APRIL 2020 Volume 6, Issue 2

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:

Raw and starting materials: troubleshooting supply, management and optimization issues

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Raw and starting materials: troubleshooting supply, management and optimization issues

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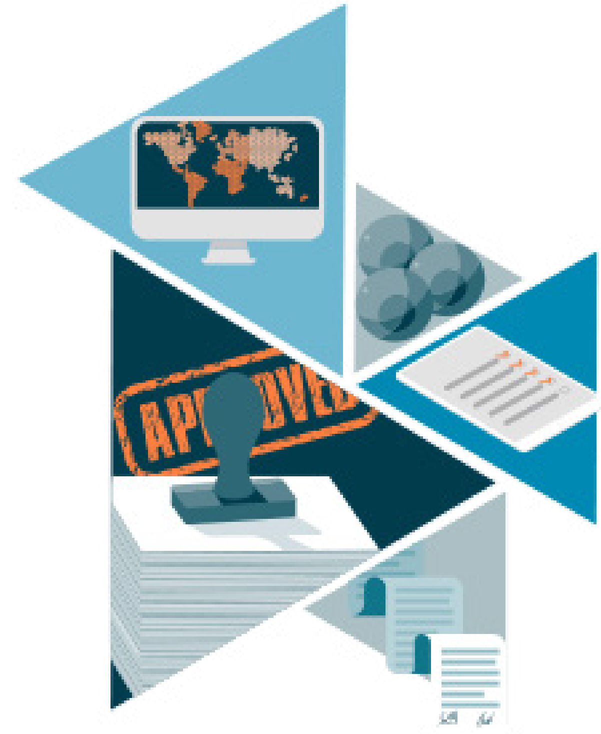

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

Broadly speaking, starting materials are key building blocks that form the foundation of the therapeutic product, whereas raw materials are typically reagents and other ancillary materials used within the manufacturing process.

FOREWORD

Steven Goodman

Attention to raw and starting materials used in the production of Advanced Therapeutic Medicinal Products (ATMPs) grows substantially with each successive year. This makes intuitive sense as the field continues to mature and more products approach or enter the commercial market. Companies have focused their development activities on clinical proof of concept and manufacturing process robustness with an eye toward establishing this new treatment modality. Now that more products have de-risked the technology and generated

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initial market penetration, manufacturers are establishing greater control across all aspects of their supply chains.

Succinctly defining raw materials and starting materials can be challenging, as it heavily depends on what type of product is being produced. For instance, plasmid DNA is usually classified as a raw material if the viral vector being produced is used as the starting material in a gene-modified cellular therapy, but is a starting material if the vector is the finished product, as is the case for in vivo gene therapies. Broadly speaking and solely for the purpose of establishing a baseline definition, starting materials are key building blocks which form the foundation of the therapeutic product (e.g., viral vector and patient/donor cells), whereas raw materials are typically reagents (e.g., media, serum, growth factors, stimulation beads) and other ancillary materials used within the manufacturing process. Out of scope would be finished products as well as general consumables such as tubing sets, culture bags, pipettes, and so forth.

The risk imposed from raw and starting materials on the quality and supply of the finished product is heavily influenced by numerous factors, for instance how the material is produced (e.g., if it is human-derived), the grade (e.g., Research, High-Quality, GMP), and the availability of suppliers (e.g., sole-sourced, single-sourced, or multiple sources). This level of risk is impacted by the level of characterization available directly from suppliers, and requirements for additional characterization and qualification increase as the therapeutic program progresses through product development. Regulators factor these considerations in when determining the expected level of control for any given material.

This Spotlight edition of Cell & Gene Therapy Insights explores a broad range of aspects of raw and starting materials for ATMPs. We hope sharing experiences from numerous innovators will benefit the entire industry as the field continues to mature.

AFFILIATION

Steve Goodman
Head of drug product manufacturing, bluebird bio

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

RESEARCH ARTICLE

A simple RP-HPLC method for the stability-indicating determination of *N*-acetyl-L-cysteine and *N*,*N*'-diacetyl-L-cystine in cell culture media

AS Prakasha Gowda, Andrew D Schaefer & Terry K Schuck

N'-Acetyl-L-Cysteine (NAC) can exist in the reduced form, containing the sulfhydryl (-SH) group, and it can exist in its oxidized disulfide form *N*,*N*'-Diacetyl-L-Cystine (Di-NAC). However, an analytical method that can separate and quantify both compounds in cell treatment supplement media is not yet available, to the best of our knowledge. A stability-indicating RP-HPLC assay method for the determination of NAC and Di-NAC in the cell culture media has been developed. The proposed method showed good linearity for NAC (R = 1.00) and Di-NAC (R = 1.00), accuracy, precision, specificity and system suitability results within the acceptance criteria. The limit of detection and limit of quantitation were found to be 0.0001 mg/ml and 0.00018 mg/ml for NAC, and 0.00015 mg/ml and 0.00045 mg/ml for DI-NAC. However, our method can be used for the separation and quantification of NAC in cell treatment media, *in vitro* dissolution studies and pharmaceutical formulations.

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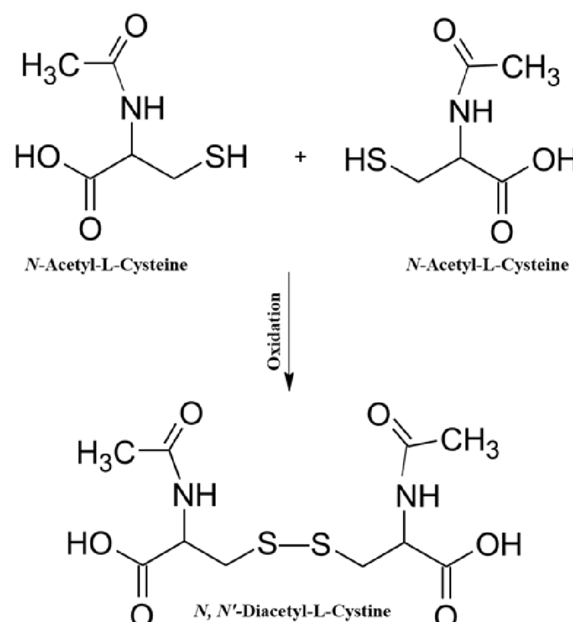
N-Acetyl-L-Cysteine (NAC), commonly known as acetyl cysteine, is the amino acid derived from L-Cysteine (Cys) with an acetyl (-CO-CH_3) group attached to the amino (NH_2) group. It is widely used in clinical application as an antidote to acetaminophen overdose induced toxicity [1], as a mucolytic agent in the treatment of respiratory disorders [2], and to treat various oxidant-derived diseases such as chronic pulmonary diseases, cardiovascular diseases, neurodegenerative diseases, and cancer [3]. Of interest in this research, is the utility of NAC as a supplement used in cell culture medium and a practical method for estimation of NAC and its oxidation degradation pathways in that medium. NAC has been elucidated to interact with numerous metabolic pathways including, regulation of the cell cycle and apoptosis, carcinogenesis and tumor progression, mutagenesis, gene expression and signal transduction, immune modulation, cytoskeleton organization and trafficking and mitochondrial functions [4,5]. However, detecting NAC in a biological setting has been a challenge to overcome for researchers. NAC is quite stable thiol molecule. It is oxidized and degraded when in solution and exposed to air (USA Patent number, 5, 691.380, US 8,148,356 B2, US 8,399,445 B2). This oxidation easily and rapidly occurs via the disulfide (Figure 1) formation to Di-NAC [6].

The acetyl group makes Cys more water-soluble, and functions to speed absorption and distribution on orally ingested Cys [7]. The acetyl group reduces the reactivity of the thiol (–SH), making NAC less toxic and less susceptible to oxidation than Cys [7]. It is a small, water-soluble [8], membrane-permeable [9] and can cross the blood–brain barrier (BBB) [10] NAC is a membrane-permeable cysteine precursor that does not require active transport to deliver cysteine to the cell [9]. Once NAC is inside the cells it is rapidly hydrolyzed, then cytosolic acylase I deacetylates NAC (Figure 2) to Cys [11], a substrate for gamma-glutamylcysteine ligase (γ-GCL; Figure 2), which is the rate-limiting enzyme of the glutathione (GSH) biosynthetic pathway [12]. NAC is a by-product of GSH, is important in restoration of GSH stimulating hormone level [13], and therefore is popular due to its role in GSH maintenance and metabolism [14].

FIGURE 1

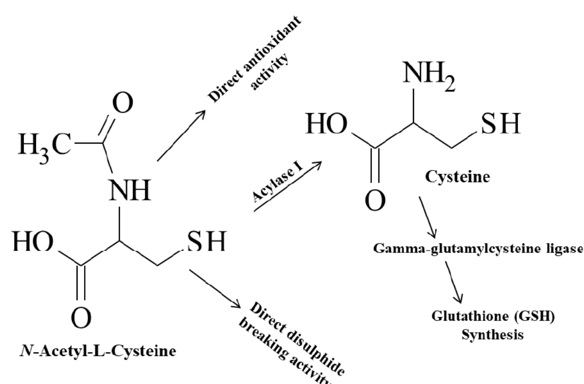
Oxidation of NAC.



N, N′-Diacetyl-L-Cystine is a sulfur-containing dipeptide obtained by the oxidation of two NAC molecules which are then linked via a disulfide bond.

FIGURE 2

Antioxidant activity of NAC.



Increasing reactive oxygen species (ROS) depletes the antioxidant enzymes in the cells. NAC act as a direct scavenger for antioxidants. NAC is converted to L-Cysteine through a deacetylation reaction catalyzed by acylase I, indirectly act as a GSH precursor and involved in GSH synthesis. NAC has a direct effect on disulphide bond breakage and helps release free thiol molecules and increase the GSH synthesis.

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Many studies show that NAC, a well-known antioxidant, has been used as an antioxidant in a wide variety of experiments and shows antioxidant activity in both cell-free and in cell systems [15]. The broad application of NAC is not only because of its well-described antioxidant and radical scavenging activity but also because, as a thiol molecule, it is quite stable, commercially available, and inexpensive. NAC exhibits antioxidant properties through the interaction of its free thiol group with the electrophilic groups of ROS [16] and nitrogen species as a scavenger of oxygen free radicals [17]. Antioxidant activity of NAC primarily acts to scavenge hydroxyl radical (HO·) and hypochlorous acid (HOCl), but also reacts with hydrogen peroxide (H_2O_2) [18] and superoxide ($O_2^{·-}$) [19]. It does not react with O_2 and nitric oxide (NO) [20]. Furthermore, it can serve as a metal chelating agent for several toxic metals such as cobalt, boron, cadmium, lead, and arsenic [21].

NAC has been used as a component of supplement and expansion medium for the treatment of cells, but at higher concentration NAC decreases cell viability [22]. Stability testing studies of NAC in cell culture media, active pharmaceutical ingredient (API), and pharmaceutical formulation provide evidence of the intrinsic stability of the molecule in response to environmental conditions, e.g., air, temperature, humidity, and light. Consequently, there is extensive established stability and shelf life for NAC [23].

In the past for the quantitative determination of NAC several analytical methods such as fluorimetry [24], HPLC [25], potentio-metry [26], spectrophotometry [27,28], colorimetry [29,30], chemiluminiscence [31], electrochemical detection [32,33], turbidimetry and nephlometry [34], liquid chromatography tandem mass spectrometry [35], gas chromatography mass spectrometry [36] and capillary electrophoresis [37,38] have been employed. It has been simultaneously quantified along with other substances like clomiphene citrate [39], arginine [40], and cefexime trihydrate [41]. Its related substances have been described by the European pharmacopoeia and British pharmacopoeia as L-cystine, L-cysteine, *N, N'*-diacetylcystine and *N, S* diacetylcysteine [42]. Among chromatographic methods in the literature, separation methods like RP-HPLC and ion pair chromatography for related substances have been used in tests of NAC in bulk products [41,42]. Other less widely available techniques like LC-UV-MS [43] and capillary electrophoresis-mass spectrometry [44] have been used for quantifying the related substances of NAC. However, to our knowledge, there is no analytical method in the literature that determines NAC stability and concentration during its use in cells treatment. A method of analysis that enables accurate quantification and stability determination of NAC under those conditions typical of cell growth, expansion and expression was therefore needed.

Various cell culture media commonly contain other low molecular weight thiols such as Cys, Cystine and glutathione. Therefore, any analytical method typically faces challenges in distinguishing between NAC and these other species, which have similar physical and chemical properties [45]. One way this has been overcome is through RP-HPLC methods, which retain reduced NAC as a stable, detectable molecule [46]. The literature survey reveals that a few stability indicating RP-HPLC methods for NAC are available [47,48] but all these methods are specific to formulation compositions which are far different from those used in common commercial formulated media and of those developed in our laboratory and used in cell treatment.

The purpose of this study was to examine the stability of the NAC content in a cell culture media. Hence, it was necessary for the present study to investigate stability-indicating RP-HPLC method for the determination of NAC in DMEM cell culture media. The present analytical work describes an accurate, specific, and repeatable. This method was validated according to International Council for Harmonization (ICH) guidelines.

EXPERIMENTAL PROCEDURES

Reagents & chemicals

N-Acetyl-L-Cysteine was purchased from Alfa Aesar (Tewksbury, MA, USA), *N, N*-Diacetyl-L-Cystine was purchased from Santa Cruz Biotechnology (Dallas, Texas, USA), Acetonitrile was received from Fisher Scientific (Middletown, VA, USA), Trifluoroacetic Acid, HPLC Grade was purchased from JT Baker (Fail Lawn, USA), Orthophosphoric acid, HPLC grade was purchased from EMD Millipore (Burlington, MA, USA) and Dulbecco's modified Eagle's medium F12 (1:1) nutrient mixture F-12 (Ham) was purchased from Thermofisher (Greenville, NC, USA). Milli-Q® water for solutions made in house with a Milli-Q® system (Millipore, Milford, MA, USA). All other chemicals were obtained in an analytical grade or from standard commercial suppliers. Mobile phase was used as the diluent.

Placebo preparation

Placebos are an important methodological tool, used in research studies testing drugs *in vitro* and *in vivo*. Dulbecco's modified Eagle's medium (DMEM) is now extensively used in culturing a wide variety of mammalian cell types, cell lines and treatment of cells [49]. It provides a buffering system and maintains the physiological pH range and osmotic balance of the culture medium. It is also a source of water, essential and non-essential amino acids, vitamins, organic and inorganic ions, and energy for cells. It is common to purchase and use commercial media. In this present study DMEM medium was considered as a placebo for NAC stability assay.

Instrumentation & chromatographic conditions

The reverse phase high-performance liquid chromatographic (RP-HPLC) method development and complete partial validation studies was performed with a Waters alliance 2695 Separations Module, comprised of a quaternary pump solvent delivery module, online degasser, thermostated, column compartment, Waters external column heater, auto sampler, auto injector (Model Code SM4) with 100 µl injection loop, and a diode-array detector (DAD 2487). Samples were maintained at 5 °C in the autosampler prior to analysis. System suitability parameters were tested to show that the system was working accurately during the analysis. The system was used in a room temperature HPLC laboratory (20 ± 2 °C). The analysis was performed on a C18 column (YMC-Pack Pro C18, 250 X 4.6, S-5 µm, 12 nm) under reversed-phase partition chromatographic conditions. RP-HPLC method development process utilized an isocratic elution method with a mobile phase composed of Acetonitrile (ACN) and water (4:96 v/v) containing 0.1% TFA at a flow rate of 1.0 ml/min. Injection volume was kept constant 20 µl and column temperature was maintained at 25 °C. The detection of NAC and Di-NAC was monitored at an UV wavelength of 212 nm. Chromatogram output, integration of peaks, calculation of peak areas, retention times and system suitability parameters such as peak asymmetry and column efficiency etc. were obtained using the Empower software, version 3.

PREPARATIONS OF STANDARD & PLACEBO SAMPLE SOLUTIONS FOR HPLC ANALYSIS

Preparation of NAC & Di-NAC standard solutions

A stock solution of NAC and Di-NAC standard for method development was prepared by accurately weighed out 50 mg of NAC and Di-NAC transferred into separate 25 ml of volumetric flasks. Each was dissolved in mobile phase, and diluted to a final volume of 25 ml with mobile phase. From these stock solutions, working standard and calibration stock solutions were prepared. The working standard solutions of 0.005 mg/ml were prepared by transferring 0.125 ml of stock NAC and Di-NAC solutions

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into separate 50 ml volumetric flasks and diluting to volume with mobile phase.

Preparation of NAC & Di-NAC linearity standard solutions

A calibration standard stock solution of NAC and Di-NAC was prepared. A volume of 100 µl of NAC and Di-NAC stock solutions was transferred into separate 20 ml volumetric flasks and diluted to the mark with a mobile phase. According to ICH [50] guidelines, for the linearity assay a minimum of 5 concentrations is recommended. Six linearity standard solutions were then prepared by diluting from calibration standard stock solutions with mobile phase to yield varying concentrations over a range of 0.0003, 0.0006, 0.002, 0.005, 0.0075 and 0.01 mg/ml. These standard solutions were used to perform the analysis of calibration curve. The linearity was established by calculating the coefficient of determination (R^2) value for NAC and Di-NAC, separately.

Limit of detection & limit of quantification

Limit of detection (LOD) is defined as the smallest amount of analyte in the test sample that can be reliably distinguished from zero. The LOD and limit of quantification (LOQ) were calculated mathematically by the relationship between the standard error (σ) of the calibration curve and its slope (S) using the multiplier according to ICH [50] guidelines.

EQUATIONS 1 & 2

σ = the standard deviation of the response.
S = the slope of the calibration curve.

1 $$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

2 $$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

This approach is mainly used in chromatographic methods. Modern chromatography programs determine this value automatically. The Calibration curve was constructed by plotting peak area against the corresponding concentrations. The LOD and LOQ were calculated by Equations 1 and 2.

Preparation of placebo sample solution for stability

To determine the placebo component's effect on the NAC stability, placebo sample stock solution was prepared by accurately weighed out 50 mg of NAC into a 25 ml of volumetric flask. Material was then dissolved in DMEM, and diluted to a final volume of 25 ml with DMEM. For stability analysis placebo sample solution at concentration 0.005 mg/ml was prepared by pipetting 0.125 ml of above placebo sample stock solution into a 50 ml volumetric flask and diluted to the mark with a mobile phase. The stability was assessed with placebo sample and NAC standard solutions were incubated at room temperature (RT) (20 ± 2 °C) and 37 °C for 24 and 48 h, whereby the effect of NAC oxidation was determined. The solutions were injected separately and the content of NAC was determined by comparing the peak area of the freshly prepared placebo sample with that of fresh NAC standard, for 24 h interval up to 48 h.

Stability of NAC in DMEM cell culture media

While much work has been done to understand the impact of NAC product formulation on stability, there is limited understanding of the link between cell culture process conditions and soluble Di-NAC formation in NAC product. Further, to understand stability of NAC in cell treatment DMEM [49], pH 7.5, accurately weighed out 50 mg of NAC into a 25 ml of volumetric flask. Material was then dissolved in DMEM, and diluted to a final volume of 25 ml with DMEM. The solution

was split into three portions immediately after preparation. One portion was stored at RT (20 ± 2 °C), second portion was stored under refrigeration (2–8 °C) and the third portion was directly incubated at 37 °C for 24 h. DMEM matrix, temperature and pH 7.5 effect stability of NAC were conducted, whereby the effect of pH and temperature on NAC oxidation was determined. After incubation all three solutions were diluted to concentration 0.005 mg/ml with mobile phase. The solutions were injected separately and the content of NAC and formation of Di-NAC was determined by comparing the peak area of the freshly prepared NAC in DMEM and immediately diluted with mobile phase, NAC and Di-NAC standards in mobile phase.

Specificity

Specificity is the ability of a method to measure the analyte response in the presence of all potential impurities and placebo components. To study whether any interfering peaks co-elute at or near the NAC and Di-NAC peaks, DMEM was diluted with mobile phase. The specificity of the analytical method was assessed by injecting a diluted DMEM (placebo), Milli-Q® water and NAC and Di-NAC free mobile phase into the HPLC system.

Accuracy

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the placebo sample. According to ICH [50] guidelines, placebo sample with 50%, 100% and 150% of the standard NAC were analyzed. Tests to determine the accuracy were performed using solutions of low, medium and high concentrations of 0.0025 mg/ml, 0.005 mg/ml and 0.0075 mg/ml of placebo sample were prepared, each one covering the entire linearity range. The method accuracy was determined by calculating percentage (%) of recovery and relative standard deviations (RSD) was calculated for each concentration.

Precision

The precision was studied by preparing six replicates at standard level of the specification. According to ICH [50] guidelines, intraday (precision) and interday (intermediate precision) studies were carried out for assessment of the assay precision. The precision was represented by RSD. The intraday of the NAC method was checked by injecting six individual preparations of standard (0.005 mg/ml) and placebo sample (0.005 mg/ml) within the calibration range. The interday was determined by preparing standard and placebo sample at a concentration of 0.005 mg/ml on different days and on different instrument (Agilent 1100 series system, Santa Clara, CA, USA, comprised of a quaternary pump solvent delivery module). The %RSDs of intraday and interday studies was calculated for assessment of precision of the method.

RESULTS & DISCUSSION

Method validation

The HPLC method was validated as to specificity, linearity, sensitivity, accuracy, precision (repeatability and reproducibility), LOD, LOQ, and stability as per the ICH [50] guidelines.

Robustness

The analytical method robustness was tested by evaluating the influence of minor modifications in HPLC conditions on system suitability parameters of the proposed method. The solution at the specification level was used to evaluate the robustness of the proposed method ascertained by minor changes of method conditions, such as the detection wavelength, column oven (±5 °C) temperature and flow rate (±0.1 ml/min) of the mobile phase. Equal concentration of standard and placebo sample solutions was injected separately, and the chromatograms were recorded. The content of NAC was calculated by comparing the

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peak area of placebo sample with that of the standard. In all modifications, good separation was achieved between NAC and placebo components, and the %RSD values of peak area obtained from repeated injections of the standard solution and assay results for analytes obtained from placebo sample solutions were all less than 2.0%. The %RSD was calculated and in all the conditions there was no significant difference from the optimum conditions. The results are as displayed in Table 1.

Development & optimization of HPLC method chromatographic conditions

The stability indicating RP-HPLC analytical method for separation and quantification of NAC in placebo (DMEM, Cell treatment media) was developed and validated. Certain information about physicochemical properties and chromatographic behaviors of NAC and Di-NAC was obtained from literature studies. An appropriate combination of the column type, column temperature, mobile phase composition and flow rate, injection volume, and detection system was studied to produce a simple, fast, economic, and yet selective and accurate assay method. 20 µl injection volumes were validated as the maximum injection volume for future applications in analysis of biological samples. In determining the detection wavelength for the analytical method, different wavelengths were tested. Studied wavelength at 214 nm produced a lower NAC signal which made this approach not feasible. Hence, the detection wavelength at 212 nm was evaluated for NAC and Di-NAC and was found to produce highly sensitive peaks with enhanced resolution between NAC, Di-NAC and placebo components. The chromatography obtained at wavelength 212 nm demonstrated peaks that were reproducible, had

TABLE 1

Studied robustness of placebo sample.

Robustness parameter		NAC standard				
		Average % recovery of NAC	%RSD	USP s/n	USP tailing	USP plate count
Wavelength change (nm)	212	100	0.4	1112	1.05	21763
	214	100	0.2	612	1.05	21625
Column temperature change (°C)	20	100	0.4	770	1.05	21784
	25	100	0.4	1112	1.05	21763
	30	100	1.8	68	1.01	24477
Change in flow rate (ml/min)	0.9	100	0.1	73	1.05	22776
	1.0	100	0.4	1112	1.05	21763
	1.1	100	0.2	324	1.05	20695
Robustness parameter		**Placebo sample**				
		Average % recovery of NAC	**%RSD**	**USP s/n**	**USP tailing**	**USP plate count**
Wavelength change (nm)	212	102.20	0.2	650	1.05	21674
	214	99.95	0.2	98	1.05	21429
Column temperature change (°C)	20	102.80	0.2	276	1.05	21755
	25	102.20	0.2	650	1.05	21674
	30	98.37	1.9	64	1.0	25248
Change in flow rate (ml/min)	0.9	103.00	0.1	93	1.05	22671
	1.0	102.20	0.2	650	1.05	21674
	1.1	102.86	0.4	100	1.06	20584

Equal concentration of NAC working standard and placebo sample solutions were injected separately, by small changing in wavelength, column temperature and flow rate. The %RSD of robustness was calculated. Experiments were performed in triplicate.
NAC: *N*-Acetyl-L-Cysteine; RSD: Relative standard deviations; USP: United States Pharmacopoeia.

minimal peak tailing with similar response factors, and had a high signal to noise ratio and high peak areas. The initial trial mobile phase composed of ACN and water (5:95 v/v) containing 0.1% TFA at a flow rate of 1 ml/min resulted in early elution of NAC and poor response from placebo. Consequently, the organic phase was optimized at a ratio of 4:96 (v/v) for ACN:water with 0.1% TFA resulting in increased retention time, resolution from placebo components and analysis time limited to 30 minutes. The flow rate of 1.0 ml/min was selected to sharpen the peaks, resulting in NAC and Di-NAC retention times of 8.9 min and 23.7 min, respectively. This flow rate was found to be optimal to aid in the reduction of the overall run time with an acceptable column back pressure. The column temperature was maintained at 25 °C to facilitate all the components in the sample solution were adequately separated. In this final optimized RP-HPLC method all the compounds of interest separated well in 30 minutes, followed by a re-equilibration to the initial condition. Our developed analytical method is very simple and less-expensive, having no internal standard, no ion pairing agents and derivatization, thereby providing economic benefits.

