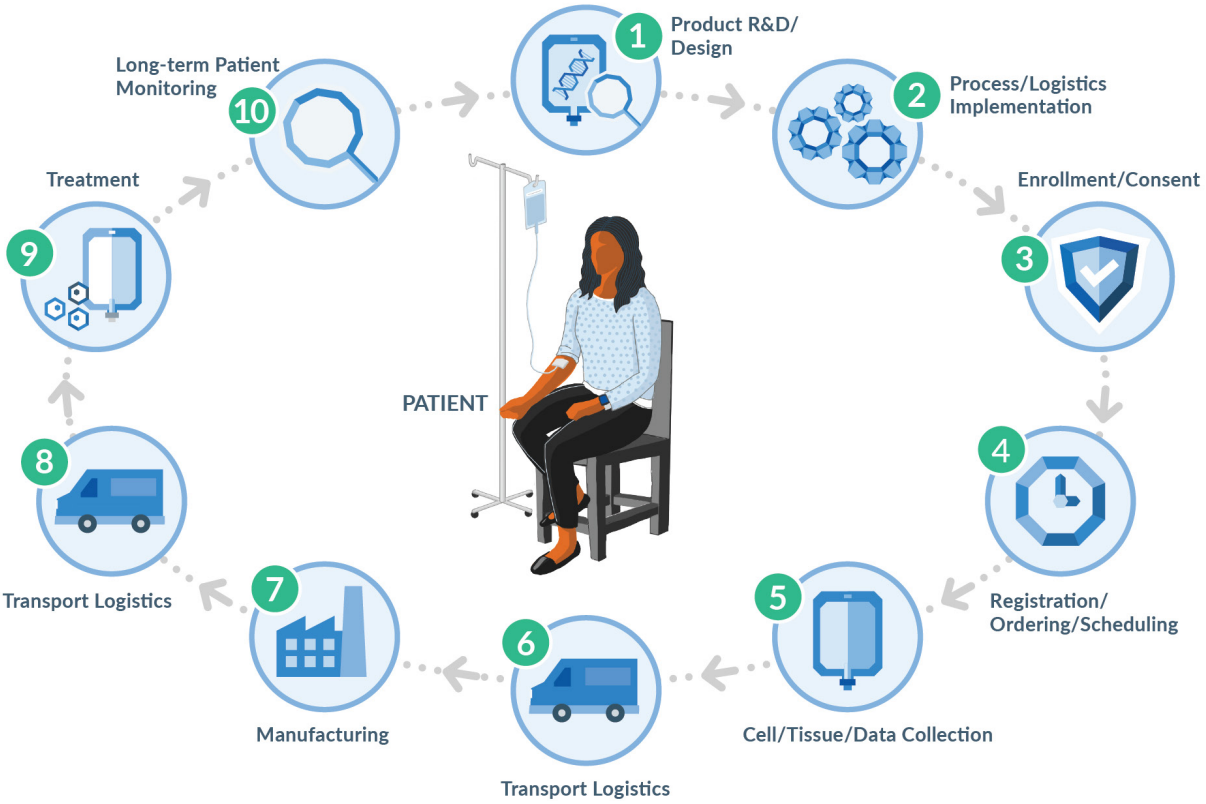
FIGURE 1

The cell and gene therapy journey.



A complex, patient-centered process delivers innovative treatments.

Similarly, personalized therapies require data for each batch – but each patient may be a batch of one. Sponsors then need to link each individual batch with each patient and pool data from all batches for overall manufacturing consistency and robustness. If an advanced therapy trial enrolls several hundred patients, the data pool thus expands dramatically.

In an effort to outline expectations, the FDA issued a CMC guidance for advanced therapies [5,1] in early 2020. The agency's actions over the course of the year indicated that regulators will apply the guidance thoroughly. As stated in the CMC Guidance for Gene Therapies, the "FDA may place the IND on clinical hold if the IND does not contain sufficient CMC information to assess the risks to subjects in the proposed studies [1]." Companies are struggling to document CMC in a way that satisfies the regulatory authorities' heightened rigor and focus on this area as evidenced by the more than 14 filings and reviews that were delayed by the FDA in 2020 due in full or in part to reasons related to CMC [6], some of which caused investors to lose more than $6B (Figure 3) [7].

With more than 1,220 advanced therapy clinical trials underway world-wide, the need to solve CMC issues and streamline regulatory approvals is acute. This article will examine six major types of CMC challenges faced by advanced therapy developers, and offer four solutions that work across these obstacles to protect patient safety, improve data collection and management, and simplify the entire CMC process for advanced therapies.

The CMC challenges in advanced therapies stem from six main areas (Figure 4):

FIGURE 2

The CGT '80/20' rule.

> "A lot of the complexity with gene therapy is in product-related issues, not the clinical issues. Whereas with normal drug review, I'd say 80% is the clinical portion and 20% is the CMC and product portion of the review. I think with gene therapy and cell-based regenerative medicine it's completely inverted. We're having to think very differently about the regulatory issues with these.
>
> — *Scott Gottlieg_2018 Bio International Convention*

In advanced therapies, CMC often becomes the dominant portion of regulatory filings [5].

- Advanced therapies are complicated products with complex, real time supply chains;
- The use of live cells and some level of personalization necessitate tight control over highly variable raw material and detailed processes, including reliable traceability systems;
- The nascent stage of the industry drives constant evolution and learning by both drug developers and regulators;
- Scientific discovery and the desire to quickly translate it to patient treatment means the product processes and analytical technologies struggle to stay on pace;
- Companies need to scale their manufacturing and clinical trial efforts to meet patient needs, meet clinical trial milestones, and match investor expectations;
- The pressure to meet stakeholder expectations and fulfill unmet patient need is heightened and requires a balancing act between speed and development.

SIX FACTORS DRIVING CMC CHALLENGES

Six themes have emerged as consistent challenges in developing robust and suitable CMC sections (Figure 5). Early awareness of these challenges and the proactive development of strategies around them will put companies on a path for successful, timely filing and approval.

It's important to remember that the fundamentals of clinical trials are the same for advanced therapies as for other drug products – Good Clinical Practice (GCP) and Good Manufacturing Practice (GMP) are still required, and endpoints are the same. The key differentiators are the uniqueness and newness of advanced therapy products and technologies, the ongoing lack of standardization,

FIGURE 3

Patient safety focus.

> **FDA - IND Sponsor Responsibilities - Drug Safety Profile**
>
> "...ensure that the IND sponsor maintains a complete view as possible of the drug's evolving safety profile...and will be expected to review, monitor, and analyze, in real time, all accumulating safety data from investigators from all clinical trials and sites."[1]

The FDA keeps the focus on patient safety from the beginning [1].

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FIGURE 4

Overview of therapy categories.

Therapy category	Product/technical features	CMC challenges
Autologous (including personalized cancer vaccines)	The patient's own cells are used as a starting raw material for the drug product, or analyzed to provide a basis for a customized drug product.	• Variability in starting raw material • Complicated, manual manufacturing process • One manufacturing batch per patient • Traceability needed every step from order to treatment • Long time-frame from collection to treatment • High volume of data • Time sensitive when fresh cells are involved
Allogeneic: matched (or patient specific) and matched-to-order	A donor's cells are used as starting raw material for the drug product and are matched specifically to a single patient.	• Same challenges as autologous • Need to coordinate both donor and patient activities • Traceability must track and link both donor and patient ("look back" and "look forward") and maintain confidentiality • Allo-specific data to capture and tie to COIs • Multiple doses may be used
Allogeneic: off-the-shelf	Source cells from donors are used as starting raw material for drug product and are either broadly matched to many patients (i.e. HLA type) or universal for all patients.	• Supply chain complexity and time pressures reduced by manufacturing drug product in batches that can be made ahead, stored and used to treat multiple patients • Traceability required to link drug product to patients • Multiple doses may be used

Advanced therapies fall into multiple categories, all with significant CMC challenges.

and new types of data resulting from the patient/donor being the central source of both CMC and clinical information. Here are the factors influencing CMC:

1. Complicated products
2. Immature process and analytical technologies
3. Speed is of the essence for patients and stakeholders
4. Manufacturing challenges around scale up
5. Adequate systems for traceability are required and must be demonstrated
6. Evolving regulatory requirements in a rapidly evolving field

To mitigate these challenges, four proven solutions can proactively address select issues and build a robust CMC package (Figure 6):

- Truly know the product and the process
- Employ modern data management practices
- Automate - adopt technology early, strategically and using a staged approach
- Collaborate with regulators early and often

FIGURE 5

Challenges in advanced therapy CMC.

New and unique drug product technologies influence CMC.

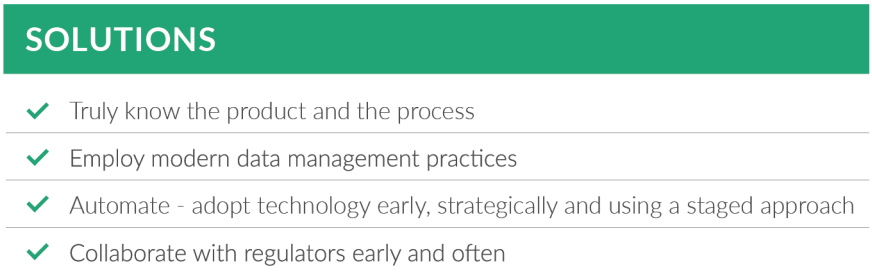
Challenge 1: complex products & processes

Advanced therapies are among the most complex and novel in modern medicine, and the underlying technologies and manufacturing processes are still being developed and explored. The patient is the product is the process, which simply means that the product and process start with the donor or patient and the live cells collected are used as a primary raw material to develop – and in many cases make – the drug product using high-touch, manual processes. These factors introduce more unknowns than are typical and have changed the supply chain, the manufacturing, treatment, and delivery processes, and the required operational and process data sets. This directly impacts CMC requirements and understanding, resulting in more complicated submissions (Figure 7).

Low variability from lot to lot is required with typical drug products and is certainly an ideal with advanced therapies. However, the biological variability of the live cells, process steps such as genetic cell modification, and lot-to-lot variability in viral vectors introduce variability beyond what is typical. This will be reduced as much as possible as process and product understanding and refinement takes place, but it cannot be eliminated and will not reach the same levels as traditional pharmaceuticals.

FIGURE 6

Solutions to advanced therapy CMC challenges.



Proven solutions to proactively address CMC challenges.

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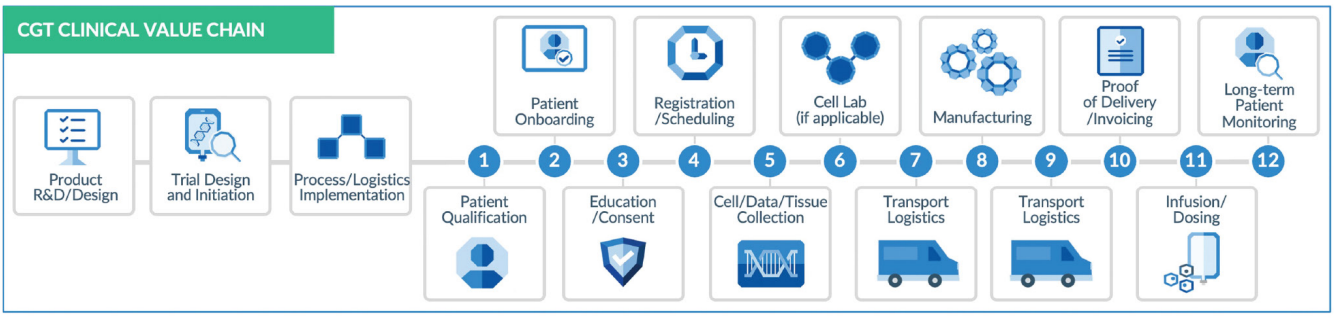
FIGURE 7

Advanced therapy clinical trial value chain.



The most complex in medicine, the CGT value chain brings a new dimension to CMC.

Clinical trials have always generated significant amounts of data, but in CGT much of the data is patient-specific and spread across a broad, fragmented ecosystem, often collected via manual methods. Compounding this issue of increased complexity is that the number of patients in any given trial phase may be much lower than in typical trials. Therefore, every bit of data for every patient counts, yet companies still have to meet material requirements for comparability and process validation – essentially, show more with less data. All patient, supply chain, and manufacturing data must be high-quality, validated, and accessible.

Challenge 2: immature process & analytical technologies

The relatively rapid evolution of the advanced therapies field means that scientific advancements are taking place and heading to the clinic before the products are well understood, before processes are optimized, and while the analytical methods are being developed. This is exacerbated by the inherent variability of some raw materials, which then extends into the products. Key areas where these issues play out are with the Critical Quality Attributes (CQAs), comparability, and potency assays.

Demonstrating, measuring, and maintaining product quality and consistent production are integral parts of CMC activities, yet understanding a cell or gene therapy product's CQAs and controlling the manufacturing process to produce repeatable product quality is difficult because of input variability and unknowns related to operating parameters (i.e. manual processes) and material parameters (i.e. live cell raw material). Additionally, developing a true understanding of what the CQAs should be, and which process parameters are critical and impactful to CQAs, can be a bit of a moving target for companies as they learn about their products and try to interpret the guidance. There is general guidance available on comparability, but no way to receive rapid feedback on comparability protocols.

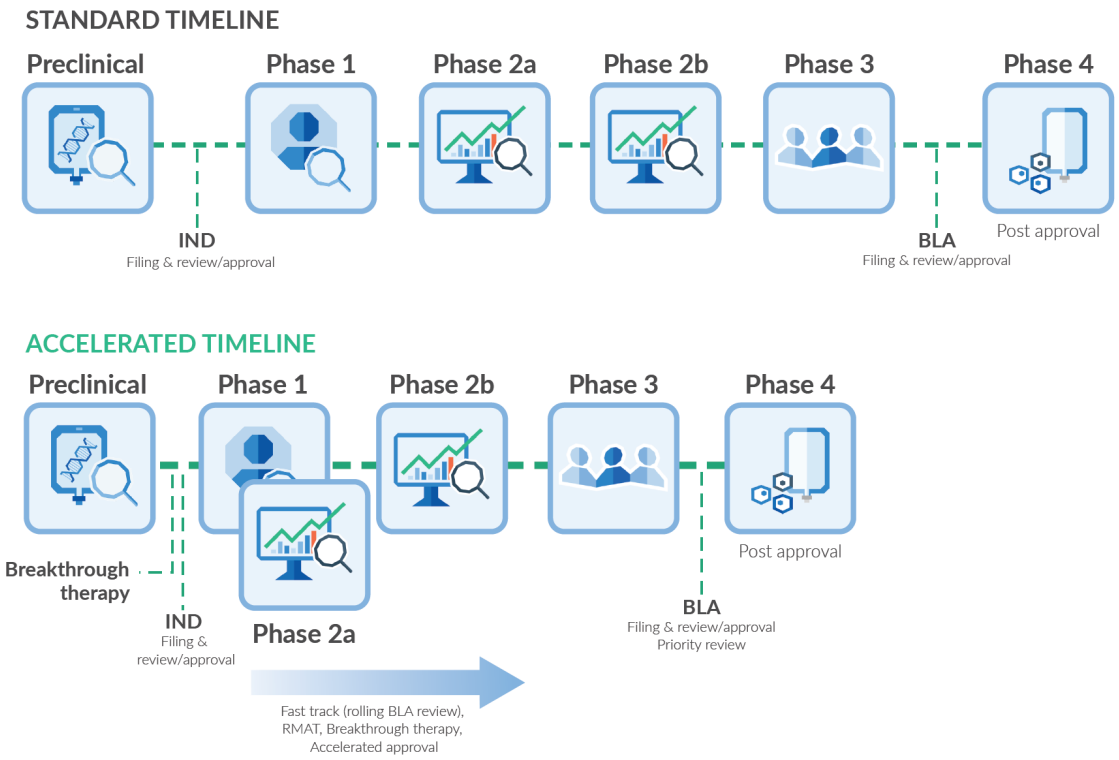
Biologically relevant potency assays are proving to be challenging from both a discovery and development standpoint as well, but also in gaining alignment with, and satisfying regulators. The gaps are typically regarding the rationale for the potency assay, level of validation required, and other feedback needed.

Challenge 3: speed is of the essence for patients & stakeholders

Patients desperately need new treatment options in disease areas with unmet need. And stakeholders, including investors, have high expectations of value generation – the sooner the better. Some investors are banking on special options which are attached to timely BLA filing. Yet the drug development and clinical trials process is lengthy, even when things go

FIGURE 8

Advanced therapy clinical progression.



Drug development is a lengthy and expensive process where opportunities for acceleration increase pressure on CMC.

smoothly and when sponsors are able to take advantage of accelerated regulatory pathways [8]. Therapies typically require an estimated minimum 7–10+ years for Phase 1 through BLA approval. The drug development and approval process is also an expensive undertaking where delays further drive up costs. Advanced therapy clinical trial costs typically run in the $10Ms, even $100Ms, and, according to an industry executive, the total cost from research to approval can be in the $800M – $1B range (Figure 8) [9].

It is time consuming to get trials open and trial sites onboarded. Patient identification and enrollment can be difficult in small and/or high need patient populations. As trials progress, having high quality data readily accessible and easily analyzed can be a challenge, especially in the complex, distributed advanced therapy ecosystem where digital technology may not be used by all stakeholders. On top of this, and many other potential delays, there is the added time and expense of really understanding and locking in CMC for a product and building a robust CMC package. And while accelerated approvals and special designations have advantages, it puts extra time pressure on CMC development and understanding. As previously mentioned, 75% of late-stage advanced therapy regulatory reviews in 2020 were delayed due to CMC-related issues [6], generally resulting in a four to 6-month minimum delay, with average delays for CMC issues being about nine months [10].

Challenge 4: manufacturing challenges around scale up

The complexity of advanced therapy products and supply chains has already been discussed,

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as has the impact of using live cells as a raw material and the immature state of the technology and processes. All of these factors lead to processes that may not scale well and have limited economies of scale (Figure 9).

Additionally, accelerated approval pathways shorten manufacturing and process design timelines, reducing the opportunity to optimize before a process is locked in, yet regulators expect companies to demonstrate a high success rate and the ability to scale effectively. It is challenging to optimize processes and then validate and control the process as the need arises to scale up in volume and out in geography and location. This has been recognized as an issue by the FDA, as Peter Marks, Director of CBER recently "observed that difficulties in establishing a reliable method for moving from laboratory production to a viable commercial process 'without a hitch' can delay product approval [11]."

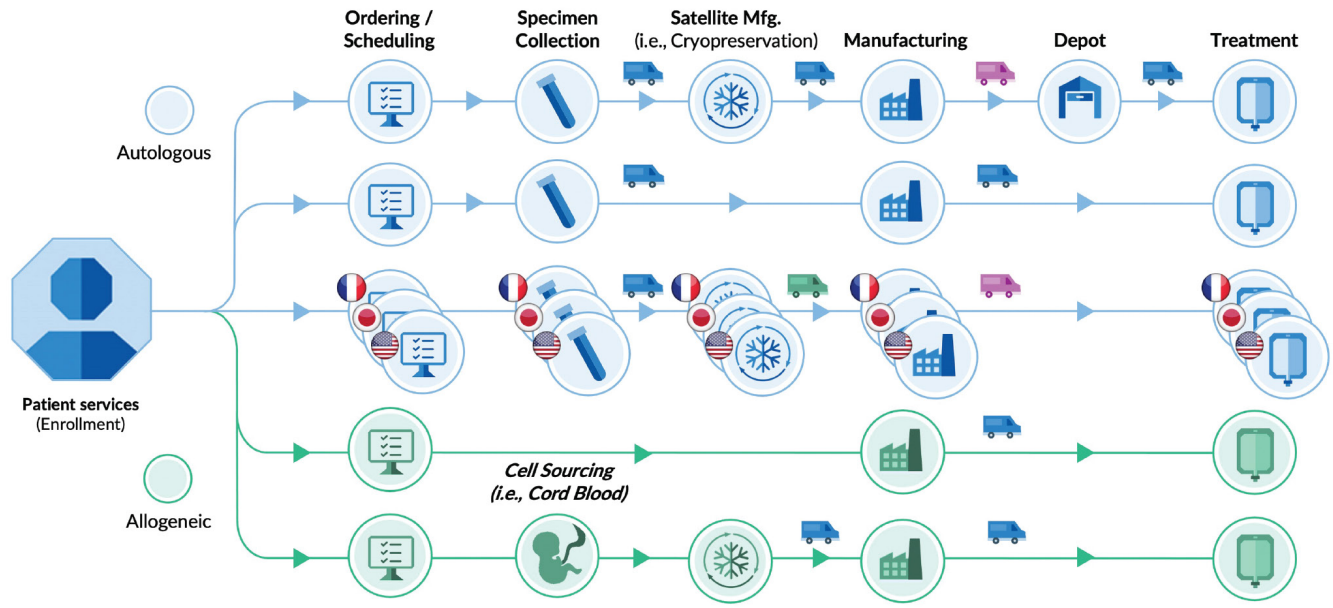
The manufacturing process for advanced therapies also creates considerably more data than a typical drug product. The complex, multi-step processes and manufacturing lots for each individual patient (rather than one lot for multiple patients) exponentially increases the volume of data. Some autologous cell therapy products have up to 20 batch records for a single patient's drug product lot, each of which is hundreds of pages long. Allogeneic products, especially those that have some level of matching, are similar and have the added twist of linking donor and patient data. All of this data needs to be synthesized and analyzed as part of building out CMC processes and preparing the filing package, which becomes increasingly unwieldy as volume increases (Figure 10).

Challenge 5: adequate systems for traceability are required & must be demonstrated

Patient safety is the number one goal of regulatory frameworks and sponsor companies. In advanced therapies there are more opportunities for patient safety issues and therefore, one aspect of ensuring patient safety – raw material and drug product traceability – receives

FIGURE 9

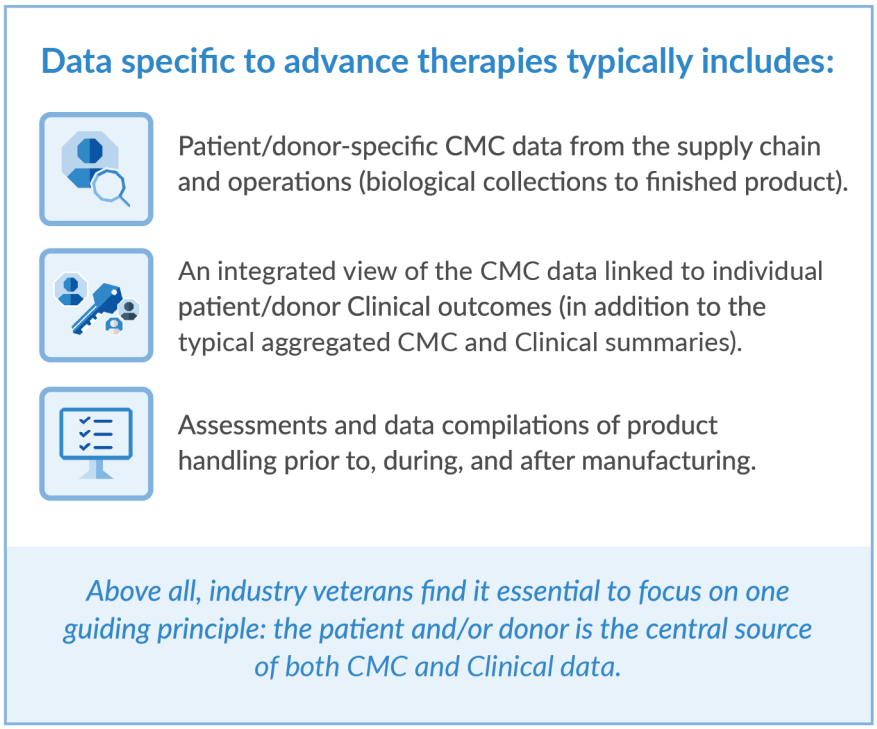
Complexity increases with scaling.



Scale and expansion complicate harmonization of CMC data.

FIGURE 10

Advanced therapies are data intensive.



Advanced therapies generate many types of data, all of which is critical for CMC.

extra attention. Both the FDA and EMA have clearly articulated the need for traceability systems to be in place during clinical trials and for these systems to be demonstrated as suitable in order to proceed with trials and ultimately gain commercial approval [1,12,13]. A detailed systems and methods description of traceability must be included in the BLA (Figures 11 & 12).