System suitability

System suitability testing was evaluated to verify that the analytical system was working as desired and can give precise and accurate results. Working standard of NAC and Di-NAC at a concentration of 0.005 mg/ml was injected five times into the HPLC system. The RSD of peak area was within 2% (Table 2), indicating the suitability of the system. Column efficiency is usually represented by the number of theoretical plates for each peak. In addition to the theoretical plates and the tailing factor is another parameter of system suitability which reflects the symmetry of the peak.

The current method shows that all the values for the system suitability parameters are within the acceptable limits, the results are displayed in Table 2. The column efficiencies were 21748 and 22409 United States Pharmacopoeia (USP) theoretical plates for NAC and Di-NAC, respectively. The USP tailing factors were 1.05 and 1.0 for NAC and Di-NAC, respectively, indicating good column efficiency and optimum mobile phase composition.

Specificity

Specificity is the ability of the chromatographic system to chemically distinguish between sample components. To understand the placebo matrix effect, specificity was evaluated by comparing the chromatograms of mobile phase, Milli-Q® water, placebo solution, placebo sample and NAC and Di-NAC standard solutions. For this purpose, 20 µl from mobile phase, Milli-Q® water, placebo, NAC, Di-NAC standards and placebo sample solutions was injected into the HPLC system separately, and the chromatogram results are in Figure 3. In selected chromatographic

TABLE 2

System suitability was determined by injecting NAC and Di-NAC standard solutions.

System suitability Parameters	NAC	Di-NAC	Acceptance criteria
%RSD	0.1	0.5	aNMT 2.0
Theoretical plates	21748	22409	bNLT 2000
Tailing factor	1.05	1.0	aNMT 2.0
USP s/n	965	574	>2–3
Retention time window	8.991–8.995	23.729–23.746	–

The %RSD for NAC and Di-NAC peak response from five replicate injections of standard solution, theoretical plate count, the tailing factor and high signal to noise were within acceptable range. Suggesting mobile phase and column efficiency are acceptable.
aNMT: Not more than; bNLT: Not less than.

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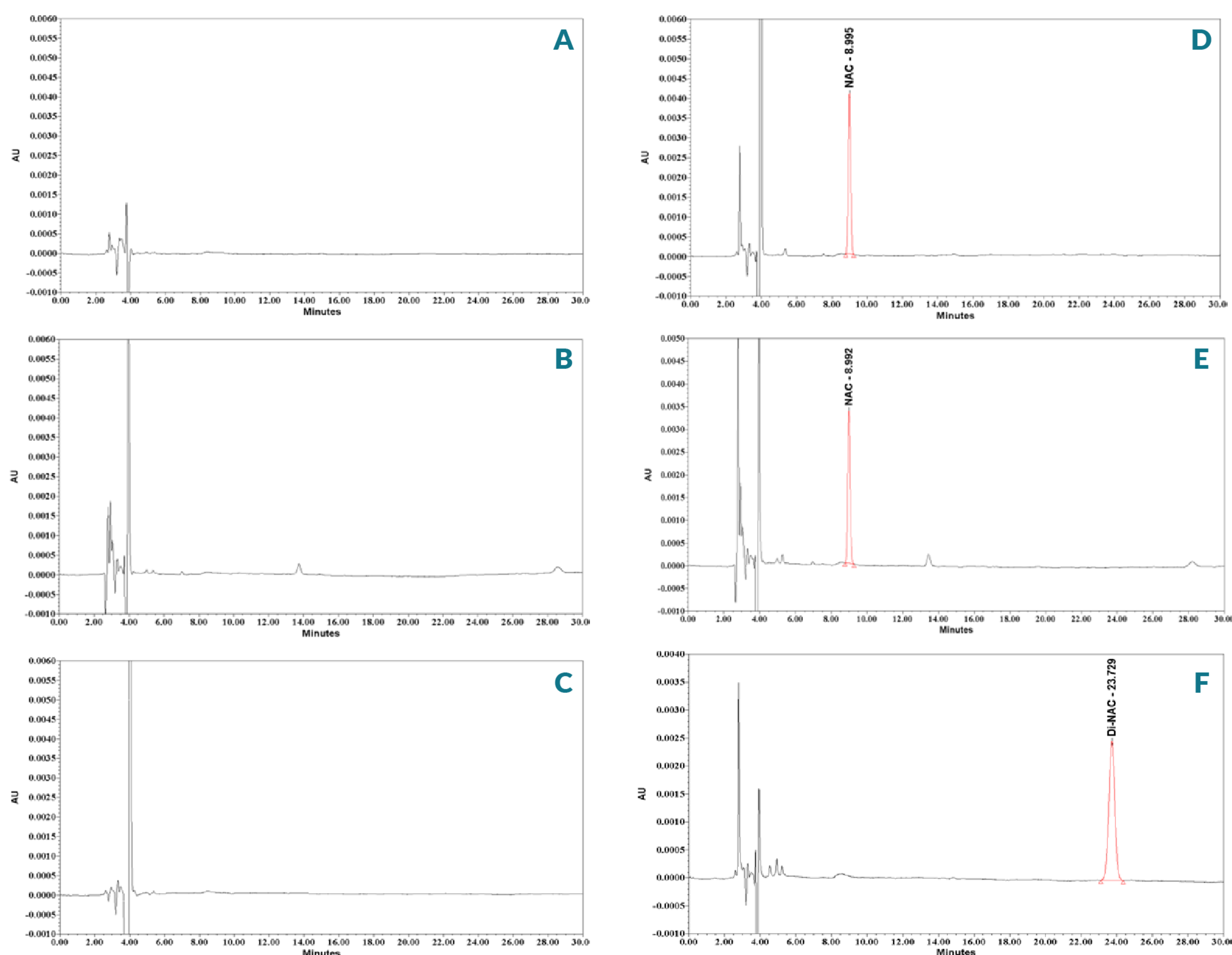
conditions, NAC was eluted in one peak at 8.9 min and Di-NAC was eluted in one peak at 23.7 min. It can be observed from the peak purity analysis (Figure 3) that there are no co-eluting peaks at the retention time of NAC and Di-NAC to interfere with the peaks of interest. This result indicated that the peak of the analyte was pure, and this confirmed the specificity of the method.

Linearity

Analytical method linearity is the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The linearity of NAC and Di-NAC was analyzed over the range of 0.0003 mg/ml to 0.01 mg/ml. The peak area obtained from the HPLC was plotted against corresponding concentrations to obtain the calibration graph. The linearity was determined by the linear regression analysis. Standard curves were constructed by plotting peak area versus concentration of the NAC and Di-NAC (Figure 4A & B). Standard curve for NAC and Di-NAC was linear over the range of 0.0003–0.01 mg/ml. The coefficient of determination (R^2) was determined for NAC and Di-NAC, R^2 = 1.0 for

FIGURE 3

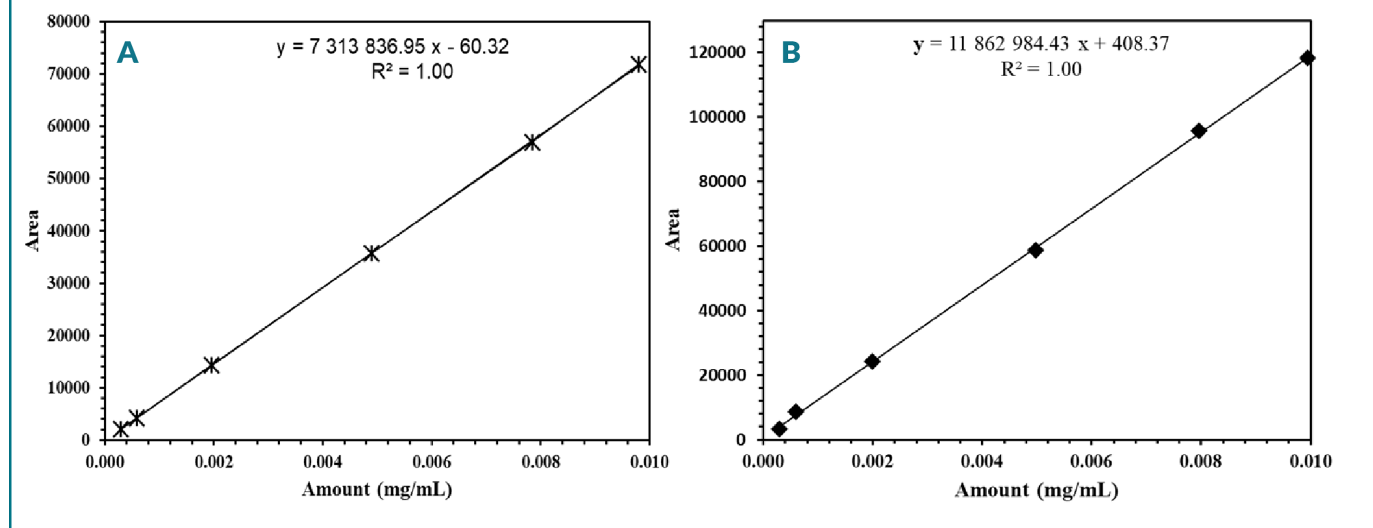
Specificity chromatograms.



20 µL of mobile phase (A), placebo (B), Milli-Q water (C), NAC standard (D), placebo sample (E) and Di-NAC standard were injected. The result of the chromatograms shows that the peak of analytes was pure and there are no co-eluting peaks at the retention time of the NAC and Di-NAC.

FIGURE 4

Linearity curves of (A) NAC and (B) Di-NAC.



NAC and R^2 = 1.0 for Di-NAC, respectively. The y-intercepts as a percentage of the analytical concentration response for NAC and Di-NAC were evaluated. The equation of the standard curve correlating the peak area (Y) to the NAC and Di-NAC concentration (X in mg/ml) in this range was Y= 7.31E+06 X – 60.32 for NAC and Y= 1.19E+07 X + 408.37 for Di-NAC, respectively. When R^2 values are greater than 0.999 it indicates that there is a good correlation of linearity through all the concentrations used.

Sensitivity study

Limit of determination and limit of quantification: The LOQ is the lowest amount of the NAC and Di-NAC in the sample that can be confidently quantified using the method. The LOD of an analytical procedure is the lowest detectable amount of an analyte in a sample but not necessarily a quantifiable value. The LOD and LOQ were calculated mathematically by the relationship between the standard error (σ) of the calibration curve and its slope (S) using the multiplier according to ICH [50,51] guidelines. A specific calibration curve should be studied using samples, containing an analyte in the range of LOQ. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation [49]. For the current method, the LOD and LOQ concentration was found to be 0.0001 mg/ml and 0.00018 mg/ml for NAC and 0.00015 mg/ml and 0.00045 mg/ml for Di-NAC, respectively. The LOD and LOQ is in a comparable range or even better than other published methods. All these results imply that this analytical method is sensitive enough for determination of NAC content in cells treatment media and formulations.

Accuracy

Accuracy of the proposed method was performed on the basis of recovery studies performed by comparing the theoretical and measured concentrations of placebo samples at 50%, 100% and 150% of working the level [50]. The accuracy of an analytical method expresses the closeness of results obtained by that method to the true value. The percent accuracy was calculated at all levels. In this study, the results of recovery studies gave the average recovery rate of 102.2% (for 50% placebo samples), 103.6% (for 100% placebo samples) and 104.9% (for 150% placebo samples). The %RSD values at each level for each analyte varied from 0.0 to 0.3%, results

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for accuracy are summarized in Table S1. These results were within the accepted limit for recovery and a %RSD of not more than 2.0%. The tailing factor and theoretical plate count are 1.05 and 21524.

Precision

The method precision of estimation of NAC by the proposed method was evaluated by replicate analysis of six standard and placebo sample solutions each carefully prepared in quintuplicate at a concentration of 0.005 mg/ml. The precision of the method is defined as "the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [50], and it is normally expressed as the %RSD. The RSD of six replicate injections was calculated and assay precision was represented as the %RSD. In terms of system precision, the %RSD of retention time, peak areas, and performance of chromatographic system, represented by the tailing factor, were all less than 2.0% and the number of theoretical plates was higher than 2000 for NAC peak, results shown in Table 3. The interday was determined by preparing the standard and placebo sample at a concentration of 0.005 mg/ml on different days and on different instruments, and the RSD of six injections were calculated. In terms of method intraday, the %RSD of assay results for NAC in evaluation of repeatability and interday were all less than 2.0%, results are summarized in Table 3. Therefore, the results of both system and method precision (Table 3) showed that the method is precise within the acceptable limits (not more than 2.0% for the %RSD and the

TABLE 3

Determined intraday and interday precision of placebo sample.

# injections	NAC standard (mg/ml)					Placebo sample (mg/ml)				
Intraday	Peak area	NAC content	% recovery	USP tailing	USP plate count	Peak area	NAC content	% recovery	USP tailing	USP plate count
1	36944	0.004964	100	1.05	21789	37320	0.00512	103.7 104	1.05 1.05	21488
2	36972	0.004964	100	1.05	21756	37631	0.00516	104.0	1.05	21548
3	36959	0.004964	100	1.05	21728	37296	0.00512	98.9	1.05	21549
4	37018	0.004964	100	1.05	21732	37272	0.00523	103.9	1.05	21515
5	37006	0.004964	100	1.05	21702	37384	0.00513	103.8	1.05	21488
6	36982	0.00496	100	1.05	21779	37262	0.00511	109.8	1.05	21566
%RSD (n=6)	0.1					0.4				
Average NAC content		0.004964		1.05	21748		0.00515	104.0	1.05	21526
Interday precision										
1	36354	0.004900	100	1.0	22882	36282	0.00488	99.95	1.1	22947
2	36499	0.004900	100	1.1	22585	36533	0.00492	99.50	1.0	22481
3	36497	0.004900	100	1.1	22599	36426	0.00490	100.02	1.0	22565
4	36374	0.004900	100	1.1	22527	36629	0.00493	99.25	1.2	23259
5	36384	0.004900	100	1.0	22608	36603	0.00493	99.76	1.1	22366
6	36318	0.004900	100	1.0	22555	36347	0.00489	99.10	1.1	22777
%RSD (n=6)	0.2					0.4				
Average NAC Content		0.004900		1.1	22626		0.00490	99.6	1.1	22733

To estimate the intraday precision of placebo sample, prepared six replicates of placebo sample solutions (0.005 mg/ml). The RSD of six replicate injections were calculated and assay precision was determined. The interday precision was evaluated with six replicates of placebo sample (0.005 mg/ml) solutions on different days and injected on different instrument and %RSD of six injections was calculated. The result shows that the method is precise

TABLE 4

Studied stability of NAC in placebo.

Samples Stability conditions		NAC standard			Placebo sample			
		% NAC recovered	%RSD	USP plate count	% NAC recovered	%RSD	% Conversion of NAC to Di-NAC	USP plate count
Fresh solution	0 h	100.0	0.3	21776	103.6	0.0	No	21529
Incubated at RT	24 h	100.2	0.3	21637	99.2	0.5	No	21726
	48 h	99.6	0.1	21736	101.2	0.3	No	21766
Incubated at 37 °C	24 h	99.5	0.7	21656	100.9	0.3	No	21679
	48 h	99.0	0.5	21704	100.1	0.6	No	21726

To determine the stability of NAC prepared placebo sample solution at concentration 0.005 mg/mL, solutions were incubated along with standard at RT and 37 °C for 24 and 48 h. The solutions were injected separately and the recovery of NAC was determined by comparing the peak area of the freshly prepared placebo sample and NAC standard. The stability results indicated that NAC is stable in placebo. Experiments were performed in triplicate.

tailing factor, and not less than 2000 for the number of theoretical plates.

Stability

To determine the effect of placebo components on NAC stability, the placebo sample and standard solutions for the NAC solution stability study were prepared. Stability was performed by injecting solutions of placebo samples and NAC standard. Samples were analyzed as a single batch upon the completion of the incubation study bench top stability at RT and 37 °C for 24 and 48 h. The percentage of recovery was within the range of 99.0% to 101.2% at all temperature and time points, and %RSD was 0.1% to 0.7%, results shown in Table 4, indicating a good stability of the NAC in a placebo solution for 24 and 48 h at both RT and 37 °C conditions. Figure 5, shows chromatogram purity of NAC peak in standard and placebo sample solutions. These results proved that NAC were stable in placebo and standard solutions prepared as described in the experimental section, indicating preparation procedure for placebo sample and standard solution was suitable for intended application of the method. This result suggested that placebo matrix and temperature did not influence conversion of NAC to Di-NAC.

While much work has been done to understand the impact of NAC product formulation on stability, there is limited understanding of the link between cell culture process conditions and of NAC conversion to Di-NAC during treatment. To study this, NAC solution in DMEM was prepared for the stability test. The solution was divided into three parts, and incubated at RT, 2–8 °C and 37 °C for 24 h since the analysis time did not exceed 24 h. All solutions were protected from light during incubation. All solutions were analyzed as a single batch upon completion of the incubation time against fresh Di-NAC standard solutions on the respective day, with %RSD not more than 2.0%. The average percentage of recovery of NAC was 96.4% under refrigerated condition, 84.4% under RT and 78.8% under 37 °C conditions. The results are summarized in Table 5 and indicate NAC was not stable in DMEM in all three temperature conditions. Amongst the three different temperature conditions the oxidation is rapid at both RT and 37 °C and slow under refrigerated temperature. The major oxidation product of the NAC at higher temperature is Di-NAC. As depicted in Figure 6, the peak height and area counts of NAC reduced, confirming the susceptibility of NAC to heat and pH conditions. The oxidized product Di-NAC was quantified with freshly prepared Di-NAC standard the results are summarized in Table 6.

DISCUSSION

NAC is considered by the World Health Organization (WHO) as a relevant medication needed in a basic health system [52]. NAC is

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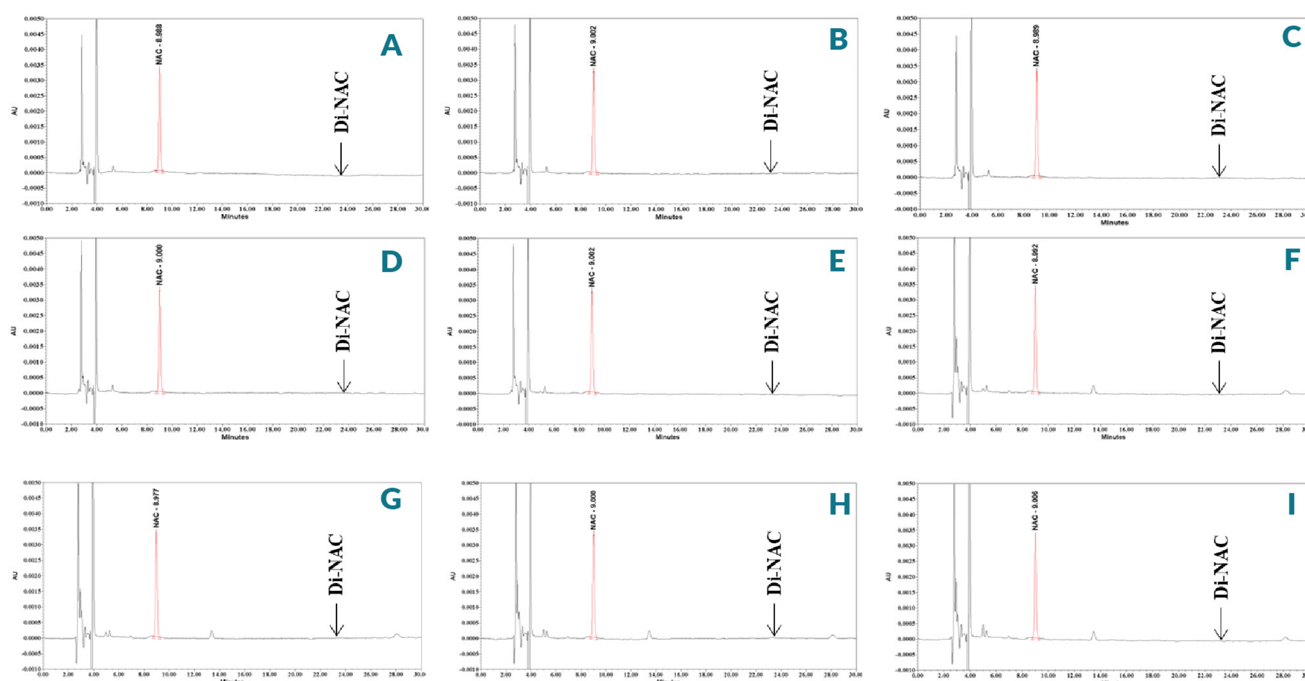
safe, even in large doses [10], and is a better source of Cys than Cys itself. However, optimum drug delivery is the key to successful treatment. Quantitative determination of NAC is one of the most important studies for the pharmaceutical industry because of the direct effect of active ingredients on human health. A sensitive and accurate analytical method allows the scientist to perform quantitative determination at trace levels without the interference effect. The main objective of method development was to determine the stability-indicating NAC in the presence of structurally similar Di-NAC and related substances in cell culture media within a reasonable run time. The RP-HPLC method was developed to select chromatographic conditions (stationary phase, mobile phase, wavelength for recording chromatogram of UV-Vis detector) and sample preparation procedure. For this purpose, preliminary trials were performed by varying the composition of mobile phase and optimizing chromatographic conditions on a C18 column. A series of trial runs were executed using different mobile phase and chromatographic conditions.

Preliminary studies involved trying different C18 columns and different mobile phase compositions for the effective separation of NAC. For optimum separation of the NAC, Di-NAC and placebo components, C18 column was chosen as the stationary phase. For selecting the wavelength for NAC, different wavelengths were studied and an absorption maximum was found at 212 nm. The mobile phase composition was developed based on the pKa of NAC. Literature was searched for mobile phase organic solvents and Acetonitrile is well known to have a higher elution capacity than methanol [53]. NAC was chromatographed with different mobile phases, consists 4:96 (v/v) ACN:water with 0.1% Orthophosphoric acid (OPA) and 4:96 (v/v) ACN:water with 0.1% TFA, and no

FIGURE 5

Determined NAC stability in placebo for 24 and 48 h at RT and 37 °C.



To evaluate the stability of NAC in placebo solution, 0.005 mg/mL of NAC standard and placebo sample was incubated at RT and 37 °C for 24 and 48 h. (A) Fresh NAC standard, (B) NAC standard at RT for 24 h, (C) NAC standard at RT for 48 h, (D) NAC standard at 37 °C for 24 h, (E) NAC standard at 37 °C for 48 h, (F) Placebo sample at RT for 24 h, (G) Placebo sample at RT for 48 h, (H) Placebo sample at 37 °C for 24 h and (I) Placebo sample at 37 °C for 48 h. These results indicate that NAC was stable during the time analysis period. Experiments were performed in triplicate.

TABLE 5

Studied stability of NAC in DMEM medium.

Solution stability condition		% of NAC content in DMEM				
		% Di-NAC recovered	%RSD	USP s/n	USP tailing	USP plate count
Freshly prepared NAC in DMEM	0 h	103.7	0.0	103.6	1.05	21518
DMEM sample solution refrigeration at 2–8 °C	24 h	96.4	0.3	107	1.05	21444
DMEM sample solution at RT	24 h	84.4	0.2	92	1.05	21450
DMEM sample solution at 37 °C	24 h	78.8	0.2	80	1.05	21473
DMEM solution at 37 °C	24 h	27.8	0.5	165	0.90	22534

To determine the stability of NAC in DMEM medium, NAC was dissolved in DMEM medium and immediately divided into three parts, part 1 was refrigeration at 2–8 °C, part 2 was incubated at RT (20 ± 2 °C) and part 3 was incubated at 37 °C for 24 h. After the incubation time solutions were diluted with mobile phase. The solutions were injected separately and the content of NAC was determined by comparing the peak area of the freshly prepared NAC in DMEM (further diluted with Mobile phase) and NAC standard. Experiments were performed in triplicate.

significant differences between the two mobile phases, regarding the separation of both NAC and Di-NAC was found. OPA has pKa values 2.14, 6.86, and 12.4 [54]. Since mobile phase containing 0.1% OPA has a higher pH than the pKa of the NAC carboxylic group and placebo components, there are insufficient protons (H^+) in solution, and NAC dissociates into its conjugate base and become ionized, resulting in reduced retention, on RP-HPLC. Moreover, found placebo components peaks were interfering and co-eluted with the NAC peak (Data not shown).

NAC, its impurities, and thiol containing placebo components, are highly polar in nature. And for their maximum retention a column with a greater non-polarity is required [55]. Consequently, the placebo components, Cys, L-Cystine and other impurities elute near the void volume. For retaining such compounds on non-polar stationary phase mobile phase modifiers like, ion pair reagents need to be used. The pH of the mobile phase is usually a key parameter for selectivity optimization when dealing with analyte molecules that have ionizable groups. Changes in mobile phase pH should be undertaken carefully as not all silica based HPLC columns are resistant to extremes of pH. As per Henderson-Hesselbach [56,57] equation, molecules above their acid groups pKa are known to exist in their ionized form and elute early from the column. According to physicochemical studies, the pKa of NAC carboxylic acid is 3–3.5 and -SH group is 9–9.5 [58]. To further increase retention of NAC, reduce the run time and maintain selectivity among structurally similar Di-NAC and placebo components, the mobile phase was optimized to a ratio of 4:96 (v/v) ACN:water with 0.1% TFA. A solution of 0.1% TFA gives a pH of approximately 1.8–2.0 in aqueous solutions [59]. Therefore, at this concentration, the mobile phase pH is less than the pKa of NAC carboxylic acid groups, and NAC and Di-NAC remains in the unionized form. Although the silane groups of the C18 column are also fully protonated the acidic environment provides sufficient protons (H^+) in the solution that the acidic NAC will retain its protons, improving retention on RP-HPLC. In addition, the NAC and Di-NAC amino groups are acetylated and the non-polar part binds to the non-polar chain on the column further increasing their retention [59].

As the NAC samples of interest were from a DMEM solution, it was important that the matrices present (amino acids, water soluble vitamins, sodium pyruvate, HEPES, glucose, minerals, sodium carbonate, sodium bicarbonate, salts, etc.) in those samples did not interfere with the NAC quantitation. TFA

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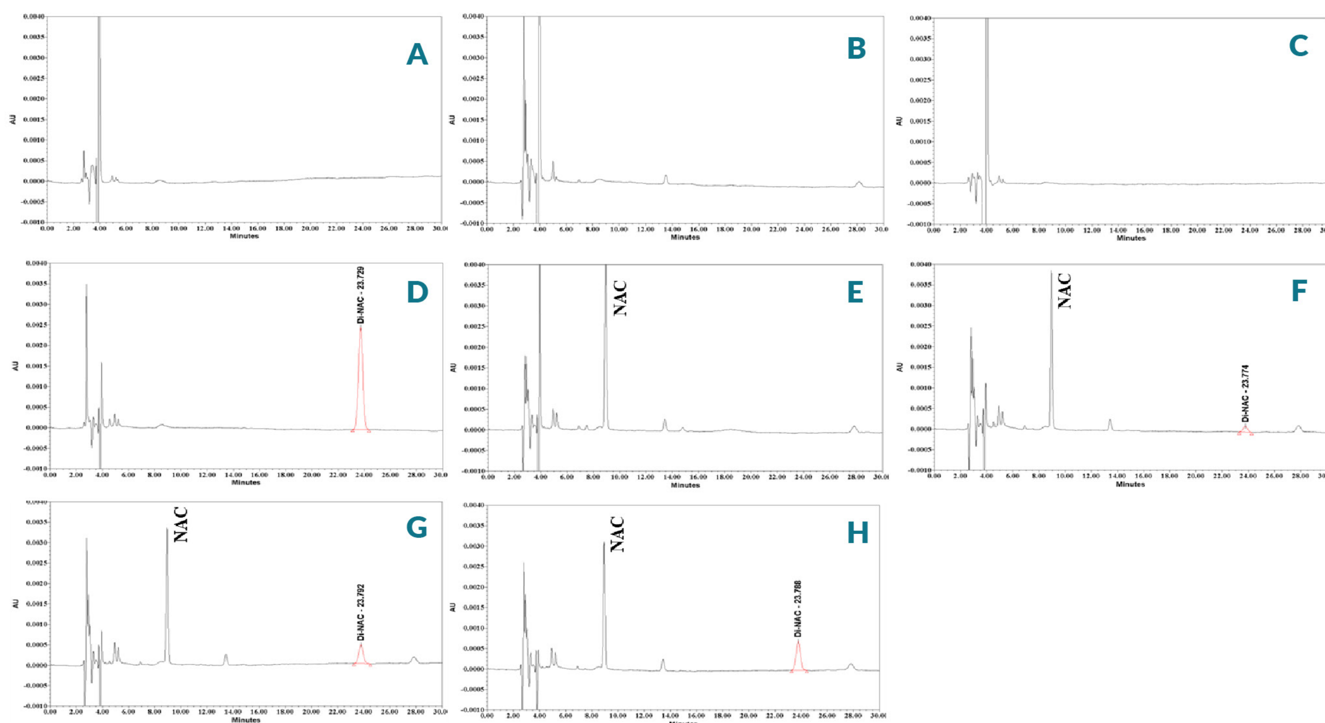
was used to provide a good peak shape and avoid the use of buffer salts that may precipitate due to innumerable interactions with placebo components. Selectivity studies were carried out to confirm that the developed RP-HPLC method had the capability to generate "true results" i.e., those tests are free from matrix interference. The HPLC chromatograms in Figure 3A–C indicate that there was no peak around the NAC and Di-NAC retention time in any of the experimental media: (i) placebo without NAC and Di-NAC, (ii) Mobile Phase and (iii) Milli-Q® water. In addition, eliminating a buffer allows the method to be easily adapted for other complex samples analysis of NAC in the future. Column temperature was maintained at 25 °C. An isocratic mobile phase was used because of its stable baseline and unvarying response factor in method development assays. Different flow rates were tested; increasing flow rate decreases retention times, but also led to interference of placebo components. A 1.0 ml/min flow rate was found to be optimal, and led to an optimal run time of 30 minutes. An injection volume of 20 µl was adequate to analyze NAC, Di-NAC and placebo components.

Stability studies provide knowledge on the possible oxidation of NAC during cell's treatment and its oxidized product Di-NAC in supplement media. NAC undergoes various transformations to form its known and unknown impurities in different stress conditions. The main impurity in NAC is Di-NAC which is formed in all the stress conditions due to the high susceptibility of the thiol moiety to oxidize and form disulphide (Figure 1). As a thiol-containing compound, NAC is readily oxidized to disulfide dimer Di-NAC [60] at 25 °C [61]. This impurity is also seen to form during treatment and storage of NAC. The amount of NAC was found to be in the range of 99.2% to 101.2% of theoretical at

FIGURE 6

Evaluated NAC stability in DMEM at RT, 2-8 °C and 37 °C.



To evaluate the stability of NAC in DMEM, NAC dissolved in DMEM was incubated at RT, refrigeration (2-8 °C) and 37 °C for 24 h. (A) Mobile Phase, (B) placebo, (C) Milli-Q water, (D) Fresh Di-NAC standard, (E) Fresh placebo sample, (F) NAC in DMEM at 2-8 °C for 24 h, (G) NAC in DMEM at RT for 24 h (H) NAC in DMEM at 37 °C for 24 h. At all three conditions during the time analysis period, decreased peak area of NAC parent peak and appearance of additional Di-NAC peak due to possible oxidation product were observed. Experiments were performed in triplicate.

TABLE 6

Determined Di-NAC content in DMEM after incubated at different temperatures.

NAC solution stability condition		% Di-NAC recovered	% of Di-NAC in DMEM			
			%RSD	USP s/n	USP tailing	USP plate count
Standard Di-NAC	Fresh	100	0.5	574	0.99	22408
Fresh DMEM solution	0 h	0.0	0.0	–	–	–
DMEM solution refrigeration at 2–8 °C	24 h	5.2	1.3	31	0.99	22982
DMEM solution at RT	24 h	18.2	0.0	98	1.02	22237
DMEM solution at 37 °C	24 h	27.8	0.5	165	0.90	22534

To determine the stability of NAC in DMEM, NAC was dissolved in DMEM and immediately divided into three parts, part 1 was refrigeration at 2–8 °C, part 2 was incubated at RT and part 3 was incubated at 37 °C for 24 h. The solutions were injected separately and the content of Di-NAC was determined by comparing the peak area of the freshly prepared NAC in DMEM (diluted with mobile phase) and Di-NAC standard. Experiments were performed in triplicate.

room temperature and 37 °C conditions of standard and placebo sample, thus proving the stability power of the method. The stability result of standard and placebo sample solutions showed that there is no instability up to 48 hours at both temperatures. No additional peaks were observed at any of the time points in comparison to zero day analysis (Figure 5). This leads us, to conclude that the standard and placebo sample in acidic solutions were stable at both temperatures. Our results suggests that the chemical stability of NAC, the active pharmaceutical ingredient, is well within the guidelines set forth in United States Pharmacopeia Chapter <795> (90% to 110% stated potency) for both temperatures (Table 4).

Stability testing indicated that the known impurity Di-NAC is on oxidation impurity which needs to be strictly monitored during stability studies. The purpose of this study was to determine the 24 h stability of NAC in DMEM when incubated in a temperature which may mimic a treatment of cell's conditions. To investigate, different temperature (RT, 2–8 °C and 37 °C) conditions were incubated for 24 h to simulate any possible oxidation that might occur during media preparation and *in vitro* or *ex vivo* experiments. All solutions were protected from light during the stability period. Samples were subsequently analyzed against fresh standard solutions using the RP-HPLC method. The results showed that NAC was subjected to oxidation (Table 5 & Figure 6) and was susceptible to conversion of NAC to Di-NAC in DMEM in all temperature conditions. The NAC sulfur atom can adopt a variety of oxidation states, for example, the NAC thiol group can behave as a potent nucleophile or reducing agent, while its corresponding disulfide might behave as an electrophile or oxidizing agent. The specific reactivity of each NAC thiol is governed by its micro and macro-environment in the solution, with its pKa and redox potential influenced by the local polarity and interactions with neighboring residues.

CONCLUSION

In the present work, a new sensitive and reproducible stability indicating RP-HPLC method was established for the quantitative analysis of NAC in DMEM, to support quality control and to assure the therapeutic efficacy of the NAC. In addition, another difference and advantage of our study is that the method of analysis has been tested in DMEM which is the transport media where permeability studies were carried out in cell culture methods. The method has been successfully validated as per ICH guidelines for specificity, linearity, accuracy, and precision, limit of quantitation

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and limit of detection, and proved to be suitable for routine quality control use. The results demonstrate that the method is suitable for evaluating the stability of NAC in cell treatment medium, pharmaceutical formulations and biological matrices products.

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AFFILIATIONS

AS Prakasha Gowda
Author for correspondence:
Department of Raw Materials, Eurofins Lancaster Laboratories, LLC. 2425 New Holland Pike, Lancaster, PA 17601, USA
PrakashagowdaAladahalliSanneGowda@eurofinsus.com

Andrew D Schaefer
Department of Raw Materials, Eurofins Lancaster Laboratories, LLC. 2425 New Holland Pike, Lancaster, PA 17601, USA

Terry K Schuck
Department of Raw Materials, Eurofins Lancaster Laboratories, LLC. 2425 New Holland Pike, Lancaster, PA 17601, USA

BioPharma
Product Testing

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *AS Prakasha Gowda conceived, designed, performed the experiments and wrote manuscript. Andrew D Schaefer and Terry K Schuck reviewed the manuscript. All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

INNOVATOR INSIGHT

Improving the quality cell yield of T-cell immunotherapies through selective pressures imparted by culture media supplements

Steven Thompson, Alex Klarer, David Smith, Steve Charlebois, Hayley Steidinger & Amanda Taylor

New T-cell based therapies use the adaptive immune system as a modality in multiple blood cancer indications and are being investigated in some solid tumor indications. This study looks at both total yield and memory character as measures of cell quality and those traits were used to evaluate human AB serum (ABS) and human platelet lysate (nLiven) as culture media supplements. Two independent labs showed statistically significant increases in both total cell yield and final T-cell central memory phenotype after expanding isolated cells in medium supplemented with nLiven as opposed to ABS. There was an additional, unexpected observation of increased donor to donor consistency when cultured with nLiven which may be a result of a more homogenous source of proteins and chemicals typically required to expand T-cells. Developing commercially viable manufacturing processes for T-cell-based therapies requires the adoption of new technologies that will facilitate process robustness. This study investigates media supplements within this context.

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Engineered T-cell therapies, CAR-T and TCR, have emerged as a highly effective new therapeutic modality in blood cancers and are showing promise in solid tumor indications in the clinic. These treatments work by co-opting the immune system's natural cancer fighting ability and targeting a surface antigen specific to the cancerous cell population. Utilizing a complicated biological system provides therapy developers with a powerful tool to direct against various diseases, however it also limits researchers' understanding of the attributes that indicate the drugs' efficacy. Historically, treatment doses are calculated based on the total number of cells presenting the surface antigen of interest with the hope that a portion of those cells would engraft in the patient and exhibit a persistent response. Recent research, however, has indicated that T cells that present a central memory phenotype (TCM) have increased efficacy over effector phenotypes across multiple disease models [1–4].

Using cell memory character as a lever to increase therapeutic efficacy could have significant downstream effects for manufacturers and patients. If the treatment is more effective on a per cell basis, patients could be treated with a smaller minimum therapeutic dose. Manufacturers could have shorter manufacturing lengths, fewer materials, and increased process consistency, while patients could see a cheaper therapy that has a more consistent efficacy.

Another concern raised by therapeutics developers who have received or are anticipating market approval is the sustainability of their chosen media supplements. Historically, human primary cell culture has been limited to a small group of investigators, but recently the space has grown rapidly. That growth has put stress on the established supply chain. Fetal bovine serum has significant regulatory concerns as an animal derived reagent, so researchers have leaned heavily on human AB serum as a source of a particular mix of proteins, hormones, and cytokines that promotes growth in T-cell manufacturing processes. However, members of the industry are acutely concerned about the long-term availability of human AB serum which has to be sourced from voluntary, male donors of the AB serotype. The source of this material is relatively inflexible as only approximately 3% of the total population have the AB serotype, it cannot be scaled to meet demand like other reagents, and AB serum is used in the culture medium of most T-cell therapies [5]. The need for an alternative xeno-free supplement is rapidly approaching.

To investigate methods for improving cell quality and shore up supply concerns, a group at the Baylor College of Medicine, led by Norihiro Watanabe, performed a small-scale evaluation of the impact of various protein sources in culture media on T-cell memory phenotype and therapeutic efficacy using fetal bovine serum (FBS), human AB serum (ABS), and a uniquely processed human platelet lysate (nLiven PR™). Their results show a statistically significant increase in T cells exhibiting central memory phenotypes and in total survival using a mouse model when cultured in nLiven PR™ versus ABS and FBS [6]. Dr Watanabe's team evaluated the *in vivo* efficacy of T-cell cultured in nLiven PR™ against both solid tumors and blood cancers. The study shows that the T cells expanded *ex vivo* with nLiven PR™ had statistically significant increases in the duration that cells were present in peripheral blood, total cells that were actively circulating, and the percent survival of the mice when compared to the same population of T cells expanded with either FBS or ABS. To further evaluate the impact of each medium supplement for therapeutic effect, the study evaluates engraftment by rechallenging the solid tumor model 21 days after the initial infusion. Again, the response by the cells expanded with nLiven PR™ was significantly more pronounced than in the cell populations expanded in either FBS or ABS.

Hitachi Advanced Therapeutics Solutions (HCATS) and Sexton Biotechnologies partnered to investigate if the human platelet lysate supplement nLiven PR™ would maintain these strong advantages when evaluated in more clinically representative culture models.

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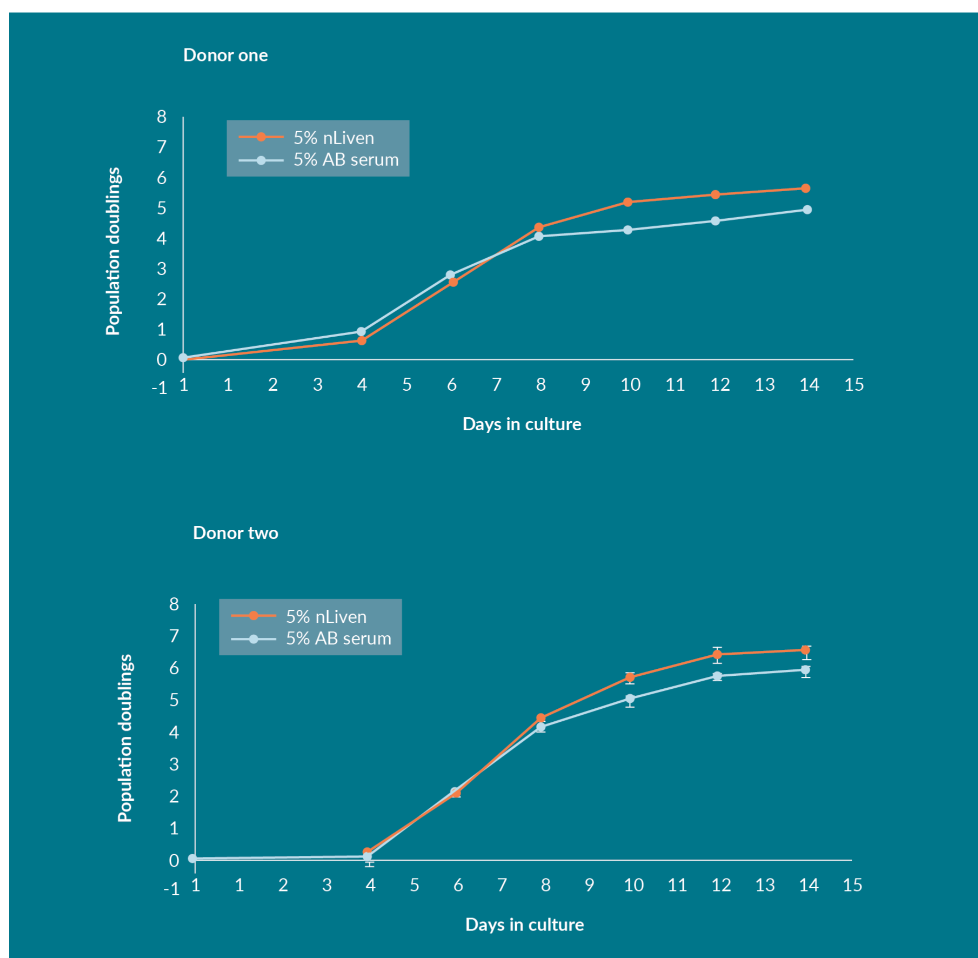
The first consideration for the experiments performed at Sexton and HCATS was to confirm that medium supplemented with nLiven PR™ produced a similar yield of total T cells to that of standard medium supplemented with ABS. At Sexton, peripheral blood mononuclear cells (PBMCs) were obtained from two donors (StemCell Technologies), activated with ImmunoCult™ Human CD3/CD28 T Cell Activator (StemCell Technologies), and cultured for fourteen days in static conditions. There was no significant change in cell expansion through the first 8 days of culture, however after day 10 the cultures using nLiven PR™ showed significantly higher expansion compared to ABS in both donors **(Figure 1)**. Part of the increase in total expansion may be the result of a promotion of T-cell proliferation over other cell populations. This is based on a FACS analysis performed on day 8 where nLiven PR™ had increased the $CD3^+$ population to 98.2% ± 0.24% and 97.6± 0.26% for donor 1 and 2, respectively, while the ABS conditions were at 94.8% ± 0.63% and 91.9% ± 1.47% (data not shown).

Negatively selected, homogenous $CD3^+$ starting populations were obtained from three donors, activated with Dynabeads® (Thermofisher), and expanded for 11 days in stirred-tank bioreactors for the work performed at HCATS. This served to assess if the impact of using nLiven in place of standard protein supplements would persist in an

FIGURE 1

PBMCs from two donors were expanded in the labs at Sexton Biotechnologies.



In both instances, statistically significant expansion was achieved after ten days in culture (p=0.002 and p=0.009 respectively by T-test). (N=3).

agitated culture system as opposed to a static one. The average population doubling across all three donors was 6.1 ± 0.58 and 6.0 ± 1.37 for the nLiven PR™ and ABS conditions respectively (Figure 2). There was no statistically significant change to the expansion of T cells when using nLiven PR™ in the experiments performed at HCATS. Despite a similar cell expansion, the nLiven PR™ media demonstrated a coefficient of variance of 10% compared to 23% for ABS media highlighting a major decrease in donor to donor variability with the nLiven PR™ cultures.

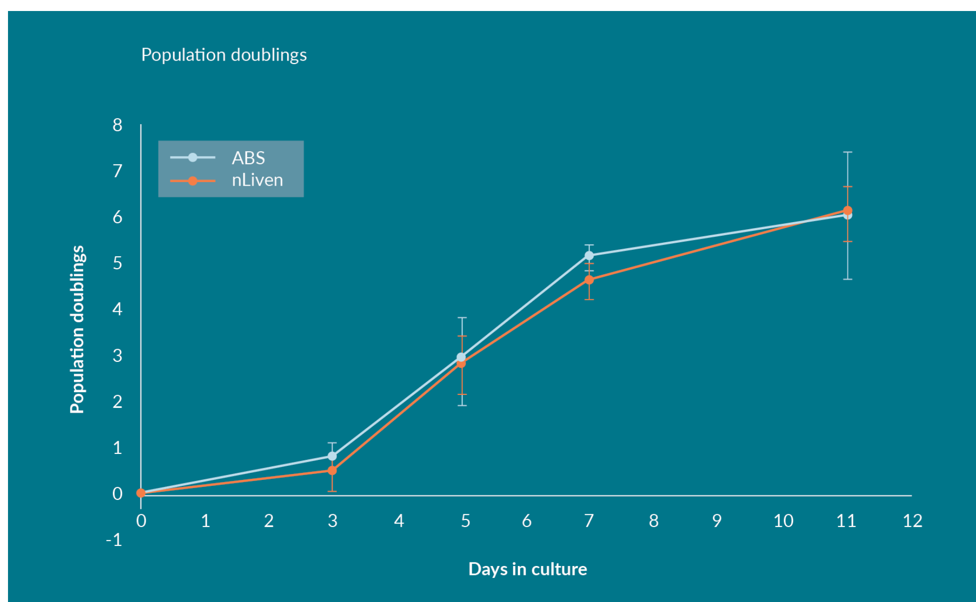
Process consistency is a persistent issue in *ex vivo* manufacture of human primary cells. Especially in autologous therapies, donor-to-donor variability forces a broadening of final product specifications and a looser understanding of critical quality attributes (CQAs). Changing to reagents that reduce variability is a powerful way to improve the understanding of those therapy characteristics. Developers may be able to produce comparable but better characterized therapies since variability was reduced in both studies when the media was supplemented with nLiven PR™ as opposed to human ABS and expansion remained consistent.

Cultures executed at both Sexton and HCATS would typically double between six and seven times during the culture period. Those results show that there was no negative impact on cell expansion by exchanging human ABS for nLiven PR™ and the substitution may result in an increase in total cell yield depending on the homogeneity of the starting cell population.

The percentage of a T-cell population that presents a central memory phenotype is typically negatively correlated to the number of times a population doubled. In two cultures that expanded a similar amount, for instance, the resulting TCM population should also be similar. What was exhibited when culturing with nLiven PR™ in static flasks, however, was a statistically significant increase in this TCM subset compared to media supplemented with ABS (Figure 3; p=0.0006 by T-test). Given similar yields of total cells, the nLiven PR™ conditions are producing a higher number of quality, efficacious TCM cells.

FIGURE 2

Similar average expansion of T cells from three donors (N=3) was observed in the experiments performed at HCATS.



Donor to donor variability decreased by over 50% when the cells were cultured in the presence of nLiven PR™ as opposed to ABS.

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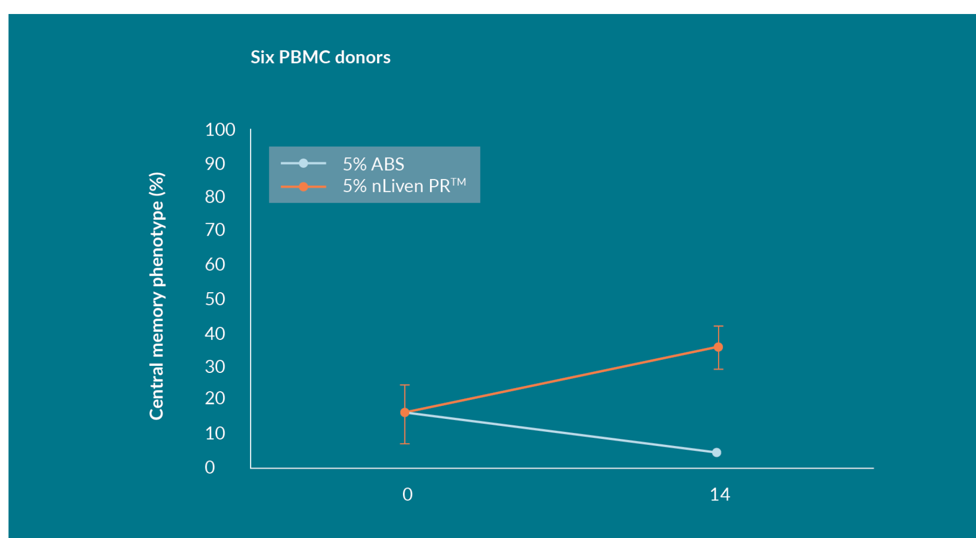
FIGURE 3

Compiled data from 6 independent donor experiments expanded in static flasks showing that nLiven PR™ consistently increases the total central memory T-cell population of PBMCs after 14 days in culture.



The increase in TCM population from using the nLiven PR™ product was statistically significant (p=0.0006 by T-test). The coefficient of variance for the ABS population is 0.25 versus 0.18 for the nLiven population.