It is critical that patients are treated with the drug product containing the cells or formulation meant specifically for them to avoid safety consequences such as product rejection or anaphylactic shock. Chain of Identity (COI) and Chain of Custody (COC) are the backbone of traceability – both for ensuring patient safety and supporting successful regulatory filings. In addition to preventing product mixups, the COI and COC data provides important product and process information.

Challenge 6: evolving regulatory requirements in a rapidly-changing field

In a nascent field with ongoing discovery and novel product technologies, it is no surprise that both the industry and regulatory bodies are struggling to adapt. CMC is complex for advanced therapies, and the industry has not yet standardized. Nor does it have a mature understanding of the new technologies, the products in development, and their links to clinical outcomes. It is challenging for sponsor companies to balance strong quality, safety, and efficacy standards as they learn about their new products, interpret new guidances, and meet development and filing timelines. Yet it is clear from the FDA's actions over the last year that they will not only expect proper application of foundational GMP and GCP principles, but that they will thoroughly apply

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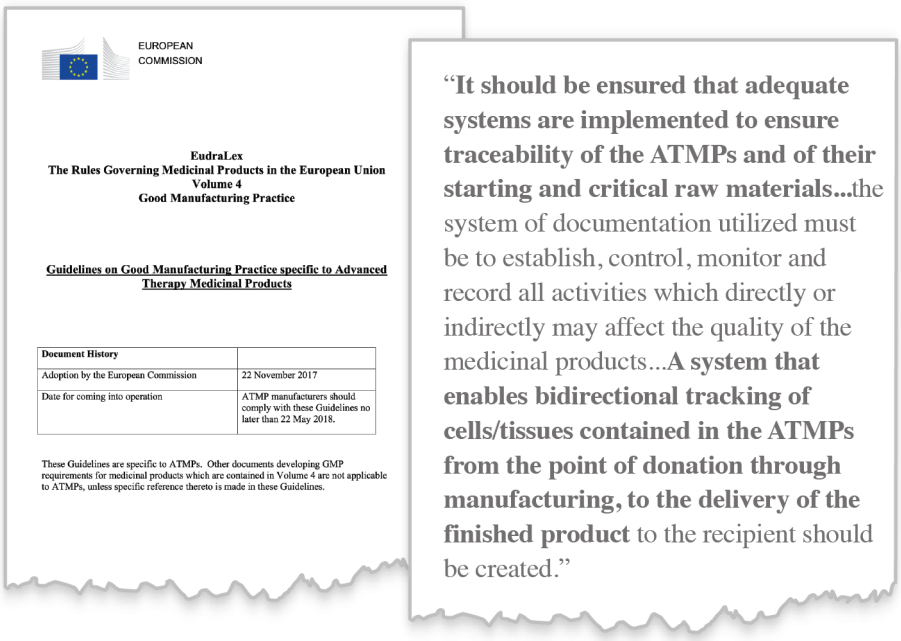
FIGURE 11

Traceability is critical for patient safety and CMC.



The FDA and EMA both require rock- solid traceability from the outset [1,12].

advanced therapy specific guidances [1,12] and provide additional scrutiny to CMC.

Industry questions around CMC regulatory requirements remain, and this is a subject of discussion among industry working groups. Some of the most common issues raised are requirements for the BLA filing versus what is required during review and inspections, clarity around potency assay requirements and validation expectations, and questions on guidance for comparability and a rapid feedback mechanism on comparability [14].

PROVEN SOLUTIONS

The issues may seem daunting, and they are complex, but there are some proven ways to proactively address select issues and build a robust CMC package (Figure 6):

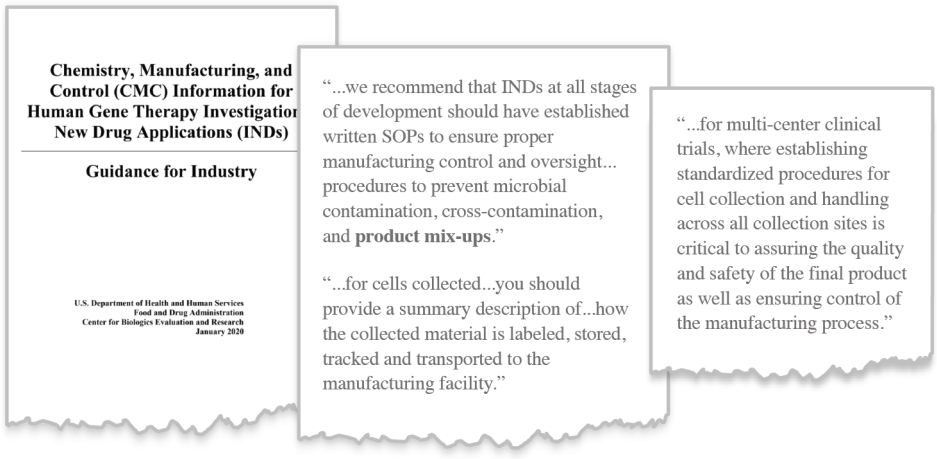
- Truly know the product and the process
- Employ modern data management practices
- Automate strategically
- Collaborate with regulators early and often

Solution 1: truly know the product & the process

Be ready for CGT's unique version of the '80/20' rule when it comes to CMC and regulatory filings and the major focus on CMC not typical for traditional medicinal products. Engaging CMC experts early in the development lifecycle is key to long-term success. The planning for all aspects of CMC, especially establishing and utilizing continuous feedback loops that provide product and process knowledge, must start early to avoid delays and product development issues. Additionally, establishing a well-defined and complete supply chain, with solid data capture, from the earliest stages will aid in product understanding, especially around CQAs and

FIGURE 12

Traceability is critical for patient safety and CMC.



The FDA and EMA both require rock- solid traceability from the outset [1,12].

critical process parameters (CPPs). And with improved understanding comes the opportunity to identify areas to optimize efficiency, efficacy, and safety and will yield more productive discussions with regulators. During development, the challenge will be balancing speed with process optimization – the foundation of which is built on data and knowing the product and the process (Figure 13).

Solution 2: employ modern data management practices

Data from every step of the patient and product journeys is needed to demonstrate a well-defined, well-characterized end-to-end supply chain and drug product manufacturing process. Modern data management should be prioritized to ensure that every patient journey and every step in the process counts. Both US and EU regulators have issued guidances calling for a new focus on data management (Figure 14) [1,12,15]. For an in-depth discussion and actionable recommendations on modern data management in advanced therapies, a copy of From Complex to Controlled: Data Management Strategies for Advanced Therapies can be found at [16].

Data capture along the entire process by a validated, real-time system provides critical information at an individual product level and in aggregate for understanding the product and its complexity, the clinical impact, and ensuring that each product stays within critical parameters. It enables maximizing limited opportunities to optimize the process within tight timelines. Additionally, telling a cohesive story with data and analytics is also the best way to inform and engage with the regulatory agencies and is critically important to demonstrate adequate traceability.

As trials scale and patient volume increases the amount of data will increase exponentially. Starting from a solid foundation of validated, high quality data that is readily accessible will support the expansion of trials, enable real-time monitoring of each process, and provide the data needed to understand CMC components and prepare a strong filing package – keeping trials and filings on time.

Solution 3: automate strategically

Companies running advanced therapy clinical trials may need to automate earlier than expected to manage the complexity of the

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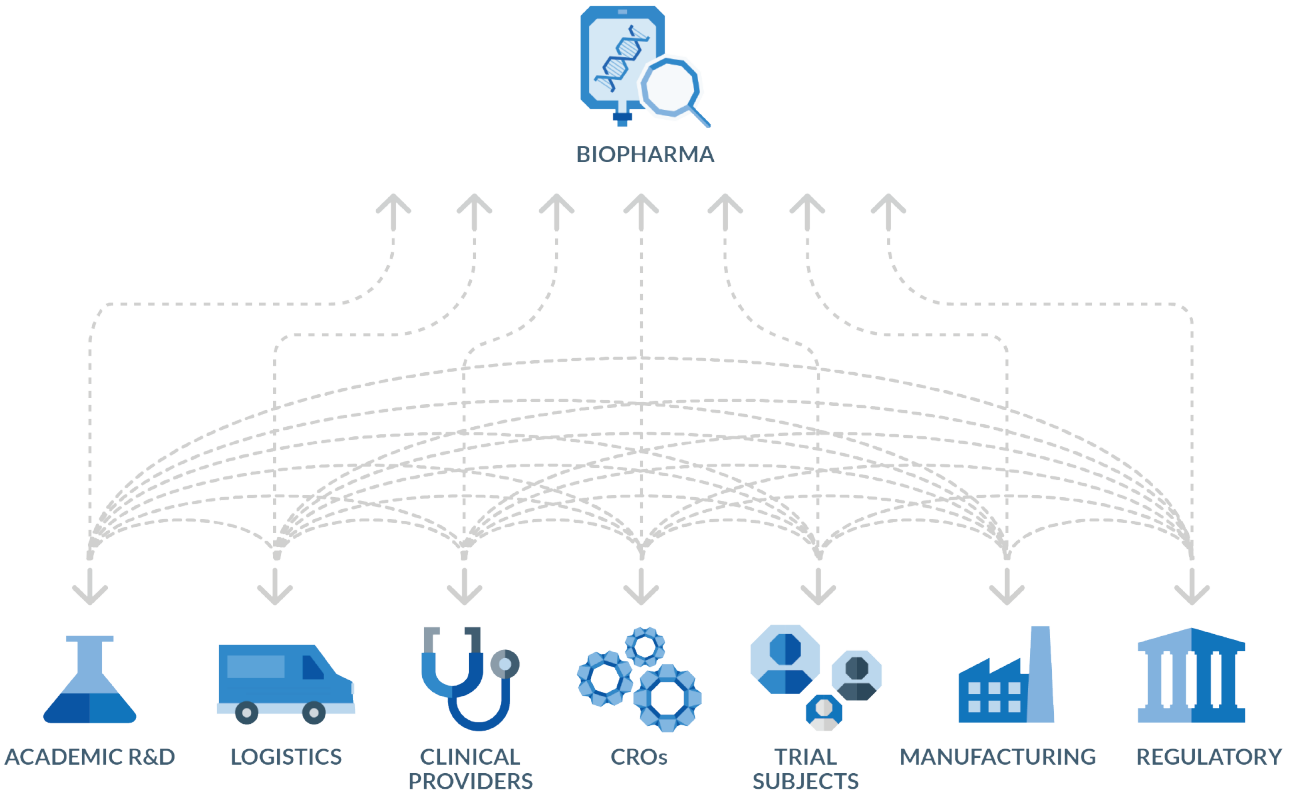
FIGURE 13

Data in the advanced therapy ecosystem.



Patient, product, and trial data is typically found in fragmented systems and varies in accessibility and quality.

products and processes and the extremely high volumes of data. And to support ecosystem partners who are not already operating to GMP standards. Industry veterans recommend automating strategically – focusing on areas of high impact first – and using a staged approach.

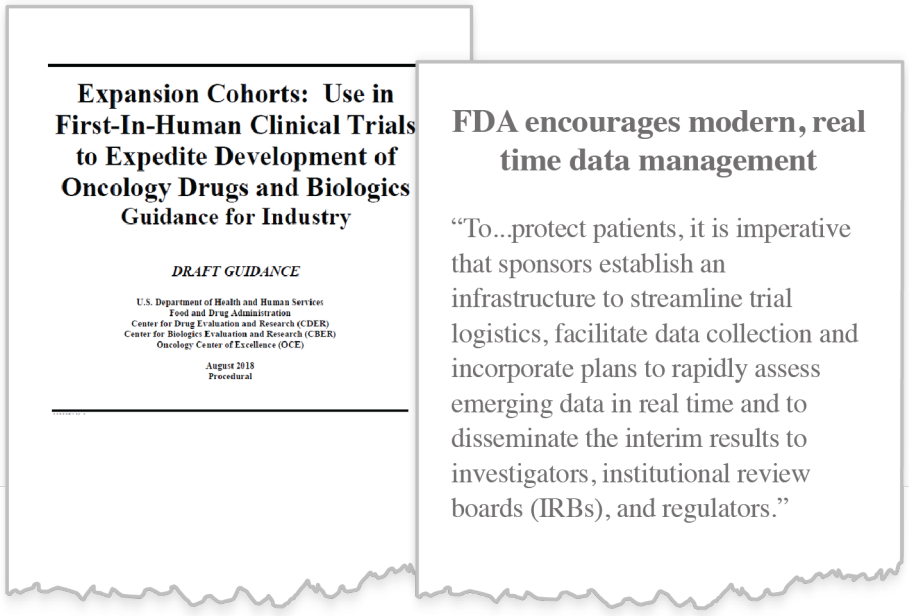
Some of the more complex products will outgrow manual systems as trials expand and move into Phase 2. This might be as early as 10 donors/patients for some autologous or matched allogeneic products. Proven, cloud-based digital workflow management systems, with key integrations such as with specialty couriers, provide the critical foundation of traceability, and also enable flexibility and maintain compliance as product and process changes are needed during the development lifecycle. The industry standards and best practices are built in, freeing teams to focus on patients and the science. Data is collected in a predictable, formatted, complete manner, and is much easier to analyze and report. This approach also provides confidence to regulators that sponsors can scale effectively, reliably protect patient safety, and maintain compliance through centralized administration. Automation also reduces errors and enables close, real-time monitoring of the product journey, ensuring the fastest, most accurate delivery possible – keeping trials on track to meet milestones.

Solution 4: collaborate with regulators early & often

Proactive, formal and informal communication and collaboration with regulators is key to ensuring that issues are identified early and that sponsors have a clear understanding of requirements and expectations, especially around CMC, for their filings. Insufficient CMC information can result in a Refuse-to-file (RFT)

FIGURE 14

FDA encourages modern, real-time data management [15].

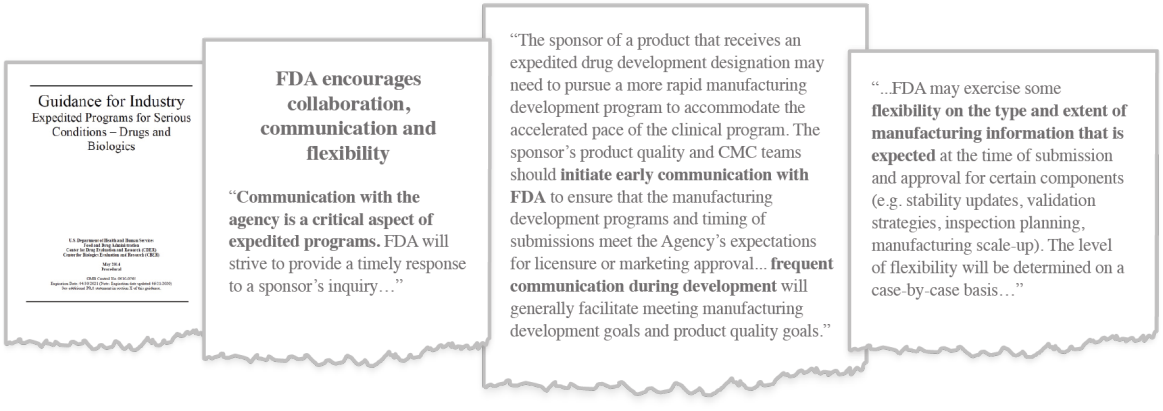


action or a Complete Response Letter (CLR), resulting in months-long delays – often 6–9 months or more [10] – in value generation for patients and other stakeholders. Both the FDA and EMA have issued advanced therapy specific guidances to assist sponsors in understanding expectations [1,12], and early, regular communication related to a number of aspects, including CMC, is especially encouraged by the FDA in the case of expedited programs (Figure 15) [8].

The agencies are striving to be flexible and progressive while keeping an eye on the important task of ensuring patient safety and drug quality. Yet agencies are struggling to keep pace with scientific innovation and therefore, an early introduction to a product and technology will help regulators assess and provide guidance from the earliest stages. In anticipation of 200 INDs per year and a predicted 10 to 20 cell and gene therapy approvals in the next few years [11], the FDA

FIGURE 15

FDA encourages collaboration, communication and flexibility [8].



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is ramping up. Both CDER and CBER are working to recruit and train 200 reviewers and scientists, although efforts are slowed by the unique expertise requirements and a tight labor market [11].

And as previously mentioned, many advanced therapies move to a multi-geography footprint relatively quickly in the development lifecycle. It's important to plan strategically with an eye on global expansion from the outset, especially around CMC. Even for products expected to stay in one regulatory jurisdiction, developing a roadmap of the path from IND to BLA will be helpful to level set internal teams, partners, and regulators.

CONCLUSION

Now is the time for the advanced therapy sector, individually and as a whole, to address CMC issues. As evidenced by the numerous regulatory delays experienced in 2020, progress on behalf of patients depends on a unified, standardized approach.

Regulatory authorities have been clear about the nature of CMC requirements, and are enforcing guidances rigorously. To streamline the collection of extremely large volumes of data and simplify development of a robust CMC package, we recommend the following four solutions:

- Truly know the product and the process
- Employ modern data management practices
- Automate strategically
- Collaborate with regulators early and often

We encourage the advanced therapy sector to work together through industry-wide groups such as the Standards Coordinating Body for Regenerative Medicine [17] to achieve greater harmonization and standardization, which will make these solutions more attainable for all. Standardized CMC baselines will allow the most meaningful drug product differentiation – including safety, efficacy, and manufacturing time – to truly emerge as fast as possible. Patients are waiting, and their faith in advanced therapies will be even more rewarded when CMC alignment allows approvals to proceed as quickly as possible.

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AUTHORSHIP & CONFLICT OF INTEREST

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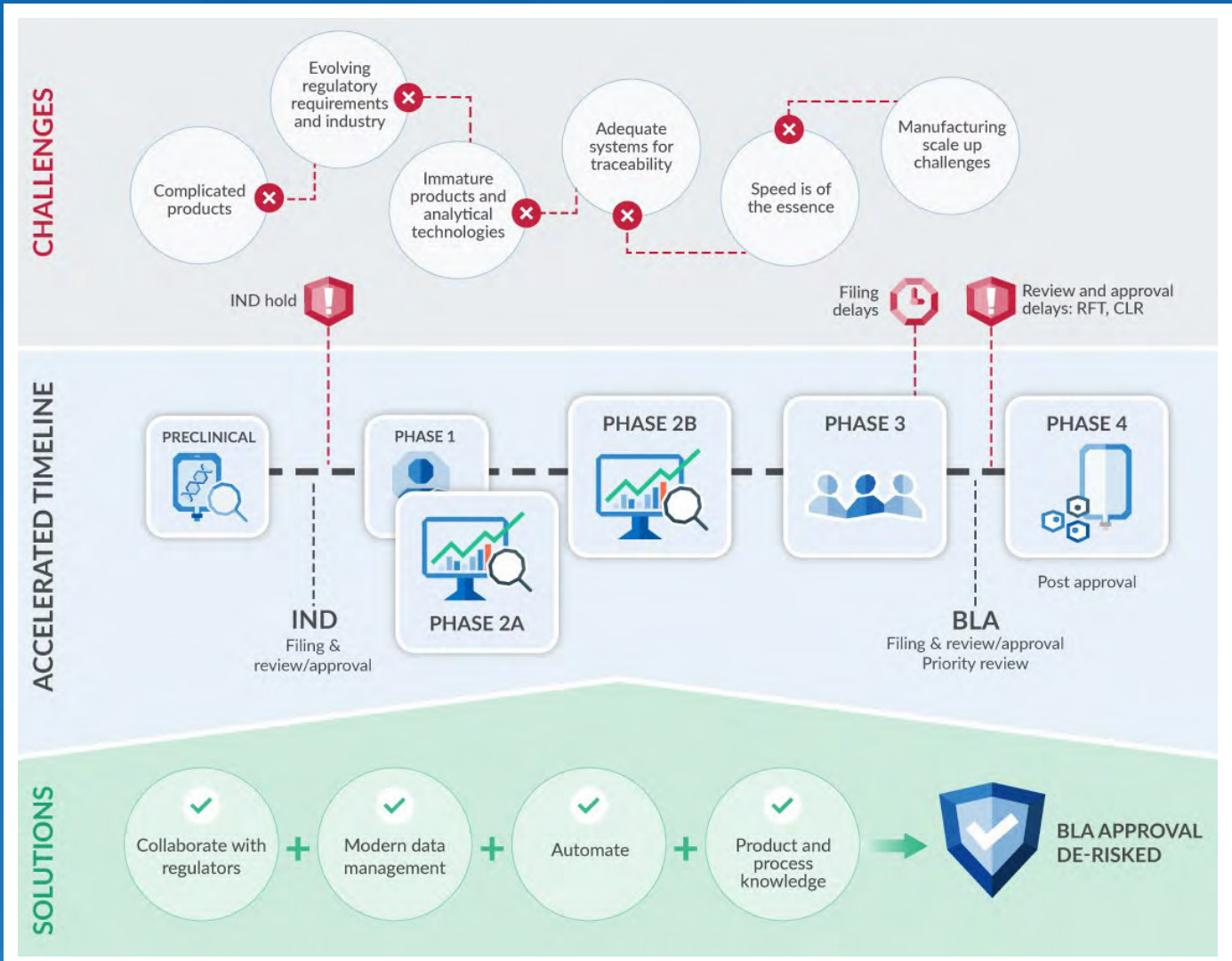
Marc focuses on leading the global sales activities for the Vineti Platform. Prior to joining Vineti Marc, held various positions at Werum IT Solutions, the leading provider of Manufacturing Execution Systems for the Pharmaceutical Industry. There, he was directly involved with sales and implementation of Werum's PAS-X MES, as well as supporting organizations as they continued to drive benefits post go-live. Marc came to Werum with ten years consulting experience as a partner focused on the pharmaceutical and biotech sectors at Tefen USA. He holds a Bachelor's in Physics from UC Berkeley and Master's degree in Industrial Engineering and Engineering Management from Stanford University.

Cell and gene therapies present serious CMC risks, but proactive systems and planning can protect your success.

Vineti

PTM ESSENTIALS



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CELL & GENE THERAPY INSIGHTS

INTERVIEW

Pursuing a fully closed cellular immunotherapy manufacturing model

ED SAMUEL serves as SVP Technical Operations at Achilles with responsibility for overseeing GMP operations for product delivery of its autologous T cell therapies, the expansion of the Achilles manufacturing footprint and the integration of automation and device technology driving the future platform for commercial delivery. Ed has over 18 years of industry and academic experience in process development, technology transfer, GMP manufacturing, QC and regulatory affairs in the field of cell and gene therapy. Before joining Achilles in December 2017, Ed was the Cell Therapy Operations Manager for Europe at Orchard Therapeutics where he was responsible for overseeing CDMO tech transfer and establishing the supply-chain infrastructure to support cell therapy manufacturing and regulatory submissions in the US and Europe. Previously, Ed held positions at Cognate BioServices Ltd as Director for Manufacturing and in post-doctoral translational research at University College London (UCL). Ed holds a PhD in T cell Immunology from UCL, and an MSc in Medical Immunology from King's College London.

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Q What are you working on right now?

ES: In my role as SVP for Technical Operations I am responsible for overseeing manufacturing for our early-phase clinical studies. We have two ongoing Phase 1/2 studies in non-small-cell lung carcinoma and metastatic melanoma, named CHIRON and THETIS, respectively. For both, we are actively recruiting, manufacturing products, and dosing patients.

In addition to looking after the end-to-end manufacture and QC release of our clonal neoantigen T cell (cNeT) products for these studies, my remit includes building our manufacturing footprint for global supply. We have dedicated manufacturing space for our Phase 1/2 studies and now want to build on that and work toward development and scale-up of our platform to deliver registrational studies, and to think longer-term about the commercial platform.

The other element I am responsible for at Achilles, which is very exciting, is looking at new technologies in terms of our manufacturing process. It is a rapidly evolving field, specifically tailored towards cell and gene therapy and autologous cell therapies. We look at opportunities to develop (not off-the-shelf) technology solutions with other partners, in addition to building internal capabilities for developing our own devices and systems.

Broadly speaking, my responsibility is to oversee product delivery: considering what the future process looks like, how short we can make it, and how we can reduce costs.

Q Can you give more details of the Achilles Therapeutics R&D pipeline?

ES: In addition to the two open Phase 1/2 studies I've mentioned, we will be introducing a checkpoint inhibitor combination arm to the melanoma study later this year and targeting an IND for a study in head and neck cancer.

We are executing our plan to begin our higher dose manufacturing process. We are moving through the required process development and technology transfer into GMP to look at generating higher cNeT doses to test in the clinic. We expect to recruit patients this year, then look at dosing patients and clinical data in a higher dose cohort next year for both CHIRON and THETIS studies.

In parallel to the higher dose process, we are looking at follow-on indications in head and neck cancer, and also renal cell carcinoma. We are generating key R&D data to support the genomics element, in terms of looking at each patient's clonal neoantigen burden, and then going through the same manufacturing processes and generating a cNeT product from the patient's tumor material in these cancer indications. Beyond that, we are looking towards additional follow-on indications and exploring other opportunities.

Another area of interest our R&D teams are evaluating is different starting materials. Today, we start manufacture with a tumor resection to manufacture our cNeT product. We are exploring using different starting materials, like blood, alongside assessing gene editing tools

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for cNeT. This is another area that enables us to diversify our pipeline of assets, and we see the potential opportunity for gene editing at multiple steps of the manufacturing process.

We are also looking at building a platform for our tumor archival program. As it stands in our clinical studies, we access material from patients who tend to be late-stage patients who have undergone multiple other treatment therapies, whether it be checkpoint inhibition, or chemotherapy. We are interested in collecting material from newly diagnosed patients who are treatment naïve and looking at how we can potentially provide a platform for banking tumor samples as well as the patient's genomic data. We know that these patients do not necessarily need to be treated in the same timeframe as a late-stage patient. Understanding and building the extensive platform to deliver an archival program is a cross-functional effort being led by our clinical supply chain team working in concert with our R&D and GMP teams.

Q And can you provide some more details of your manufacturing process?

ES: One of the things we have been focused on from the very beginning at Achilles is ensuring that we have a platform that can be fully closed. Most manufacturing processes in the cell and gene therapy world have been transferred out of academic labs and have been developed for Phase 1/2 clinical studies treating small numbers of patients. These processes have not necessarily been developed with the view of taking a process and product to market. Our current clinical manufacturing process includes a manual dissection of the patient's tumor sample in an open step, but we have engineered an entirely closed process downstream of that. We currently have on-going engineering projects to fully close the entire process.

Like other cell therapy manufacturing process, our proprietary VELOS™ manufacturing process contains many elements. Our patients come into the clinical sites for surgery, and we procure a surgical resection of the tumor, which is the starting material for making our drug product. We also collect a sample of whole blood from the patient. Both samples are generally collected at the same time and shipped to our manufacturing facility.

When these materials arrive at our manufacturing facility, the first thing we do is an assessment of the tumor samples for manufacture followed by dissection into fragments for cell culture. We currently procure tumor samples where there is variability from patient to patient, both between indications and within indications. We take regions of the tumor samples to perform DNA and RNA extraction for sequencing in order to generate genomic data, which then gets sent to Achilles for bioinformatic analysis.

A lot of these steps happen in parallel. We collect samples from the tumor for sequencing and at the same time, we take the tumor through our fragmentation process.

> "We are executing our plan to begin our higher dose manufacturing process ... We expect to recruit patients this year..."

"...each T cell clone in each patient can behave slightly differently – technology that allows us to understand the metabolomics of cell culture is very exciting."

This essentially involves putting very small tumor samples into cell culture to isolate the tumor infiltrating lymphocytes (TIL) over a two-week pre-rapid expansion process, which are then frozen down. In parallel, on the same day, we are generating monocyte-derived dendritic cells (DCs) from the blood in a closed process. This is a five-day process and those DCs are then frozen down as well.

It is really important for the current process to have the flexibility to allow all of these elements to come together. Freezing our TIL and DCs gives us the freedom to do that without having to rely on sequencing data reading out within a certain timeframe.

Once the sequencing data arrives, it is then run through our PELEUS™ bioinformatics platform, and that generates the patient-specific peptide list of clonal neoantigens. Each patient will have a different list that results in a unique set of peptides to be used in the manufacture of a personalized cNeT product. Currently, an external supplier synthesizes the peptides and once we have those peptides shipped to our manufacturing facility, we can go back to the frozen TIL and DCs and initiate manufacture of the final cNeT product. The manufacture of the patient's peptides is a step we plan to bring in house in order to support a shorter manufacturing process. Once we have the patient's peptides, DCs, and TIL, we can bring all three together and load the dendritic cells with the peptides. Then, we co-culture them with the TIL.

At the moment we are not necessarily driven by vein-to-vein time, *per se*, unlike many CAR-T therapies where the patient is often waiting for the therapy following procurement. Our patients, from the time we collect tumor material at surgery, will tend to go on to other therapies. In the clinical trials, we are making a product ready for when they either relapse or there is a clinical need for our product.

Traditional TIL therapy approaches generate products that contain multiple different types of T cells, including tumor-specific T cells, regulatory T cells, and possibly other antigen-specific T cells. In the Achilles process, TIL represent the starting material that contain the subset of clonal neoantigen T cells, or cNeT, which represent our target cell population. Through the use of DCs and antigens in the form of patient-specific peptides, our process is able to selectively expand the cells of interest. When a cNeT recognizes its cognate antigen presented by a dendritic cell, that is one of the required signals, together with cytokine stimulation, that will enable that T cell to expand. The expansion of cNeT is performed in a co-culture period of about two weeks, prior to freezing the final drug product. The freezing step gives us the flexibility to perform the appropriate QC testing, QP certification, and then get the drug product shipped to the clinical site when the patient needs it. It is quite a complex process that we have now reduced to practice in a clinical setting.

Q Stepping back for a moment, what do you view as they key trends in cellular immunotherapy manufacturing strategy at the moment

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– for instance, in terms of the ongoing debates around in-house vs outsourcing, and centralized vs decentralized manufacturing models?

ES: The sands do shift from time to time. What we know today is that there are models for both decentralized and centralized manufacturing. In my view, when you are developing autologous cell therapies, control is the most important thing at the early stages of clinical development. You want to retain control to enable the continued development of the process in concert with generating translational science data that will inform the key product attributes that drive clinical efficacy.

Once you start to manage the roadmap towards commercial manufacturing with a global footprint, there are a number of opportunities. For us, we don't see one single central facility necessarily supplying global product, because the logistics around shipping tumor and blood, and shipping drug product the other way, are challenging. That doesn't mean it cannot be done – it is something we are beginning to do for our open IND in the US. However, it doesn't represent the most attractive commercial proposition.

In my view, a successful model for delivering our products at scale will include having key manufacturing sites in the US, and at some stage possibly Asia-Pacific, that could manage the end-to-end manufacturing process from the starting material all the way to the drug product. We have also seen the hub and spoke model utilized for the management of distinct elements of the process, especially in the manufacture of CAR-T, where the option of local, decentralized facilities that can take care of the starting material, freeze down a leukapheresis, and then ship it to the central facility to finish off the manufacturing have been deployed.

However, the field is still maturing, and we only have a handful of cell therapy products on the market so the need to continuously assess all the available opportunities for de-risking manufacturing and product delivery is critical. In the field of allogeneic therapies where single batches can treat a large number of patients, the future model might look very different. Achilles are currently focused on autologous cell therapies where every single starting material represents a drug product and maintaining ownership and full control of that is a key driver.

When you get to commercial manufacturing, again, having full control for a complex manufacturing process is an attractive option. You see some companies that will perhaps bring on board CDMOs or other manufacturers at some stage along the clinical roadmap or development pathway. But once you get to a commercial setting, the tendency is to want to take full control of that manufacturing process. To be able to do that, you are going to have to think strategically in terms of timing for committing the necessary investment to build a manufacturing footprint that can deliver thousands of doses, because the lead times are so long.

Building the infrastructure to deliver these medicines is not a 12-month exercise, and the field is still learning. I think Kite Pharma is certainly showing already what the model could be. They have built out their facility in the Netherlands to service Europe, alongside two facilities in the US to enable global supply.

Another area of interest is new technology and this one is getting very exciting. Lots of the early T cell therapy companies, even ones with products on the market, have had to rely on

manufacturing processes that were developed more than 10 years ago and prior to cell and gene therapies being considered as medicines. Technology platforms have developed significantly in this time, in terms of closed manufacturing systems, cell culture bioreactors and smart technologies to enable in-line monitoring and facilitate better understanding of the kinetics of cell expansion. We are already making personalized therapies, but each T cell clone in each patient can behave slightly differently – technology that allows us to understand the metabolomics of cell culture is very exciting. We are seeing many more companies with new and innovative platforms coming into the advanced therapy space. As more investment has flowed into pre-clinical and clinical stage cell and gene therapy developers, the breadth of external technology providers has also grown alongside more established life science companies who are also investing in delivering solutions for commercial manufacture that have the potential to be more cost effective.

The technology around building clean rooms is evolving too. We are seeing a change from the traditional stick-built model to other flexible ways of building facilities with modular pods, and that is certainly interesting to Achilles as we look to our own future manufacturing footprint.

Technology also has the potential to have an impact on manufacturing time as well. New technologies that provide tools to enable smarter manufacturing processes can translate to a reduction in the manufacturing times and also a reduction in the cost of goods at scale. In every conversation I have on the subject of manufacturing of cell therapies, someone tends to ask about vein-to-vein time and costs, so I don't think these topics are going away just yet.

Then there is digitization. Traditionally, because cell and gene therapies have been considered quite boutique, some of the systems available until recently for managing things like electronic batch manufacturing records, labelling, supply chain and electronic quality management have been tailored more towards the manufacture of biologics and small molecules where the processes are perhaps more mature and well understood. These systems can be challenging to transfer into the field of cell and gene therapy where processes are still at an early stage and the variability in starting material exists. There are now several companies developing electronic systems tailored towards advanced therapies that take us away from mountains of paper and into a digital format. Again, that enables us to shorten the vein-to-vein time, and to be more fluid and agile. And it is not just about the manufacturing itself, but digitizing tracking and traceability as well from procurement to patient treatment.

A wider consideration for taking full control of the manufacturing process is to develop your own technology and devices, in concert with controlling critical raw material supply. Achilles is keenly focused on establishing control of that side including peptides, which are a critical raw material, thus removing the need to rely solely on third parties, through building redundancy in the supply chain. We are also getting much smarter in thinking about vendor or supplier relationships to avoid being in a vulnerable position with any one company.

Another thing we are seeing more and more, especially in the autologous cell therapy space, is how to de-risk the transition from pre-clinical to clinical in terms of the development of the manufacturing process. The requirement for human samples both to develop a process and validate it before taking it into the clinic represents a challenge in biotech. To address this specific gap, we have built a material acquisition platform (MAP) that gives us access to real-world matched tumor and blood samples from patients who aren't going to get treated; they are just

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"...as we currently require tumor samples that can be quite difficult to access in sufficient volume from commercial suppliers, our material acquisition platform (MAP) continues to be a key strategic resource. MAP also provides an opportunity to look at new indications ... to generate proof of concept and GMP-scaled data before we commence clinical studies."

consenting to give their material to help us develop our process and continually develop our genomics and bioinformatics capability. With this approach you are doing a number of things: you are de-risking the manufacturing process itself, because you are not relying on tumor cell lines or healthy donor materials that don't represent an optimal surrogate for the starting materials procured when you enter into clinical trials. You are also de-risking the supply chain, by engaging early with clinical sites to build procurement models ahead of time. The earlier you can start taking sites through those processes outside of the clinical study, the more time can be allowed for pressure testing all these areas from procurement to shipping and therefore avoiding many of the surprises you encounter once you get into the clinic.

Early-stage companies will need to invest time and resource to think about what platforms they need to develop to de-risk this, particularly for autologous cell therapies. For Achilles, as we currently require tumor samples that can be quite difficult to access in sufficient volume from commercial suppliers, our material acquisition platform (MAP) continues to be a key strategic resource. MAP also provides an opportunity to look at new indications like head and neck, or renal cell carcinoma, to generate proof of concept and GMP-scaled data before we commence clinical studies.

Q What will be the next steps in terms of delivering further manufacturing improvements?

ES: As I mentioned earlier, one of the areas of focus for us at the moment is finalizing development of our tumor collection and processing device, which will enable us to fully close the manufacturing process – that is a very important project for us, and we aim to incorporate the closed automation technology in our trials towards the end of next year. Fully closing the process removes issues like dependencies on manual operator steps, whilst reducing the operational expenditure required for the type of clean room environment and equipment needed for open processing steps. These are further key drivers for reducing COGs. The investment in developing a closed system tumor collection

and processing device was a key strategic decision given that there was no available platform technology commercially available on the market, in part due to the complexity and the market opportunity.

At Achilles, we decided almost from the inception of the company in 2016 that we wanted to focus on some of these devices that de-risk the process, close the process, and give us a competitive advantage. We are also collaborating with Ori Biotech, a fairly early-stage technology developer, to look at automating our cell co-culture step, where we combine TIL, DCs, and peptides.

The challenge with developing autologous cell therapies, is that you don't always know the quality of the starting material as each patient is different. There is often a need to iterate the process over time and develop a deeper understanding of incoming material specifications. At the same time, you want optionality. Working with a partner like Ori has given us the ability to look at how we can tailor a developing technology towards the Achilles process, as opposed to taking something off-the-shelf that has been built generically to fill a gap in the market.

The field of advanced therapies has talked for many years of concepts like 'GMP-in-a-box' systems – you open a box, load up your starting material and consumables then out pops your product several days later. The field has yet to realize these solutions for autologous cell therapies, and it is difficult to believe it will materialize in the next few years, but that doesn't mean there isn't an appetite to get smarter across the whole process. We start with blood, tumor, and bring together patient-specific peptides in a final cell co-culture step: our ultimate aim is to integrate all these processes in the most efficient way possible. The end result might be that instead of 6 pieces of equipment, we can reduce this down to 2. These are key areas we will focus on as we think about the commercial process and future processes from alternative starting materials, together with controlling the COGs and reducing vein-to-vein time. Emerging technology also allows us to establish greater reproducibility in the process, so that we know every time we collect a patient sample that we have a high chance of successfully making a patient's product.

Q What specific disruptions has COVID-19 brought you, and what for you are the keys to addressing them effectively on an ongoing basis?

ES: I suspect lots of companies like Achilles will have similar stories and like the majority of people, we have found it challenging; there is no getting away from that. It has impacted patient recruitment into our clinical trials as clinical sites have to deploy resources elsewhere, which has affected many companies.

Coming out of that early lockdown phase, we started to see more patients coming back into our studies. The fact we have been able to recruit and treat patients during the COVID pandemic is remarkable. We are very pleased to have been able to keep things moving. We have kept very active with our clinical sites and kept our manufacturing, clinical and R&D teams moving. We have had to be very agile in the way we have done that. At the same time, we have been cognizant of the COVID environment for all our employees. We have introduced access

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to lateral flow testing and PCR testing to ensure we are providing a safe working environment for our staff. We added this testing capability into our overall COVID secure approach which included shift work, employee bubbles, change of hours as well as the fundamentals at the heart of all COVID secure workplaces like social distancing, mask wearing and enhanced cleaning regimes. These measures have enabled us to keep moving, and we are now starting to see things pick up again and look forward to welcoming more people back into our sites post the next Government announcement.

As a company, it has been right at the front of every Senior Leadership Team meeting – how is COVID going? What does the landscape look like? What are we doing for our staff? What are we doing to provide the resource and environment they need, so that they can continue to do their jobs safely and enable us to keep operations moving to treat patients?

Q Can you summarize your chief goals and priorities over the foreseeable future?

ES: Generating the clinical data for our monotherapy in our high-dose cohort is something that we expect to report in the second half of 2022. Additionally, we are looking at clinical data this year with the current manufacturing process, and starting to dose patients in our combination study with checkpoint inhibition therapy.

We will be opening additional clinical sites in the US and in Europe, and then preparing to file an IND in one of the follow-on indications I described earlier.

In terms of our manufacturing footprint, we have a cleanroom suite at the Cell and Gene Therapy Catapult Manufacturing Centre (CGTC-MC) in Stevenage. We are currently in the tech transfer phase, setting up and validating equipment ahead of process qualification and preparing for clinical readiness. A key area of focus is for operations to commence at the CGTC-MC so we can start manufacturing clinical batches for patients in our high dose cohort. So, it is a combination of the clinical data and building on the manufacturing footprint.

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CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNO-ONCOLOGY: OVERCOMING MANUFACTURING & DEVELOPMENT OBSTACLES TO COMMERCIAL SUCCESS

SPOTLIGHT

INNOVATOR INSIGHT

Navigating regulations: novel cell therapy platforms and their path to clinical manufacturing

Nina Bauer, Natika Calhoun, Anthony Davies & Matt Muldoon

Manufacturing strategy can have a critical impact on cell therapy development programs, and as regulatory and manufacturing trends evolve, and the need for more efficient manufacturing technologies increases, question of when to introduce novel equipment into a process can pose a challenge for cell therapy manufacturers. Merck KGaA works with a broad range of customers in the area of gene editing and novel modalities and is currently preparing the ekko™ Acoustic Cell Processing System for commercial market entry (Box 1).

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In this current pre-commercial phase, discussions with customers have revolved around de-risking, regulatory acceptance, and available documentation. Navigating these aspects as an equipment provider requires very close collaboration with both therapeutic developers and regulatory authorities, and the complexity of introducing such novel equipment into therapeutic manufacturing has sparked many discussions. In the following expert roundtable, industry experts discuss the rapidly maturing regulatory environment, and the critical questions of when to introduce novel equipment, and how to de-risk technologies during therapeutic development.

BOX 1

The ekko™ Cell Processing System (Figure 1) is based on the physical properties of a so-called 'standing wave'. It is generated by a transducer and a reflector, and essentially creates an 'acoustic mesh' that can capture and retain cells in suspension, while removing liquids and smaller particles. The approach functions similarly to a traditional membrane filter, but without the disadvantages of a porous, rigid filter material.

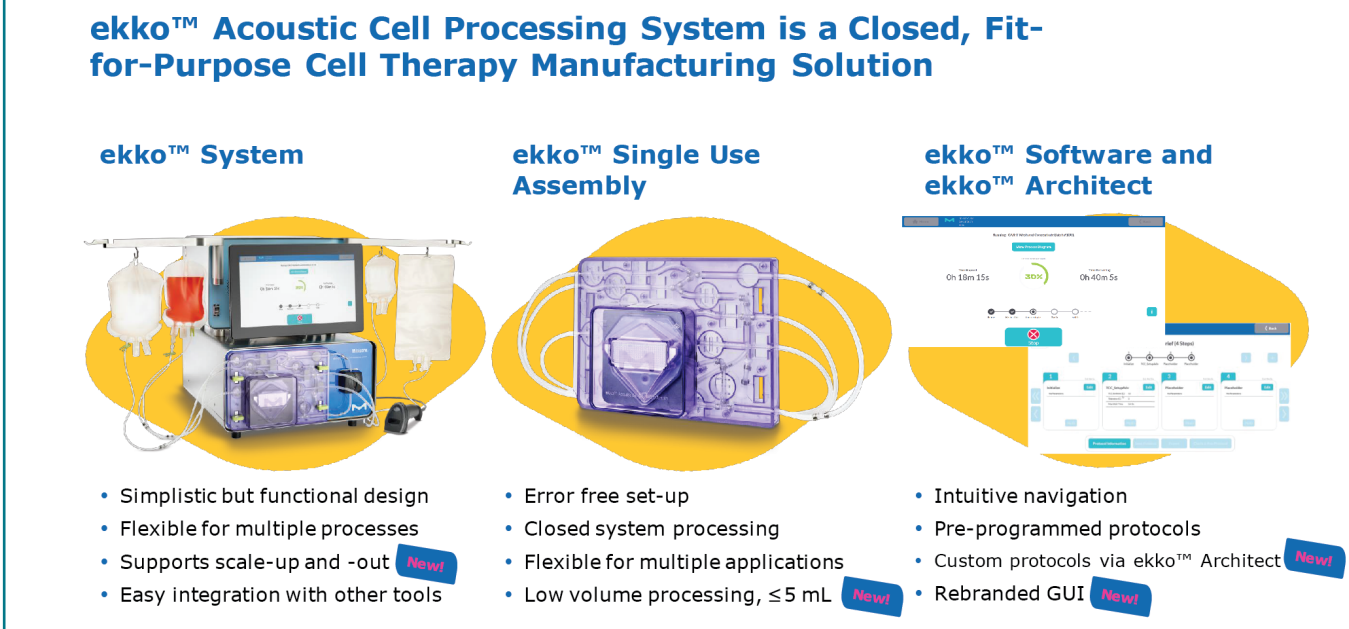
The ekko™ System is a closed, fit-for-purpose cell therapy manufacturing solution comprised of benchtop equipment. It comes with an easy-to-use user interface operated via touchscreen, as well as a desktop application, ekko™ Architect, which enables full custom programming.

The accompanying ekko™ software supports 21 CFR Part 11 compliance and was designed according to GAMP5 guidelines. The technology has been independently audited, and Merck KGaA is now in the final stages of compiling validation datasets to ensure compliance with GMP guidelines.

Given the high flexibility of this technology, it is uniquely suited for unit operations across the entire manufacturing process. Using a CAR T manufacturing process as an exemplar, the system can perform all of the typical wash and concentrate steps, along with media exchanges as required during longer cell expansion phases, as well as buffer exchanges that are required for final formulation. A next-generation system for cell selection, the ekko™ Select, is currently in the final stages of development and will be launched next year.

FIGURE 1

The ekko™ Acoustic Cell Processing System.



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EXPERT ROUNDTABLE DISCUSSION

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Q (NB): Welcome, everyone. I have lots of questions for our panel today, and we will also be taking questions from the audience. To begin, we will discuss the regulatory space for cell therapy in general. We have recently heard of more pushback from the FDA, and other regulatory authorities, on investigational new drug (IND) submissions and registrations. There seems to be a tightening of regulations with regards to Chemistry, Manufacturing, and Controls (CMC). Is this something that you would agree with?

AD: We certainly are hearing a lot more about this. At Dark Horse, we hear from a lot of people who have that hypothesis, if you will, that things are tightening up, and that cell and gene therapy is receiving an increased level of scrutiny.

We actually disagree with this hypothesis. We believe that the overwhelming majority of what is happening in the regulatory dialogue is that the field as a whole is shifting towards the commercial end of the spectrum, and away from the early-stage developmental end. This part of the spectrum has always been tightly regulated, with relatively little flexibility around the GMP guidelines that the FDA and other regulatory bodies provide.

For example, since we all last spoke while preparing for this discussion, Iovance has further delayed their BLA submission. This is the second successive delay and will make their cumulative delay at least 18 months. Almost all of this delay seems to revolve around their potency assay.

Last time I checked, a fully validated potency assay has been an absolute requirement for any biologic since the inception of the Center for Biologics Evaluation and Research (CBER). There were some very choice remarks from Peter Marks on this subject the other day, which I interpreted as an expression of irritation and frustration with the field more than anything else. I have the quote here:

"Pick something, pick some quality of the cell, pick something you think might correlate, and measure that. We'll take any offers that are reasonable."

Our position is that the FDA is losing patience because the field is entering a space that has always been strictly regulated. We see this as an increasing threat to the field.

Q (NB): Natika, you deal with both the viral vector space and the cell therapy space through the equipment lens. Any thoughts on what Anthony just described?

NC: I think there is an increase in expectations, given guidance that has been put out for manufacturers. I do agree that these regulations have been written and they are there for us to follow. The expectation is that we catch up and provide all of the data that is expected.

Speaking from being inspected as a viral vector manufacturer, the expectation on risk assessment is evolving. We have documentation and risk assessment for the most key and critical pieces you would expect – for example, how you mitigate cross-contamination – and that has always been there. But there are so many other layers now around risk assessing numerous parts of the process, numerous equipment additions, and having documentation around all of that, and also the need to have it put in a formal package. For example, the requirement to have a contamination control strategy where all of these things are put together in one place. That is very handy, but that language wasn't written years ago. We have been in the same location since 2004, and these things weren't written.

When I think about how regulators will put out guidance, it is generally because they are seeing that the industry may need some help so that we are collectively submitting the same things, and there aren't gaps from one sponsor to the next. The difference I am seeing is that expectations on risk assessments are much broader, and we need to be much more detailed.

Q (NB): Matt, you are on the therapeutic developer side of things, and deal with a lot of supply chain questions. Any thoughts from that angle?

MM: From a manufacturer perspective, and considering path to market, I think there is a really traditional trade-off here of speed versus a more robust manufacturing operation, and trying to build the best product possible.

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The promise of allogeneic cell therapy is that it offers a reduced timeline to make a difference for patients, and the opportunity to potentially commercialize products after approval from a pivotal Phase 2 with positive clinical data.

As a result, process improvements or enhancements, such as closing a process from an aseptic manipulation standpoint, or dual-sourcing key materials, can be compromised on or may have to represent that trade-off, and then the timeline is condensed. But this is not a new trade-off.