This analysis gives credence to the idea that yield should be evaluated as the resultant population of T cells with a favorable TCM phenotype. This quality cell yield could serve as a surrogate for lengthy and expensive *in vivo* potency assays that are out of reach for many groups without a vivarium or for imprecise and target-dependent *ex vivo* potency assays that do not account for the persistence of response. Evaluating culture performance by the final population of TCM may provide a more realistic determination of therapeutic relevance than total T-cell yield.

Regardless of the starting percentage of Central Memory T-Cells by the end of the 14-day culture period approximately 30% of the total population were classified as TCM – this seems to be a consistent and reproducible effect of growing PBMCs in nLiven PR™. Equally, expanding PBMCs in ABS consistently results in a significant reduction of the TCM population.

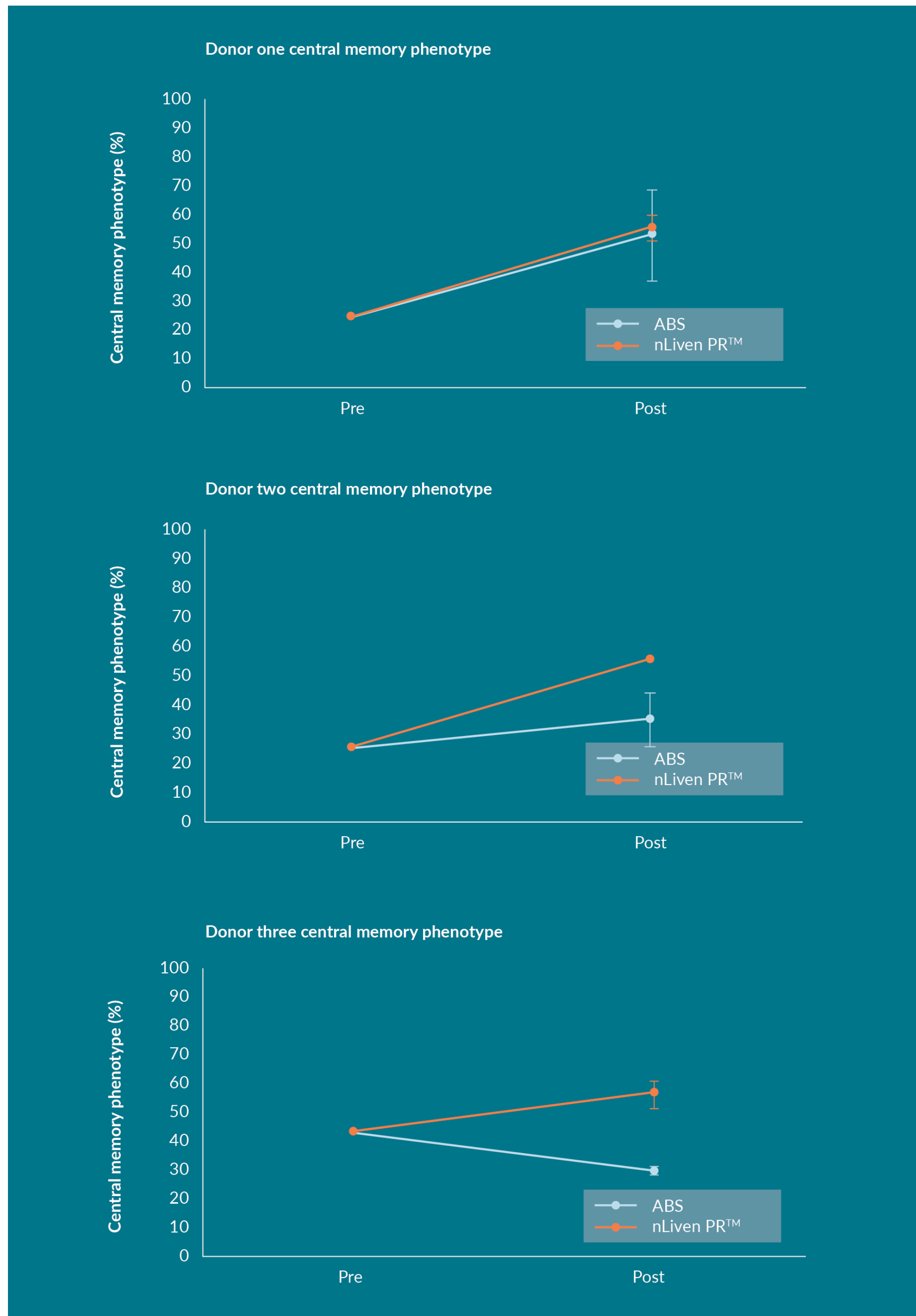
Keeping with the results from Sexton's experiments, the three donors tested by HCATS finished the culture period with about 50% of the T-cell population classified as TCM (Figure 4). This again shows high donor-to-donor consistency with a standard deviation of only 0.2% between donors. Additionally, two of the donors showed statistically significant increases in the TCM population post culture when compared to ABS (Figure 4; donor 2 p=0.02, donor 3 p=0.002 by T-test).

In both Sexton and HCATS studies the T cells cultured with nLiven PR™ saw a net increase in their TCM population after *ex vivo* expansion, which is contrary to the expectation that expanding primary, human T cells drives differentiation toward an effector phenotype. The same result was not duplicated by the cultures supplemented with human ABS. That suggests that the addition of nLiven PR™ selectively promotes the maintenance of a TCM phenotype throughout *ex vivo* cultures.

These independently conducted studies discovered a tendency for the memory phenotypes of the final T-cell population to favor subsets that were correlated with improved therapeutic efficacy and that the resulting expansion of the TCM populations were consistent between donors. In autologous therapies, final product specifications attempt to account for variation in starting material

FIGURE 4

Culturing T cells in stirred tank bioreactors with nLiven PR™ increased the total central memory population in all three donors.



The impact of changing from ABS to nLiven PR™ caused a statistically significant increase in the population for donor 2 ($p=0.02$) and donor 3 ($p=0.002$) when analyzed by a student T-test.

collected from individual patients, this factor was mirrored in these studies as Sexton's starting populations were unpurified and HCATS studies utilized $CD3^{+}$ selected cells. Regardless of starting material the final cell products showed remarkable similarities. Driving down lot-to-lot differences is a major consideration when developing an autologous manufacturing process, and nLiven PR™ is showing promise as a contributing factor to

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improved process robustness. Consistency itself is a valuable trait to develop into the production of an advanced therapy. As a derivative of human platelets, nLiven benefits from a more homogenous combination of biological chemicals than pooled human sera. This difference may be the reason for a more consistent response from primary, human cells.

Typically, the main consideration when evaluating a potential reagent change is the relative yield of viable cells throughout the process, and the results show a comparably high total expansion between the two protein supplements over the evaluated culture periods. However, there is an emerging mindset that focuses on attributes of cell quality in addition to bulk yield. For T cells, memory phenotypes can indicate a therapy's ability to provide a persistent *in vivo* response. The resulting memory phenotypes trend toward the conclusion that TCM phenotypes are promoted in cultures that include nLiven PR™ in the media formulation. Multiple donors from two separate labs had significantly higher TCM populations post expansion. Consistency across a group of donors is another important consideration when investigating if a process impact can be translated into the clinic [7]. When subjected to commercially relevant expansion nLiven PR™ supplemented media resulted in a lower level of variance in total cell yield or central memory phenotypes vs ABS – this indicates more consistent culture conditions that could translate to more robust product manufacturing. The results from the work performed at our labs lead to the conclusion that incorporating nLiven PR™ into T-cell manufacturing processes could lead to a high yield of quality therapeutic cells.

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AFFILIATIONS

Steven Thompson
Vice-President, Sales and Product Management, Sexton Biotechnologies

Alex Klarer
Hitachi Chemical Advanced Therapeutics Solutions

David Smith
Hitachi Chemical Advanced Therapeutics Solutions

Steve Charlebois
Vice President of Engineering, Regenerative Medicine
MED Institute Inc.

Hayley Steidinger
Research Scientist, Regenerative Medicine, MED Institute Inc.

Amanda Taylor
Cook Research Incorporated,
1 Geddes Way, West Lafayette, Indiana

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

EDITORIAL

Raw material risk

"...as companies accountable for bringing life-changing products to patients, we must monitor and mitigate risk in our supply chains wherever it exists."

TOM WALLS, Associate Director Supply Chain at bluebird bio

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The cell and gene therapy industry is an exciting and challenging area to work in. Every day it seems there are new companies announcing exciting therapies and cures for some of the most insidious diseases. We are all making the world a healthier and better place. Our industry has rightfully focused on the amazing science which enables these therapies. Quality and process development are making improvements to manufacturing across the entire manufacturing chain. With any rapidly growing industry, there are growing pains and there are many great articles and insights on manufacturing processes; one area that is slowly getting more attention is raw materials and the supply of raw materials from vendors who are trying to manage exploding growth for their items.

Manufacturing today is largely executed by Contract Development & Manufacturing Organization (CDMO), which largely has had the same focuses noted above (science, quality & process development). An area which is now getting more attention as companies move from development to clinical and commercial production is materials and inventory management. I am tempted to call this 'old school supply chain management' because almost every business in the world – from commodities to manufacturing to warehousing to retail must get the very basics of balancing supply (including inventory) and demand correct in order to survive. The simplest seeming businesses have these processes down; think about your local convenience store, if they are constantly out of a favorite item, or invest too much cash in slow selling items, they will be out of business quickly. Because of the unique nature of our very young industry, and because of the cash infusions many of our companies enjoy – we haven't had to work much on these processes. That is rapidly changing.

Because of the growth in our industry, there are new capacity constraints being introduced beyond our production sites, down to their vendors. You may see the effects of this in extended lead times from vendors, or infrequent (or frequent) backorders or missed shipments. As an industry we are creating more demand on vendors who may not have enough capacity to fulfill all of our needs; and because many of these vendors are inexperienced themselves in these areas, may not know they don't have capacity long term to supply demand.

Beyond simple capacity issues there are other types of risk at the raw material level which should be considered – financial risk, for example: is your vendor solvent enough to remain in business years into the future? Consider the 2011 Tōhoku earthquake and tsunami, which caused many ripples across supply chains beyond Japan - are your vendors located on a geographically unstable part of the globe? There may also be political risks, if your vendor is based in a country that is unstable or is a potential target for tariffs, which could make use of that part economically unfeasible. Most of the monitoring processes noted below do focus more on capacity issues, but if you collect the basic data prescribed you may also monitor many types of risk.

Please note – when I refer to 'raw materials' in this piece, I am referring to all inputs consumed to produce an output/product – chemicals/reagents, tubing, assemblies, ancillary materials, etc.

SUPPLY CHAIN STEWARDSHIP

I am a big believer in supply chain stewardship, meaning that even though we may ask a third party to manufacture a product for us, our company is truly accountable for that product. We are accountable to make sure it is safe, we are accountable to ensure the product is imported and exported correctly, and we are accountable to ensure supply of the product and understand any risk to that supply.

When a company employs a CDMO, it is acquiring more than their scientific, manufacturing and regulatory know how; it is purchasing their expertise in other areas including materials management. Even though the physical tasks are carried out by CDMOs, all companies should view the supply chain as their accountability, and act as its steward. When monitoring risk to the supply chain, you will have to reach out to your CDMO Partners to get key data, which we will review later. This may feel invasive to your CDMO Partners, so make sure you communicate clearly with them about requirements and why you are looking for this data. It is important that CDMOs know they can execute their processes unencumbered.

If manufacture of your goods is completely internal, which is a true rarity in this space; you will need just as strong a handle on the data outlined below. Of course, there are different challenges with internal manufacturing versus outsourcing, but these processes

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for monitoring risk are vital to your supply chain.

BILL OF MATERIALS MANAGEMENT

Per the APICS Operations Management Book of Knowledge, the definition for bill of materials (BOM) [1] is "The BOM is the document that specifies the components needed to produce a good or service. It lists the parts, raw materials, sub-assemblies, and intermediates required by a parent assembly. A BOM specifies the quantity required to make one item, specifies units of measure, and quantifies phase-in and phase-out dating. Other names for the BOM are formula, parts list, and recipe or ingredient list."

The BOM may reside in many different types of documents (Batch Record, ERP system) at the manufacturing site. Wherever they reside, as for any critical large data set, it is important to be able to report, categorize, search and aggregate the data within the BOM. I highly recommend translating all BOM data into an Excel, Access, SQL database or other ERP system. We will review how the data can be used further on in this article.

The BOM is the key data for managing risk of raw materials. To expand on the definition above, these are the key pieces of information for every item in the bill of material for a manufactured product (Box 1).

Other groups may want you to capture other pieces of data in the BOM database, including specification, material of construction, sterilization method, incoming testing requirements and others. I suggest capturing the basic data mentioned in Box 1 before getting this information.

If you are working with a CDMO Production Site; it is best to put together a simple template (in Excel) with each of the items above as columns, to reduce the workload on the CDMO and also to ensure the data you get back is complete and in the format you require.

BOX 1

- **Production site** – where the raw material is used/ where the product is manufactured
- **Platform/product type** – type of product being manufactured at the Production Site
- **Manufacturing BOM/sampling BOM** – is the raw material used for manufacture of a product? Or for sampling of the final product?
- **Program (if your company manufactures more than one product)** – Name of manufactured product
- **Production site part #** - what is the part number or Stock Keeping Unit (SKU) # at the Production Site
- **Description of item** – Per the production site (or vendor to the productions site)
- **Units consumed** – number/quantity of raw material that is used to manufacture one batch/lot of the manufactured product
- **Unit of measure** – for the raw material item in the bill of material (i.e. milligram, ounce, each, milliliter)
- **Vendor name** – business entity for vendor to the Production Site
- **Vendor address** – shipping location
- **Manufacturer/distributor** – if a vendor manufactures and sells its own product, then it is a Manufacturer; if it sells another company's product, it is a Distributor
- **Vendor SKU/catalog #** – SKU# at vendor
- **Minimum** – Minimum amount that must be ordered
- **Sole/single/multiple Source** – Sole sourcing – only one vendor for this item, and the item can only be bought from one location (usually because it's a custom material). Single sourcing – only one vendor for this item, but it could be sourced from other vendors. Multiple sourcing – the Production Site can buy this item from multiple vendors/locations.
- **Vendor manufacturing site** – if the vendor is a Distributor, then this is the site where the raw material is manufactured
- **Vendor manufacturing address** – if the vendor is a Distributor

Please note, if the item is Multiple sourced; at least two vendors' information should be captured.

Putting this data together can be very time consuming internally or with a CDMO. Cell and gene BOMs can be well over 100 items, so for one BOM, if you only capture the key sixteen items above, there are 1,600 data points for one BOM. Allow enough time,

answer questions when they come to you, and remember – patience is key!

Once you have a database with all your BOM information, you can begin reporting and categorizing the data based on any information you capture. You will be able to better show where certain items are used quickly, and which vendors are key across your entire supply chain. While certain groups and individuals may have this information in documents which aren't easily accessible, it is important to have this information in an easily searchable format.

During process development and clinical studies, BOMs may change many times as you and the Production Site discover better ways to run the batches. It is critical that if any of the BOM data changes that you are made aware as soon as possible. Change controls are the best way to keep BOMs up to date.

ITEM RISK SCORING

As mentioned above, BOMs for cell and gene products can be very large, and if you are managing more than one product or Production Site the amount of data to focus on can be daunting. Determining item risk scores is a key step to ensure that you are focusing on the items that are most important to the process. I will review two types of risk scoring below, and two dimensions to each type; you may choose to add other ratings/dimensions based upon your experience or process. The focus here is to develop an objective way of measuring risk. I suggest you review this scoring twice per year, since the dimensions in this scoring may change.

Using the risk types, you can develop a scoring system to help rank all items' risk scores. The actual numerical scoring can be determined by you – I will show some potential scoring in the examples listed below.

Material based risk – this risk type focuses on the material used in the process. The dimensions are Intellectual Property/Custom Goods and Process Contact. If the material is your IP or custom made, then obviously, it would be very hard to replace quickly if something happened to the vendor from which you get the material. The other dimension is process contact: obviously, if the raw material touches the final product, then there is likely to be a regulatory aspect to changing the material which makes any issues more complicated.

Vendor based risk – is the material sole, single or multiple sourced? The difference between sole and single sourcing is if there is only one vendor who makes the raw material to your knowledge (which is likely with custom/owned IP items) – then it is sole sourced; if other vendors can make it, but you simply aren't using them now, consider it single sourced. The other vendor-based dimension is current lead time (LT) – this is a figure which will change, daily sometimes. We will talk later about LT monitoring, but to help define criticality, take a snapshot of LTs when you are going to do this criticality scoring. You will have to give some scoring based on grouping of LTs (i.e., LT less than 4 weeks gets a score of 1; LT between 4 and 10 gets a score of 5; LT between 10 and 20 gets a score of 10; LT over 20 gets a score of 15).

After you develop the risk scores you can begin focusing mitigation efforts on items with scores in the top 10 or 20% of all raw materials. I will refer to these items as 'critical' items moving forward. Note, you can also change the weighting for each risk type. For instance, you may say that process contact scores should be weighted more than the LT score.

Table 1 provides a quick look at what scoring for two items may look like. In this example, all scores are weighted equally.

It can take a long time and a lot of effort to put all this data together, and you may want to get started on certain mitigation efforts before you have collected all the information. In that case, you can simply interview people in your company who have institutional knowledge of items which have 'caused problems' in the past. Memories may be faulty and the interviewee's judgement will likely cloud

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TABLE 1

Example risk scoring system

Category/ dimension	Scoring rules	Assembly 123		Alcohol Pads	
		Result	Score	Result	Score
IP/custom goods	Y = 15, N = 3	Y	15	N	3
Process contact	Y = 6, N = 1	Y	6	N	1
Sole/single/multiple	Sole = 10, Single = 6, Multiple = 1	Multiple	1	Single	6
Current LT	LT less than 4 weeks gets a score of 1; LT between 4 and 10 gets a score of 5; LT between 10 and 20 gets a score of 10; LT over 20 gets a score of 15	26 weeks	15	8 weeks	5
Total risk score			**37**		**15**

LT: Lead time.

responses, but this type of subjective rating may be helpful at the start of your program. By identifying these items as 'Critical' you can get started on some mitigation activities; of course, it is better in the long run to get to objective risk scoring as soon as possible.

SUPPLY CHAIN MONITORING

Once you have the BOMs captured and you have determined which items are critical based on risk scoring, you can begin working with production sites and vendors to monitor your supply chain.

Inventory monitoring is reviewing, on a monthly basis, the inventory of any Critical raw materials. This inventory should be compared to future demand and consider the current lead times. The inventory file should be reviewed between you and the Production Site after they update the current inventory, and you have updated the future demand for production. Any increased lead times or potential shortages should be discussed during the meeting and there should be a stated mitigation process, and escalation process if initial mitigation efforts don't support stated production dates. The purpose of the inventory review is to understand risks at a high level. You don't want to give the impression that you want the Production Site to abdicate their responsibility for inventory management, or want to override their ERP/MRP systems for inventory management; although you may suggest changes to safety stock as an input to their ERP/MRP system.

Another key way to monitor supply chains is to take a survey of critical items' lead times. This can be done during the monthly meeting with the Production Site; and with the vendors to the Production Sites themselves. We will discuss your relationship with vendors of raw materials more below, but developing communication channels with raw materials vendors is key to understanding potential problems. Meet with critical item vendors quarterly or twice per year. One of the key talking points should be lead time updates and capacity monitoring.

As you get more savvy with these basics, there are other types of monitoring your company should engage in. Market Intelligence reports from third parties can be a powerful tool. These reports can give you insights into financial risk, geopolitical risk and even commodity-based risks (i.e. – for silicone or other commonly used base materials).

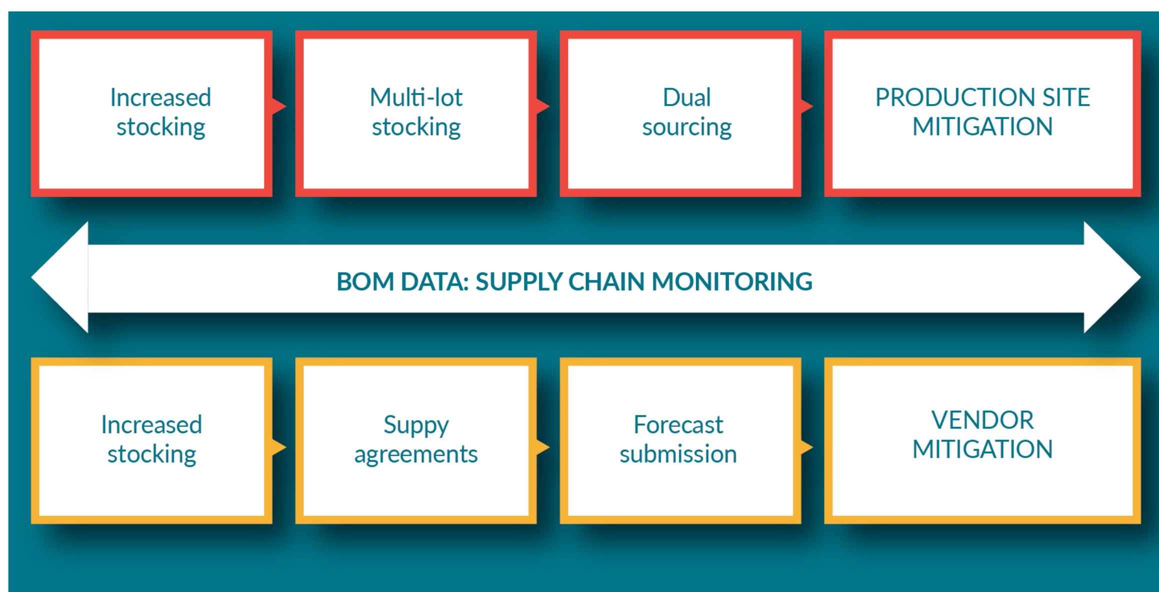
MITIGATION

If, through supply chain monitoring processes, you determine that a certain raw material, or group of raw materials is at risk, there are a few tools which can be used to mitigate that risk (Figure 1). These tools can be used in coordination with the Production Site/CDMO or with the raw material vendor.

Inventory is one of the key tools in risk mitigation. If lead times are growing or if

FIGURE 1

Tools to mitigate raw materials risk.



BOM: Bill of materials.

there is a known capacity constraint looming, adding inventory will cover you through those time periods. There are numerous tools online which can help you determine the correct amount of stock (Safety stock or reorder point calculations) to carry given factors such as upcoming demand, current lead time, confidence of supply (how often does the vendor deliver on time?) and variability of demand. Capturing all of this data can be tough, especially if you have multiple Production Sites, so you may have to use a more basic calculation. But putting some rigor around this calculation is important to make the case to carry more inventory. Inventory can be a big investment and while it is technically considered an asset, practically that cash outlay is also a liability - there is therefore likely to be pushback from your Production Site, especially if the site is a CDMO. You may have to amend you Supply/Quality agreement with a CDMO if you are proposing this change specifically, and make sure any requested inventory increase is fully vetted internally before approaching a CDMO with this request.

Another tool for risk mitigation is dual sourcing at the Production Site. This involves getting another manufacturer approved, or if it is your Intellectual Property, working to get another manufacturer to make the item. Obviously, this takes a lot of time and effort, but for key items it should be strongly considered. This should be considered especially if you are concerned about a vendor's financial stability or long-term capacity versus overall demand.

Microbial issues with reagents at your Production Site can always be mitigated by asking the site to carry more than one lot of reagents or other chemicals. This doesn't necessarily increase the amount of inventory held at the Production Site but will cut down on reaction time if a batch is found to be out of specification.

Sharing total forecast information with raw material vendors is a relatively easy way to mitigate risk without increasing inventories. This is especially valuable if you use more than one Production Site. Providing a total demand for critical items across the entire supply chain gives the vendor a complete view of your demand. In order to correctly forecast total demand, you will need up to date BOM data, and your production

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forecast. Individual production sites will only know about their demand, and not all production sites may have the capability or band width to provide forecasts. Any of the proposed vendor mitigation processes will require an NDA or other type of supply/quality agreement. Forming relationships with raw material vendors will likely have many other benefits beyond risk mitigation.

You can also ask vendors to hold or set aside inventory for you; which can be drawn upon by your production sites. Vendors are likely to be savvier at inventory management than production sites and may not charge you anything for this service, if they know they are securing your business.

CONCLUSION

As previously noted, the above tools aren't meant to supersede Production Site material management processes. However, as companies accountable for bringing life-changing products to patients, we must monitor and mitigate risk in our supply chains wherever it exists.

Our industry is growing rapidly and many of our companies are drawing from the same vendors and materials. The ability to provide accurate forecasting of demand and proper reduction of risk for those materials will ensure that the industry can do its important work with less chance of disruption due to raw material shortages.