It does need a risk-based approach, as Natika mentioned. What is unique to the cell therapy space from a regulatory perspective, and maybe furthering Natika's point, is that you have to apply those same principles across a much broader spectrum, and in particular this applies to starting materials. This is where the evolution or maturation of regulatory principles is still coming into focus as it applies to vectors, mRNA, plasmids, donor cells, and so on.

The old adage that everyone wants to be first and fast, but nobody wants to learn the hard way, is probably more applicable than ever in the cell therapy space.

NB: To follow up on that, you are also in charge of evaluating equipment. In cell and gene therapy we have been repurposing existing equipment from the blood industry, and often relying on technologies that are mainly used in the research space. This is one of the areas where we find ourselves in a bit of a gray zone, where we as an equipment provider are trying to develop material and equipment that is GMP compliant, and specifically designed for the cell therapy manufacturing environment. This is a bit of a shift for a lot of our customers when bringing in completely new technologies like acoustics.

Q (NB): Matt, from a therapeutic development side, and with everyone pushing as hard as they can to get to market and treat patients, any perspectives on how you make decisions on when to introduce, and how?

MM: First and foremost it starts with the technical capabilities of any piece of equipment. At Allogene we are fortunate to work with a very talented research and process development organization that can assess those capabilities.

Leading the sourcing and supplier management function at Allogene, I have a vested interest in ensuring that supply chain has a seat at that table, and is thoroughly involved in the process to look for particular pitfalls.

In the cell therapy space there are two key areas regarding commercial setup and expectation of some of these suppliers, as well as the management of their IP and confidential information. Here I would point to a traditional supplier-manufacturer relationship that most of us are used to in this space, and to the example of bioreactors and monoclonal antibodies (mAbs).

There has always been an exchange of confidential information for the sake of process optimization, and in some cases, comparable information around how the equipment works is not readily shared with manufacturers today in the cell therapy space. The space is certainly new and developing, and as a result, there is novel IP, but manufacturers don't have any interest in this confidential information beyond the purpose of improving our process to make the best products for patients. This is no different than the mAbs and bioreactors of years prior.

Unfortunately, this setup means that process development and characterization can be hindered, but it is my strong opinion that this will not last in the short- to mid-term as competition increases. Traditional relationships will return, and suppliers will be sought that help manufacturers create the best product – period.

For now though, when considering new partners in this space, it is paramount to ensure terms and conditions around commercial expectations are clear and reasonable.

Q (NB): It is definitely my opinion that providing the broadest access possible will ultimately help everyone. Natika, as I mentioned earlier, you have two hats. You have responsibility for our gene therapy and viral vector manufacturing business, where you have brought the facility to commercial approval. Any thoughts or experiences from that perspective?

NC: We have had the benefit of going through the process of becoming a commercial manufacturer, validating and implementing all of the equipment in use, and sharing that and explaining the depths of those qualifications to the regulators. We have been successful in that.

One of the things that now is a benefit to us a few years later is that for all of our clients, who are for the most part in their clinical phases, we are prepared, as their manufacturer, for that commercial endpoint that they are looking towards. We already have these things in place in terms of our facilities and our process control expectations, and we bring those to benefit the client.

However, the expectations for early-stage clinical manufacturing aren't the same, in terms of validation. We deliver interim levels of support based on phasing, but we are prepared, and we provide that roadmap to commercial success.

In terms of a case of novel equipment that we have implemented, we have had equipment where we had some failures in the earlier stage and had to go back and work with the supplier of that equipment to map out and make sure we had taken care of failure modes. As a service provider, we would do that on our own proprietary equipment, but we have also brought in equipment that clients requested to use in their process, and then we helped them navigate through making sure that could be qualified sufficiently for consistent processes.

Q (NB): We see that every day in the CDMO space, where we have very bespoke equipment and that needs to be managed. Anthony, to speak to your point earlier on everyone gunning for the endgame of commercial, and also picking up on what Natika just said about regulations not being as tight in the early stage: people often use the term phase-appropriateness. If you think about working with your clients, what recommendations do you have on how to go about that?

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AD: It is a very important phrase to bear in mind, because obviously you are filing an IND or a Clinical Trial Authorization when you are at that point, not a Biologics License Application or a Marketing Authorization Application.

There is phase-appropriate, and there is phase-appropriate. There are certain things you do in early-phase that you will live with forever.

I will go back to the potency assay as an example of this, then I am going to circle through to equipment. The potency assay does not need to be validated for your IND, your Phase 1, your Phase 2, or even – if you are foolish enough – your Phase 3. But there is an expectation that it will be validated when you go for the ticket.

It is phase-inappropriate to develop a validated potency assay for your IND. However, if you get to the endgame and you discover to your surprise that your assay is not validatable, it turns out not to relate sufficiently to your mechanism of action, or it turns out not to be the critical quality attribute mirroring the CQA you thought it would, then that is a problem. You either have to have an abundant supply of retains to test everything that has gone in the clinic previously with your new potency assay if you are going to have to replace the old one, or you are going back into the clinic. That is a very tough thing to say, but that is how it is. There is no time machine to go back and repeat and re-release lots if you have to switch an assay.

That is a good example of what is and is not phase-appropriate. The same thing rattles through to equipment: if you have manufactured your early-stage material with a piece of equipment that then turns out not to be commercial-grade, then the early-stage material's quality will be called into question and there will be a vigorous discussion of that with any regulator.

I have to say, it is so refreshing to see brand new technology like the ekko™ System coming into the field. It is quite rare, and I think more events like this will occur, and need to occur, to bring down the cost basis of these products and increase their quality. But the biggest pitfall is doing your earlier stage manufacturing one way and then landing yourself with a nasty big comparability issue further down the road.

Q **(NB):** The question of supplier qualification and validation feeds into providing our customers such as Matt and his team, with the right documentation. Natika, with that second hat you wear where you support us in qualifications, validation, and documentation, how do we go about this? How do we support and ensure customers get what they need?

NC: We have development teams that are working on not just equipment, but platforms, and we are looking at how to improve our processes every day.

For example, suspension cells in vector manufacturing: most of the processes now are in adherent cells, and so the early approvals are based on adherent cell processing. That speaks to Anthony's point that when you have developed all this data, you don't want to have to go back with a huge comparability issue at the end. For a client to start with an adherent cell process

and then get to market with a suspension cell process, that is going to create issues and a big delay.

We are not seeing that the first in the pipeline gets the change that has the new equipment, for example. It is not the folks that are far ahead in their trials and have locked down their process, it is those with early stage pipeline candidates, or more broadly those earlier stage companies, that we can talk to and say that we have a piece of equipment that is going to really speed up the process, and we also are supplying validation that goes along with that. I'd point the audience to our Emprove™ Program, for example.

For a lot of these novel therapies we are using research-use types of equipment. We will then proceed to having a conversation with regulators that this is the only equipment we have that serves this purpose, and it is not available from the supplier as a fully validated, mature piece of equipment. For our own equipment, such as the ekko™ System, which is not yet fully validated, we help by putting together the plan for that piece of equipment. Our approach is to work with the customer and provide as much data on this equipment as we can, from whatever research use that we have, and provide expected timelines for when we will have datasets available for a fully validated system.

So, there is a spectrum where we tell customers that we know that this is the equipment they need to use right now, and in the future, for their next candidates, we have suggestions for them. We are working early with the customers who are developing the technology, we are committed to working with them, and we are working together to get to a GMP compliant piece of equipment. There is an expectation of what it needs to look like commercially, but we have been able to work with the agencies and say it is not quite there yet, and they have been accepting of that. As we are now maturing, we are really trying to bring in equipment that is as far along on that path with all of the qualifications and validations that we can get, and offer that.

Q (NB): Anthony, any thoughts on how you have supported clients in bridging the gap between us as suppliers, the therapeutic developers, and the regulatory authorities?

AD: The best and only way to do it is with really open channels of communication.

There are bad equipment manufacturers, who will remain nameless, who blackbox stuff. There is a bit of paranoia about trade secrets. But this is a field where, in a very real sense, a rising tide lifts all ships. Likewise, the regulators do not like surprises.

There is a lot of game theory we see from very seasoned regulators, and we see it from law firms too. We get involved with compliance and remediation cases where people have already lawyered up. The lawyers are good, and half of them are ex-FDA themselves. But there is a tendency for game theory, and an attitude of "we don't want to tell them this because they might not like it, and it might sow problems down the road"

It is one thing if you are in a pre-approval inspection from the FDA Division of Manufacturing and Product Quality (DMPQ), because those inspectors are in uniform, and have no sense of humor and lots of clipboards. But if you are still dealing with the scientists and the medics at

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the FDA, I think they enjoy proactive and honest upfront communication. They thrive on it. They see things that you do not know they have seen, and they cannot supply you with those pieces of information, but they can use them in their response to you.

Bring more information rather than less to regulators – and here I am talking to the therapeutic developers as well as to your people, Nina – and you will be surprised how helpful that is in the long run.

NB: To add to that, one of my experiences in a pre-Merck KGaA life, when I was promoting and working on a new piece of equipment, was exactly that. I once gave a presentation with FDA people in the audience; they approached me afterward and were extremely excited about the equipment. They invited me to come in and talk about it, and bring them up to speed on all the new stuff that is coming up. From a supplier perspective, that was a really interesting experience for me and something that I have always valued very much.

Q (NB): Matt, you are on the end-user side of things, and you have seen things come through that are research-use-only, but serve the purpose to a tee. How do you support your internal programs when dealing with the likes of Merck KGaA, or other equipment suppliers?

MM: We made the earlier reference to phase-appropriateness, and this certainly can occur if the roadmap exists. Suppliers that have the right experience can be extremely valuable in that capacity.

Within Allogene we have a common saying: "right size for right now." As a company, we need to continually check ourselves to make sure we are staying at the forefront, but also that we are not out over our skis, as we don't have unlimited resources.

In that regard, phase-appropriateness in this space, if you have the opportunity to potentially commercialize in Phase 2, is a bit of a myth. It is really best to start with suppliers who have been there before, even if it is in other modalities. They know what it takes for a product to be commercialized, and this is where a risk-based approach comes in again and is so important.

Working with some of the niche suppliers in this space that have really exciting technology, they certainly don't intend to have lower quality or regulatory standards, but it is absolutely a risk. Larger organizations like Merck KGaA, or some of your competitors, can step in and show their value and experience here, particularly as it applies to equipment or reagents. On the equipment side, I would call out validation packages, software, and hardware in particular.

Q (NB): A question has come in from the audience that is perfect for this line of discussion. When making automation and process closure improvements post-approval, is it generally advisable to group as many of these process improvements together as possible, to make comparability studies and associated regulatory dialogue more efficient?

AD: So basically should you batch up your CMC amendments, or drip-feed?

There are pros and cons, obviously. The drip feed sounds worse than it is, and enables you to bring amendments in a timelier manner to the agency. The one-at-a-time approach is riskier because if you put in six amendments, statistically there is a bigger chance that something is going to trip up. When you have got all six bundled together, and if number five of six is the problem child it is going to hold back the rest of the class, potentially. On the other hand, if you batch them up, it is a more complete up-rev, and if there is comparability to be done, it is only one set of comparability.

Another argument for the drip feed approach is that time is money, and if you have got an improvement to the process, getting it into the plant that is spitting out either your commercial drug or your pre-commercial drug faster is a good thing. On the other side of the coin, batching does reduce the regulatory burden, and it does make things more efficient.

For truly significant amendments, which require deep comparability, I think it is important to get them in front of an agency as soon as possible. For lots of little ones, or LOLO as we call it here, batch them.

Q (NB): A very broad question that essentially goes back to the overall regulatory environment – do you think that international harmonization of requirements between various regulatory agencies is a strong trend, and would such harmonization be more viable in specific disease areas? Anthony, any thoughts on international harmonization?

AD: We recently hired Don Fink from the FDA, and in the last 5 or 10 years he has been involved in a lot of harmonization discussions between the FDA and other regulators. We also have a lot of clients for whom we often take the US material to the MHRA, and we take the EMA trials to the PMDA in Japan.

It is a strong trend, and it is increasing. There are great things around the world, like the EudraLex and the International Organization for Standardization. It is increasingly international, and I think this is the way it is going to go.

Especially with the COVID vaccine situation. It is kind of nuts to have the MHRA approve a vaccine first, out there in London, then the FDA with a very fast second, and the EMA just struggling, frankly. There is no need for that, and I think things like the pandemic will just reinforce that trend. These products are either gene therapies like the AstraZeneca and J&J vaccines, or basically a gene therapy, like Pfizer and Moderna. I think that will reinforce the trend. I would say it is a strong yes to the question.

NC: I echo the strong yes.

The good news is that as our clients are submitting their BLAs, or getting new products that they want to get approved; the agencies are asking us first for our inspection reports. They want to see which agencies have inspected the site, referring to our viral vector manufacturing facility. The first question, for example if we have had the FDA come, is can we see your inspection

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report? Other agencies are definitely looking at those and making acceptances without necessarily coming to do an on-site inspection themselves.

I will also bring up COVID. In 2020 we had several inspections from different agencies planned. Of course, restrictions for travel, for coming on-site, for the safety of our operations, and having a 2-week quarantine for regulators to come from another country and sit in a hotel for weeks before coming on-site, just made everything entirely unfeasible.

They looked at what the other agencies had reported on before, and we were able to move forward, or have extensions, and so on. That was inter-agency, and is a direct benefit of the harmonization that is happening.

MM: Maybe it goes without saying, but obviously manufacturers clearly benefit from that harmonization. As much as possible, trying to follow a single set of clear guidance is an extreme advantage, especially as some of the players coming up in this space are smaller companies, and quite frankly don't have the resources to look at regulatory guidance from many different places.

As much as that industry trend continues, it significantly helps the industry as a whole to push these therapies from the clinic to a commercial setting.

Q (NB): Another audience question – Daniel from Allogene is postulating that some equipment manufacturers have been less than transparent regarding operating ranges that are embedded deeply in recipes, and this is creating a validation and process troubleshooting challenge. Do we as the panel see this changing in the future? I can only speak for us, but we try to be as transparent as we can. We are creating our documents in such a way that there is as much information as we can provide, and create the datasets that we share with all our customers that we work with. Any comments from the panel?

AD: Daniel, we must have been dealing with the same equipment manufacturers!

It is exactly what Daniel has articulated here, a classic example of the sort of thing I was mentioning before, where people are a little bit Wizard of Oz about their equipment, and want to keep it blackbox.

Everyone is going to try and bring out competing products. There will be competition to the ekko™ System, either literal competition that will end up in IP court or similar, or non-literal competition where some other orthogonal physical method will try and eat your lunch. Fundamentally, competition is a good thing, and cream rises to the top. It doesn't help in the long run to take a short-sighted view.

Q (NB): Another question that tags on to that one by talking about the adoption of new equipment, automating processes, and speeding things up. This has most likely, or pretty certainly, happened in the

mAb space in the past, which has got us to where we are right now where monoclonal antibodies can be manufactured in large quantities and at a very reasonable price. Learning from the mAb space, how does the panel think this might play out for cell therapy? Will adoption speed up and innovation slow down, or will there always be a mismatch between the two?

MM: This is a really challenging question, and maybe from a scale perspective we will always have that challenge.

We talk frequently in the industry about scaling up versus scaling out. Many of the processes in CAR T manufacturing today can still be very much be characterized as benchtop manufacturing scale. It is essentially a research-scale operation that we are scaling into a commercial phase.

Considering what we do in mAbs today, with 2,000 liter bioreactors, or even much larger, I don't see that as being a driving trend in the near term for cell therapy. I see it more as a scale-out process that we would follow. From the standpoint of a manufacturer working with suppliers, the point we discussed before regarding an openness on how we make the process the most efficient, and deliver the best product for patients, needs to be the primary goal and focus.

NC: I have one thought regarding this mismatch of having the right technology, or the most efficient technology, and the speed that we need. A direct example that we have, because we also have that process development side, is that we have looked at new client's processes, for example, and recommended certain changes, and done studies for them to translate their process into our facility with X equipment.

What we have seen in the last three years or so is that the clients have been, and still are, a little bit hesitant to change things when they are on their path with their first candidate. Part of that mismatch is that they have told their investors here is what our process is, and here is what we are doing when. We come along and make suggestions and they say yeah that is great that we could get better yield, but we are sticking with what we have got. I think there is still going to be some of that.

Q (NB): The last question today goes back to scale out. How do you see the role of a modular cleanroom facility with a quality management system (QMS) in relation to its operations, in helping to expedite its biotech client's cell therapy development pipeline? The facility is not a classical CDMO, but one that provides the necessary manufacturing facilities for multiple small and medium enterprise (SME) companies at the same time.

AD: We like them. There have been pioneers in the field, and I think there are now a lot of fast followers.

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It is a spectrum. There is the idea of the manufacturing facility on a truck and the idea is not as dumb as it sounds – the blood donation industry has been just fine doing this for decades.

You can have modular: "QMS in a box" if you like. You can have semi-modular, where the walls and ceilings and floors arrive on a truck and they are assembled IKEA-style inside the aircraft hangar of a building, and there are a lot of facilities around the world that have basically done that. They partially come with an associated QMS; they certainly come with their own facilities, utilities, and metrics.

There will always be good old-fashioned stick-built and purpose-built, and so on and so forth.

Looking at the question carefully, the part I disagree with is saying that that facility is not a classical CDMO. It doesn't really matter that much. As I have said on previous forums, we sometimes see the facility as being more the lumen of the two. That is what is under GMP – in a truly closed system, the facility is inside that closed system. The box, the room, the pod, whatever it sits in, is somewhat on the periphery of where regulatory focus is going to be down the road.

NC: In my experience, over some decades of coming up with ways to manufacture these cell therapy products without the huge expense, having something that is modular has been a very useful answer. They have helped bring things to where they are today.

Sometimes we are looking at aseptic processing, and at whether to have a closed system. Those are considerations we have to have, and it is easier if you have a closed system. They are helpful, because if you can get your batches made, and you have some data you are collecting, when you get further along and you move to other pipeline candidates, this is data that is necessary. Talking about modular cleanroom facilities, those do meet the requirements.

Obviously, I like our purpose-built facility and our years and years of experience, but it is certainly a feasible way to go.

Q (NB): Matt, as a therapeutic developer, any thoughts on whether you would go with that for your manufacturing?

MM: There are really good arguments for both modular, cleanroom-in-a-box type solutions, and more traditional stick-build. I think is a worthwhile debate today.

Speaking from Allogene's perspective, we have just completed our own internal manufacturing facility. The need to have control over the operation at this level and stage in development is paramount. It is less about the design of the facility, and more about ownership of that and being able to oversee the key operations we see as an important lever to success.

NB: Thank you to everyone who provided questions, and a very warm thank you to the panel.

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Head of Commercial, Gene Editing and Novel Modalities, Merck KGaA

Dr. Nina Bauer is the Commercial Head of Cell Therapy at MilliporeSigma, the life science business of Merck KGaA, Darmstadt, Germany. Nina joined MilliporeSigma through the acquisition of FloDesign Sonics in October, 2019, where she was the Chief Commercial Officer. The core of her activities within MilliporeSigma is the launch of FDS' first commercial product, the ekko™ acoustic cell processing system, and related products and services. She has developed a passion for solving cell and gene therapy manufacturing bottlenecks during her time as Lonza's Head of Autologous Cell Therapy business. As part of that role, she was also in charge of establishing novel manufacturing technologies, most notably the Octane Cocoon™ platform. Previously, Nina held business development roles at the Cell Therapy Catapult (London), and the University of Edinburgh, and worked as Life Science Consultant for regenerative medicine businesses. Nina holds a Master in Neurobiology from Stony Brook University (NY), a PhD in Neuroscience from the University of Oldenburg (Germany), and an MBA from the University of Edinburgh (Scotland).

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Natika Calhoun is a regulatory Subject Matter Expert for biological APIs, virus and gene therapy products, antibodies, linkers, and antibody-drug conjugates, based in California, USA. In her current role, she consults on regulatory topics and works directly with customers as a Regulatory liaison to support technical writings, filings, and submission success for biological products. She previously served as the site Head of Quality for MilliporeSigma's viral vector manufacturing site in Carlsbad, California, USA, where she was responsible for leadership of the Quality unit, and for preparing the facility for commercial and inspection readiness to support its first FDA and EU licensed products, which resulted in successful FDA and EMA inspections. With over 25 years of Quality and Regulatory experience, Natika brings a strong background in aseptic processing, GMP manufacturing, and leading regulatory inspections. Natika holds a B.S. in Biology from UC, San Diego, M.S. in Biology – Molecular Cell Science from U. of Memphis, and Certificate in Biotech Management from Vanderbilt Univ., Owen Graduate School of Business.

Anthony Davies

Founder and CEO, Dark Horse Consulting

Anthony founded Dark Horse Consulting in 2014, bringing 20+ years of leadership experience in product, process and manufacturing development to cell and gene therapy companies in need. Anthony has a proven track record in managing pharmaceutical pipelines, is a skilled liaison with international regulatory agencies, and has an intense familiarity with a wide range of biologics, and cell and gene therapies. He is a highly sought-after keynote speaker and chair of national and international conferences and seminars, noted for his provocative, thoughtful and sometimes contrarian presentations.

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Matthew Muldoon is the Sr. Director of Sourcing and Supplier Management at Allogene Therapeutics. He is responsible for organizing supplier engagement activities to ensure

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supply of the equipment and materials utilized at Allogene's internal allogeneic cell therapy manufacturing plant in Newark, CA. In his previous role as Director of Biotech Sourcing for Bayer in Berkeley, CA, he was primarily responsible for external vendor interactions supporting the recombinant rFVIII franchise, Kogenate®, Kovaltry® and Jivi® and various radiology medical devices. Earlier in his career, Matt worked in Operational and Manufacturing Sciences capacities at Merck's Westpoint, PA site producing bulk Zostavax® and Varivax® vaccine.

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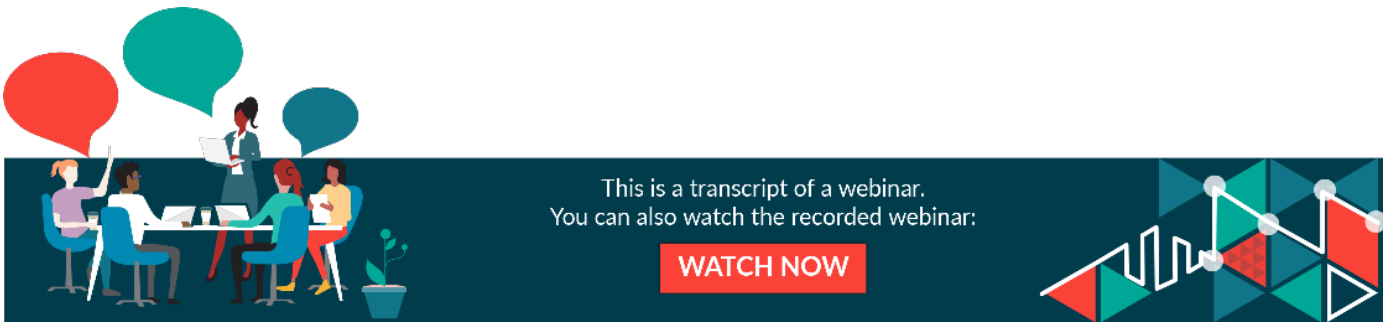


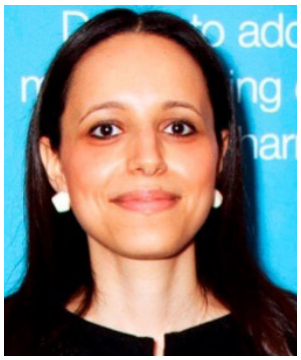
CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNO-ONCOLOGY: OVERCOMING MANUFACTURING & DEVELOPMENT OBSTACLES TO COMMERCIAL SUCCESS

SPOTLIGHT

INTERVIEW

Reflecting upon the GSK–UCL collaboration on viral vector bioprocess economics for *ex vivo* gene therapy commercialization



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Q Firstly, can you frame for us in broad terms the requirement for Cost of Goods (COG) reduction in the *ex vivo* gene therapy field? What are the most significant pain points - and the most obvious targets for improvement?