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AFFILIATION

Tom Walls
Associate Director Supply Chain
bluebird bio

CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

EXPERT INSIGHT

Considerations on impact of raw material variability in gene therapy manufacturing: applying lessons learned from biologics manufacturing

Aaron Mack, Thomas Matthews, Bryan St. Germain, John Kerwin, David Kolwyck & Greg Stromberg

As the number of cell and gene therapy products grow it is important to consider aspects of gene therapy production process robustness, and one key characteristic is the variability of incoming raw materials. Understanding and controlling raw material variability is an important aspect of process development, characterization and commercialization. In the recent history of therapeutic protein production with cell cultures, many problems caused by raw material variability were only discovered post-commercialization, and failure to understand and address key raw material variability during process development led to costly underperformance and batch failure. In this article, we consider some known sources of raw material variability and specifically, those that have been known to impact cell culture production processes. Trace element impurity variation, particularly iron, copper, manganese and zinc, are candidates for high process impact risk. Compositional variation of undefined material (i.e., fetal bovine serum [FBS]) is another important potential failure mode. This includes known nutrient components, such as amino acids and cholesterol, as well as unidentified components, such as growth factors. Cell and gene therapy processes also include relatively new materials for cell culture, such as plasmid DNA and the transfection agent polyethyleneimine, the understanding of the variability of which must be considered.

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Commercial-scale production of gene therapy treatments is relatively new and in support of a disruptive technology. Adeno-associated virus (AAV) is a common gene therapy expression and delivery platform for the treatment of monogenic diseases. Novel approaches to inoculum expansion, production bioreactors, and low yields have increased the potential impact of raw material lot-to-lot and supplier-based variability on the gene therapy manufacturing process. As these processes are scaled-up to meet commercialization requirements, the associated increase in the quantity of raw material used compounds the risk of impact from lot-to-lot variability.

Gene therapy production processes are like protein production processes in that mammalian cells are utilized to produce an active molecule in an adherent or suspension cell culture system. The cells can be either genetically modified (producer cell line, or PCL) to express the target molecule, as is well established in mammalian recombinant protein (mrProtein) manufacturing, or expressed transiently using plasmid DNA (transient transfection), which is not typical in commercial mrProtein production processes. A demonstration of a PCL platform in gene therapy has been accomplished through stable integration of the transgene and rep/cap genes into the host cell line, and helper genes are introduced through the infection of a wild-type adenovirus (wtAd), which initiates viral vector production [1]. Cell line selection is a vital aspect of the gene therapy production process and cell type and modification will influence process parameters, such as productivity and cell viability [1,2]. A nuance of cell line selection is sensitivity to raw material variability, as material requirements are typically program specific. These issues are more prevalent in cell therapy processes where highly specialized raw materials may not be readily available under the required conditions for cell modification [3,4]. Special consideration should be made at the in the early stages of a project to account for sustainable raw material sources.

Both PCL and transient transfection systems can produce an active molecule in an adherent or suspension (continuous or batch-fed) cell culture system. However, PCLs are typically made for suspension processes. Adherent cell cultures are processes wherein cells adhere to a substrate, such as a petri dish, in a monolayer using structural proteins to adhere to the substrate surface, and passaging cells requires enzymatic dissociation of the cell from the substrate. These processes exist in GMP manufacturing spaces as roller bottles, high surface area culture chambers, fixed bed reactors and other modalities that produce relatively high titers of virus per liter of batch volume compared to current suspension processes for gene therapy. However, scaling these processes adequately for high titer patient dosing requirements for intramuscular therapies and similar treatments is currently challenging. Suspension cultures are grown in wave bags, shake flasks and large stirred tank reactors that maintain constant fluid motion and keep cells from adhering to fixed surfaces. Suspension cultures excel at producing high viable cell count but produce relatively low titers of virus per liter of batch volume with current large-scale methods. [1,5]. Further productivity optimization through process and raw material understanding is expected and this article aims to provide areas to focus for raw material optimizations.

TRACE ELEMENT IMPURITIES

Impactful raw material variability has previously been observed in biologic drug substance production processes, wherein variability in the trace element contaminant profile of some cell culture nutrients impacted the drug substance product quality in terms of glycosylation and galactosylation profiles [6]. Historically, cell and gene therapy processes have few defined trace element additions in the process and are therefore at higher risk for natural variation in serum and media lots. Because gene therapy batch sizes tend to be small compared to current therapeutic

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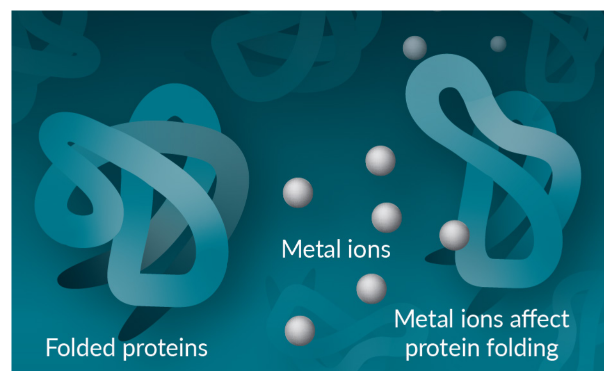
protein production, significantly fewer lots of raw material may be consumed through process qualification and commercialization, leading to an incomplete understanding of raw material variability and process impact at the commercialization stage.

Current gene therapy manufacturing processes are similar to mrProteins production processes, and learnings from mrProtein batch failures and investigations can be applicable in gene therapy viral vector production. That being said, in the case of large molecule production, cellular machinery is genetically instructed to produce a ~150kDa molecule with appropriate placement of sugar moieties, which is a complex process shown to be sensitive to changes in raw material trace contaminants [6]. In the case of gene therapy production, cells are genetically instructed to produce 60 monomers, comprised of three different viral proteins of different molecular mass in a molar ratio of 1:1:10. The mature assembly of the monomers is a functional viral capsid, capable of cellular transduction and delivery of encapsidated 4.7 kilobases of DNA, with the full capsid assembly with an average molecular weight of approximately 3800kDa [7]. With additional complexity of the viral assembly process, the potential for aberrations due to known and unknown impactful raw material variability increases.

In mrProtein processes, from experience it is known that animal-sourced material and plant-derived hydrolysates pose the greatest risk to product quality, and some unexplained variability can be attributed to the use of these chemically undefined raw materials. Additionally, variation in naturally occurring trace elements, in particular in chemically undefined materials such as serum and hydrolysates, has been shown to be impactful when below or greatly exceeding threshold levels [6]. Copper is an essential cofactor for proteins involved in cellular respiration, and varying trace copper levels can impact cell culture lactate levels and cell growth [8]. Zinc and manganese affect protein expression and glycosylation [9–11]. For virus production in gene therapy applications, it has been shown

FIGURE 1

Metal ions impact protein folding

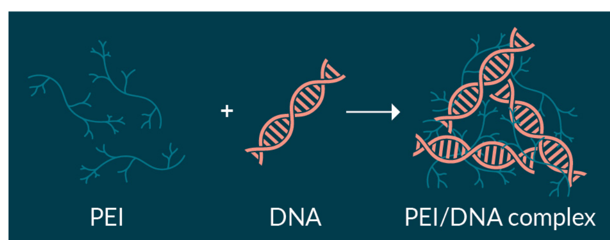


that zinc and cadmium can negatively impact transfection assay efficiency through indirect impact, and metals have previously been shown to influence DNA repair in cells, which could prove impactful at threshold levels both in process and in expression in a patient. Media and culture conditions are known to impact transfection efficiency, and more work is needed to understand the potential impact from trace element contaminants. As additional gene therapy processes are commercialized and more material variability experienced, the knowledge base of impactful elemental variability will grow [12,13].

PLASMID DNA & POLYETHYLENEIMINE

Many of the raw materials used in large molecule and gene therapy processes overlap, but there are some unique critical raw materials, such as plasmid DNA (pDNA) and polyethyleneimine (PEI). These two materials pose unique variability risks that are impactful to transfection efficiency during the production phase, viral potency, and the function of the target gene when expressed in target cells. The potential for unwanted plasmid mutation does exist, and since any pDNA mutation could directly affect viral capsid construction and package contents, it is imperative that plasmid sequences are verified [14–16]. Moreover, only the impurity analysis of plasmid DNA has the potential to impact transfection

FIGURE 2
DNA/PEI complexation



efficiency – endotoxin and residual DNA/RNA. DNA supercoiling, a product quality metric for plasmid DNA manufacturing, does not necessarily impact transfection efficiency for AAV production [17,18].

Branching and electric charge characteristics of PEI can impact transfection efficiency. PEI suppliers have optimized formulations in terms of formulation salt concentration, volume, incubation time, order of addition and ratio of plasmid to PEI. These optimized formulations of transfection reagent are produced using GMPs and are commercially available. Transient transfection poses a challenge to both the process and cost of AAV manufacturing. The transfection complex is a sensitive mixture and large-scale transient transfection production poses scalability challenges related to the pump rates, mixing times and other process variables. Additionally, plasmid DNA and proprietary formulations of PEI are costly critical raw materials, impacting the per-batch cost at scale [19–24].

Alternatively, stable transfections made by genetically modifying PCLs can be pursued as a path forward over transient transfection methods, and this approach could prove more attractive for scale up of suspension cultures. While the initial genetic modification of the PCL requires more development, it has been shown in HeLa and HEK293 cells that once the PCL is stable, vectors can be made through 60 population doublings, with reasonably high vector yields and full capsids [2,25]. While other polymeric materials such as High Density Polyethylene (HDPE) bags, filters, resin, tubing and other process aids are used in the gene therapy manufacturing process, these items and their associated variability are beyond the scope of this work.

FETAL BOVINE SERUM

At the time of publication, most current late stage clinical and commercial gene therapy processes are in adherent cultures and require fetal bovine serum (FBS). FBS is an animal-derived raw material sourced from isolated bovine populations during peak breeding seasons and can vary based on environmental factors, location and harvest timing. The inherent variability of the harvest process, from age of the fetus through harvest and purification methods can negatively impact serum performance and composition [26,27]. Due to the nature of this material, it is difficult to source, expensive, there are adventitious agent/biosafety concerns associated with its use (e.g. Bovine Spongiform Encephalopathy [BSE]/Transmissible Spongiform Encephalopathies [TSE] risks), and it has a concentrated supplier base with limited options available in the worldwide supply of FBS [28]. Concurrently, with a high incidence of phase three and commercial gene therapy programs requiring FBS, increased demand will put additional strains on the current global supply of this commodity material, particularly from low BSE/TSE-risk regions such as Australia and New Zealand. It is for these reasons that understanding critical factors in FBS composition and working to reduce the overall need for FBS through identification of critical components and optimization of lot use will reduce both the supply and variability risk from the use of this material in gene therapy programs.

One component of FBS identified as critical is cholesterol, which is present in several different forms and can vary in concentration among lots of this material [29]. It has been observed during mrProtein production that cholesterol is a critical component, in that it is a key constituent of plasma membrane lipid rafts and has previously been shown to be a critical component. Variability of cholesterol concentration in FBS could create challenges

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in predicting cell culture growth kinetics. Transfection is a process that is dependent on the cell density where inaccuracies in predicting the growth kinetics could result in lower transfection efficiency and lower AAV productivity. Understanding the nuanced impact of these types of materials on AAV gene therapy production process is imperative for process optimization [30,31]. It has been previously demonstrated that cholesterol supplementation in lentiviral vectors can increase potency by a measure of 4-fold to 6-fold, and can rescue infectivity in virus production processes with depleted cholesterol with addition of exogenous cholesterol [31,32]. Through a deeper understanding of which cholesterol esters directly contribute to viral infectivity, the material selection process could allow for sourcing of non-animal derived GMP-grade materials [33].

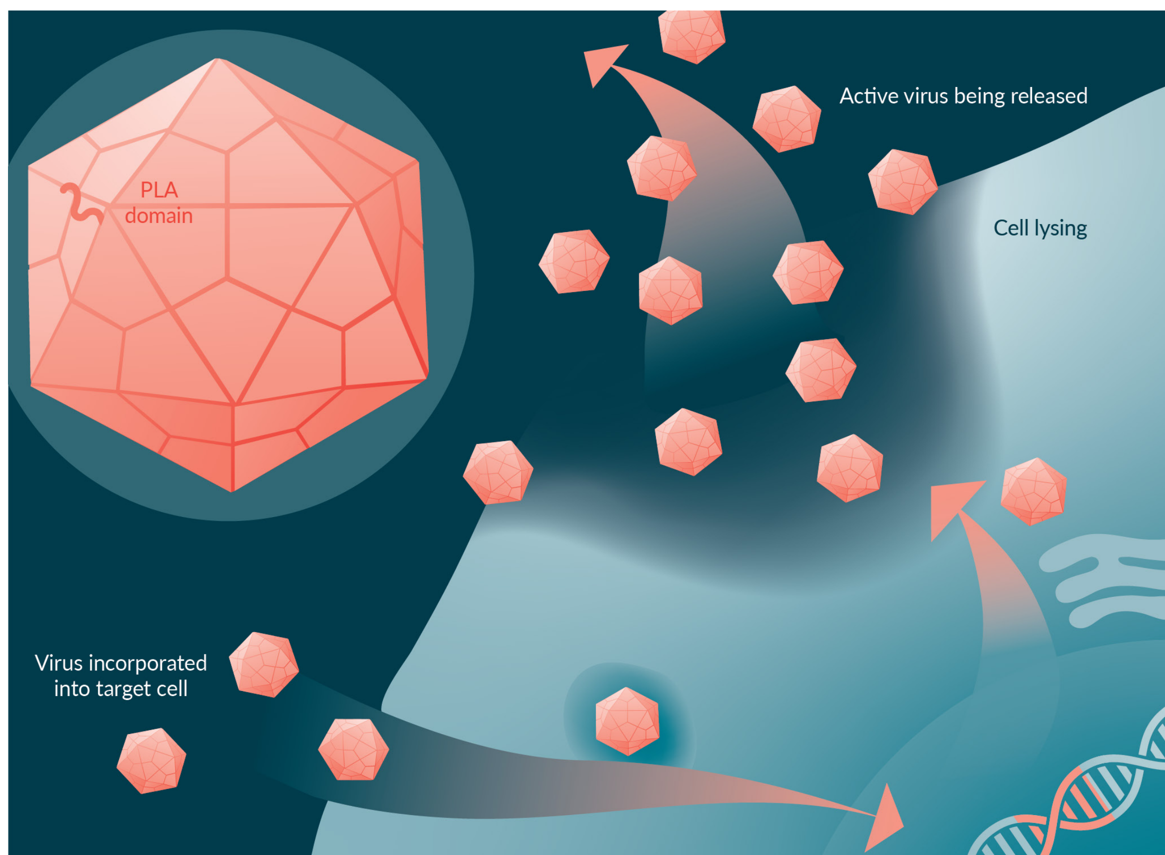
Another aspect of FBS is the contribution of this material in terms of free amino acids. When a single component such as FBS comprises 5–10% of a media formulation, changes in the free amino acid profile of this material can negatively impact viral production on the cellular level. One such observed impact from the known degradation path of L-glutamine into glutamic acid and ammonia. L-Glutamine is a component in media that degrades relatively quickly once hydrated, and degradation products can impact viral production on the cellular level if ammonia levels reach concentrations at or above 10mM. Even at levels of 1–2mM, ammonia can have a negative impact on viral production through increased cell apoptosis [34].

PATH FORWARD

Pharmaceutical manufacturing associations have issued guidance and application

FIGURE 3

Virus production



documents on how to assess raw material variability risk in small molecule and mr-Protein manufacturing based on health authorities and standards publications. Some of these associations are updating these guidance documents to include cell and gene therapies, as there are some specific raw material risks associated with these processes that are not inherent in other pharmaceutical manufacturing, and this guidance can be valuable in assessing raw material variability risk [35–38].

Most existing gene therapy processes establish process robustness using analytical measurements with poor precision, which can lead to inconsistency and extra expense, and can potentially exhibit more variability at large scale when multiple lots of raw material are consumed across a campaign. During development, it is best practice to perform analytical and performance testing upon multiple lots of each raw material if multiple lots are available. To further investigate the impact of raw material variability, analytical characterization of raw materials is important, and the choice of testing should be based on known material variability issues impacting viral production and other cell culture processes, investigations into process variability and hypothesized impactful contaminants based on the raw material manufacturing process. Upon identification of variable components in a raw material, rescue studies should be performed to assess mitigation methods to reduce the impact of variability in gene therapy manufacturing processes.

DISCUSSION & CONCLUSION

Manufacturers are inclined to enhance process control through understanding key raw material attributes and application-based requirements in order to constantly supply the market with safe and consistently potent gene therapy treatments. Enhanced processing with dependable potency will enable lower cost, efficient and reliable dosing and packaging of gene therapy drug product. Improved understanding of variability and the limits of process performance will allow for the targeted control of the critical parameters of key components, such as FBS, that allow for strategic sourcing of this material to meet technical and quality requirements. An improved understanding of the role these parameters play in the consistency of drug substance from gene therapy processes will allow for lot screening initially, but ultimately, process control, optimization, and reduction of animal derived components used in gene therapy manufacturing processes. As with all biologics manufacturing, a formal risk assessment should be performed to identify critical raw materials and critical process parameters. The output is a robust control strategy to ensure safety and reproducibility of a gene therapy manufacturing process. It is ideal to implement these types of studies as early in the process as possible, but these types of raw material impact assessments may not be practical until a program reaches early clinical stages. Improvements to the scalability of gene therapy production process will drive enhanced patient access to this revolutionary therapeutic modality that offers the potential of a permanent cure for patients.

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AFFILIATIONS

Aaron Mack, Thomas Matthews, Bryan St. Germain, John Kerwin, David Kolwyck & Greg Stromberg
Biogen, NC, USA

AUTHORSHIP & CONFLICT OF INTEREST

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EXPERT ROUNDTABLE

Overcoming Raw Material Challenges in Cell & Gene Therapy Manufacturing

DAVID DIGIUSTO
Chief Technical Officer,
Semma Therapeutics.

David has over 28 years of experience in the scientific, clinical and regulatory aspects of cells as therapeutic agents including the isolation, characterization and genetic modification of hematopoietic stem cells and T-cells for clinical applications. He has been instrumental in the creation of six GMP compliant biologics manufacturing facilities and associated quality systems, production and QC testing programs. Under his direction, plasmid DNA, CAR-T-cells, regulatory T-cells, engineered stem cell grafts and gene modified hematopoietic stem cell products have been manufactured and released for use in Phase I/II clinical trials.

BERND LEISTLER
Vice President Production,
CellGenix

Bernd has a long track record as protein specialist. He joined CellGenix in 2003, and is currently responsible for all GMP and preclinical cytokine products for further manufacturing use, as well as process development for protein production which includes new packaging formats. Following his degree in chemistry he completed his dissertation on the structure, function, folding and assembly of oligomeric proteins. His professional career started at a leading manufacturer of diagnostic autoantibody immunoassays, where he managed the Biotechnology Department and developed it as a corporate service unit for recombinant and conventional human autoantigens and allergens.

TOM WALLS
Associate Director of Supply Chain,
Bluebird Bio

Tom Walls has over 15 years of supply chain experience in life sciences. He has led initiatives in business process management, production capacity management, global trade compliance and global planning. He has experience in cell & gene therapies, as well as small molecules, branded, generics, commercial and pre-clinical stage companies.

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Q What risks should you look at when choosing a material supplier?

TW: You want to look at their physical capacity; what is their ability to produce to your forecast, not only in the short-term and in development, but in five or even ten years in the future? They need to have the technical ability to scale up to what is needed as you move into clinical and commercial applications.

You also need to have an understanding of financial risk, and of whether a supplier is financially healthy. Geographic risks must be considered too – are they built on the side of a volcano? Probably not, but are they in a location that may be subject to earthquake or tsunami issues?

And then there is political risk; whether there a chance the supplier may be subject to embargoes or increased tariffs. We all know tariffs have been up and down over the last two or three years, so understanding that sort of political risk is also key.

BL: The first risk I would like to mention is your sole source suppliers with single source materials. You should have a second source available whenever possible. But of course, typically this is not the case for customized products.

Next, it is good advice to think ahead in terms of quality. The quality of a material in use may be sufficient for early clinical trials, but you need to think ahead to the quality needs for a licensed medical product, and if your supplier is prepared for that.

Thirdly, think about supply security, and think about your needs a few years ahead. My recommendation would be to exchange rolling forecasts with your supplier on a regular basis.

> "The quality of a material in use may be sufficient for early clinical trials, but you need to think ahead to the quality needs for a licensed medical product, and if your supplier is prepared for that."
>
> **- Bernd Leistler**

Q What information is needed to understand the risks related to supply, demand, and material supplier capacities?

DD: First you need to establish the manufacturer's production requirements. In order to assess risk, you need to know what you're asking of the supplier; in terms of volumes, cadence of delivery, and cadence of provision, as well as your raw materials specification – I think a lot of people underestimate the value of the raw material specification. For example, a supplier may say that they can give you 95% purity in a material, and that's okay for your purpose, unless the other 5% will interfere with your product.

Really understanding the critical quality attributes of your material, and the need for that material upfront, is going to be the basis for what we subsequently do, which is perform the equivalent of a failure mode analysis. We look at all the possible ways things can fail – on my list of considerations I have

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the size of the organization that's supplying, their financial history support, whether they are a stable company, and the turnover rate of employees too. If every time you call for a reagent there's someone new on the phone, it causes concern about the continuity of service.

Regarding capacity, a big issue is if you take a small company that makes an esoteric reagent, and they can supply you with 500 micrograms, and all of a sudden you want to move to 100 x scale, they may or may not have that capacity. You need to have an understanding of their ability to scale. And if they can scale, can they make the same product at scale? Oftentimes when you scale up a product the attributes of that product change.

These are all things you need to understand about the supply and demand of materials. Another way to look at the quality of the history of the materials to understand where they've had materials out of specifications. How often do they make a lot that doesn't meet criteria? If they quote a capacity but 30% of those lots fail, it impacts that capacity.

TW: This may be a smaller point but when you're talking with vendors, or talking internally, I think it's very important to use a common language for capacity, whether it's batches, milliliters, micrograms, and so on. This allows you to better compare and contrast both vendors, and your entire network situation, when you use common language.

"if your supplier is a sole source provider, meaning they are the only ones that manufacture an esoteric or custom reagent, it adds significantly to your risk profile."

- David DiGuisto

BL: I would recommend asking about the production capacity, and whether stock levels are prepared for future demands while maintaining a high level of quality.

Next, I would ask about production lead times. Combining these, I would recommend exchanging regular rolling forecasts of both demand and capacity, in order to speak the same language as far as quality standards, in order to be prepared well in advance.

DD: A consideration we haven't brought up is that if your supplier is a sole source provider, meaning they are the only ones that manufacture an esoteric or custom reagent, it adds

significantly to your risk profile and the considerations become different. In these cases you do not have the option of identifying backup vendors. This comes into play more often than you might think, and it's a major factor in risk assessment.

Q What do you think is the best approach to contingency planning in situations where there is a single supplier of a critical raw material – and how would you manage unique situations, such as the ongoing pandemic?

DD: The most important approach to contingency planning is to review the performance of a vendor, and then have a plan for what happens if that vendor goes away. What is the impact?

You can't control the vendor being there and also being able to meet capacity, but you can have a backup plan for your process – even if it's a challenging backup plan.

I've heard people having back up plans up to and including the purchase of the company, or an arrangement with the company that if they go out of business, the product line becomes the property of the client. That way, if they're unable to manage a business but they have a production technology you require, you can inherit the production technology. That's one way you can deal with a sole source provider – of course, not everyone will agree to it. But if they're small and esoteric, they may not be around in 3 to 5 years.

TW: Absolutely – especially for single or sole source materials, having a backup plan written into your contract is key. Speaking specifically to the situation today with COVID-19, we use a lot of CDMOs to manufacture our products, and we have some internal manufacturing. That presents a special challenge because we rely on the CDMOs to manage their inventory and understand risk. But as the IP holders, the company that is delivering these lifesaving therapies for patients, we have to be stewards of our own supply chain. Knowing your biller material, even if it's through a CDMO, is critical, and knowing where there may be risk is critical.

That means diving down into your biller materials: knowing of course what materials are there, but also going two or three steps further and being aware of what is being sourced from potential hotspots. That's very difficult, although there are some software solutions that can help.

BL: I consider contracts such as supply agreements and quality agreements to be very important elements. This can include stock levels and

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supply schemes, and on top of that as part of our mitigation plan and supply security plan we have a stock of product intermediate at each processing step to shorten the lead time, apart from what we have agreed with customers in terms of finished product forecasts.

Q How can the sterile connectivity between GMP raw materials and closed automated cell processing systems be improved?

BL: This is a frequently posed question. The ultimate goal is to have a sterile connectivity solution that can be operated outside the cleanroom. Today, I think weldable tubing is possibly the most broadly accepted and applicable technology. Secondly, there are sterile connectors, but the problem here is it requires standardization at both ends of the process, standardization of the raw materials and the cell culture system at the same time.

DD: We run into this all the time, where we might get something in a vial, and so it's an open process. What we've done where applicable is work with manufacturers on custom packaging. This involves identifying a unit of a material that we're going to use in a process, identify how we're using it in the process, and then asking them to package it in a way that allows us to make a sterile connection.

For example, we've had small bags of reagent with a segment of tubing that can be welded on to the automated production system. You may have to enter a supply agreement to justify the change to the manufacturer, but I think it's going to become more important for manufacturers to recognize that simply vialing may not work for all intended purposes, and that custom packaging – both in the size of the unit and the ability to do sterile connection – is extremely helpful to the client.

Q How can you streamline manufacture by reducing raw material handling requirements?