SF: For autologous *ex vivo* gene-modified cell therapies such as CAR T and HSC we have identified the key COG drivers. These include factors such as materials, quality control (QC) and labour costs and hence they all represent key targets for COG reduction. Strategies for reducing costs include reducing culture timeframes, lowering viral vector cost contributions, centralising QC activities and the ability to decrease the dose of these therapies through a better understanding of mechanism of action. Of course, the alternative being explored at the moment is allogeneic or off-the-shelf therapeutics, which have the potential to benefit from scale-up approaches to manufacturing and economies of scale that will translate to lower COG. If some of the current challenges relating to immune response can be resolved, then that is obviously an attractive approach in terms of helping with the COG dilemma.

Q Can you give us some background on the lentiviral vector (LVV) process economics study you performed in collaboration with GlaxoSmithKline (GSK) - how did it come about, and what were the specific goals of the study?

SF: We began an Engineering Doctorate (EngD) collaboration between UCL Biochemical Engineering and GSK on "Supply Chain Economics of Clinical and Commercial Autologous *Ex Vivo* Cell/Gene Therapy Manufacture" about 5 years ago. EngDs are industry-sponsored doctorates focused on training future bioindustry leaders responsible for the manufacture of next generation complex biological products. This EngD was part of our world-leading UCL Centre for Doctoral Training in Bioprocess Engineering Leadership funded by the UKRI Engineering and Physical Sciences Research Council (EPSRC) and industry.

When setting up the collaboration, GSK was expanding its cell and gene therapy portfolio with autologous *ex vivo* gene-modified cell therapy candidates for rare diseases and oncology. Given that these therapies require the management of a complex supply chain that includes three manufacturing processes (for plasmid DNA, viral vector, and the gene-modified cell therapy), GSK was keen to address future supply chain challenges for products in the pipeline. More specifically, the need for systematic methods to explore innovative manufacturing and supply chain solutions was identified to enable increased patient access and feasible business models, especially for the higher demand patient-specific therapies. This need aligned very well with research expertise at UCL Biochemical Engineering, where we pioneered the development of decisional tools to address cost-effective bioprocess design, portfolio management, and capacity planning decisions. These decisional tools have been applied to explore the best route to commercialization for various ATMPs such as allogeneic MSC cell

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"Strategies for reducing costs include reducing culture timeframes, lowering viral vector cost contributions, centralising QC activities and the ability to decrease the dose of these therapies through a better understanding of mechanism of action."

therapies [1–5], iPSCs for drug screening [6], CAR T-cell therapies [7], and lentiviral vectors for *ex vivo* gene-modified cell therapies [8].

The collaborative EngD project with GSK aimed to devise a tool integrating cost modeling at the process, facility, and enterprise level, so as to assess the cost and risk implications of manufacturing strategies for autologous cell therapies and the associated viral vectors and thus facilitate decision-making. The EngD researcher working with me between UCL and GSK was Ruxandra-Maria Comisel, with significant and invaluable support from our industrial supervisors from GSK, Bo Kara (currently at Evox Therapeutics Limited) and Fritz Fiesser. Following the EngD, Ruxandra–Maria has attained a role as Product Translation Engineer at eXmoor Pharma, a leading technical and strategic consultancy specialising in ATMPs and biopharmaceuticals.

Q Can you share any key findings from the study?

SF: We have recently published some of the findings from the collaboration between UCL and GSK related to LVV bioprocess economics for cell and gene therapy commercialization [8]. The work was driven by the sector's renewed interest in industrializing viral vector manufacture, given reports of limited capacity and capability for GMP manufacture of viral vectors and high COG, and this interest was further heightened by the recent successes of *ex vivo* gene-modified cell therapies such as CAR T. We set out to build and apply our decisional tools to address a number of industrially-relevant questions for LVV manufacture:

- What is the ranking of the cell culture technology options in terms of cost of goods (COG) across different types of viral vector products and titers?
- What are the COG savings achieved when moving away from cell factories?
- What are the cost drivers for each of these technologies?
- What is the target harvest titer required to lower the COG/dose to a specific threshold?
- Does switching from transient transfection to a stable producer cell line impact the technology ranking?

We explored a matrix of scenarios with different demand, dose, and titer combinations to reflect different disease indications and market penetration for the *ex vivo* CAR T, TCR, and HSC therapies requiring LVV, and differing degrees of upstream optimization for the LVV process. For each scenario, we ran an optimization to pick out the most cost-effective manufacturing strategy. We compared adherent 2D cell culture technologies ranging from ten-layer cell factories with limited scalability to more scalable options such as hollow fibre bioreactors, fixed bed bioreactors (e.g., iCELLis™), and rocking motion bioreactors with microcarriers (e.g., Wave™ with Fibra-Cel®). In addition, we explored suspension culture using single-use stirred tank bioreactors (i.e., SUBs).

The key findings can be summarised as follows:

1. The SUB was the most cost-effective technology across most scenarios when a suspension-adapted cell line was available, while the fixed bed bioreactor (FB) was the most cost-effective when adherent cell culture was preferred instead.

2. At large scale, the COG reduction achieved by switching from cell factories to suspension culture in SUBs or adherent culture in fixed bed bioreactors was at least 90%.

3. The raw materials cost drivers were the single-use components in the case of cell factories and hollow fibre bioreactors, and pDNA in the case of the more scalable technologies (fixed-bed bioreactors, rocking motion bioreactors with microcarriers, and SUBs).

4. To drive down viral vector cost contributions to cell therapy costs, we identified harvest titers need to increase by approximately 3-fold for CAR T, and 30-fold for high-dose HSC therapies.

5. COG values with stable producer cell lines can be 15-30% lower than with transient transfection owing to the removal of pDNA.

We have an upcoming paper that extends this study to determine the process change costs when switching from transient transfection to stable producer cell lines and to weigh up trade-offs such as the reliance on costly plasmid DNA supply with the transient transfection system versus the longer cell line development times and potential delays to market with the stable producer cell line.

"...the findings from the collaboration fed into live projects, supported business cases, and influenced decision-making at the sponsor site."

The benefit of the EngD mechanism, as opposed to a conventional PhD, was that it helped ensure the uptake of our modeling results by GSK to deliver real impact on strategic planning in this emerging sector. More specifically, the findings from the collaboration fed into live projects, supported business cases, and influenced decision-making at the sponsor site.

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Q What for you would be the important targets for further streamlining and COGs reduction in LVV manufacture moving forward - and how might it be achieved?

SF: Our research has helped prioritize targets for COG reduction in LVV manufacture. Most imperative is for the sector to shift away from the lab-scale methods that are commonly relied upon nowadays to scalable alternatives, such as the fixed bed bioreactor or suspension culture in SUBs. This will produce the cost significant COG reduction. Titer also has a major impact on COG and efforts to improve titers will help drive COG down. Given that pDNA can also be a significant cost contributor, the ability to switch from transient transfection to stable producer cell lines will remove the reliance on pDNA and result in cost reductions.

Q Looking further ahead, what does the future hold for LVV in cell therapy? What are its prospects for remaining the predominant method for cell transduction in light of recent safety concerns and the development of alternative platforms?

SF: LVV are likely to continue to dominate gene-modified cell therapies for a while. However, we are exploring non-viral routes in our Future Targeted Healthcare Manufacturing Hub, a large research centre hosted at UCL Biochemical Engineering in collaboration with academic and industrial consortia. We are carrying out experimentation with non-viral alternatives to identify their potential performance and will feed that into our decisional tools to determine the cost comparison and pinch points between the two routes. Given the nascent nature of non-viral routes, there may still be hurdles to overcome before they are used more routinely in clinical products.

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CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNO-ONCOLOGY: OVERCOMING MANUFACTURING & DEVELOPMENT OBSTACLES TO COMMERCIAL SUCCESS

SPOTLIGHT

VIEWPOINT

Allogeneic T-cell therapies: from R&D lab to production facility

Jakob Dupont, MD & Joe Newell

The allogeneic cell therapy field is exploding, with T cells attracting particular attention. While autologous T-cell therapies have been life-changing for many patients, allogeneic platforms hold the promise of "off-the-shelf" cells manufactured at scale and delivered to the patient within days.

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In this article, we will discuss the benefits and challenges of Atara Biotherapeutics' unique allogeneic Epstein-Barr virus (EBV) T-cell platform, from our perspectives as Head of Global R&D and COO at Atara.

THE EBV T-CELL PLATFORM

Most of us have been exposed to EBV by adulthood and never fully clear the virus – instead, it lies dormant, kept in check by the immune system, including EBV-specific T cells. Since 90 percent of healthy adults produce EBV T cells, they are readily available from donor blood and have several properties that make them ideal for cell therapy applications, including a good safety profile, the ability to persist in the body, and the potential for rapid scale-up.

We either use the donor-derived EBV-specific T cells in an un-modified state to treat EBV-associated cancers or autoimmune disease like multiple sclerosis, or we genetically modify the donor-derived EBV T cells to create CAR-T cells for the treatment of cancer.

Moreover, by using T cells specific to EBV as the basis for our therapies, we eliminate the need to genetically edit out the T-cell receptor using CRISPR Cas9 or similar methods to

ensure product safety and/or make cells suitable for use in non-related people. Instead, we keep the native structure of the cell intact but add elements such as co-stimulatory domains to enhance function.

T-CELL THERAPIES TO TREAT EBV-ASSOCIATED DISEASES

The natural biology of EBV T cells gives us the flexibility to use them in several different ways. Firstly, we can use them to treat diseases associated with EBV, including a number of blood cancers and autoimmune diseases. The goal here is simple: attack and destroy the EBV-infected cells that are causing the disease.

Currently in late-stage clinical development, tabelecleucel (tab-cel®) is one such allogeneic EBV-specific T-cell immunotherapy. Originating from research at Memorial Sloan Kettering Cancer Center (MSKCC), tab-cel® targets an aggressive type of EBV-driven lymphoma that occurs in transplant patients, known as post-transplant lymphoproliferative disorder (PTLD). Immunosuppressant drugs prescribed to prevent rejection can cause activation of dormant EBV, leading to a dangerous overproduction of immune cells.

Tab-cel® consists of healthy donor EBV T cells, processed with a goal of seeking out and killing EBV-infected lymphoma cells. Working with MSKCC, we have treated close to 300 patients with this product and clinical data so far suggests that it is a potential transformative therapy. We have completed an interim analysis of our Phase 3 trial data for currently enrolled patients and we are now preparing our BLA submission to the FDA, which pending alignment with FDA, we hope to complete by Q3 2021. If tab-cel® is subsequently approved, it will be the first allogeneic T-cell therapy approved by a regulatory body.

Another candidate drug based on EBV-specific T cells from healthy donors is ATA188, which is being investigated for the treatment of progressive forms of multiple sclerosis (MS). More than 2 million people worldwide are affected by MS, and there is increasing evidence that EBV may play a role in the underlying pathophysiology of the disease. We are currently treating patients with progressive forms of MS (primary and secondary progressive MS) in a randomized Phase II trial and early data suggest that a substantial number of these patients are having disability improvement with treatment. We plan to have interim analysis data in the first half of 2022 and final data from this study in late 2022/ early 2023.

NEXT GENERATION ENGINEERED T-CELL THERAPIES

We are not limited to diseases where EBV plays a causative role. By adding chimeric antigen receptors (CARs) or T-cell receptors (TCRs) we can target specific cells, whilst retaining the properties that make EBV T cells an attractive therapeutic approach. We are currently developing several allogeneic CAR T-cell therapies including product candidates that target mesothelin in solid tumors and CD19 in blood cancers.

First-generation CAR T-cell therapies were designed to treat liquid cancers, such as B-cell malignancies; treating solid tumors has presented and will bring new challenges. To solve for that, we are equipping engineered T cells with receptors that improve persistence and survival in the hostile tumor microenvironment. For example, many tumors produce an immunosuppressive factor, PDL1, so our T-cell therapies have been engineered to express PD-1 dominant negative receptors to counteract immunosuppression. Gaining a better understanding of the tumor microenvironment is a major research priority for the field to allow cell therapies to move from liquid to solid cancers.

ADDRESSING REMAINING DEVELOPMENT CHALLENGES

From an R&D perspective, key challenges include:

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1. Developing the most effective allogeneic off-the-shelf cell therapy platform. This allogeneic therapy needs to be safe and the donor-derived cells must be highly efficacious.

2. We need to develop approaches that enhance the persistence and potency of these cells in patients—the longer they persist, the more efficacy they are likely to deliver.

3. We also need to innovate 'armoring' technology where the CAR T cells are engineered to overcome the immunosuppressive tumor microenvironment (TME). Some examples of TME-associated immunosuppression include PD-L1 and TGF-beta.

The cell and gene market has focused heavily on analytical development in recent years. Companies are finding it easier to achieve regulatory approvals because they better understand the mechanism of action and performance of their product. But there are still many challenges to be addressed in characterizing these complex products. In future, the field is likely to move away from more traditional cell markers and towards measuring gene expression in single cells. These analytical tools are available today but have not yet reached the production environment.

ACHIEVING LARGE-SCALE MANUFACTURE OF ALLOGENEIC T CELLS: NOW & IN THE FUTURE

In a successful cell therapy company, R&D and manufacturing excellence are two sides of the same coin. One of the benefits of an allogeneic approach is that it allows us to plan production well in advance and build an inventory of cells that we can pull out of cryogenic storage and deliver to the patient in three days. Our ability to store cells over time was of huge benefit when donor donations had to be suspended due to COVID-19. However, we saw virtually no interruption to our supply chain due to the flexibility the platform provided us.

Currently, both autologous and allogeneic cell therapies are manufactured at a relatively small scale, which drives up costs. We have spent the last few years looking at how we can scale-out and scale-up our processes for conditions that affect millions of patients around the world, such as MS. We are now very close to commercialization and have proven each element of the supply chain, so we feel confident in our interactions with regulators.

A key step has been taking our process into the stirred-tank bioreactors typically used in biotech. Using bioreactors, a single donation can produce tens of thousands of doses of ATA188 for those living with MS. The ability to scale-up in this way brings down production costs, and – importantly – allows us to hire personnel from the wider biotech sector.

Scalability on a global level is not just about technology but also being able to hire enough qualified staff. This is such a new field, and it is moving so fast, that finding skilled staff risks becoming a bottleneck. It is vital for the future of the field that we have a pool of talent with expertise in cell therapies, genetic engineering, tumor biology, and how to work effectively with academic centers and investigators. Looking ahead, we also need to attract collaborators with disease-specific expertise to help us apply T-cell therapies to new, non-cancer indications.

Finally, as more companies move towards commercialization it is important to be prepared for compliance evaluation. Some suppliers along the supply chain are not entirely ready for that evaluation at a commercial scale. The supplier base needs to mature in the GMP space to enable companies like ours to move to commercialization quickly.

PREPARING THE COMMERCIAL SUPPLY CHAIN

We worked very hard to establish each element of our commercial supply chain in preparation for our Phase 3 pivotal trial. One

of the major advantages of our allogeneic platform is that we can build product inventory that can be stored and delivered within three days. We have evaluated each element from operational, quality and compliance perspectives to ensure that expectations for a successful commercial product launch are met. What gives us a high level of confidence is that we have been leveraging the exact supply chain that will be used to deliver our first commercial product candidate.

our T-cell manufacturing process by adopting a bioreactor-based system capable of delivering several thousands of doses from one lot. As we and other manufacturers move closer to large-scale commercialization, there are several challenges that need to be addressed. Key areas of focus for the future include expanding the pool of skilled and experienced staff, developing better analytics for more accurate characterization and ensuring suppliers are ready for compliance evaluation.

SUMMARY

Atara Biotherapeutics is developing allogeneic EBV-specific T-cell therapies for a range of indications, including hematologic cancers, solid tumors and autoimmune disease. We are using healthy donor-derived EBV T cells both to treat EBV-associated conditions, and as a starting point for next-generation CAR and TCR T-cell therapies. We have scaled up

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CELLULAR IMMUNO-ONCOLOGY: OVERCOMING MANUFACTURING & DEVELOPMENT OBSTACLES TO COMMERCIAL SUCCESS

SPOTLIGHT

INTERVIEW

A bright commercial future for off-the-shelf NK cell therapy

JAN SPANHOLTZ is currently CSO of Glycostem Therapeutics, a Dutch biotech company, focused on developing allogeneic cell products for cancer therapy. Jan qualified in medical biology at Radboud UMC in Nijmegen, the Netherlands, and developmental stem cell biology at Heinrich-Heine University in Düsseldorf, Germany. Jan has 20 years of expertise in stem cell research at universities and within biotech companies. Jan has experience of biotechnology scientific research development programs, leading to patent strategies around product and business development. He is author of multiple peer review research articles and inventor of various patent applications and within Glycostem responsible for research programs, patent strategy and early clinical translation, as well as coordinating several R&D collaborations with international partners from academia and biotech businesses.

TROELS JORDANSEN started his career in healthcare at LEO Pharma. After four years with Johnson & Johnson Orthopaedics he was one of the initial Genzyme Europe hires to focus on commercializing Carticel and Epicel in 1996. Over the past 20 years Jordansen has worked for five different cell therapy companies including Dutch IsoTis NV, Australian Clinical Cell Culture Pty Ltd. and British Azellon Ltd. where he was co-founder. His roles have covered sales, marketing and general management; for the past 15 years he has been managing director and/or chairman for private and public listed companies. Jordansen has been part of award-winning management teams that have raised over €175 million in funding. He became Chairman of Glycostem in January 2014 and CEO in July 2016.

VOLKER HUPPERT is a graduate bioprocess engineer from RWTH Aachen University. Among his achievements are participation in the set up of a quality system for a medical device/biotechnology company and development of several clinical-level reagents, disposable tubing sets and process software for cell separation and cell culture medical devices. He contributed to both the tubing set and process software development of a leading cell therapy-manufacturing device. Additionally, he managed projects and teams developing cell-manufacturing procedures for hematopoietic stem cells and Natural Killer cells. Volker has published 12 papers in peer reviewed journals over the past 20 years while working for a leading biotechnology company and is co-inventor of 9 patent families, including methods for T cell depletion of hematopoietic stem cell products, NK-cell transduction and NK-cell proliferation.

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Q What are you working on right now?

It is a really exciting time at Glycostem. We are treating AML patients in our WiNK trial at up to 8 clinical centers in 5 different countries. We have worked for some years towards being able to provide products for clinical trials from our own GMP certified manufacturing facility, which has involved all our departments – a real company team effort. We are currently adapting our manufacturing plans to soon be able to provide oNKord® for US clinical trials. It is very exciting for a young pharmaceutical manufacturer to work according to the similarities but also important differences between US and European regulatory standards.

In addition, we are busy with optimizing our CAR-NK and TCR-NK products in pre-clinical tests. We are seeing some really fascinating data coming through, so the future looks bright. We have grown our company to 50+ employees so we keep getting new inspiration to do better and move faster.

Next to those promising product pipelines, we are also focusing on NK cell combination therapy. One aspect of this work is that that cultured NK cells often have a lower number of CD16 receptors; the receptor that is needed to activate antibody dependent cellular cytotoxicity (ADCC) as mediated by antibodies connected to CD16 receptor on NK cells. In December '20, we in-licensed a technology from the University of Gent that will not only give us an abundance of CD16 receptors after culture, but also has the potential to reduce our production time by half.

Q The NK cell therapy field has certainly grown apace in recent times – what is your high-level analysis of the current state-of-play in the field?

NK cells always have been in the shadow of T cells and recently, in the even bigger shadow of CAR-T cells. However, the stakeholders have now realized what we first thought 15 years ago – namely, that cell therapy will only have blockbuster potential when we can use it in a universal way through having access to allogeneic off-the shelf products, which are ready to use and where patient donor matching, or mismatching, is not needed to provide safe treatments to patients.

That is why recently, more and more NK cell companies have been founded. In general, they differ from Glycostem in that they either use older cell culture methods with feeder cells, or multiple variants of IPSC-derived NK cells. It is very interesting to see that not a lot of preclinical functionality data has been published to date, and that often only genetic manipulation is needed to get those NK cells to work; our published results on hematological and solid cancers show superior killing also related to low expression of inhibitory receptors on oNKord®.

We observe that not every NK company has a clear idea around starting clinical trial activities with manufacturing processes that have the potential to be used in Phase 3 trials and beyond. We believe this a key discriminator which makes Glycostem differs from those newcomers mainly focusing on complex genetic modification concepts, rather than understanding the basic potential of their NK cell product and having a solid, up-scalable manufacturing concept for more than 10k infusion doses a year.

Q Can you tell us more about Glycostem's NK cell therapy platform and approach – what are the chief benefits or differentiators?

We basically started developing our manufacturing process with the intention of having a chemically defined, animal component-free cell culture process, without complicated manufacturing steps such as the introduction of feeder cells. Glycostem's NK cell manufacturing technology has been designed with pharmaceutical manufacturing standards and quality control in mind. It does not require us to translate an in-licensed, academia-derived process to manufacturing and QC requirements. Moreover, we always aimed to have a completely closed cell culture process with a high level of automation to reduce labor and manufacturing costs. The development of our own serum-free cell culture medium 13 years ago enabled the innovative cell culture process to grow NK cells from CD34 hematopoietic stem and progenitor cells enriched from umbilical cord blood. In order to have an easier process during regulatory clinical trial initiation filings for IND and EMA-guided European multicenter trials, we chose not to use feeder cells, which are bound to create regulatory tension due to impurities and side effects. The use of feeder

"Glycostem's NK cell manufacturing technology has been designed with pharmaceutical manufacturing standards and quality control in mind."

"...we are amongst the first in the world to have a truly off-the-shelf cell therapy product needing no HLA matching or mismatching, a product with no known severe side effects, and a production cost that will allow for serious market penetration."

cells also complicates the manufacturing process unnecessarily. These are issues that synthetic media simply do not create. Last but not least, we have developed a closed manufacturing system, which with minor adaptation, can manufacture both non-manipulated and manipulated products – this gives us total production flexibility. Most importantly, the small but very clearly defined footprint of the manufacturing process allows for robust up-scaling to serve the needs for pharmaceutical cellular therapy manufacturing.

The benefits are really multiple. For instance, we are amongst the first in the world to have a truly off-the-shelf cell therapy product needing no HLA matching or mismatching, a product with no known severe side effects, and a production cost that will allow for serious market penetration.

Q What were the considerations for utilizing a cord blood cell source?

First and foremost, we wanted to have a potent product that could make a real impact on cancer. After researching various options, we chose umbilical cord blood several years ago. The cord blood stem cell-derived NK cells have unique phenotypic profile, which also results in a superior cytolytic profile if compared to other NK cells, such as activated NK cell from peripheral blood. There is a steady global supply and the $CD34^+$ stem cells with which we initiate our manufacturing process grow fast and have high potency. We have access to enough cord blood from one supplier to generate 25 million doses of CAR-NK products; so we are certain that we have more than sufficient supply.

Q How and where has the COVID-19 pandemic had an impact on Glycostem's clinical development and supply chain – and what are the key learnings you will take forward from this enforced stress-testing?

We have generally seen a delay of several months on some of our projects due to COVID-19. We had supply issues for production, which delayed the initiation of our clinical trial. Even today, we are seeing how companies claiming to be involved in COVID-19 work get priority in supply making it more troublesome to re-stock for disposables. With this in mind, we have increased our stock levels and raw material sourcing options. We are not anticipating more delays due to COVID-19.

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We have learned that an allogeneic off-the-shelf product is less sensitive to supply challenges when final drug product is on stock.

Q What will be the key considerations at the strategic level as Glycostem's next-generation CAR NK cell therapies enter and progress through the clinic?

CAR-NK cell therapies should be off-the-shelf, cryopreserved products, providing sufficient stock to serve the needs of a clinical trial. CAR-NK should combine natural NK cell functionality with CAR-mediated specificity. Target selection is essential, providing the required specificity for tumor antigens as well as broad coverage. Glycostem's has made its choices in target selection with a network of highly experienced NK cell and cancer scientist.

Progressing through the clinic requires availability of the product, safety, and efficacy. And soon after Phase 1 trials, scalability of the manufacturing process will be essential again.

Q On the more technical/scientific level, what trends do you expect to see emerging or developing that are particularly relevant to NK cell therapy, moving forward?

We see the need emerging for feeder cell-free and cancer cell line-free approaches. Manufacturing platforms need to be scalable to thousand of patients per year, and closed systems with a high degree of automation will be the standard. Engineering platform will allow for product batches for multiple patients, and they will maintain the broad functional repertoire of primary NK cells. Genetic engineering platforms will be cost-effective, allowing manufacture of cell therapy products for many patients while keeping healthcare costs under control. Genetic engineering platforms will allow us to access the full potential of chimeric antigen receptors and regulatory proteins such as cytokines.

We anticipate that we will also be able to combine the potential of highly specific T cell receptors with allogeneic, off-the-shelf NK cells to be able to cover nearly all relevant cancer-related antigens and their mutations. Combination therapies (NK cells and antibodies, NK cells and conventional drugs) will have a solid technology platform and will have passed the first (non-technical) challenges of novel partnering and licensing approaches within the industry.

Q How is Glycostem seeking to build flexibility into its R&D and manufacturing/supply chain models, given the current state of rapid technological and regulatory evolution in the field?

As discussed earlier, Glycostem is building platforms such as closed manufacturing systems. We are carrying this philosophy over into our CAR-NK/TCR-NK research, so that once we have one solution in place, we can easily expand to research and develop more

products. Our closed manufacturing system is the perfect example; we can use it for all our products – the raw materials, disposables, and equipment are the same. This will also allow our regulatory affairs department to recycle reports and data sets from non-manipulated to manipulated product applications. Overall, a great use of resources that will save time and money.

We are working closely with partners that are at the technical forefront in their specific areas to enable step-wise improvement of our platforms. The platform technology allows us to have multiple iterations up and running, always using the best option for the specific scaling, regulatory, and clinical stage. Information technology for data and process control is part of the portfolio of improvements.

Q What are the most important preparatory steps that you are taking now with commercialization in mind – particularly in light of today's often substantially reduced development timeframes?

With conditional approval expected by late '23, we need to think about how to satisfy the demand for our product. To this end, we have been working with a consultancy company to help us understand the time and funds needed to realize this milestone. With this effort comes hiring of significantly more staff, training them, and so on.

We are also getting the best out of every batch, enable scaling of batches, increasing the level of automation, making documentation review and approval more efficient, and generating performance data in preparation for process validation and extended assay validation.

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

In biotech you are constantly fundraising, so this is a repeat priority, but with a clinical trial going on and more indications to come, clinical development plays an increasingly important role. The same goes for manufacturing and quality, ensuring that we have the products with which to treat patients. Looking to the horizon, we will need to take advantage of the unique features of NK cells and ensure that we stay at the forefront in all disciplines.

We want to see the first NK cell product approaching approval and being available for all patients who need it. We want to see cell therapies starting to be used as a standard therapy, and patients starting to be treated with an off-the-shelf CAR approach. Having stumbled into NK cells in 1998, we have been working with many great partners, sharing a vision of NK cells making an impact – so we want to see that happen, for all of us: for the patients and for the healthcare system.

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CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNO-ONCOLOGY: OVERCOMING MANUFACTURING & DEVELOPMENT OBSTACLES TO COMMERCIAL SUCCESS

SPOTLIGHT

INTERVIEW

Optimizing manufacturing processes to develop novel cell and gene therapies

KERRY INGALLS Kerry Ingalls is Chief Operating Officer of Poseida Therapeutics, Inc., a clinical-stage biopharmaceutical company utilizing proprietary genetic engineering platform technologies to create cell and gene therapeutics with the capacity to cure. He brings a long history of operating experience in pharmaceuticals and biotechnology. He has held numerous leadership roles including the oversight of clinical and commercial manufacturing for Amgen, leading its corporate engineering and then biologics manufacturing operations in Colorado, Ireland, California, and Puerto Rico. Ingalls has extensive professional background and training in the United States Navy spanning 30 years, including advising senior defense leaders on critical national security issues and leading elite deployed submarine operations. He received a Master of Arts in International Law and Diplomacy from Tufts University as well as a Bachelor of Science in Mechanical Engineering from the United States Naval Academy.

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"Our non-viral DNA delivery system, piggyBac®, has the benefit of large cargo capacity and is active in virtually any cell type with broad applicability. We can use piggyBac alone or in combination with our highly precise Cas-CLOVER™ site-specific gene editing technology, which can efficiently make multiple edits with little to no off-target activity."

Q What are you working on right now?

KI: **Poseida is constantly driving to develop therapies with the potential to achieve better patient outcomes with low toxicity, and ultimately single treatment cures for cancers and genetic diseases.** While developing our rich portfolio of proprietary and highly differentiated gene engineering technologies, we are also applying these breakthroughs in our clinical program candidates. Our leading candidates are CAR-T therapies in both hematology and solid tumors. We are developing treatments for patients with multiple myeloma, prostate cancer, and soon other cancers like breast, ovarian or lung as well. Our technology also enables the development of in vivo gene therapies, specifically in liver-derived diseases. We have significant work ongoing in all these areas now.

Q Can you give us some more background on Poseida Therapeutics' technology platforms and R&D pipeline candidates?

KI: **We utilize specific proprietary technologies to develop our product candidates.** Our non-viral DNA delivery system, piggyBac®, has the benefit of large cargo capacity and is active in virtually any cell type with broad applicability. We can use piggyBac alone or in combination with our highly precise Cas-CLOVER™ site-specific gene editing technology, which can efficiently make multiple edits with little to no off-target activity. We use both technologies in the development of our upcoming fully allogeneic CAR-T product candidates. PiggyBac's ability to deliver large capacity genetic cargo and permanently integrate into DNA enables us to extend our technologies into diseases beyond the reach of more standard, transient viral-based delivery methods.

Poseida is focused on migrating to a fully allogeneic approach that will modify T cells from healthy donors and turn them into ready-to-use or "off-the-shelf" therapies to treat cancer patients. The manufacturing process for our allogeneic product candidates is nearly identical to the process for our autologous product candidates, except for the gene editing and related purification steps. This approach offers significant benefits, including much more rapid availability

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of treatment when prescribed, reduction in the cost of manufacturing, and expanded patient access to potentially lifesaving treatments.

The first version of our fully allogeneic CAR-T, P-BCMA-ALLO1, is expected to enter the clinic later this year. We also are developing a pan solid tumor version of a fully allogeneic CAR-T, P-MUC1C-ALLO1, expected to enter the clinic this year as well. We are optimistic that fully allogeneic CAR-T, featuring a high percentage of stem cell memory T cells (Tscm), will be the answer for accessible single treatment cures for these cancers and beyond.

Q What overall manufacturing business model/strategy has been selected with these product candidates in mind, and why?

KI: We currently partner with Contract Manufacturing Organizations (CMOs) until it makes sense to make significant investments in such capabilities after we have better visibility on our finalized processes and associated manufacturing needs. At that juncture, we will evaluate whether it makes more sense to manufacture some or all of our products ourselves. We look forward to that development.

We also have diversified our CMO partners for additional risk mitigation and alignment with their specific areas of manufacturing expertise.

Last year, we completed construction of a Pilot Plant on our campus here in San Diego that promises to accelerate our exciting pipeline into the clinic. In fact, we recently introduced P-MUC1C-ALLO1, our second allogeneic program candidate, into the plant and intend to file that IND application later this year. We will leverage the plant as much as possible for preclinical and early clinical stage programs.

Manufacturing encompasses a lot more than just being the proud owners of a facility, as you know. A manufacturing system includes a phase-appropriate quality system, well-trained and qualified manufacturing, quality, process development, analytical development and regulatory teams, reliable raw material and equipment suppliers, and so on. Those are some of the pieces, and I would add that a strong quality and compliance *culture* is critical as well. We are developing all of these elements now.

Q In concert with many in the cellular immunotherapy world, Poseida has a number of allogeneic therapies following autologous lead candidates into the clinic - can you go into more depth on the main challenges and opportunities you see on the manufacturing/supply chain side for each one at the moment?

KI: Whether we are talking about autologous or allogeneic programs, our manufacturing challenges are probably similar to those experienced by other companies in this field.

First on my list is the urgency of Poseida's mission: to deliver the next generation of cell and gene therapies with the capacity to cure patients with cancers and genetic disease. These

patients are very ill and, almost by definition, running out of options to treat their disease. We are working urgently to reliably deliver therapies with the capacity to cure those illnesses in ways that will also differentiate Poseida from our competition. We are blessed with brilliant scientists, and I really admire the application of their education, experience, and raw intellect as they come forward with new discoveries, insights, and approaches. The only thing I find more motivating than "We don't understand the biology yet," is when I hear, "We just figured it out!" But disease does not take a day off – there's no time to waste.

"We continuously work to optimize process development without compromising speed to clinic."

Before joining Poseida, I led manufacturing operations in big biotech where raw materials, manufacturing processes, and even the regulatory space were typically well-characterized and mature, usually resulting in a high degree of predictability in outcomes and reliability of supply. That situation is not always the case in cell and gene therapy. For example, some of our raw materials are unique and occasionally reveal a need for better characterization. In the case of autologous approaches, the patient leukopak itself is a critical starting material in which we see significant variability between patients and between disease states as well.

We continuously work to optimize process development without compromising speed to clinic. Our processes are always being refined and, while appropriate for early-stage trials, they can and will benefit from continuous improvement as we approach commercialization. Also, process steps are sometimes open and operator intensive, demanding better solutions in the near term.

Some manufacturing hardware used in this space is novel or at least relatively new in industry, which can make manufacturing operations highly dependent on vendor availability and willingness to share proprietary information when problems arise.

And of course, manufacturing throughput is a challenge throughout the cell and gene therapy arena, particularly for autologous programs. It requires a tremendous amount of manufacturing space and staffing, especially when significant commercial demand is foreseen. Leaps forward in process closure, automation, and accelerating cell expansion are common topics in the field. Like many companies, at Poseida we are focused on continuous improvement in manufacturing operations.

So, you can see we are working to transform this complex package of variables into a reliable manufacturing output. It is not easy. In the field of cell and gene therapy, the regulatory space remains both a challenge and an opportunity as well.

Regulatory agencies play a critical role in our ecosystem. They too have the mission to serve patients. Success for patients requires us to bring regulators along in understanding and safely deploying novel therapies based on scientific breakthroughs. Interactions to educate, train, and partner with regulators – appreciating the importance of the natural tension between manufacturers and regulators – are critical if we are to move smoothly into the clinic and ultimately commercialization. This challenge was present 40 years ago with the arrival of monoclonal antibodies and remains present today with the explosion of gene engineering modalities.

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Regarding opportunities, the allogeneic approach will fundamentally transform the cell therapy space, and we are confident in this future. Some clear advantages include:

- Readily available starting material. Healthy donor leukopaks should be on the shelf, ready to support timely manufacturing starts without the vulnerabilities inherent in autologous processes – patient screening, logistics challenges, and so on.
- Higher likelihood of manufacturing success. In our experience, healthy donor material manufactures with a very high level of reliability.
- Near immediate availability of product. There are several supply chain strategies that will facilitate this goal.
- Significant reduction in cost of goods. Imagine reducing the current cost of an autologous product by a factor of 10 or even 100. That day is coming, and it will be a game-changer.

Q What would you pick out as the key next steps for the allogeneic cell therapy field in particular in terms of capitalizing on the inherent advantages of off-the-shelf products?

KI: We are very excited about allogeneic cell therapy and what it will mean for patients and for the industry. As you know Poseida is working on several allogeneic candidates and plans to submit two INDs just this year, one for liquid tumors and one for solid tumors. More such candidates are in our pipeline as well.

Key next steps include:

- Define, identify, and secure a supply chain of healthy donor material. Many in the industry are working on this key resource pool. Poseida is no exception.
- Work closely with regulators to build confidence in the pathway to approval.
- Generate compelling data showing safety and efficacy.
- Drive down cost of goods by maximizing yields.
- We are also keen on stabilizing a platform approach for allogeneic program candidates so that we can accelerate discovery-to-clinic timelines.

Q What for you will be the key learnings that the cellular immunotherapy sector will carry forward from the COVID-19 pandemic?

KI: First, I'd like to say how proud we are of our team for their patience, cooperation, and agility during the pandemic. Because of them, we have been able to continue our mission with great success over a historically challenging period.

"We must continuously improve, capturing these lessons and leveraging them to make Poseida even more prepared for future challenges. We owe that to our colleagues and the patients we serve."

Related, as is always the case in times of crisis, is that we learned a lot about how we might work differently going forward. Internally, we have even better ideas about how to make our workplace healthier and more productive in flexible ways. Externally, we are better informed about the capabilities of our partners and vulnerabilities in the supply chain of raw materials and transportation sectors, especially across international borders.

It should come as no surprise that I think the COVID experience highlights again the advantages of an allogeneic approach to manufacturing. Several clinical sites across the country were diverted to COVID response and could not support clinical trials to the same degree they otherwise would have. Lockdowns undoubtedly discouraged some patients from seeking investigational treatments. Many flights were cancelled or delayed due to a significant drop in numbers of the flying public, placing a big strain on delivery of leukopaks for autologous manufacturing. Allogeneic therapies, ready and waiting on the shelf, would have been a big help in this crisis.

I served for many years in the US Armed Forces. One saying that always stuck with me was, "If war comes tomorrow, you fight with the plan you have, not the one you wish you had." To me that means we must continuously improve, capturing these lessons and leveraging them to make Poseida even more prepared for future challenges. We owe that to our colleagues and the patients we serve.

Q Finally, can you summarize your chief goals and priorities in your role over the foreseeable future?

KI: "Foreseeable" is a bit of a moving target! In the near term we are focused on the two autologous CAR-T programs we already have in the clinic, advancing two allogeneic candidates for IND submission and clinic entry later this year, and pressing forward with our first gene therapy candidate targeting Ornithine Transcarbamylase (OTC) deficiency.

Meanwhile, a rich pipeline is advancing immediately behind these lead programs; our Pilot Plant is beginning to deliver the value for which it was intended; and our brilliant scientists are advancing exciting technologies every day. We are growing our team at a responsible rate, and we will consider partnering and collaboration where helpful to advance this promising science as rapidly as possible. It is an exciting time to be at Poseida!

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CELL & GENE THERAPY INSIGHTS

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INTERVIEW

Finishing strong: evolving fill and finish technology for the cell and gene therapy field

JEAN-SEBASTIEN PARISSE, is Commercial Director at Aseptic Technologies, a company he joined in 2007. His role is to manage and direct global sales efforts of Aseptic Technologies; accelerating growth and creating tighter connections between customer requirements and innovation, along with increased service levels, with a special attention to ATMPs since 2009.Jean-Sébastien is member of the Process and Product committee of the International Society for Cell Therapy (ISCT).

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Q Why do you feel evolution in fill-finish technology is required for the cell and gene therapy space? What are the key drivers at the strategic level?

J-SP: The most important factor here is the necessity of reducing the cost of goods of these therapies, to allow broader access to patients. Fill and finish has a role to play

in that goal, as it is an important step in the manufacturing of the finished drug product.

> "Because of the use of cryoprotectant, there is a limited time window between final formulation and freezing. This means the fill and finish should be done fairly rapidly. You have to have a relatively high output of your equipment, even though the total batch may be small."

Q … and at the operational level?

J-SP: Fill and finish for cell and gene therapy has some specificities. The most important, of course, is the fact that you need a final container that is compatible with cryoprotectant, as most of these products will be formulated with a cryoprotectant.

You have to ensure that the container will provide an uncompromised container closure integrity during storage, which is mostly at -70°C , or in the vapor phase of liquid nitrogen.

Because of the use of cryoprotectant, there is a limited time window between final formulation and freezing. This means the fill and finish should be done fairly rapidly. You have to have a relatively high output of your equipment, even though the total batch may be small.

Formulation with cryoprotectant also affects the product at room temperature, so you also have to have a process which is extremely robust, in order to ensure that as soon as you start production you will be able to run it smoothly until your batch is completed.

Q … and finally, at the technical level?

J-SP: The price of these drug products remains very high, and there are various aspects that need to be managed to avoid losing this extremely high-value product.

If possible, you should aim to:

- Limit overfill in the container, which is possible if your container allows complete collection of the product out of it at the clinical site.
- Reduce the dead volume in the drug product path – meaning the holding container, the tubing, and all of these elements of your equipment.
- Move towards zero-defect production, as you want to avoid any rejection during your batch.

Q Why is it especially important to have an integrated or automated fill-finish solution?

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J-SP: Reproducibility during a batch is key – it has to be completely homogenous. Between batches you also need consistent production, and therefore you want to avoid operator-dependent factors.

The main risk factor for contamination during a production is the operator. You want to limit human interaction and interventions. Having an automatic fill and finish solution will also enable you to validate your critical process parameters, and to run those during all your subsequent batches.

Q Can you tell us specifically about the utility and value of having a built-in weighing device for the CGT field?

J-SP: We are bringing a solution of having 100% in-process control of weight, so that we weigh the vial before and after filling. By doing so we make sure that all the vials are within specifications.

By having very accurate weighing, and doing it on each and every vial, you will have very accurate filling throughout a batch. Because you weight 100% of your containers, that will also allow the equipment to do self-dose recalibration throughout the batch.

Q How are you responding to growing calls from the industry for enhanced configurability of bioprocess steps and devices?

J-SP: Aseptic Technologies has been active in the field since 2009, so we have a lot of experience and a comprehensive understanding of the industry needs.

We are a solution-oriented company, and flexible to implementing new systems and processes our customers are willing to integrate. We are working with all of our users, who number more than 350 today, to understand their needs and to validate new concepts with them.

Q What key trends do you anticipate for cell and gene therapy manufacture, and how is Aseptic Technologies innovating to accommodate those?

J-SP: As I mentioned earlier, the most important issue is cost of goods

"The main risk factor for contamination during a production is the operator. You want to limit human interaction and interventions. Having an automatic fill and finish solution will also enable you to validate your critical process parameters, and to run those during all your subsequent batches."

reduction, so that cell and gene therapies can be offered to a wider patient base. Cell and gene therapy needs to become a commodity. We are working with our customers so that we can reduce cost of goods with them.

In terms of future developments, we are also working on drug product homogenization. This has been requested by many of our users, and so we are working with them to develop a device for this.

Q You have seen an exponential growth of users – how do you explain that trend?

J-SP: We have been active in the field since 2009 – so almost from the beginning. We are gaining experience every day by working with our users to improve our solutions, and develop new ones where needed.

Most importantly, our technology is the AT-Closed Vial, which ensures 100% container closure integrity at temperatures of -70°C, or even lower in the vapor phase of liquid nitrogen. This solution is very easy to implement in the early phases of drug development, and by design is ready to be scaled up as needed towards commercial productions of these drugs.

AFFILIATION

Jean-Sebastien Parisse,
Aseptic Technologies

AUTHORSHIP & CONFLICT OF INTEREST

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“THE MOST IMPORTANT ISSUE IS COST OF GOODS REDUCTION, SO THAT CELL AND GENE THERAPIES CAN BE OFFERED TO A WIDER PATIENT BASE. CELL AND GENE THERAPY NEEDS TO BECOME A COMMODITY”
- Jean-Sebastien Parisse, Commercial Director
Member of SKAN Group
At Aseptic Technologies, we enable biopharmaceutical companies to deliver cutting-edge therapies to their patients, by introducing innovations to the fill & finish operations.
Discover our scalable AT-Closed Vial Technology® widely used for the cell and gene therapy products since 2009.
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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Supporting development of mRNA-based therapies by addressing large-scale purification challenges

Kelly Flook

The field of mRNA-based therapies is a rapidly emerging area with increasing real-world applications. The potential of these therapies is being demonstrated in various fields. Although the potential of mRNA in therapies is seemingly endless, obtaining the quantities of synthetic mRNA needed for clinical treatment remains a challenging obstacle, and current methods for mRNA purification are creating a bottleneck in large-scale manufacturing. Particularly for vaccine development, obtaining the quantities of synthetic mRNA needed for clinical treatment remains an obstacle. As a result, a robust, scalable and easy-to-use platform to support all mRNA therapies is needed. To support the development of mRNA-based therapies, Thermo Fisher Scientific has developed an affinity resin for the purification and isolation of mRNA from *in vitro* transcription (IVT) manufacturing processes. The following article and case studies will highlight how the Thermo Scientific POROS™ Oligo (dT)25 affinity resin can enable efficient and simplified mRNA purification.

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THE RISE OF mRNA THERAPEUTICS

Whilst mRNA now offers a new therapeutic paradigm, mRNA itself is not a new modality. The first concept proposal and successful study was published over 30 years ago, and

the first clinical trial began nearly 20 years ago – and today, the growing applications of mRNA as a therapeutic have been greatly spurred on by the success of novel mRNA-based vaccines being made available for emergency use against the novel coronavirus.

The rapid growth of mRNA as a therapeutic can also be attributed to the fact that the action of mRNA is relatively simple and well understood, making it a promising candidate for the development of platform technology. Synthetic mRNA has many applications – it can be used to create induced pluripotent stem cells, or induce cell differentiation into desired cell types by introducing proteins that stimulate these processes. It can be used to create secreted proteins such as antibodies, and to express a homing receptor to improve cell migration to specific areas in the body. Additional uses include vaccination of rare and common diseases, and synthetic mRNA can also be used for gene editing using TALENs or CRISPR.

THE PURIFICATION CHALLENGE

For a platform technology to fully succeed, a corresponding purification platform is key. Traditionally, purification of mRNA is achieved by a variety of methods (Table 1), but each option brings disadvantages. Many scientists try to scale up tried and tested methods from the research laboratory – but when moving from micrograms to grams, and potentially even kilograms of mRNA, this may not be the most successful, or optimal approach. Scalability is not the only challenge to tackle – other important considerations include purification efficiency, ease of use, recovery, selectivity, and the option to integrate an affinity resin as a platform solution for various mRNA molecules.

Reverse phase purification

Reversed phase purification is highly effective and achieves high resolution. It offers some selectivity for product related impurities, but

TABLE 1

Methods of RNA purification.

Method	Advantages	Disadvantages
Reversed phase	▶ High resolution ▶ Some selectivity for product impurities	▶ Limited column capacity ▶ Use of expensive/flammable/toxic chemicals ▶ Column fouling impacts resolution
Ion exchange chromatography	▶ Native purification possible ▶ Scalable	▶ Column capacity and recovery (HPLC) ▶ May need toxic chemicals for denaturation ▶ Purified product can contain traces of elution salts
Size exclusion chromatography	▶ Native purification possible	▶ Separation efficiency affected by alternative folding ▶ Flow limited
HIC	▶ Native purification possible ▶ Scalable ▶ Replacement for reversed phase	▶ Non-selective
Affinity chromatography	▶ Native purification possible ▶ Scalable ▶ Platform solution for wide range mRNA molecule sizes – selective to polyA	▶ Requires additional polishing step to remove product-related impurities

Affinity chromatography can be used as a scalable platform solution for mRNA purification.

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when considering this approach from a scale up perspective, there is limited column capacity. An additional challenge is the need for flammable and toxic solvents that pose safety concerns for operators and necessitate intrinsically safe suites which are not commonplace in biotherapeutic manufacturing. These suites are costly to set up, and bring additional cost implications related to disposal of organic solvents. In addition, ion pair reagents add a toxic component that then requires additional purification steps to remove.

Without very stringent cleaning protocols, fouling from smaller proteins and enzymes can impact the selectivity and separation efficiency of the column over time.

Ion exchange chromatography

Ion exchange chromatography is a common approach when working with smaller nucleic acids, and is effective for native purification. When working with increasingly larger constructs, capacity and recovery issues arise – due to the multiple charges on the mRNA, it binds very effectively to ion exchange resins, and in some instances eluting the mRNA molecule from the column with good recovery can prove difficult.

Hydrophobic interaction chromatography

Hydrophobic interaction chromatography (HIC) is a common chromatography technique that is also being used for the purification of mRNA. It allows for native purification, and the resins are scalable. Similar to reversed phase, HIC takes advantage of the difference in hydrophobicity of mRNA and its impurities, and is commonly used by the industry as an orthogonal purification method. It has the potential to replace the traditional reversed phase method as no toxic chemicals are needed. But as with reversed phase, selectivity can be a challenge to remove specific product impurities.

Now that mRNA therapies and vaccines are making their way to the clinic, the need for a robust purification platform becomes apparent – and affinity chromatography can overcome the challenges the field is currently facing. The method allows for native purification, is scalable and highly selective as it uses the poly-A tail to purify the mRNA molecules. Any impurity lacking a poly-A tail will not bind the column and is easily flushed away, allowing all impurities without a poly-A tail to be removed in a single step. Product related impurities containing a poly-A tail such as double stranded RNA can be removed with a second polishing step. Alternatively, it is possible to engineer out the formation of double stranded RNA during upstream synthesis. This approach allows the use of affinity chromatography as a single step purification solution that can be scaled up as manufacturers move through the clinic.

THE POWER OF AFFINITY CHROMATOGRAPHY

Affinity chromatography offers many benefits beyond a selective approach, and is applicable regardless of which modality is being used. It has earned credit in therapeutic antibody development and more recently also in viral vector manufacturing. Depending on the molecule, as well as the process and product related impurities, multiple purification steps may be needed to reach the desired purity. This means that each purification step added to the process will result in lower overall yield.