> "Knowing your bill of material, even if it's through a CDMO, is critical, and knowing where there may be risk is critical."
>
> \- **Tom Walls**

DD: As we just discussed, packaging with sterile connections certainly helps, as does packaging in unit operations. For example, if I buy 100 milligrams but use 100 micrograms per reaction, I don't want to have to do that allocation myself. If I could instead buy the 100 milligrams in units per lot or per batch run, providing those specific package increments would be extremely helpful.

BL: One possibility would be to provide liquid reagents, specifically when considering freeze dried versus

lyophilized versus liquid cytokines. The big advantage of lyophilization is of course long-term stability – but as long as a shelf life of one or two years is sufficient, this could help to significantly reduce the workload of reconstituting and diluting the cytokines.

Another possibility would be customized mixes of reagents. It could be mixes of cytokines for a particular application. It could even be complete media by supplementing basal media with cytokines. This is of course very specific to the particular process or process step, but it is an achievable way of reducing manual workload in manufacturing.

DD: We've actually gone down that path, and two things came up: one was ensuring no interactions when you combine something formulated individually versus something stored and formulated as a compounded material. It may not have any interactions, but it's a question worth asking.

We also ran into an issue with lyophilized reagents. They have a stability as a lyophilized product of two years, and that's fabulous. But if we need to take that and compound it or formulate it to use it, we've now committed that lot, because we've made a new formulation. This means that stability of the compound changes completely and you have to rerun stability assays on your formulation, and the manufacturer cannot anticipate what your excipients or your matrix is going to be. So it may be less handling but it adds time and effort in re-establishing stability. When you're looking at reducing handling any impact it has on stability or interactions has to be considered.

Q What approach would you take to manage raw material variability in order to minimize its potential impact on bioprocessing?

> "...when scaling up you want to have a test lot at scale to make sure that the material has the same properties as when it was made at small scale"
>
> **- David DiGuisto**

DD: It is important to understand the quality attributes of your raw material outside of what's provided on the Certificate of Analysis (C of A), because you may have to include additional testing to what is provided by the manufacturer if it impacts the material. When it comes to consistency of quality of raw materials, there may be a burden on the user to analyses things that are not part of the C of A.

As I mentioned earlier, when scaling up you want to have a test lot at scale to make sure that the material has the same properties as when it was made at small scale. Properties often change upon scale up, or if you change the way you produce it, for example from an *E. coli* to a baculovirus, or some other change in methodology. You want to be sure that you have the same material by testing it in your production system.

BL: From a supplier perspective, you should really look at having a robust manufacturing process, and as David said, this has still to be true after scale-up and after any process changes. The critical steps have to be challenged

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> "From the user perspective, I would recommend that you agree on appropriate specifications: you can negotiate adding one or two additional specifications, and agree that each new batch is tested against the new specifications."
>
> **- Bernd Leistler**

by process validation, and these data must be available. If you have a significant process change, you have to revalidate the relevant process steps.

From the user perspective, I would recommend that you agree on appropriate specifications: you can negotiate adding one or two additional specifications, and agree that each new batch is tested against the new specifications. As I said earlier, this can all be written down in quality or supply agreements.

Q What particular issues have you encountered relating to the stability of critical reagents and other raw materials, and what is your advice on managing this particular aspect?

BL: Comprehensive stability data is a must and is required by all users. We have a multistep program – first we gather stress, accelerated and real-time stability data to demonstrate consistent quality over a long time, meaning several years. Secondly, additional supporting data are essential, such as in-use data, stability after reconstitution, and stability after a number of freeze-thaw cycles. This is tremendously helpful to the users in developing a robust and efficient manufacturing process, because they don't have to do it themselves.

DD: We try to ensure a supply chain by predicting our manufacturing capacity for a year at a time, and then acquire all those and hold all those supplies. This only works as long as the expiration date on the supplies does not exceed the year, of course. So one of the challenges is balancing your rate of purchase or acquisition of supplies with their intended use and expiry date. If I buy a years' worth of something and it expires in 6 months, it doesn't do me any good.

The other issue is shelf life. For example if with media you buy the raw components, but then you compound and test the media, you have to know what the shelf life of that media is once compounded.

So it is a supply issue, but it's downstream of the receipt of the supply, and more about the in-process life of something once compounded. You might not want to make media daily for a run that requires media exchanges but make a month's worth of media instead. The question becomes whether that compounded media is stable for the entire duration of its use.

Q Customization or standardization of raw materials: what are the pros and cons of either fundamental approach, and which do you prefer and why?

TW: I'm going to speak mainly to the pros on this one – as someone with a background in procurement and supply chain, of course I'm going to choose

standardization. That doesn't necessarily mean off the shelf or one size fits all, but within your process, I'm a big believer in standardizing wherever possible. It makes forecasting to the vendors simpler. It also makes dual sourcing more attractive for the second source if you have something that can be used across your process – and if it is off the shelf, so to speak, it makes it a lot more attractive for a secondary source because they're not making something specifically for you.

On the other hand, I do understand that there are needs for customization in certain types of packaging, but wherever possible, I would choose standardization.

BL: **I think there is no generalized answer – the answer is highly process-specific, and there are always pros and cons.**

Customized products are easier to use and may reduce labor, but you usually lose flexibility. Typically, you have a sole supplier, and you might be locked in to a particular reagent mixture which you cannot manipulate any more. Moreover, I think you should have a very stable and robust cell process to consider this option. Typically, customized articles are more costly due to additional development costs, and due to smaller batch sizes, so you should also have savings at the other end. The lead times should also be considered, as they may be longer than for off-the-shelf products which are usually always in stock.

It really depends on the maturity of the process and the state of development when you are considering whether customization is applicable or not.

DD: **I agree with Tom that there are a lot of pros to standardization.** If

you are looking at customization versus standardization, the caveats are understanding your requirements. When you say you want to standardize, what is the bandwidth of that? If you're too specific and too stringent, you're going to run into a few issues. One might be cost for your raw materials, and another consideration is the failure rate. Say you're using a material and you want to tighten the specs, and you go from 90% of the lots passing to all of a sudden 60% of the lots passing – this means your supply is diminished.

I think there's got to be a lot of care and understanding of the impact on materials when you standardize, of the stringency you impose, because it does have financial and supply chain effects in terms of availability.

Q Turning to the topic of regulation, what trends do you see evolving in terms of regulatory guidelines that impact the raw materials area? Are there any related national or global initiatives that may help with their ongoing development?

BL: I think the first generally accepted guidance document was the USP <1043>, which is currently being revised for the first time. This document established the idea of risk-based selection of raw materials, which is still today's thinking. The European Pharmacopeia is a bit more recent, with chapter 5.2.12 giving particular attention to biological raw materials. This has gotten binding in a way, in that it is referred to in part 4 GMP for ATMPs. This is another important step.

The first global initiative is from the ISO, which has launched a technical standard (ISO/TS 20399) which is today being transferred into an international standard, to gain more acceptance and to move towards being able to be certified against that.

The last trend I see, which is where we are contributing, is the initiative of the Alliance for Regenerative Medicine, which is trying to achieve a master file reference system for raw materials within the EU like the one that has existed for a long time in the US, the possibility to submit a DMF to the US-FDA and offer the possibility of cross-referencing.

These initiatives are fortunately growing in parallel and show the same thinking: risk assessment, risk mitigation, and particular attention to biological raw materials. This is all good to see – but we're still far from being harmonized globally.

TW: This doesn't speak to trends, but I will say that in previous labs in immature companies, people have sometimes mentioned vendor or brand names as part of raw material regulatory filings. It's not entirely applicable to this question, but this makes dual sourcing or multiple sourcing very tricky. I have found it's much better to be as generic as possible, keeping vendor and brand names out of any filings.

DD: In cell and gene therapy, oftentimes a raw material is a cell product harvested from a patient that then becomes part of the supply chain. And that is regulated: for example in the US 21 CFR 12 71, which addresses the requirements for donor material that are applicable to using that material. For example, if you're going to create an allogeneic cell therapy and you're using cord blood or some healthy donor product, the donor requirements help guide the standards and requirements for that type of raw material. This hasn't come up in our

discussion, but it's incredibly important for autologous and allogeneic cell based therapies where cells from a human are the starting point of the production.

Q When working with a CDMO partner, how can you manage supply risk without being overbearing?

TW: I spoke about this earlier in regards to the COVID-19 coronavirus situation, but I'm a big believer that as a biomanufacturer, someone who owns the IP and who is the ultimate steward for our patients, that you're also stewards of your supply chain. It's your absolute duty to monitor, measure, and mitigate risk, whenever possible.

> "as a biomanufacturer ... It's your absolute duty to monitor, measure, and mitigate risk, whenever possible."
>
> \- Tom Walls

This may feel a bit intrusive to a CDMO. When you get into a contract with a CDMO you are buying their expertise on supply chain management, and some are better than others. But again as a steward you have to monitor that risk and mitigate risk wherever you see it. We've talked about increased inventories – it may be a case of asking the CDMO or their tier 2 vendor to hold more inventories.

It may also mean reaching out directly to the tier 2 vendor to share forecasts across your entire network. We use a number of CDMOs, and some are very good at sharing forecasts while others are not that great. But when we present a forecast to a tier 2 vendor, we're presenting the entire universe of our demand, and that's a very powerful tool. This may involve non-disclosure agreements or confidential disclosure agreements or other types of supply agreements with vendors, because it's not necessarily your purchase order to the vendor, but you are sharing important information.

You don't want to be overbearing and you don't want to step in too much, but you need to understand the inventories of the CDMO, and you may sometimes want to reach back one link, at least, in the supply chain to talk with the vendor to your CDMO.

DD: If I'm going to go to a CDMO and put my production in their hands, and they have to manage supply chain, I'm going to audit them for their QMS system, how they manage supply chains and how they ensure continuity. This is the best way to come to an agreement a priori. That might then affect the supply and/or quality agreements for that CDMO.

I think having a written agreement upfront with a CDMO about obligations and expectations really helps clear the water, so there's not a discussion after the fact about what you thought they were going to do. Doing your due diligence up front is going to mitigate a lot of risk.

Q How are collaborative business models between cell and gene therapy developers and material suppliers evolving? What future trends and developments can we expect to see in this regard?

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DD: We've touched on a lot of these. The collaborative business models we've been working on include things we've been talking about like supply and quality agreements being absolutely essential, probably more essential for sole source providers than if you have multiple choices. And also service agreements with the CDMO outlining the expectations for service.

One possibility is to arrange for dedicated resources for a client. If you have a company and there's a product in high demand, and the CDMO has to make a large number of lots to service clients, you might ask to set up a production unit at the company to service your operation in particular, or ask to have a portion of their capacity dedicated to your efforts. That way you know you're not in a potentially variable position within a bigger queue. You might be able to pay for priority, for example by paying a premium for the service or supply to get a priority on distribution.

Finally, like I said earlier, another option is outright purchase of the supplier. The collaborative business model here is saying to them you're going to run as long as you can, and when you can't anymore, you become us. That's not always available to smaller biotechnology companies, but for larger biotechnology companies and smaller biopharmaceutical companies I think these are realistic emerging trends. We've seen people buy entire CDMOs to ensure their supply chain, so it's already happening now.

BL: As I said earlier, a very simple mode of collaboration is via supply agreement. What I predict is off-the-shelf and customized new raw material formats; in particular those that are adaptable and attachable to a sterile connectable closed system. This can be done in a co-development mode and be very customer specific. I predict co-development agreements for complex raw material products, for example media supplemented, or new innovative primary container systems.

> "Build a trustful partnership by exchanging regular forecasts, and by being prepared in terms of production capacity for future demands."
>
> **- Bernd Leistler**

TW: These are great points by all parties. To circle back, sharing information with those key critical sole vendors, sharing the forecasts, speaking common languages, and understanding their capacities are all crucial steps – and also considering their ability to flex capacities, and what that may cost.

As a start-up industry sometimes we may be a little short-sighted, but it's important to start thinking longer term and get ahead of problems by thinking years into the future, instead of just weeks and months.

What are the key elements of best practice for trouble shooting and securing supply? Particularly as both manufacturing scale and overall demand for raw material increases?

TW: Manage and mitigate risk wherever you see it in your supply chain. Work with vendors and CDMOs to share information. The cheapest way to mitigate risk is to share forecasting information. If you have to buy extra inventory, do it, but understand the dating and stability implications. And where you can, look to dual source.

BL: Build a trustful partnership by exchanging regular forecasts, and by being prepared in terms of production capacity for future demands. I would recommend auditing your suppliers on a regular basis and keep in regular contact. This helps you to understand each other's needs and capacities.

DD: I think that having a dedicated supply chain infrastructure or group at your company is really important. Asking manufacturing or quality to do it by themselves is not going to work, you need a dedicated supply chain group whose job it is to ensure this.

Knowing your book of business and knowing what you need before you go to a supplier is also key. Finally, it's important to be flexible and start early in securing your supply chain. Don't leave things until you're in the middle of production and suddenly start to have supply chain issues. Get all these contracts and agreements and supply chain specifics, such as raw materials specifications, worked out as far ahead of production as possible.

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

INTERVIEW with:
Jason C Lin, Director of Global Supply Chain, FUJIFILM Irvine Scientific

"The situation with the coronavirus ... is an example of a situation both directly and indirectly impacting supply."

Meeting the evolving challenges of media supply to the cell and gene therapy space

Cell & Gene Therapy Insights 2020; 6(2), 275–280
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> "What the supplier wants has to be balanced against what we need as a company in the long term … to ensure quality."

Q What are the major challenges in media production and supply to the cell therapy supply chain?

JCL: Generally the challenges are not directly supply related. In terms of the business aspect, it's more about getting the best value and service from the suppliers. We also want to ensure consistency of supply; sometimes that is related to single sourcing, or potential shortages coming from the market.

The situation with the coronavirus in China is an example of a situation both directly and indirectly impacting supply. Some of the crude or raw material sources are coming from China, in particular the amino acids that go into the products that we produce. On the other hand, we are in position to prepare against this by having robust inventory, and having dual sources with multiple sites capable of producing material globally. It's definitely a headache, but we have a plan to address it on an ongoing basis.

Q How do you manage the disconnect that sometimes exists between media suppliers and the cell therapy industry?

JCL: Suppliers are eager to be part of the process from early on, and they hope to see business growth and be part of continuing collaborations by getting into the R&D phase of the business. But on the commercial side, we would not make a decision too early unless we're able to see the value we hope to achieve from it. For example, say that a supplier wants to be part of the formulation ingredient we produce, we would have to see a demonstrable track record, and ask ourselves if we are aligned in commercial value-adds. Ultimately we need to be seeing quality and consistency from suppliers before we can commit to a partnership.

What the supplier wants has to be balanced against what we need as a company in the long term, because we are in the business to ensure quality, and also the most effective and robust pricing of the products we produce.

Q What are FUJIFILM Irvine Scientific's procedures for the qualification of raw materials such as media?

JCL: The starting point is always to have the right documentation. This could include the Certificate of Analysis, the TSE/BSE statements, which concerns whether the ingredient is animal-derived or not, and any other relevant documentation on the materials side. On the supplier side this is anything about the manufacturing site itself, the production history, and what quality system the suppliers have in place.

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In addition, we ask for samples of new raw materials; typically three separate manufacturing lots, to push through our quality system for testing and evaluation. Finally, a decision would be made on whether to qualify the material for use or not.

What is your approach to ensuring continuity of supply?

“Whether we’re dual sourced or not, the first approach to ensure continuity of supply is to have a robust supply agreement.”

JCL: That’s always a hot topic for supply chains in general. Whether we’re dual sourced or not, the first approach to ensure continuity of supply is to have a robust supply agreement. We have terms and conditions to protect us as the customer, just in case the supplier decides to divest or close the business. This gives us the right to procure materials up to a certain point in time, typically 9 months or a year, until we have a chance to find an alternative source.

With a supply agreement in place, the next thing we would do for a single sourced material is look for alternatives and qualify the new supplier in the portfolio as early on as possible. This can be a challenge sometimes, because there may not be other suitable alternatives in the market for the same type of products – that’s an issue we’re always dealing with.

Lastly, building a relationship with the supplier and R&D teams is crucial. You have to have good relationships with the supplier, so that when you’re in a time of need or there is a global shortage, you are considered an essential customer at the top of the list to have your needs fulfilled.

From an R&D perspective, you can look at the process early on and aim to avoid using a supplier or type of material that is going to put you in a single sourcing situation or a tough supplier situation. This becomes more difficult to address later on in the commercial production phase. It’s easier to make these decisions early as they can have a big impact on your decisions or your business further down the road.

“From an R&D perspective, you can look at the process early on and aim to avoid using a supplier or type of material that is going to put you in a single sourcing situation or a tough supplier situation.”

This is the process we like to follow if we can – it’s not always possible, but our philosophy is always to employ good decision-making from the beginning.

How is demand for media for cell therapy production evolving worldwide, and what are the keys for you in meeting the needs of this global sector?

JCL: The need for cell culture media globally is currently robust, and seeing very positive growth. In addition to this worldwide growth, from my time working in the Asia Pacific market I see the cell therapy industries booming there, too. All the existing industrial cell culture businesses such as Roche, Genentech, Amgen, and so on are continuing to have a high volume of media needs from various suppliers in the market.

What are the future media or critical raw material supply trends you foresee for the cell therapy space? How is FUJIFILM Irvine Scientific mobilizing to prepare for them?

JCL: We plan to focus on the ingredients that are driving the core volume to our business. Meaning that as we get requests for different formulations, whether it's a customer product or our own product, we really have to dig into all the sub-components and determine and rank the risk assessment level for each material, and make preparations for each. As mentioned above, some of these raw materials could be in sole sourcing situations, meaning nobody else in the world makes it. It's important that we manage to ensure supply.

We are confident about continually meeting these challenges from the supply chain perspective through robust management of the supplier, the supply, and also contractual understanding from both sides. And then lastly to have robust inventory, safety stock, and any other value-added activities we can create in the supply chain space. We feel optimistic about our success, both due to our proven track record and our continued efforts to be prepared for what the future brings.

BIO

Jason C Lin

With two decades of experience in global pharmaceutical/biotech material and product supply chain strategic settings, Jason Lin applies his expertise as the Director of Global Supply Chain with FUJIFILM Irvine Scientific. He oversees end-to-end raw materials planning, procurement, and materials management for media products across various business units, as well as drives Cost of Goods reduction actions such as manufacturing dispositions, raw materials/finished goods inventory control, and long-term materials pricing contractual agreements. As FUJIFILM Irvine Scientific expands globally, Jason will oversee the build-up of new manufacturing site supply chain functions, as well as continue to manage global network manufacturing optimization and production allocation for Japan, the USA, and the EU with the goal of meeting customer needs by region with the lowest manufacturing cost and best efficiency. Jason holds a Master of Science degree in Industrial Management and a Bachelor of Science degree in Industrial Engineering, both from Clemson University.

FUJIFILM
Value from Innovation

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

WEBINAR TRANSCRIPT

Ensuring source material consistency and continuity for commercialization of advanced therapies

Dominic Clarke, Christopher Good & Amy Shaw

A critical aspect to ensuring patient access to cell and gene therapies (CGT) and continued growth of the industry is having a proper awareness for managing the source material quality and supply chain continuity. The combination of rapid growth, individual product and process complexity, and limited industry-specific guidance or awareness presents ongoing challenges for transitioning from development to clinical and commercial manufacturing scale. For allogeneic therapies, having access to consistent and reliable donors and high quality, GMP-compliant starting material, coupled with the ability to consistently deliver this clinical source material to the required point of use, will be key to long-term success.

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INTRODUCTION

The cell and gene therapy industry continues to grow and progress at an exciting rate [1]. More and more products are advancing from research and preclinical development into the clinic. However, it is

also very much a pivotal period, as we look to venture to the next phase of industry growth and maturity: as clinical successes translate to commercialization, bioproduction resource demand has the potential to reach unprecedented levels [2].

There are notable aspects to this expected trend. Firstly, it is important to realize that the ever-increasing number of clinical trials that we are so accustomed to seeing remains just a small fraction of the anticipated demand. Secondly, despite the fact that autologous products will continue to build from their early success, it will be allogeneic therapies and products that will really drive future growth and resource demands in the cell therapy arena. (Figure 1)

Over the next 5 years, we could be experiencing a 15–20-fold bioproduction growth in the cell therapy segment. This type of growth requires continued industry collaboration and innovative strategies. A key component in accommodating the forecasted resource demands is developing a reliable and consistent supply of critical raw materials including therapeutic starting materials.

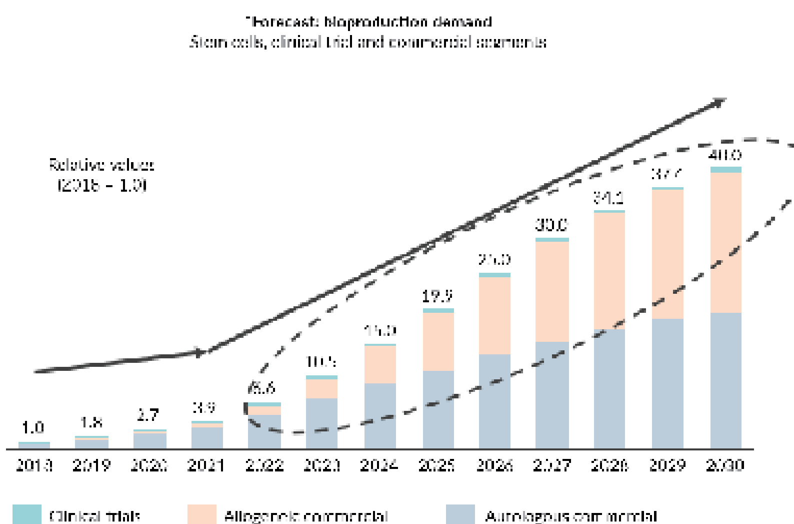
CONTRIBUTING FACTORS TO CONSIDER FOR DONOR-DERIVED STARTING MATERIAL

Donor starting material is traditionally a necessary component for development of both autologous and allogeneic cell therapies. It can be easy to take something complex like cellular starting material and simplify it, especially when the industry progresses through development at such a rapid pace. However, taken from a different perspective, the actual starting material used in downstream processing is a complex combination of different components, including donor management, physical collection, processing steps, and shipping. Understanding and managing each of these contributing factors will help to achieve a more consistent and sustainable source – especially in the context of anticipated industry scale-up demands.

Any cell therapy process typically becomes a balancing act between meeting the immediate, early bioprocess development needs versus the clinical and commercial requirements: short-term versus long-term considerations; highly variable versus highly controlled processes; small-scale versus

FIGURE 1

Growing industry landscape.



Increasing demand for managing the starting material.
*Forecast highly dependent on commercial outcomes of new stem cell products.
Source: Cambridge Biostrategy Associates (Michael Jacobson) and Black Swan analysis.

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large-scale; research use versus full GMP compliance (or feasibility versus safety and consistency). Given this balancing act, it's easy to see why development practices can hide potential quality, sourcing, and logistical challenges that will be faced eventually with the clinical translation of cell and gene therapy products. The same is true for the starting material. Considering donor management as an example, it is critical to factor in the impact of eligibility and recruitment – not only for safety but also availability – on collection and processing needs.

With cellular starting materials, we often think immediately about the variability. Given the nature of these therapies, variability may be a desirable trait at times – when it can be controlled, of course – but quality and compliance become major factors as the therapeutic product progresses into and through the clinic. Finally, shipping (as discussed in greater detail below) is always critical. The inherent stability challenges with cellular products require careful consideration early in development, as they will impact availability. But one must also be aware of a product's packaging and transit needs and ultimately, it's traceability – the assurance that the material will arrive where it needs to be, when it needs to be there, and in the specified condition.

Donor management & eligibility

Donor management is a central aspect to any normal healthy donor-derived starting material. From a regulatory perspective, donor eligibility requirements are very well defined with screening and testing designed to minimize the risk of infectious disease transmission. Box 1 lists the standard tests required, which are generally aligned across the major regulatory bodies (e.g. the US FDA and EMA). Unfortunately, full global regulatory alignment does not currently exist [3,4]. Therefore, care must be taken, especially for material being used for allogeneic purposes and the development of cell banks [5]. Awareness and traceability are always important, particularly when considering how to manage new disease concerns like COVID-19 for example.

Less well defined, but equally critical, are the eligibility or suitability criteria for the specific clinical protocol defined by the sponsor. During development, one may set restrictive limits around age or body mass, for example. Some stringency may be required, but these restrictions can significantly reduce the preferred donor pool resulting in challenges to donor or product access. Therefore, developing and implementing a robust donor strategy with the starting material supplier is needed.