The graph in Figure 1 demonstrates the number of process steps against product yield. Even with a high step yield, for example 85%, after four process steps the overall product yield is reduced to 50%. Affinity chromatography can address this challenge. Due to high affinity for the target molecule, a higher purity and yield is achieved in the first step alone. This helps to reduce the number of purification steps needed in the overall process, increasing the overall product yield. A simplified purification process

also reduces bioprocessing development time, allowing manufacturers to get to the market faster, and decreasing the overall cost of goods.

THE THERMO SCIENTIFIC POROS™ OLIGO (dT)25 AFFINITY RESIN

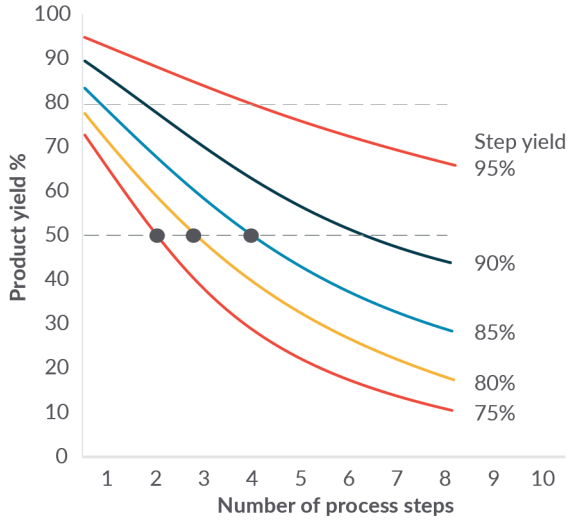
In 2020, Thermo Fisher Scientific launched a new affinity chromatography resin specifically designed for the purification and isolation of mRNA from IVT manufacturing processes in order to address the challenges associated with the purification of mRNA for therapeutic use. Figure 2 shows a schematic of the POROS™ Oligo(dT)25 resin. The resin is comprised of a 50µm porous poly(styrene-co-divinylbenzene) base bead with a polydeoxythymidine (poly-T) 25-mer (dT-25) conjugated to the surface using a proprietary linker.

A poly-T ligand on the surface of the resin allows for simple mRNA capture through AT base pairing. To load the mRNA IVT mixture on the column, salt is added. Once the mRNA is bound to the resin, the column can be flushed to remove process related impurities. To elute the mRNA from the column a low concentration of buffer, or simply water, is used.

The resin has a high binding capacity in comparison to the laboratory-based techniques discussed above, with a dynamic binding capacity of up to 5 mg/mL for 4,000

FIGURE 1

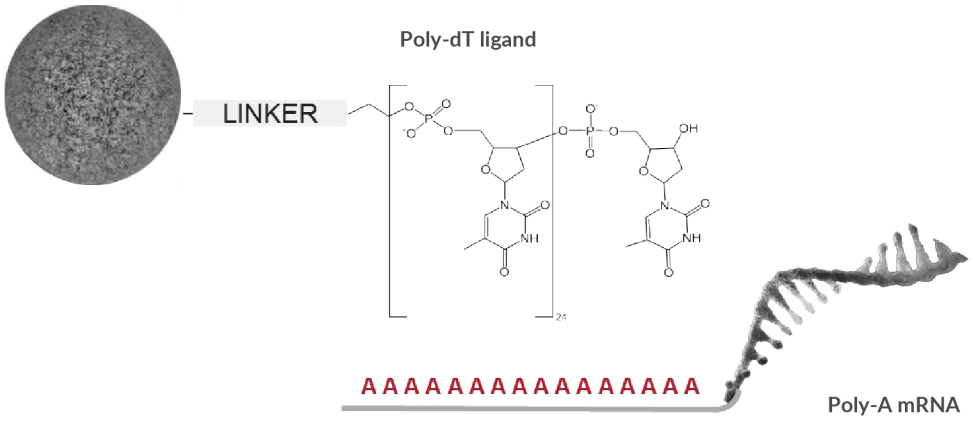
Product yield declines with increasing number of process steps.



Affinity capture can reduce the required number of purification steps, thereby increasing yield.

FIGURE 2

Mechanism of action of the Thermo Scientific POROS™ Oligo (dT)25 affinity resin.



The resin is comprised of a 50µm porous poly(styrene-co-divinylbenzene) base bead with a polydeoxythymidine (poly-T) 25-mer (dT-25) conjugated to the surface using a proprietary linker.

The poly-dT ligand will bind to the poly-A tail of the mRNA through simple AT base pairing.

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nucleotides (nt) RNA. Across a wide range of mRNA construct sizes, the recovery in the first step yield has demonstrated to be greater than 90%, and in most cases, greater than 96–98%.

As the POROS™ Oligo (dT)25 Affinity Resin is a chromatography resin, it is easily scaled, with the ability to pack columns anywhere from a few milliliters or liters, up to hundreds of liters. Like other bioprocess resins offered by Thermo Fisher Scientific, it is a 100% non-animal derived, pharmaceutical-grade reagent, suitable for the manufacturing and purification of clinical therapeutics. The POROS™ Oligo (dT)25 Affinity Resin provides a simple solution to maximize workflow efficiency and reduce the complexity of any subsequent polish steps required.

THE POROS™ BEAD

There are three main attributes that differentiate POROS™ from other chromatography resins (Figure 3).

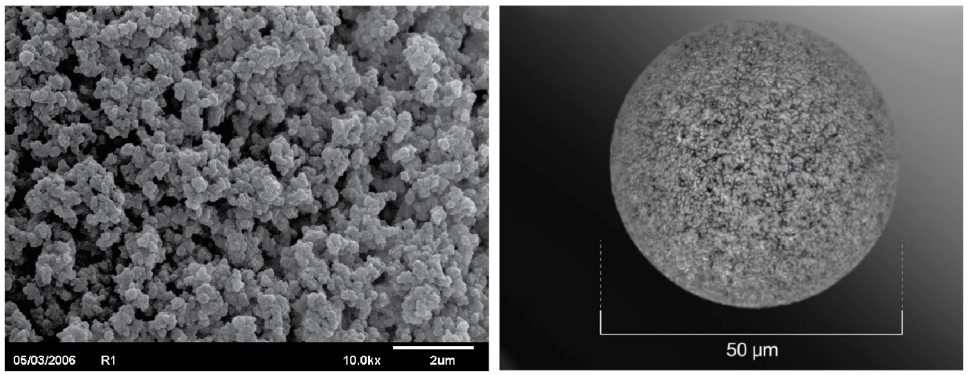
1. **Poly(styrene-co-divinylbenzene) backbone.** The beads are rigid and incompressible compared to agarose type resin. This results in stable column beds as well as linear pressure-flow profile over a wide range of column dimensions, allowing the user to maintain high operational flow rates with a modest pressure drop.

2. **Large pore structure.** The open pore structure of the beads makes POROS™ resins ideal for the purification of larger molecules such as mRNA or viral vectors. The large pores effectively increase the surface area available for interaction between the target molecule and the resin increasing both capacity and resolution. In addition, the larger pores result in reduced mass transfer resistance, which helps to improve process efficiency and productivity.

3. **50-micron bead size.** The average particle size is 50 µm, and this small particle size allows for less band broadening in packed beds, improving the ability to separate proteins and obtain effective impurity removal. Due to the reduced mass transfer resistance mentioned above, this superior resolution is well maintained and independent of linear velocity. In practice, this results in narrower peaks and smaller elution pool volumes which overcomes tank size limitations at large scale.

FIGURE 3

Scanning electron microscope images showing a POROS™ bead (left) and the large through-pores of the bead surface (right).



POSITIONING THE POROS™ OLIGO (dT)25 RESIN IN THE mRNA PURIFICATION WORKFLOW

Ideally, having just one purification step can fully maximize the productivity of the workflow. Purification with the POROS™ Oligo (dT)25 affinity resin will remove process related impurities, such as DNA template, nucleotides, enzymes, and unwanted buffer components. If some product related impurities remain such as double stranded RNA or uncapped mRNA, an additional polishing step can be used.

Affinity purification can also be used in a polish step. Some users may want to retain an initial non-affinity first step, then implement a second affinity polishing step to remove any unwanted components that are left over from the IVT reaction. One advantage of this approach is that it can also be used as a buffer exchange step, as the mRNA can be eluted directly into water.

PROCESS DEVELOPMENT & RESIN PERFORMANCE STUDIES

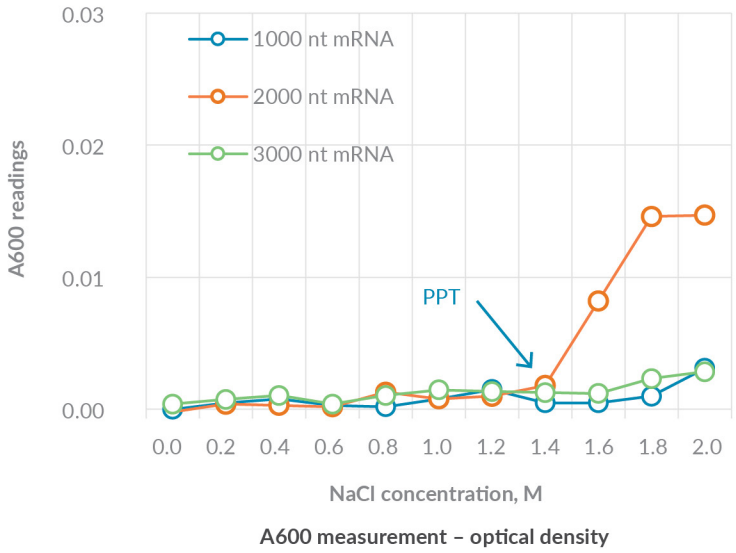
The goal of process development was to first understand how a range of mRNA molecules behaved, in order to more effectively optimize binding capacity without impacting the mRNA. Utilization of a high throughput screening approach allowed rapid optimization over a range of conditions. Once favorable conditions were found, methods were transferred to column format for further optimization.

SALT TYPE & CONCENTRATION EFFECT ON mRNA BINDING

To better understand the stability of the mRNA, and to determine favorable initial loading conditions, various conditions were examined using a 96-well plate design (Figure 4). Three different mRNA construct sizes were studied ranging from 1,000 to 3,000

FIGURE 4

Effect of salt concentration on mRNA stability.



To determine the mRNA precipitation point (PPT) for three sizes of mRNA construct, the optical density (A600) was measured at increasing salt concentrations. Precipitation of 2000 nt mRNAs occurred at lower salt concentrations than 1000 or 3000 nt mRNAs, suggesting that structure, as well as size, plays a role in stability.

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nucleotides using increasing salt concentrations and various salt types. Since the overall structure of these mRNAs is different, different behaviors are expected.

When increasing the sodium chloride concentration up to 1.4 M, precipitation began to occur for the 2,000 nt mRNA. Interestingly, this effect was not seen with the 1,000 or the 3,000 nt mRNAs, which demonstrates that the effect is not related purely to size, but to construct design. When switching from sodium chloride to potassium chloride, the 2,000 nt mRNA was not affected in the same way. Depending on the mRNA sequence being used, it may be necessary to optimize not only the loading salt concentration, but also the salt type used to neutralize the backbone.

Using the information from the 96-well plate precipitation experiment, salt concentration was then studied to determine optimal binding capacity in relation to salt concentration. A decrease of mRNA was seen in the elution pool as salt concentration was increased, demonstrating the promotion of binding – whereas at low salt concentrations, the backbone is not fully neutralized in order to promote annealing. The profile of binding capacity was again different across the three different constructs, indicating that this is another tool that can be used to optimize binding conditions.

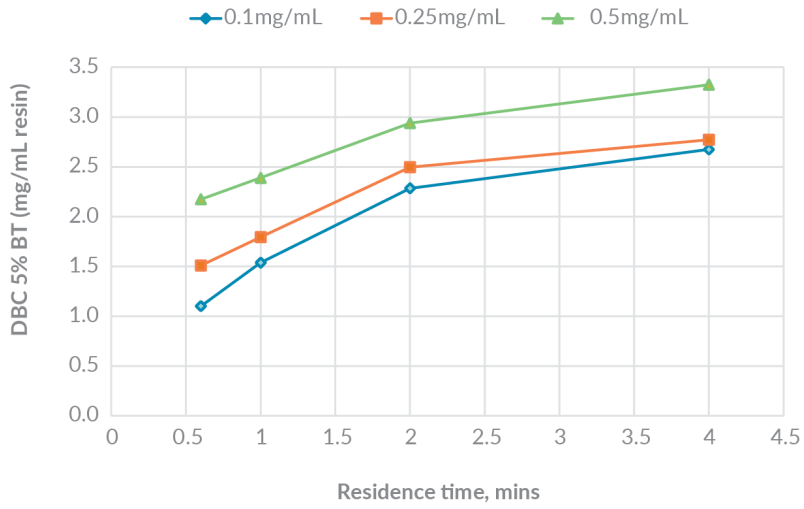
When considering buffer choice, the impact of binding across a range of pH in Tris buffer was studied. Again, optimal binding conditions were not consistent across the range of mRNA sizes used. These differences can be used to further optimize later column experiments, which will in turn assist in optimizing load concentration and flow rate.

DYNAMIC BINDING CAPACITY

The binding capacity of a capture step is an important parameter to determine how much product can be loaded on the column. In a study of binding capacity compared to flow rate, it was observed that increasing residence time resulted in increased binding capacity (Figure 5). This is due to the diffusional effects of the large mRNA molecule, and is common for larger biomolecules. In addition, higher concentrations of mRNA in the load pool better enabled the mRNA to reach the surface of the resin

FIGURE 5

Dynamic binding capacity (DBC) of 3000 nt mRNA at three different feed concentrations.



Residence time for mRNA load (Flow rate)
0.6 min (300cm/hr)
1.0 min (180cm/hr)
2.0 mins (90cm/hr)
4.0 mins (45cm/hr)

DBC increases with higher mRNA concentration and longer residence time.

due to improved binding kinetics at higher concentrations at lower flow rates. However, when considering productivity gains, benefits began to diminish beyond a 2-minute residence time. As a result of this study, a 2-minute residence time was selected for further experiments.

INFLUENCE OF MOLECULE SIZE ON BINDING CAPACITY & RECOVERY

Next, the effect of mRNA size on binding capacity was studied. To study comparative differences this experiment was not optimized for each individual mRNA size – load concentration, flow rate, and column dimensions were all kept constant in order to observe the direct effects of mRNA size. As expected, the size of the mRNA has an impact on the binding capacity and the smaller the mRNA, the higher the binding capacity achieved (Figure 6). As the mRNA constructs gets larger, steric hindrance becomes an issue, and the mRNA lacks the physical room to reach the surface of the resin.

Looking at recovery of the different construct sizes, consistent recovery well above 95% is shown, and is independent of the size of the mRNA.

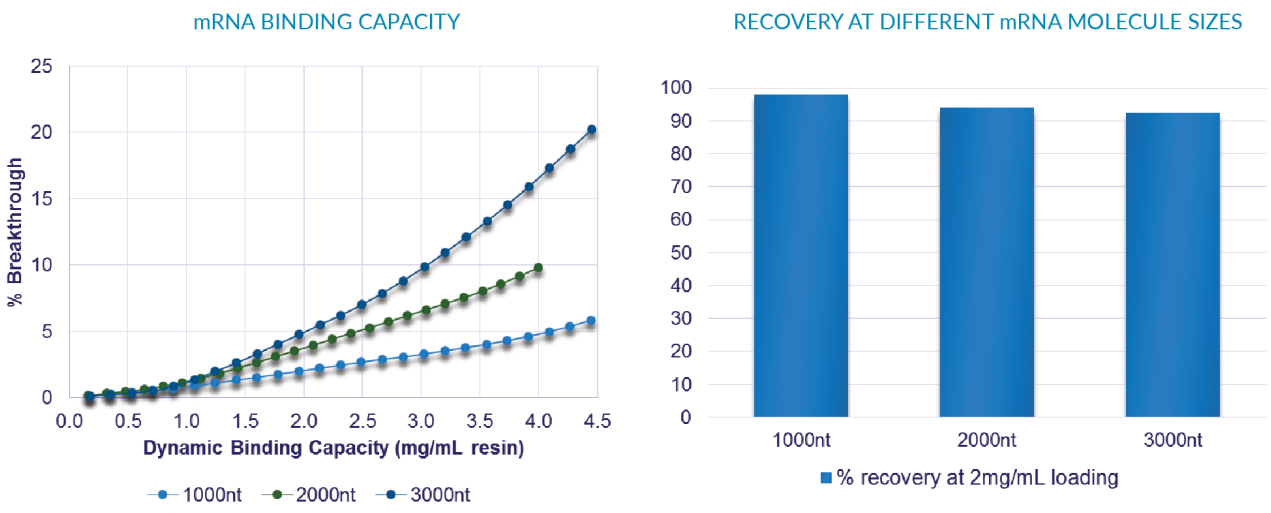
REUSE, CLEANING & STABILITY OF THE OLIGO (dT)25 AFFINITY RESIN

A 2,000 nt mRNA was used to assess the ability to reuse the resin (Figure 7). Multiple purification cycles were performed. The mRNA was bound and eluted over 10 cycles, with a cleaning step at the end of each cycle. Before the first cycle and after the 10th cycle, a blank buffer run was performed to monitor if any mRNA was eluted in the final blank run. The overlays of the blank runs appeared identical, demonstrating no carry over of mRNA from subsequent runs. In addition, this experiment demonstrated that the recovery, measured based on peak area, was consistent over the 10 cycles.

To study the effects of cleaning and sanitization with NaOH, incubation with different concentrations of NaOH was studied.

FIGURE 6

Binding capacity and recovery of three sizes of mRNA construct (1000, 2000, and 3000 nt).



mRNA molecule size impacts binding capacity but not final recovery

Smaller mRNA has a higher binding capacity (left) but size does not impact final recovery (right).

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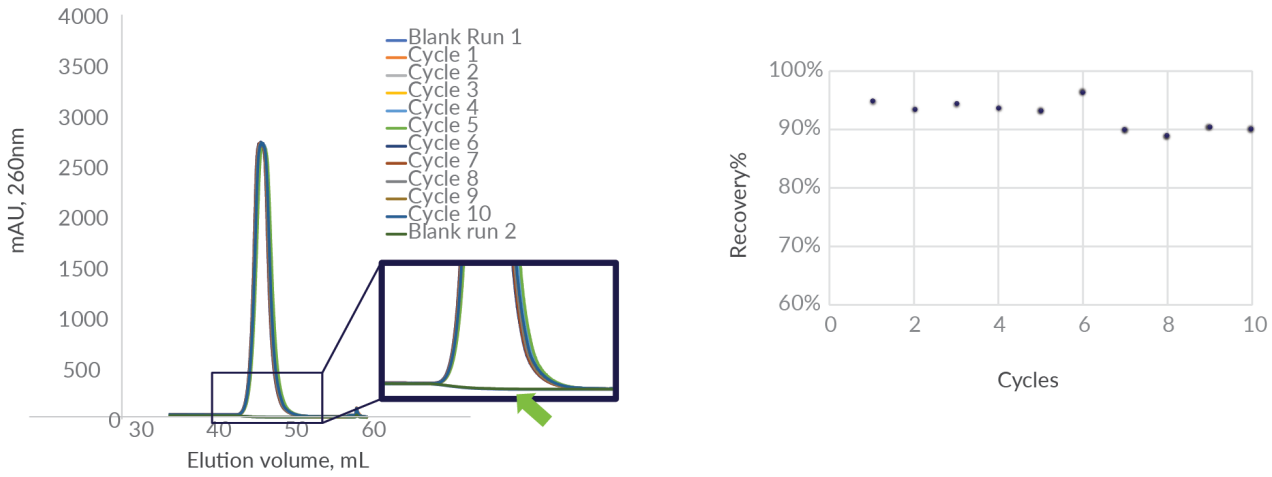
FIGURE 7

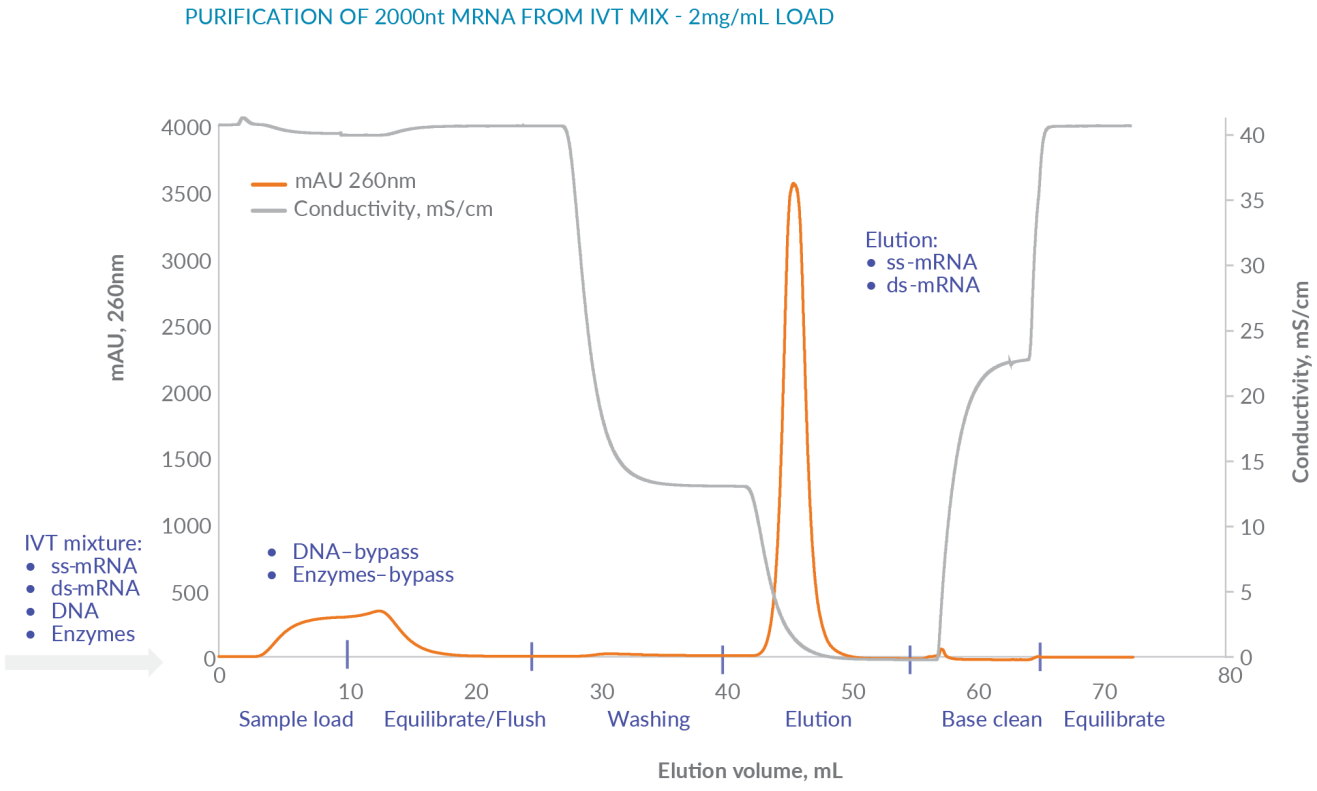
Effect of resin reuse and cleaning on mRNA purification.



Recovery is not impacted by resin reuse and cleaning. Left: Multiple cycles of mRNA (1809 nt + polyA 120 nt) purification from IVT mixture. Chromatograms from blank buffer runs carried out before cycle 1 and after cycle 10 were identical (green arrow), showing that there was no carry over of mRNA. **Right:** Recovery rates for each cycle, showing consistency between cycles.

FIGURE 8

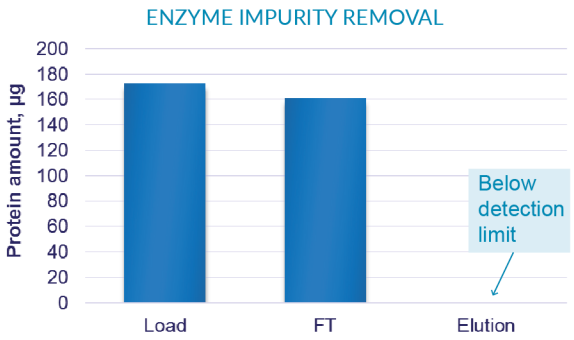
Output of a chromatographic purification run.



Conductivity (representing salt concentration) is shown in gray, while the chromatogram is shown in orange.