Managing starting material variability

While donor management is a key aspect of both safety and supply continuity, perhaps the most commonly referenced challenge is that of starting material variability. Figure 2 represents over 2,000 leukapheresis collections performed at the HemaCare facility over the past 2 years. These were all collections from normal, healthy donors. Nevertheless, the white blood cell yield from donor to donor ranges from over 30 billion to instances below 5 billion. This same variability is also observed at the cellular level, as the percentages for subsets including $CD3^+$ T cells, NK cells, and B cells demonstrate

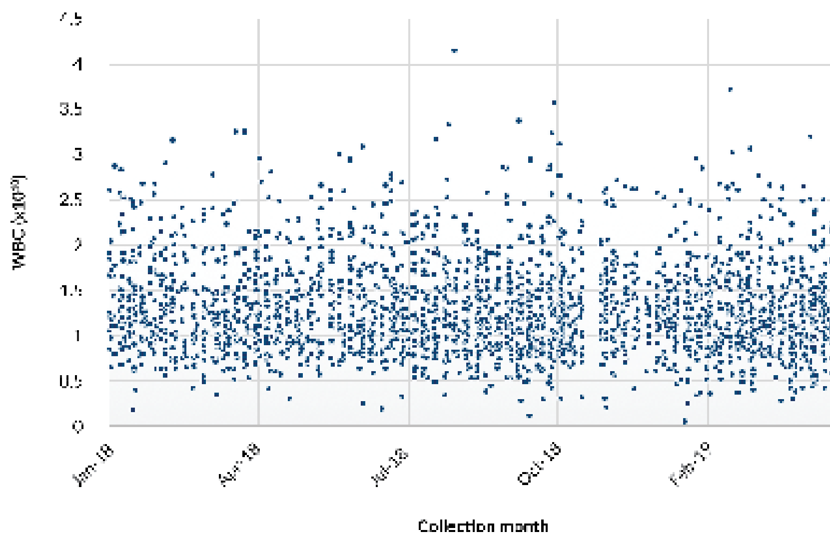
BOX 1

Standard infectious disease testing

- Hepatitis B Core Antibody (Anti-HBc EIA)
- Hepatitis B Surface Antigen (HBsAg EIA)
- Hepatitis C Virus Antibody (Anti-HCV EIA)
- HIV Antibody (HIV 1/2 + O)
- Human T-Lymphotropic Virus Antibody (HTLV-I/II)
- HIV-1/HCV/HBV Nucleic Acid Testing
- WNV Nucleic Acid
- Trypanosoma cruzi Antibody
- Zika Virus by Nucleic Acid Testing

FIGURE 2

Starting material variability.



Intrinsic unpredictability impacts downstream processing and scale-up consistency.
Data represents the common collection and associated cellular composition variability observed.
This data demonstrates importance of developing robust methods to address variability.

significant variation between donors. Understanding this variability can be an important aspect for process development and manufacturing feasibility for autologous therapies, given the wide range in patient-derived material.

For allogeneic therapies where the donor starting material is used for the final product, variability can also be problematic for manufacturing consistency. Regardless of the product type, developing a robust method or strategy to address variability is critical.

Having access to reliable and recallable donors is a highly effective method for addressing starting material variability and developing a robust supply chain. Examining cellular subsets of reliable and recallable donors, reveals an additional key benefit. A certain process may be reliant on having specific biological characteristics, such as a high percentage of CD3 or CD4 positive cells. By harnessing data from repeat donors, developers may obtain the starting material that best fits their manufacturing requirements. This leads to improvements in the management of starting material variability and thus, to an improved process and final product consistency (Figure 3).

Quality/stability

Obtaining the starting material and managing its inherent variability are challenging in their own right, but given the nature of biologicals, a keen awareness of the overall product stability is also necessary. Often in early process development, starting materials are collected and shipped fresh for downstream processing. Fresh, cell-based starting materials have limited shelf-lives. The potential loss in starting material quality and stability over time further exacerbates the variability and can greatly impact process and product consistency due to overall cell loss, functional limitations, or both. Furthermore, the stability limitations require the collection and shipping to occur without delay. This presents a challenge for all involved as delays will inevitably occur, and the level of risk only increases with scale [6].

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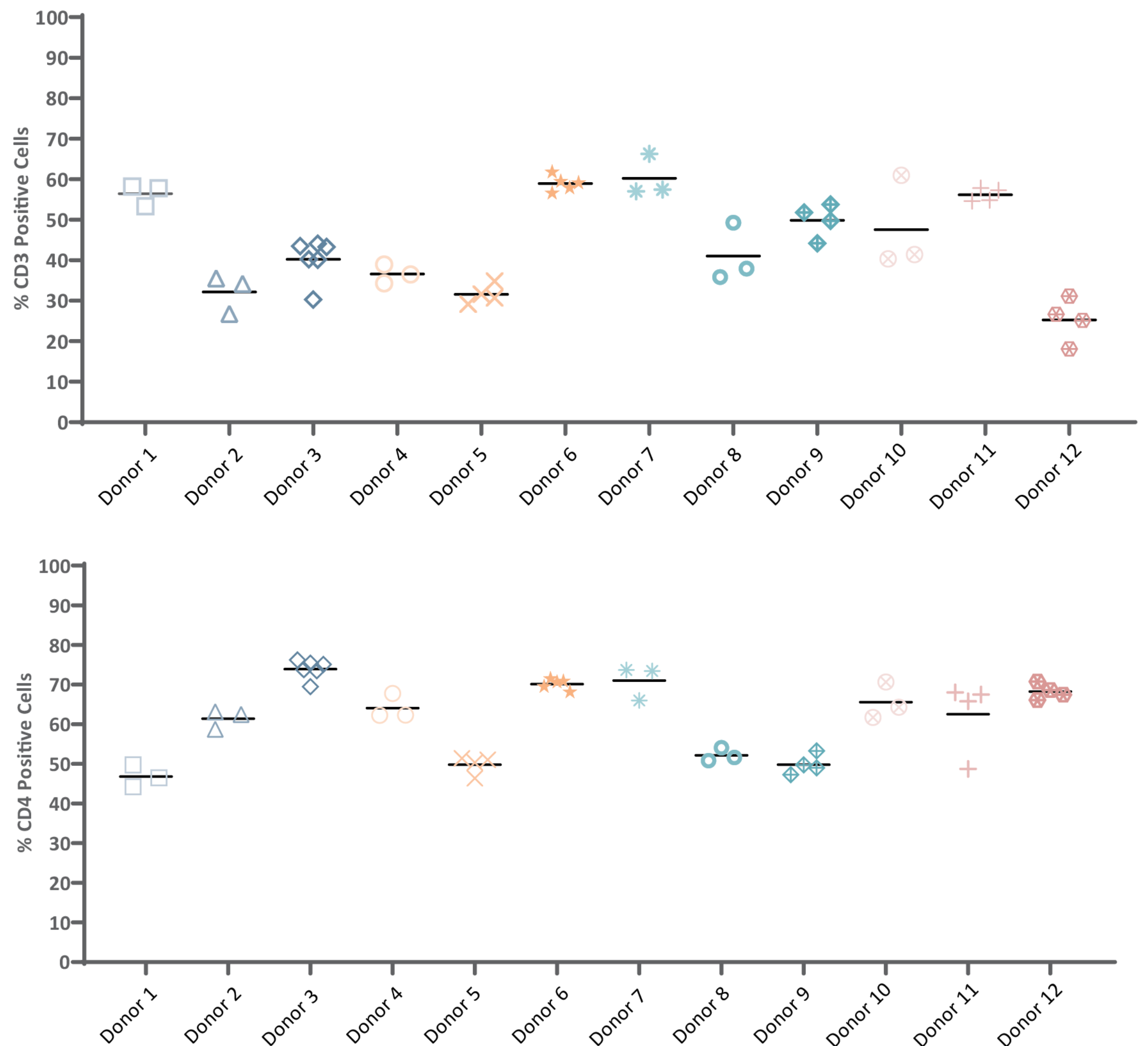
FIGURE 3

Donor management directly impacts product consistency.



Ability to select, qualify, and repeatedly source similar donors in support of target cell subsets.

Optimizing post-collection processing and shipping requirements early in development is necessary to maintain starting material quality, and a clearly defined strategy in this regard will be essential for long-term clinical and commercial efficacy of the final therapeutic product. Cryopreservation of the starting material, whether it is the entire leukapheresis or an isolated sub population of cells, can reduce or eliminate the stability limitations. However, the success of such a strategy is in part dependent on working with a partner that has the resources and capabilities to perform these steps onsite and immediately post-collection, in order to maintain the highest potential quality. The appropriate stability needs to be maintained at scale, so defining an end-to-end starting material strategy will go a long way towards simplifying donor management and collection demands, thus ensuring appropriate staffing and scheduling at both the collection site and the downstream manufacturing site. It is also ideal for all parties to work with a logistics partner that can manage the considerable shipping demands involved in enhancing product consistency and integrity, which range from the pack-out and

BOX 2

Focus on packaging

Not only does packaging physically protect the material from a variety of external factors, it also maintains the correct environment, can facilitate use, and most likely also includes some aspect of recording and/or location device. Various risk factors can affect packaging performance and consistency, from the basic design of the packaging itself to its preparation, handling, adherence to a specified pack-out, and even how the source material itself is provided – for example, warm fresh material that is to be maintained at a refrigerated temperature.

As products move to GMP supply chains, the associated packaging often needs to be demonstrated and documented as appropriate, as part of the developer's GMP obligation. In most cases, a commercially available temperature-controlled package is utilized as the best option. This provides a high performance, secure and cost-effective method of protecting the product during transit, as well as being appropriately scalable. This packaging may contain bespoke elements – for example, product-specific inserts to provide additional protection or aid use – and have completed general qualification and validation by the vendor and service provider. With the move to clinical and commercial products and associated GMP requirements, the drug developer is obligated to identify what qualification and validation work is needed to prove control of the critical aspects of their operation.

Individual products will each likely have a specified stability and associated transport temperature range, as well as a defined primary or secondary packaging type and routing profile. Vendor and service provider validation reports will often be based on generic product loads and temperature ranges. In extreme cases, these may differ greatly from the specific developer's needs based upon expected product load/volume/temperature range. Ultimately, it is the drug developer's responsibility to determine what is acceptable under their GMP obligation and this may require additional specific testing to be completed.

Before completing any specified testing, a detailed protocol should be agreed and approved to make sure that the resulting report is accepted. When designing the protocol, consideration should be given to the expected usage and worst-case-scenario options should be identified and tested. For instance:

- What will be the maximum or minimum product load?
- What is the specific preparation and loading process?
- What potential variables need to be controlled and measured – and what is deemed acceptable?
- What climatic regions and route profiles will be experienced?
- Location of any measuring devices for testing and also for subsequent use
- The potential benefits of Dynamic or extended routing testing vs Static temperature chamber testing

When completing testing, it is vital that documented processes are followed so that a true reflection of expected use is measured. When creating the summary report, any deviations from the protocol must be clearly recorded and documented along with detailed results and measurements taken during the test.

handling of materials for process development, to meeting the critical compliance requirements of the starting materials for clinical and commercial use.

KEYS TO TRANSLATIONAL SUCCESS

As previously mentioned, the starting material needs for development purposes are often not completely aligned with the clinical requirements. To define a strategy for translational success, it is important to consider all aspects of the starting material – including donor management, collection and processing, and shipping – from an early stage of development, especially for allogeneic cell products.

Clinical translation begins with donor management and careful planning around donor eligibility. Knowledge of the intended clinical regions will help ensure that appropriate screening and testing for regulatory compliance is performed prior to collection, and also for any long-term cell banking plans. A further important consideration for consistent starting material access relates to including potentially excessive restrictions, which can result in significant limitations. Access to well characterized, reliable and recallable donors can alleviate some of the risks associated with donor management.

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BOX 3

Case Study: Beam Therapeutics

Beam Therapeutics is developing precision genetic medicines through base editing. Unlike other modalities of genetic engineering, Beam's novel approach to gene editing allows the replacement of single base pairs without initiating double-stranded breaks in DNA; if other gene editing platforms can be considered to act as scissors, Beam's platform is more akin to a pencil and eraser. The double-stranded break mechanism of editing can result in nonhomologous end joining in the DNA strand, which leads to cell disrepair and death. However, Beam's technology allows for a narrow focus on a single base pair, and the ability to change that base pair without disturbing the integrity of the DNA. Beam is applying this technology in a wide array of indications. However, this case study will focus on the company's two leading cell therapy platforms: in the autologous setting for patients with sickle cell disease and beta thalassemia, and in the allogeneic setting for patients with leukemia.

The cell therapy supply chain is a new and unique supply chain that presents its own challenges. For autologous therapies, cells are collected, shipped to manufacturing sites, processed into drug products, and shipped back to the patients. Depending on a given therapy's manufacturing site, cells may need to be shipped globally both from and then back to the patient, leading to the requirement for timely, robust, and traceable logistics.

Overall, the allogeneic therapy supply chain is similar to that of autologous products. Instead of the patient's cells being collected, a healthy volunteer donates cells to be shipped to the manufacturing site. Those cells are processed, and the drug product may then be shipped to multiple patients in need. Although one element of the supply chain is eased through the collection of cells from a single donor, the dependence on a living start material still exists: the quality of this material could be compromised easily by low quality collection, delayed shipment, or reduced viability of the cell product.

Beam's autologous hematological disease programs rely on fresh, mobilized apheresis. While the composition of the leukopak will differ between healthy donors in development and patients during clinical trials, we have identified the dosing regimen required to achieve the starting population needed for development. Beam is leveraging HemaCare's expertise to improve the definition of our collection requirements for our clinical trials.

Meanwhile, to maximize our ability to address the challenges surrounding the shipment of fresh apheresis, we are working with Biocair as our shipping logistics and chain of custody partner for this program. Biocair has reliably delivered these living products across the US without diminishing their quality. We've benefited from the real-time tracking of shipments that Biocair provides. For example: recently, a leukopak was shipped to our contract manufacturing organization. However, after the certificate of analysis was released, we realized the cell count was too low for our intended purposes there. We were able to update the shipping address in real time to ensure the product was delivered to our process development lab instead. Despite this re-routing, Biocair still managed to deliver the leukopak before 9.00 a.m.

Beam has leveraged HemaCare's GMP pipeline for apheresis, identifying donors who met our collection requirements and performed well in our research process, and banking them for manufacturing. The scope of the GMP platform for apheresis collection has been critical to our campaign, allowing us to find strong donors whom we can fully characterize in process development, and then have them recollected in the GMP setting at a later date for manufacturing purposes.

In order to have a large pool of donors to select from, we started with minimum donor criteria. As we received input from clinicians, we then narrowed the donor criteria, which means that consistent screening of our selected donors is required. These requirements of our selected donor pool, along with the ever-present possibility that a donor could be sick or ineligible, or drop out of the donor pool completely, are concerning in the long-term. To overcome this issue, we aim to create a donor pool that is twice the size of that which we anticipate needing. To further secure our supply of starting material, we've built our manufacturing process around frozen apheresis, thus securing the front end of the supply chain and ensuring we can access apheresis at any time from our selected donor bank.

As the cell therapy field continues to grow, we expect regulatory guidance to evolve. We will need to continue to adapt to the input of regulators as this occurs and because of this, we will need to keep strong records of our previous donors. This will in turn allow us to keep our starting material and our lots of already produced, 'on the shelf' drug product compliant with regulation. Collecting leukopak with batch records and well-documented processes will help us as developers to keep the appropriate records of our donor pool, to hopefully keep ahead of growing guidance, and ensure evolving safety measures for our patients.

In cell therapy, the largest source of variation is the donor. Variability in the apheresis collection yields variation in the manufacturing process, which yields variation in the drug product. With allogeneic therapies, we can overcome some of this issue by pre-screening donors against our defined critical quality attributes. Unlike autologous therapies, where manufacturing is at the mercy of the incoming starting material, there's an opportunity to treat apheresis like any other starting material and find the best suited donor for your process. Beam has utilized HemaCare's network of recallable donors to bring in a variety of donors, test them against key process parameters, and assess their overall quality within our system. Once identified, we can request those donors be put on hold for drug product manufacturing.

BOX 3 (CONT.)

Case Study: Beam Therapeutics (Cont.)

We have tested both fresh, and frozen apheresis, and have found that for some key readouts, there is no impact from beginning with frozen material: there is no difference in the starting viability of our incoming cell product, whether it has been shipped fresh or thawed from frozen apheresis. Furthermore, post-processing, we maintain that same high viability regardless of whether the starting material was fresh or frozen. These results, and other critical quality attributes we have determined, led to the implementation frozen starting material for our development and manufacturing processes. The inclusion of frozen apheresis has also allowed us to bank our selected donors and save them for future manufacturing campaigns, further securing our supply chain.

From a development perspective, we believe our best path forward towards enabling the successful manufacture of Beam's cellular therapies relies on building a process around robust starting material. It is in the developer's best interest to define collection procedures and to minimize incoming starting material variation, regardless of whether the therapy is autologous or allogeneic. Particularly in allogeneic therapies, we can build processes around high quality, frozen apheresis to secure the supply chain and allow for preservation of starting material that is specific to our process.

Lastly, Beam feels it is important to take learnings from the industry by using validated shipping methods that preserve temperature-sensitive starting materials and provide chain of custody, so that the manufacturing process has the greatest chance of success. Through these learnings we can guarantee that the best possible drugs are getting to patients in need.

The collection and processing requirements of the starting material for clinical use are critical to all cell and gene therapy products. For any immediate post-collection processing of the starting material, the facilities and processes need to be qualified and validated for GMP-compliant manufacturing. For allogeneic therapies, working with a starting material provider that can perform the GMP-compliant collection and processing onsite eliminates risk and provides greater consistency.

DELIVERING LOGISTICAL CONTINUITY OF SOURCE MATERIALS FOR COMMERCIALIZATION OF ADVANCED THERAPIES

Shipping represents the final piece of the puzzle and although various options exist for managing the shipment of GMP-compliant starting materials, ultimately having a trusted logistics partner is key to success.

It is vitally important to properly consider the impact that logistics may have on the success of each new advanced therapy, particularly as products move from early development firstly to clinical and then to commercial scale. As volumes and scale increase, any weaker points in the supply chain are liable to become more exposed and therefore, an awareness of the impact on the required logistical solutions for the supply of source material is key to allowing the correct planning to be undertaken. This awareness may assist in decisions around the supply of frozen or fresh material, planning the timing and location of donor visits, or even manufacturing scheduling or location.

Maintaining consistency as scale and associated volumes increase is essential. Throughout this transition and on an ongoing basis, risk must be identified and effectively managed. The key areas of risk are likely to evolve throughout this transition, in line with the increase in scale and volume, and it should not be assumed that the same solutions should be rigidly adopted throughout. Some high-level areas that require effective control include traceability, packaging, routing, as well as regulatory and quality assurance.

GDP is the legal standard for logistics companies distributing medicinal products to ensure their safety, quality, efficacy, and traceability throughout the supply chain. GDP controls many areas from Quality Management, Premises & Equipment, Documentation and Record Keeping, through to Change Control, Cold Chain, and Training. Specialist logistics providers should already be operating to GDP as standard, providing reassurance of preparedness for the move away from the development stages. Where non-specialist /

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non-GDP providers have been utilized, significant additional planning and change should be anticipated.

Although a specialist logistics provider should already operate to GDP, some further controls also now need to be considered: GDP sets out the framework and requirements but should be seen as a minimum when moving GMP starting material, as additional control and knowledge are often needed. The differing requirements of individual products can require a tailored approach to effectively manage risk.

As well as effective risk management, there are certain aspects that are key in delivering continuity at any stage. Firstly, a clear understanding of ongoing needs is vital to provide the optimal solution from the outset. This may involve specific product sensitivities, controls or impacts on the donor, medical professionals, or manufacturer. While the core specialist logistical service may provide a 99% solution, the additional 1% tailored to the specific product can make all the difference, from controlling the packaging, through regulatory knowledge and production of associated paperwork, to the retrieval of temperature results. By taking total ownership of the whole process, the logistics provider is not only able to allow you to focus on your core tasks (and not logistics) but also better able to manage the transition from development.

As volumes increase, finding an intuitive solution that is easy to operate at each touchpoint is essential. From collection site to use at the clinic, there is a balance to be found between complexity and risk. Training and control through a quality system with effective CAPA management and suitable quality culture is another key aspect, ensuring that the whole network is working to the same processes. Technology must be utilized correctly as volumes grow, removing risk of human error where possible, but simpler solutions should not always be immediately dismissed.

Ultimately, a logistics provider must be trusted to safely collect, transport, and deliver to the correct place on time. These fundamentals must not be overlooked when managing and planning for increasing scale.

In summary, the importance of considering the impact that logistics will have and how this could influence decisions as scale increases must not be overlooked. Of further importance is ensuring the effective control of risk, bearing in mind that the major risk areas may change and evolve as a product progresses towards commercialization. Maintaining total ownership of processes facilitates management and control by aligning each step from packaging and collection though to final delivery is strongly recommended, as is involving a logistics specialist in order to help navigate and understand the impact of key decisions.

CONCLUSION

To help smooth the transition to commercialization for cell therapy developers, HemaCare and Biocair are pooling knowledge and developing universal solutions for the supply of GMP materials to the point of required use. The aim of this is to:

- Facilitate the move to GMP supply chains
- Provide clear pathways for managing increasing scale
- Limit additional workload of developers through product-specific solution qualification and validation
- Ensure the consistent and timely supply of starting materials throughout the lifecycle of any given product

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AFFILIATIONS

Dominic Clarke
Global Head of Cell Therapy, Hemacare

Christopher Good

Director, Cell & Gene Therapy Logistics, Biocair

Amy Shaw

Lead, Cell Therapies Engineering and Automation Development at Takeda (Formerly: Senior Scientist, Cell Therapy Development, Beam Therapeutics)

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

PODCAST INTERVIEW with:

Hélène Pora, Vice President Technical Communication & Regulatory Strategy, Pall Biotech

"Gene therapy usually addresses small patient populations, so batch sizes tend to be small and relatively few in number. This is a 'sweet spot' for single-use technology."

Maximizing speed to market: flexibility benefits of single-use technology for gene therapy manufacture

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You have long been involved in the development and application of single-use technologies in biological manufacture. Why is this type of innovation so important in the gene therapy sector?

> "Single-use technology allows you to plan and build facilities relatively quickly."

HP: The gene therapy sector is booming. On a daily basis you hear about new manufacturing sites being constructed and new drugs moving along the funnel in terms of clinical phases. Single-use technology is very well matched to this type of application, for several reasons.

Gene therapy usually addresses small patient populations, so batch sizes tend to be small and relatively few in number. This is a 'sweet spot' for single-use technology. In addition, the population the gene therapy segment is addressing is usually lacking any type of medication, which makes speed to market particularly important. Single-use technology allows you to plan and build facilities relatively quickly. It also allows you to retain flexibility until the last moment enabling last minute changes, and permitting a single factory to produce multiple types of gene therapy products with reduced risk of cross-contamination.

What are the key challenges gene therapy manufacturers may face with the design, assembly, and implementation of processes for single-use systems and facilities?

HP: Often the process has been largely developed in universities or hospitals, and not built with GMP-manufacturing- and process scale-up considerations in mind.

The other big issue with such a rapidly growing sector, particularly in the USA, is a lack of experienced workforce. When you are considering how you will implement new processes, you need to think about how it can be done efficiently and reliably, while ensuring regulatory compliance.

How has Pall Biotech sought to alleviate these issues, both collaboratively and in-house?

HP: Let's look at the collaborative aspect first. I mentioned that one of the big issues is scale-up; how are you going to transfer a process which might have been designed in a hospital or university and make it reliable?

That's why we have set up our process development services, which will allow us to industrialize both the development and manufacturing process. We will work with the initial provider or developer of the process to deliver a process that can then be transferred to the people who are going to manufacture it. We do this with tools which enable the scale-up required as the product moves through the clinical phases.

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> “When you are considering how you will implement new processes, you need to think about how it can be done efficiently and reliably, while ensuring regulatory compliance.”

A second aspect is standardization. We are paying a lot of attention to the use of standard solutions and standard design, keeping mindful of the speed of implementation and supply chain aspects. Demand is huge, but the best service we can give to the industry is a standard system with standard technology, in order to ensure robustness and security in the supply chain.

Lastly, it is still very important to keep in mind that the technology needs to be a good fit for the gene therapy sector. That's why we have developed the iCELLis® fixed-bed bioreactor, which has extremely good performance in the production of viral vectors. This type of unique technology is equally important to ensure that the yields required to support the growing market can readily be achieved.

Could you tell us about the Allegro Central Management System (ACMS)?

HP: It's a development which started a few years ago, when we were really starting to grow significantly in single-use systems. We realized that the way we were initially managing our competence was not sustainable, because we were dealing with big Excel spreadsheets and we had data being stored in different places – it was simply becoming too complicated.

We started developing the ACMS with the idea that it would be a web-based system where we would store all of the information related to our components. It has evolved over the years into a very complex but interesting system. We don't just manage all the component information, but also our entire process, including engagement testing, and the enquiry process. We have an elaborate SUS design configurator which allows us to select the information from the components, as well from the junction test, ensuring we can make fit for purpose designs.

It's an incredible system – and our end users are highly appreciative of it.

Could you walk us through how the ACMS works in practice in a single-use assembly project, highlighting the specific supply chain benefits its capabilities can bring?

HP: We store all project details in the ACMS. To some extent it's very close to what the BioPhorum Operations Group (BPOG) Single-Use Systems User Requirements Specification (URS) is requesting, so we will have all the details we need related to the URS.

Next, we move to the design phase: we take the input from the URS and put it in the configurator. For example; temperature and pressure. As we design the system using the configurator, the

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configurator will only allow us to select components, which can operate within the design parameters. In addition, it will only allow us to use components where the engagement testing has been validated. However, there may be a case where we have a new application, and we have to assemble two components where the junction test has not yet been validated. In this case, the system will trigger us to run a new validation related to that junction test, and the design cannot be finalized and approved until the engagement testing has been fully validated.