FIGURE 9

Enzyme impurity in load, flowthrough fraction, and elution pool.



The amount of enzyme (protein) is high in loading (load) and flowthrough (FT) fraction, but undetectable in the elution pool.

Constant incubation was studied up to a total of 48 hours, which is equivalent, depending on the residence time of the NaOH, to potentially hundreds of cleaning cycles. The experiment demonstrated that the resin can withstand up to 0.5N NaOH, allowing for stringent cleaning and sanitization. In addition, the resin demonstrates good stability over a wide range of pH conditions (1–13).

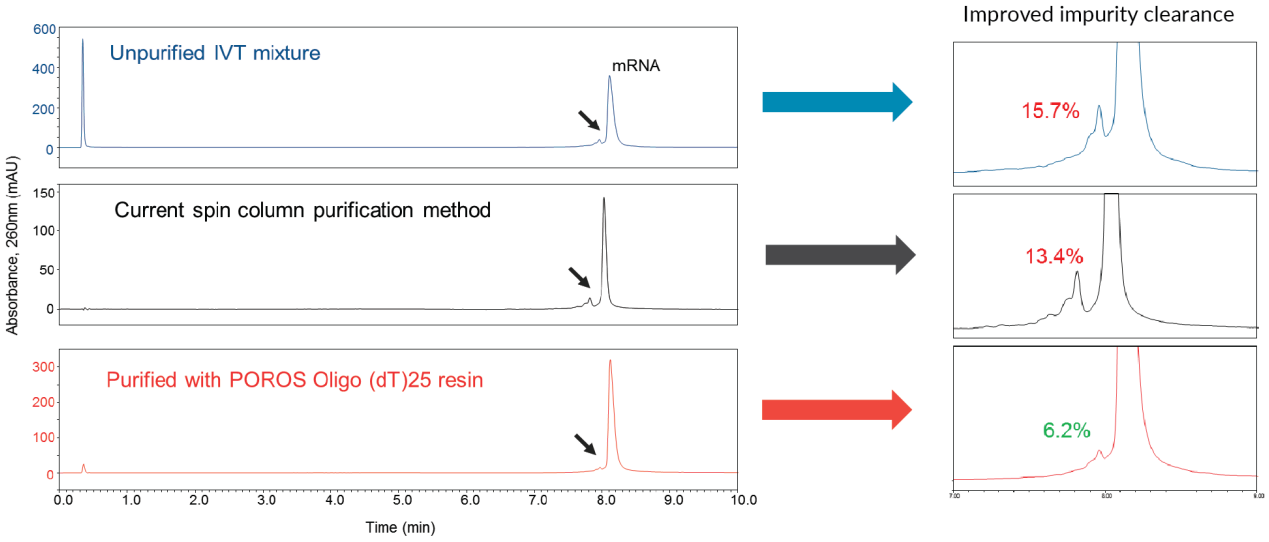
PURIFICATION VERIFICATION

Shown in Figure 8 is the output of a chromatographic purification run. The conductivity trace across the run, salt concentration measurement during the load, a step wash, and then elution and subsequent cleaning is shown in grey. The orange line is the UV 260 nm absorbance measurement and shows the chromatographic profile. At the beginning, an increase in absorbance is seen, which is indicative of DNA and other components flushing through the column. The step elution down to 150 mM NaCl helps to elute smaller truncated poly-A components that bind weakly to the column, as well as components bound to the mRNA itself, and the subsequent transition into water gives a sharp, narrow mRNA elution peak. A small peak is seen in the base cleaning step using NaOH, indicating some residual components were still on the column and are removed by this cleaning step.

The purification run was performed twice – first with already purified mRNA, where excellent recoveries of about 96% were seen. When run again with an unpurified portion

FIGURE 10

HPLC of IVT mixture after no purification (top), spin column purification (middle) and POROS™ Oligo (dT)25 affinity resin purification (bottom).



Purification with POROS Oligo (dT)25 leads to a significant reduction in impurities.

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of the IVT mixture, the same recovery was achieved. This was a key finding, as it demonstrates that the concentration of components present in the IVT mixture does not impact mRNA binding. This is important when considering resin reuse.

IMPURITY REMOVAL

Enzyme impurity removal was also studied using the IVT mixture (Figure 9). A relatively high concentration of protein was initially present in the loading pool, as measured by a BCA assay, and again a large amount of enzyme was present in the flowthrough fraction. When protein was measured in the elution pool, any enzyme present was below the limit of detection.

In addition, a comparison was done between a silica-based spin column method known for efficient removal of IVT components and the POROS™ Oligo (dT)25 resin. The results are shown in Figure 10.

The top trace shows the unpurified IVT mixture, and the peak on the far left represents enzyme, DNA, and smaller components. The impurities eluting the left (before) the main mRNA peak account for almost 16% of the main peak group. As shown in the middle trace, using the current spin column method, smaller enzymes are eliminated, but over 13% of the impurities remain in the main peak.

Applying an affinity resin (bottom trace) significantly decreased the amount of impurity to close to 6%, giving a significant reduction in impurities compared to the spin column method. Further study to identify the remaining components is ongoing, initial data (not shown) suggests the remaining impurities are polyadenylated. Earlier retention also suggests a smaller size than the full-length mRNA.

CONCLUSION/INSIGHT

Affinity chromatography offers a highly efficient and scalable method that has already proven its worth in the development of biologics, and it offers a powerful tool to help address the current bottlenecks in commercial manufacturing of mRNA therapeutics. With high affinity for the target molecule, it can deliver higher yield and purity in the first purification step, helping to reduce the number of purification steps in the overall process, and increasing total product yield. By reducing bioprocess development time, it can result in a decrease in overall cost of goods, and ultimately, a faster time to market for innovative mRNA-based therapeutics.

Q & A

Kelly Flook
Senior Product Manager, Thermo Fisher Scientific

Q Do you need to use heat to elute the RNA?

KF: For purification, we developed this resin so you wouldn't need to use heat. With more traditional, R&D types of mRNA extraction from cells, heat is typically used because the mix in the cell extract is a lot more complex, so it is used to break down a lot of the higher order structures that can bind to those resins and therefore heat aids elution. But in the case of purification, and with this resin, we see a lot of customers using it successfully at room temperature.

Q Does temperature have a negative effect on the stability of mRNA in the chromatography step – and what do you recommend to try and stabilize mRNA?

KF: If there is a stability effect with temperature, it is more related to the construct sequence versus the chromatography. We see people adding EDTA to their buffers in order to help with that stabilization.

Q What sizes of RNA can be purified, and is there a construct size limit?

KF: When we developed this resin, we had relatively small mRNA sizes in mind, typically anywhere from a 1,000 up to about 5,000 nucleotides. We were not really focusing on those larger, self-amplifying RNA up to the 10,000-12,000 range.

What we do see is an impact on binding capacity, as I discussed earlier. With smaller mRNA, you will see a larger binder capacity than you will with something that is significant bigger.

Additionally, the amount of salt you need to neutralize those charges will also be slightly different, because the larger the RNA, the more charges you need to neutralize. You would expect more salt to be needed to achieve that and maximize your binding.

Q How many cycles can you typically get out of the resin?

KF: In this case we looked at cycling just up to 10 cycles. However, we have seen some customers using this resin that are getting 30, 40, 50 cycles, so it is robust. They have a cleaning step in between those cycles as well, this is also a quick sanitization step between cycles.

Q What would you advise for salt concentration to get optimal binding?

KF: We have seen good success starting at about 0.5M sodium chloride in the initial instance. Then either increasing that slightly to increase binding, or simply decreasing that down to the minimum level you need to achieve binding.

Q What is the maximum operating pressure for the resin?

KF: The resin has a robust poly(styrene-*co*-divinylbenzene) core, so the resin itself can withstand pressures over 100 bar. As far as operating and packing for a purification set up, your pressure limitations are really going to be limited by the hardware, and not necessarily the resin.

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Q How can you separate single stranded mRNA from double stranded, and do you have any particular products that fit this goal?

KF: As I mentioned earlier, one of the great things about the dT is that it will bind poly-A well. This also includes double stranded RNA. We recommend our HIC resin range – we have a POROS™ Ethyl, Benzyl and Benzyl Ultra, that can be used to separate the double stranded RNA from single stranded.

BIOGRAPHY

Kelly Flook
Senior Product Manager, Thermo Fisher Scientific

Kelly Flook is Senior Product Manager for Purification products within the Bioproduction Division at Thermo Fisher Scientific. Kelly has a Ph.D in Polymer and Analytical Chemistry from the University of Durham, UK. During her 15 years at Thermo Fisher, Kelly has gained extensive experience in product development across all scales of chromatography and related biological workflows. Kelly has a strong expertise in bead technology and bio-separations. Drawing from a diverse technical background, in her current role Kelly is responsible for new product development and commercialization of solutions across the downstream workflow.

AUTHORSHIP & CONFLICT OF INTEREST

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FASTFACTS

Overcoming challenges of rapid stem cell expansion

Catherine Siler, Field Application Scientist, Corning Life Sciences

Mesenchymal stromal cells (MSCs) are a varied and promising source of cellular therapies, but the large batch sizes involved present unique culture challenges. Corning® High Yield Performance (HYPER) technology is designed to meet the need for more efficient large-scale adherent cell production, allowing users to grow more cells in the same footprint as traditional systems.

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HIGH YIELD IN A COMPACT FOOTPRINT

MSCs are an attractive target for cell therapy, but the number of cells required for therapeutic efficacy (108 for an adult patient) poses a challenge for manufacturers. With hundreds of clinical trials now in progress using MSCs, there is a strong demand for technologies that allow rapid expansion.

Corning HYPER technology allows users to perform adherent cell culture in a compact space. Corning HYPERFlask® and HYPERStack® vessels consist of multiple thin layers ('stackettes') in which the cells grow on gas-permeable membranes. Stackettes are separated by air gaps to allow optimal gas exchange and are protected by solid top and bottom plates.

As seen in Table 1, HYPER technology offers significantly greater surface area within the same footprint as conventional vessels, allowing fewer vessels to be used per batch, and increasing efficiency and consistency.

Table 1. Surface area comparison between vessels.

Vessel	Surface area	Equivalently sized vessel	Increased surface area
Corning® HYPERFlask®	1720 cm^2	T-175	10x
Corning HYPERStack®-36	18,000 cm^2	Corning CellSTACK®-10	3x

QUICK AND EFFICIENT SCALE-UP AND SCALE-OUT

MSCs can become senescent over time so it's important to harness their capabilities before growth slows. Scaling up and scaling out quickly is crucial to achieve the highest cellular yield with minimum passages.

To demonstrate how HYPER technology can be used in a seed train for quick and efficient scale-up, umbilical-derived MSCs were thawed and grown in a T-175 vessel until they reached 90% confluence. After passaging, the cells were expanded into a HYPERFlask vessel at a density of 3000 cells/cm^2. After 5 days, the cells were passaged into a HYPERStack-36 vessel, with 18,000 cm^2 of growth area. The HYPERStack-36 is a closed-system vessel, which allows users to transfer liquids without contamination by external environments and can be used in GMP production – vital for risk mitigation in clinical applications.

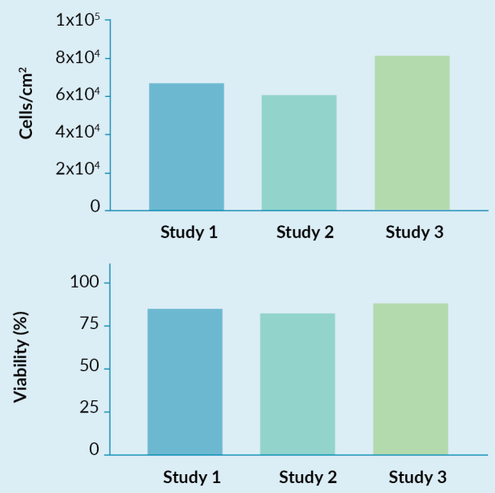
After 5 days of culture, approximately 40,000–50,000 cells per cm^2 were recovered (Figure 1). The average across three studies gave a total MSC yield of more than $8.7x10^8$ cells per HYPERStack-36 vessel, with consistent viability at 90%.

Marker expression after scale-up was consistent with the starting material and met the International Society for Cellular Gene Therapy (ISCT) criteria of 95% expression of CD105, CD73, and CD90 and lack of expression of typical differentiation markers (Figure 2).

SCALE-OUT MADE SIMPLE

For research-scale or development work, T-flasks, Corning CellSTACK® and Corning HYPERFlask vessels provide a convenient means to produce millions of cells consistently. For clinical-scale production, Corning can provide scale-out schematics for HYPERStack vessels, including automation platforms, as well as microcarriers for work with bioreactors.

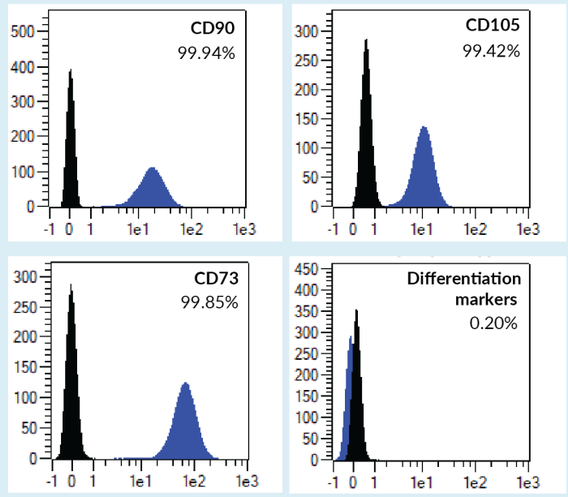
Figure 1. Viability and cellular yields of umbilical-derived MSCs in three studies.



TAKING ON THE CHALLENGE OF MSC CULTURE

HYPER technology from Corning offers high-yield cell culture in a compact footprint and consistency across vessel platforms from R&D to manufacturing scale. In particular, Corning HYPERStack vessels use closed-system components to mitigate risk in cell therapy bioprocessing.

Figure 2. Umbilical derived MSCs retain marker expression across passages. Representative MSC marker expression from one study. Sample in blue compared to isotype control in black. Differentiation markers are a cocktail of CD45, CD34, CD11b, CD19, and HLA-DR.



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EDITORIAL

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"As fifth generation CAR-T cells reach their limits for modifications facilitated by genetic engineering, new enhancement strategies independent of genetic modification are in development."

CAROLYN PORTER, CEO, Cytoseek

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INTRODUCTION

In March 2021, the US FDA announced approval of Abcema™ (idecabtagene vicleucel); a new CAR-T cell treatment for adults with multiple myeloma and the first approved for this indication [1]. With this latest approval, there are now five FDA approved CAR-T cell therapies targeting hematological malignancies and most recently multiple myeloma, which have been approved within the last 4

years. The CAR-T development pipeline is robust, with existing therapies seeking indication expansions, and fifth generation CAR-T cells are in development [2] albeit clinical efficacy has been greatest with 2nd generation CAR-T [3]. However, the majority of new cell therapies in the pipeline are continuing to target hematological malignancies, while CAR-Ts targeting solid tumors only represents a fraction of the overall clinical pipeline [4]. CAR T cells face many obstacles in the solid tumor microenvironment (TME) including homing to and penetrating into solid extracellular matrices (ECMs), overcoming tumor antigen escape, and surviving within the hostile tumor environment of low pH, hypoxia, and numerous other immunosuppressive factors [5]. This has led investigators to develop novel CAR engineering strategies to overcome these obstacles, which are being explored in clinical trials for the treatment of malignancies, such as breast cancer, sarcoma, and neuroblastoma [6]. The majority of these strategies have focused on optimizing the molecular design of the CAR through variation or enhancement of its constituent protein domains [7]. However, current genetic engineering strategies enable only a limited number of genetic modifications to enhance CAR-T cell function. While individual genetic modifications demonstrate efficacy to combat the obstacles facing CAR-T in the TME, the challenge for the field is to develop new strategies to combine a greater number of mechanisms for enabling individual CAR-T cell products to overcome the potent solid tumor inhibitory environment. As we seek to layer additional functionality to CAR-T cells to address multiple mechanisms, new innovations will be required to overcome the gene cargo size limitations associated with viral delivery of CARs and their enhancements to T cells. Approaches to overcome the limitations with adding additional enhancements include the use of non-viral vector systems for gene delivery such as specialized nanoparticles coupled with electroporation [8] and the use of non-genetic strategies that modify the surface of cells to enhance functionality.

NON–GENETIC STRATEGIES TO DRIVE A CAR-T: BACKPACKS, SCUBA TANKS & OTHER MODIFICATIONS

Several strategies involving surface modification of T cells to enhance functionality are emerging that will complement existing genetic engineering approaches. One strategy is to load CAR T cells with nanogel 'backpacks' capable of delivering protein to the tumor microenvironment upon CAR recognition of target antigen. Proteins of interest are packed into nanogels which are cell surface conjugated and release their 'packs' after an increase in T cell surface reduction potential triggered by antigen recognition. Investigators pursuing this approach loaded CAR-T cells with IL15 super-agonist complex backpacks and illustrated a triggered expansion of T cells by 16-fold in tumors, where higher doses of this cytokine could be administered using backpacks without toxicity, when compared with systemic delivery [9]. This cytokine has been delivered systemically as combination therapy with CAR-T and genetically expressed on CAR-T to enhance proliferative capacity with systemic approaches demonstrating toxicity in patients [10]. The backpack approach could therefore be used to provide additional functionality of proliferative cytokines to these cells, minimizing toxicity while leaving room to utilize genetic modification to overcome other CAR-T obstacles. Another approach uses supercharged proteins that self-assemble on the surface of T cells to add multiple different types of functionality to these cells. These supercharged proteins are bifunctional with an oxygen carrying anchor region conjugated to surfactants which facilitate stable membrane insertion and fused to a functional domain of choice. Developed originally to facilitate hypoxia resistance of stem cells [11] these supercharged artificial membrane binding proteins (AMBP) provide 'scuba tanks' with oxygen reservoirs to the cell surface potentially enabling T cells to avoid hypoxia mediated exhaustion in the TME. In addition to oxygen carrying function the

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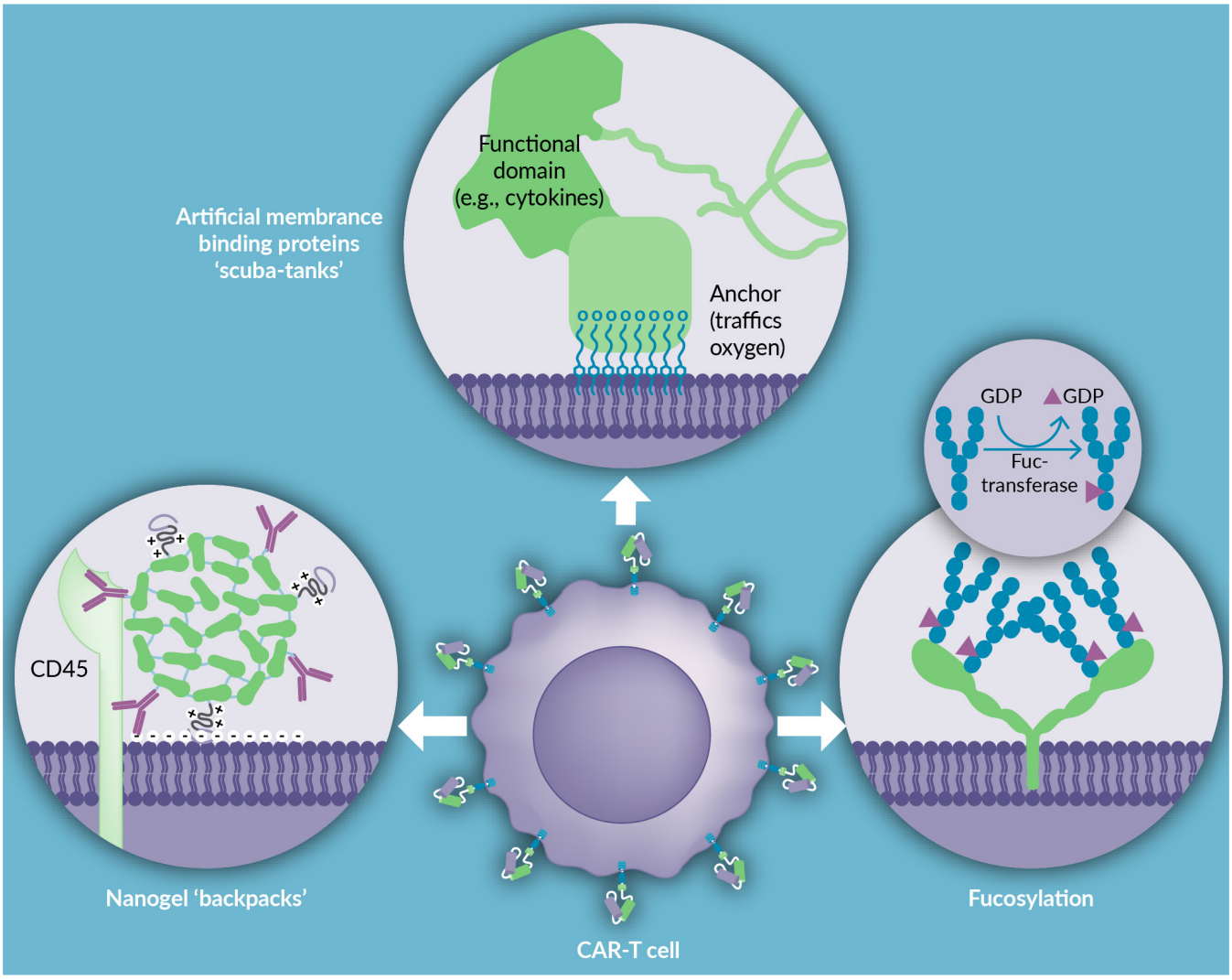
AMBPs can also be engineered to add multiple functionalities to cells including homing [12], proliferative cytokines and immunomodulators interrupting checkpoint mediated immunosuppression.

Other surface modifications such as *ex vivo* enzymatic glycoengineering using fucosylation and the use of antigen targeting homing nanoparticles are being tested preclinically to improve homing of CAR-T cells to solid tumors. In the glycoengineering approach a sugar (fucose) is added to certain cell surface molecules which interact with selectins found on the lining of blood vessels and epithelium to direct fucosylated cells to the site of the tumor and improve their retention. This approach has shown enhanced tumor activities of fucosylated cytotoxic T lymphocytes (CTLs) against leukemia, breast cancer and melanoma in murine models [13]. Target homing nanoparticles have also been used to modify T-cells, referred to as prosthetic antigen receptors (PARS), that take advantage of both hydrophobic insertion [14], as well as bispecific multivalent antigen binding [15]. The PAR T-cell approach has demonstrated the ability to carry out specific tumor cell killing, as wells as solid tumor eradication in a murine model of breast cancer [15]. These approaches comprise a subset of nanomaterials being investigated to overcome clinical barriers to T cell-based immunotherapies [16]

FIGURE 1

Non-genetic based strategies to enhance T cell function.



that do not require genetic modification of the target cell. These offer the potential to deliver a variety of enhanced functionality to these cells that have either been too toxic or too non-specific to mediate their effects in systemic delivery strategies (Figure 1).

While these approaches can address some of the current challenges in the field, modification of the surface of cell therapies such as CAR-T without genetic engineering is self-limiting and over time the effect of modification decreases via endocytosis of the surface enhanced functionality and dilution via division of the modified cells. This does not preclude some durability in effect, as evidenced by the previously mentioned IL15 backpacks that continued to stimulate T cells for a least 9 days [9] and our own investigations illustrating enhanced T cell proliferation and activation over 7 days with T cells coated with AMBPs. Self-limiting dosing can also be viewed as an attractive built-in safeguard against excessive stimulation of T cells or on-target-off-tumor T cell activation, leading to undesirable toxicities [17]. However, this will limit the types of functional enhancements enabled by these approaches to those where relatively short-term effects are desirable.

FUTURE CONSIDERATIONS

As fifth generation CAR-T cells reach their limits for modifications facilitated by genetic engineering, new enhancement strategies independent of genetic modification are in development. These will provide complementary avenues to enhance the function, persistence, longevity, and phenotype of these cells. Many of these approaches have focused on improving efficacy and safety of CAR-T cell therapies for the treatment of solid tumors, and are also applicable not only to other T cell-based therapies such as TIL, CTL, and TCR-T therapies but could be used to modulate other therapies based on immune cells, such as NK cells, macrophages or B-cells. They represent further mechanisms that cell therapy developers can deploy to overcome the complex mechanisms of immune evasion employed in the tumor microenvironment.

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