> "...the best service we can give to the industry is a standard system with standard technology, in order to ensure robustness and security..."

As we are designing systems, for each component we pick we consider the lead time, the impact on cost and so on, so you see how the design is linked to supply chain issues. This allows ACMS to direct the user to what we call the preferred component, meaning the component is held in stock and has the strongest level of validation data.

How does the ACMS support standardization?

HP: The ACMS acts as a library of designs. It contains not only all the customized designs we have generated for our end users, but also all our standard designs. We have also built other tools into ACMS, including things like electronic signature software, such that the approval of designs is now handled electronically without having a hand-off using email. Everything we are doing within ACMS is really about using standard components, using standard process flows, and ultimately streamlining the whole business process.

Why are the ACMS's capabilities relating to training facilitation so important for the gene therapy space?

HP: As I mentioned above, the lack of a robustly-educated workforce in this sector makes training crucial. If you are using a standard solution, you can also use standard assets for training. In particular there are web-based training assets such as training videos, e-training courses, e-knowledge checks and so on. All of these tools, which may already exist with the supplier, can be prepared up front and be implemented as you are validating the manufacturing site. This means that the end user doesn't need to create their own training assets, and can instead leverage the experience and prior knowledge of the supplier.

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You're also currently involved in developing a regulatory portal – how will this further support end users?

HP: We are involved as a company in an industry working group on this topic, and the issue of how to provide transparency to detailed information on all of the components. This is something, which comes up on a daily basis. But here is the problem. we are managing thousands of components in order to build single-use systems.

The only way to be capable of addressing market demand is to build a regulatory portal, through which users can access information pertaining to these components. The aim of the regulatory portal is to address this requirement from the end users, provide transparency, and to be capable of supporting them on a 24/7 basis.

How and where do you expect to see further innovation in single-use technology development bringing benefits to gene therapy manufacture in the future?

HP: As discussed, more standard solutions is an obvious one. But the other thing to consider when creating reliable technology is automation. We all know that operator errors are often a major issue in manufacturing. I expect we will see the development of systems capable of being run in an automated way, reducing operator-dependence.

Another thing we have must always bear in mind is that whatever technology you're developing, it needs to be fully scalable. This is especially important for gene therapy. At the moment we're looking at the larger indications for gene therapy, but there will come a time when we tackle the market of personalized medicine, and this may require further miniaturization.

What will be the next steps for both Pall Biotech and the gene therapy sector as the development and integration of Industry 4.0 tools and practices continues?

HP: We will continue to develop and extend ACMS and will be looking at tools that can be used to improve facility design – such as virtual and augmented reality. We are using the Hakobio platform internally, and seeing how this can help beyond simply the facility design stage. Through the use of twin facilities, you can allow the end user to navigate in the plant before the plant is even constructed, allowing you to gain time ahead of implementation.

Training and capacity support can also benefit from the use of digital tools such as augmented reality. As we develop these individual platforms, we need to consider communication between the different tools and platforms the customer is using. Interlinked communication in digital systems will be very important going forward.

"Interlinked communication in digital systems will be very important going forward."

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BIO

Hélène Pora

Dr Hélène Pora is Vice President Technical Communication & Regulatory Strategy within Pall Biotech, where she leads technical training and regulatory support improvements. Hélène has been instrumental in the development of Pall single-use technologies for the last 20 years, while getting heavily involved in manufacturing, quality and regulatory aspects. Hélène has over 30 years of experience working for the biopharmaceutical industry, the last 29 years within Pall Corporation. She speaks regularly at conferences about single use technology with a strong focus on validation and overall process integration aspects. She is also involved in a different industry interested group with a strong focus on BPOG, and she sits on the board of BPSA.

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

REGULATORY INSIGHT

Regulatory FAQs and common concerns for cell and gene therapy raw and starting materials

Kasey Kime, Jerrod Denham & Christopher Bravery

In cell and gene therapy, materials matter. However, misconceptions abound, exacerbating a lack of harmonization and standardization in key areas. For example, uncertainty around quality grades at the various stages of R&D is commonplace, and everyday terms are frequently misinterpreted or misunderstood, with potentially damaging ramifications for advanced therapy development, manufacturing and commercialization. Here, we aim to debunk some popular myths, provide practical guidance based upon long experience in the field, and clarify key regulatory considerations and requirements across the cell and gene therapy raw and starting materials area.

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BASIC DEFINITIONS

Beginning with definitions of some important common terms that are used for regulatory submissions internationally, the following all come from ICH guidelines:

- Raw materials are described as components or reagents used during the manufacture of a therapeutic product;
- Source or starting materials are raw materials, intermediates, or active substances that are incorporated as a significant structural fragment into the structure of the Active Pharmaceutical Ingredient (API);
- An excipient is an ingredient added intentionally to the drug substance which

should not have pharmacological properties in the quantity used. In other words, an excipient is everything that is used in the final formulation of the therapeutic product except for the active substance and the labelled container closure.

A review article published last year in the *New England Journal of Medicine* provides a high-level example of how this terminology is used in practice [1]. It concerns an *ex vivo* gene therapy product and its manufacture. On day 0, cells are harvested from mobilized peripheral blood using apheresis. $CD34^+$ hematopoietic stem cells are isolated from the apheresis unit using an antibody conjugated to a dextran-coated iron bead and an instrument that comes equipped with a magnet. The isolated cells are cultured overnight in growth medium, supplemented with cytokines, and after overnight culture the cells are transduced with the viral vector that inserts the gene into the DNA of the cells. Following this, the cells are again cultured overnight. On the last day of processing, the transduced cells are harvested, washed, formulated, filled, finished, and cryopreserved.

In this example, the source materials for this product are the apheresis unit and the viral vector to produce the final product. Examples of raw materials are the cell culture medium, cytokine supplements, or even the transduction reagent used. Excipients are the reagents used to formulate the final therapeutic product prior to filling into the final container closure. In this case, this is a bag that is then cryopreserved as the final drug product before being thawed and administered to the patient.

TROUBLESHOOTING MISUSE OF TERMINOLOGY AND ITS REPERCUSSIONS

Firstly, given the differences in basic terms utilized in different regulatory jurisdictions ('ancillary materials' according to the USP, versus 'raw materials' elsewhere, for instance), and the regularity with which internal company-specific terms and acronyms routinely make their way into dossiers for regulatory submission, it is recommended that ICH terminology should be used wherever possible. Broadly speaking, if a cell or gene therapy developer uses the language of the regulators as much as they can, it will facilitate assessment.

A number of specific terms are commonly applied to raw materials inaccurately. One of the chief offenders from the regulatory point of view is "GMP grade". In fact, GMP isn't a grade, it's a quality system (or more accurately, part of a quality system – Good Manufacturing Practices). Suggesting that GMP is a grade is an oxymoron, because a grade is a set of test methods and acceptance criteria that fully characterize the material, i.e. a specification.

There are neither general nor legal requirements in either the EU or the US for raw materials to be manufactured to GMP. The most that can be expected is that they are manufactured to the principles of GMP, because no regulatory agency has the legal remit to inspect a raw materials manufacturer. They may inspect a manufacturer for other reasons – because they are producing licensed materials on the same premises, for instance – but they won't look specifically at the details for other materials manufactured.

It is up to the individual cell or gene therapy developer as to whether they choose to take a risk-based approach to this issue. However, there are a number of reasons why one might want to have a quality system in place for these types of products in particular. For example, there may be a greater need to ensure the traceability of materials that come into contact with the cells or viral particles. Additionally, one may also want assurances regarding material quality. In this instance, GMP does not necessarily need to be the quality system in question. A preferable approach is to consider the nature of the given material and its use, and then consider what level of quality system is adequate for it. This is the typical approach taken by pharma regarding excipients, which are arguably of far

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greater concern because they are administered to humans.

The origin of the raw material has an impact on safety, but its complexity effects how quality is actually defined. The more complex a material is, the greater the need for a robust quality system (i.e. to follow the principles of GMP).

One further example of a potentially misleading term applied to raw materials is "chemically-defined". Taken at face value, this may mean the material is purely a mixture of small molecule chemicals. However, some include highly purified and homogeneous recombinant proteins in this definition, whereas proteins purified from natural sources such as animals, humans, and plants are excluded due to their natural heterogeneity. This is confusing because all biological materials, including all proteins, are inherently heterogenous. Indeed, if one considers the number of potential post-translational modifications for a glycoprotein such as a monoclonal antibody, there is the theoretical possibility of up to 108 different forms of the same protein in a protein mixture. Of course, in practice, the heterogeneity would not be so high because the method of purification used should reduce it, as would other methods that we use during the preparation. Nonetheless, the point is made that both highly purified, homogeneous recombinant proteins and naturally-occurring proteins are isolated by a purification system of some sort, and so it is difficult to make the argument that one is more or less heterogenous than the other. It would very much depend on how each protein was prepared.

Therefore, it is highly questionable whether "chemically-defined" is a particularly useful definition. Equally, there is no particularly compelling reason to use recombinant serum albumin, for instance, over naturally isolated human serum albumin. (Recombinant human serum albumin may be slightly safer as long as suitable viral reduction elimination steps are included).

Actual chemical raw materials have the advantage of fully defined structure and quality. For example, there is a pharmacopeia monograph that fully defines the quality of dimethyl sulfoxide (DMSO). This greatly simplifies the market authorization dossier as it becomes a case of simply citing that the material complies with this monograph. In most case, the monograph will provide compendial test methods, meaning what might be considered the minimum requirement of an identity test is de facto validated. There may be no need to provide further information about the test method or its validation, nor to name the material supplier. It may also be possible to change one supplier for another (of a compendial grade of the given material) without the need to demonstrate comparability or seek regulatory approval. If a chemical molecule isn't covered by a monograph, one may still be able to follow the same approach, to a degree. However, the therapy developer will have to fully define the chemical grade themselves (including potentially developing the requisite test methods) and to justify the particular quality of material chosen.

In contrast, biologically sourced raw materials (or very highly complex chemical materials, such as polymers) will not have a full monograph. They cannot be fully-defined due to their natural heterogeneity, and because the quality (e.g. impurities) is dependent on the manufacturing process used for their preparation, which cannot be envisaged by any pharmacopeia. It is important to bear in mind that pharmacopeia general monographs for materials such as fetal bovine serum do not provide a complete specification. While it is certainly desirable for one's supplier to comply with the monograph, additional testing will be required, particularly to measure the biological activity of the given raw material. The developer may find themselves needing to develop and validate this test, and having to provide all the details for the dossier complete with a full (and fully-justified) specification that includes the tests that the supplier carries out. The developer may also need to assign a shelf-life for the material, which could involve undertaking additional stability studies.

The upshot is that because complex biological materials will vary depending on who manufactures them, one cannot simply substitute for another source without undertaking comparability studies. The extent of those data very much depends on where (in the process) and why the material is used. Additionally, it will be necessary over the course of the product development process to try to understand the critical material attributes (CMA) of any complex materials (not to be confused with critical quality attributes, which relate to the active substance). Batch-to-batch variability will need to be studied and managed, and in some cases, it may be necessary to work with the supplier to improve the material quality if it is insufficient.

Generally speaking, raw materials are not regulated products. Regulatory guidance suggest materials should be made according to GMP, but the type of quality management system is not specified further than an "appropriate quality management system". Therefore, it is important to consider the basis of a supplier's quality claim. A good first step towards understand a supplier's GMP claim is to request evidence of independent quality management systems certification, such as an ISO certificate. This will clarify what particular standard the supplier is certified to, and whether that aligns with the therapy developer's expectations of GMP for raw materials used in cell and gene therapy. To further guard against any misconceptions, it is also recommended to confirm the supplier's GMP claim – for example, by conducting an onsite audit of the supplier to make certain that true alignment of GMP levels or principles exists.

WHERE DO YOUR MATERIALS COME FROM AND WHY IS IT IMPORTANT TO KNOW?

Understanding where materials come from is vital for knowing the right questions to ask of a supplier.

There are numerous examples from the wider world of the unsuspected presence of materials of animal origin in everyday items – new plastic banknotes (and other plastic objects) containing animal fats, for instance. DMSO is a by-product of the paper industry. Various amino acids are isolated from sources such as hair, feathers, hides or skins, and even basal culture media is likely to contain amino acids. Plant extracts may seem harmless, but they may have been grown in locations where they are open to interaction with rodents or birds. Whilst there may well be nothing inherently risky about these materials, providing they are correctly prepared, it is nevertheless important to know where they come from in order to know which questions to ask.

One of the key questions relating to source is whether or not it is acceptable to use raw materials that may contain human/animal origin components. Firstly, it is important to note that there are several levels of animal origin, including at the product level, which means there might be animal materials present within the raw material, and at the production level, which means animal/human origin materials might have been used during the manufacturing process (but not intended to be present in the final raw material). In some cases, it may be necessary to go further back. While the general advice is to avoid human/animal origin components when possible, it is not always possible to do so. Therefore, a risk-based approach to the selection of raw materials is critically important. For one thing, viral reduction/elimination steps cannot be applied to cell and gene therapy products, making it absolutely essential to mitigate risk as far as possible and identify any human/animal-derived materials.

If a raw material composition or manufacturing process does utilize human/animal origin materials, items to consider include:

- Country of origin. (This is important to consider for ruminant-derived components due to transmissible spongiform encephalopathies risk in certain countries, as well as for some viral and parasitic disease risks related to human blood-derived materials);

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- Whether viral inactivation is feasible for the given material or process;
- Material grade (quality);
- Where in the process the material is being used (e.g. in upstream or downstream processing);
- Whether a lower risk option is a possibility (e.g. could a biologically derived protein be replaced by one from a recombinant source; and would that be preferable, e.g. viral risk, performance?);
- Available supplier traceability and testing documentation to help support risk assessment.

However, while it may seem a very straightforward decision to switch from a human/animal-derived to a human/animal-free material, there are a number of potential issues to consider.

For example, manufacturers of mesenchymal stromal cells (MSC) have been keen to move away from fetal bovine serum (FBS), and some have begun to consider human platelet lysate. From a viral safety perspective, they both pose a risk and arguably, FBS may actually pose a lower viral risk because the viruses are animal not human. Furthermore, in some jurisdictions such as the EU, it is basically mandatory to irradiate FBS, whereas many sources of human platelet lysate have no viral inaction steps. (FDA guidance and the USP also suggest that irradiating FBS is desirable, although recent experiences suggest that this is not enforced by the FDA to the same extent that it is in the EU – a point of regulatory disharmony which can lead to an unwanted requirement for unnecessary comparability work for some US developers). A further consideration for using human platelet lysate is that it is preferable to use pooled platelets rather than individual platelets to reduce batch to batch variability. However, this brings with it the question of how many platelets to pool together from how many donors? (The Paul-Ehrlich-Institut in Germany suggests it should be fewer than 16 [2], unless a viral reduction step is to be implemented, but even this number might be considered risky with market authorization in mind).

Pooled human AB serum provides an example of the importance of knowing about the preparation of a material. It is pooled from multiple donors and not usually subjected to viral reduction elimination steps in its manufacture, meaning it will also likely need to be irradiated, or similar. Furthermore, one must consider that the human serum may have been made from plasma, which necessitates use of an anticoagulant. There have been recent examples of (non-medicinal grade) heparin being used as the anticoagulant in this application – a material derived from pig gut.

The key lesson here, in addition to knowing a material's source, is to ensure any likely material changes are identified and made as early in development as possible.

RAW MATERIALS QUALIFICATION & CERTIFICATION

When it comes to the testing of raw materials, ultimately the user is responsible for the quality of the materials used in their process, but they need to work in cooperation with the supplier to achieve this. An end user may choose to accept the supplier's Certificate of Analysis (COA), if the raw material is fully characterized and the COA is sufficiently detailed. However, if the end user is qualifying a material intended for research use, they may need to perform additional quality control (QC) testing to determine suitability, and if the material is considered suitable, may also need to implement some routine testing.

For biological raw materials, sterility, residual host cell DNA, endotoxin, mycoplasma, and 9 CFR–compliant, species-specific adventitious agent testing may all be recommended.

We have already established the importance of traceability and regulatory documentation to support raw material risk assessment. Some common examples of key documents

for risk assessment include the COA, Certificate of Origin (CO), Material Safety Data Sheet (MSDS), Certificates of Compliance (if available), and whether or not the supplier provides access to regulatory support files or master files. Some suppliers may offer master files, which are confidential documents filed directly with the regulatory agency. However, not all regions will have the ability for suppliers to submit raw material master files, as master files are not available for the end user to review. In those situations, suppliers may offer regulatory support files, often under a Confidential Disclosure Agreement (CDA). Therefore, the regulatory support mechanism will depend on the level of propriety information and the region in which the supplier is operating.

A raw material supplier can significantly reduce the end user's qualification burden by designing highly characterized products, meeting the various pharmacopeia requirements, CMC guidelines, and ISO requirements as applicable.

RAW MATERIAL RISK ASSESSMENT FROM ANY EARLY STAGE

Acting early and decisively is key when evaluating raw material risk, with the ultimate goal being development of a material qualification program. The purpose of this type of program is to establish the source, identity, purity, biological safety, and overall suitability of a specific raw material. As part of this qualification program, a structured risk assessment strategy should be employed to evaluate overall safety risk of using the raw material in a given manufacturing process.

A failure modes and effect analysis (FMEA) approach considers severity, probability, and detection of failure related to a raw material. This allows for prioritization of what and when to mitigate. This is often useful, as one can evaluate risk pre- versus post-implementation of any mitigations that have been put in place, thus showing how overall risk will be reduced once these mitigations or hazard controls have been implemented

Regarding the identification of worst-case residual levels related to a certain raw material, it is often possible to gauge these early in product development by initially using worst-case estimations of process related residuals (process-related impurities) that are essentially calculated using simple wash-out numbers. However, the need to formally characterize through testing (depending on the nature of the raw material used) must be kept in mind, even for early-phase products. For example, a gene editing step may involve raw materials that could have a significant effect on the final product even at very low levels.

Depending on the raw material, mitigations might include the therapy developer's raw material process intermediates, or final drug product specifications and testing, as well as any material supplier information: for example, the supplier's production processes, their own specifications and testing, quality systems, or overall policies. Within the US, while some groups do still use USP-1043, which does have information related to materials for cell and gene therapy products, this is a general guide only (not a monograph, just guidance).

While many developers tend to focus on safety risks, it is important not to forget about business and supply chain risk. Changes to raw materials are generally easier to implement earlier in development, in part because the extent of evidence for comparability is lower earlier in development.

Last, but not least, it is necessary to communicate clearly and frequently with the raw material supplier from the earliest stages of developing of a material qualification program.

SOURCE OR STARTING MATERIAL TESTING

For source/starting materials, a general rule of thumb is that for straightforward cases

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- for example, an apheresis unit for autologous collection – it is simply a case of looking up the regulations. With respect to donor cells, two key regulations that describe the requirements are 21 CFR Part 1271 from the US FDA, and the European Tissues and Cells Directive (2004/23/EC). Both of these texts outline the key requirements for controlling donor cellular materials and include information such as procurement, donor eligibility, screening, processes, and other requirements that are necessary to control donor cellular material. However, for a source material such as an established human embryonic stem cell (hESC) line, which is used as the starting material for a product that is going to be used to treat many patients, it is often more complicated. For this type of more complex case, additional testing may be required that can often exceed what is considered standard for typical biologics cell substrates.

Turning to viral vectors, many groups are using the FDA guidance for gene therapies. Key messages from this guidance include the importance of an understanding of the impurity profile of your viral vector, and the need to characterize the biological activity early. It is also important to note that viral vectors have their own starting material, including both plasmid DNA and the cell line or cell bank that is used to generate the viral vector. For plasmids, it is recommended to avoid beta-lactam antibiotic resistance even early in development. (The majority of groups today have either already transitioned or are going to transition to kanamycin). Furthermore, testing of the bacterial bank should not be overlooked. Finally, as characterization is key, one must have complete understanding of the identity of the plasmids as well as the viral vector. Regarding cell line and cell banks, a good understanding of the cell line's history is important, but so is a strong focus on viral safety, as this information is critical even for early stage programs.

Many cell lines in use today were derived prior to establishment of the current regulations. (In the US FDA example, that is prior to 2005 when 21 CFR Part 1271 was established). Consequently, not all donor eligibility or testing was performed according to the formal regulations that are in place now. In addition, because of the nature of the material that is used to derive hESC lines, the donors are often not tested exactly according to the established donor eligibility or screening regulations, because that is not required for the donors' own IVF needs. This issue typically only comes into play once the parents decide to donate the embryo for research purposes. As a result, in these more complex cases, it is vital to establish a strategy that allows for additional testing on the back end to make up for any tests that may be excluded upstream.

Additionally, there are difficulties when one begins to delve into the fine details around the testing done on such cell banks. For example, on one level, there is an agreed testing regime across ICH regions. However, supplementary to this are the various texts from individual regional and national jurisdictions, some of which may be guidelines from the regulatory agencies, while others could be texts within pharmacopeia. It is easy to assume that they all add up to the same thing (and to some extent, this is true), but careful study reveals many discrepancies between the various texts. For example, US FDA guidance relating to *in vivo* testing in suckling mice states a preference for 28 days, whereas the EU is happy with 14 days. Similar differences arise around the *in vitro* indicator cell lines. To some degree if you're working globally you may have to test to the highest standard. Both US and European agencies state they are happy for cell and gene therapy developers to suggest non-*in vivo* cell bank testing, despite the fact that most existing guidelines for what does need to be included feature *in vivo* testing. Although it may seem a somewhat difficult conversation to have with the regulator, it is nonetheless encouraged.

In between the two extremes of autologous cell therapy and cell therapy based on pluripotent cells, lie allogeneic cell products.

Some are much nearer to autologous, in that each allogeneic donor can only be manufactured into a few doses. Others can be used to prepare thousands or hundreds of thousands of doses. This raises the question, is it really necessary to do full ICH testing of all allogeneic banks? There is arguably room for maneuver here at the present time. The degree of risk carried by the allogeneic cell therapy in question, which may be defined by the number of patients who will be treated by the given cell donor, should dictate whereabouts on the spectrum one should aim for: from ICH-level full testing through to very minimal autologous therapy testing. Of course, the eventual degree of testing required will be the result of case-by-case negotiations with the agencies.

RAW MATERIAL SUPPLIER SELECTION & PARTNERING STRATEGY

It is important for an end user to calculate their likely future demand and select an experienced raw materials supplier that can scale its manufacturing to meet it. Security of supply is also desirable, as is the ability to customize product configurations and testing based on an individual end user's manufacturing needs - for example, the ability to adjust pack sizes of certain buffers or reagents, or to conduct additional characterization testing on a custom basis. In order to help facilitate the end user's risk assessment and set them up for clinical manufacturing success, securing a supplier offering highly characterized raw materials with quality manufacturing evidenced by independent quality management system certification is optimal.

The supplier-end user relationship is also key. There will be numerous instances where it is critical for the end user to work closely with their supplier – for instance, when requiring the supplier's support regarding detailed raw material traceability questions.

PARTING ADVICE

Kasey Kime

I would reiterate the importance of taking your time, and really focusing on your raw materials risk assessment upfront. This will save a huge amount of time and energy later, and help you avoid any kind of clinical hold due to an inadequate raw material safety issue. And when possible, choose highly characterized raw materials with strong evidence of GMP manufacture, which are specifically designed for use in cell and gene therapy manufacturing.

Jerrod Denham

Even for the most complex raw materials and reagents, try to keep the description, explanation and analysis as simple as possible, especially initially. It makes it far easier to provide that information to the regulators and they can then get a hold of what is really important. As you then advance further and further, develop and enhance your programs – both as the reviewers deem necessary, but actually more importantly, as you gain a greater understanding of what you're trying to do with regard to manufacturing and controlling your product.

Christopher Bravery

When you write a document and then you re-read what you've written, you find some mistakes. If you hand it to someone else when you think it's perfect, they immediately spot typographical errors, missing punctuation, spelling errors, almost immediately. It's the first thing they see. The same is true of your dossier, and the same will be true of your risk assessments. Make sure you've got a diverse array of people looking at these documents, including some people from outside of the group developing the product. That final point is really key - one of the things with risk assessments is it's very easy to talk yourself out of risks. "Oh no, that will be fine because..." Get a third party to have a look as well, and make sure you've thought of everything.

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AFFILIATIONS

Kasey Kime
Thermo Fisher Scientific

Jerrod Denham
Dark Horse Consulting

Christopher Bravery
Consulting on Advanced Biologicals Ltd

VIEW RESOURCES

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Regulatory FAQs and common concerns for cell & gene therapy raw & starting materials: **Dos & Don'ts**

DO look at all the supplier documentation that comes with the product

Compare it to the regulatory guidance to check if there are any gaps, or there is any further evidence you might need from your supplier. For example, the supplier may mention that a product has been virally inactivated, but you might need evidence of that viral inactivation report.

DON'T take product marketing claims for granted

For example, if a supplier claims animal origin freedom, research and verify that claim before choosing a raw material. Investigate multiple levels of processing when assessing risk associated with the use of a material of animal origin.

DO start doing material qualification and risk assessments early in development

Ideally start before clinical manufacturing. Determine a phase appropriate strategy that allows you to start working through mitigations for key materials. This will allow you to show reduced risk in your supply chain going into your clinical process.

DO educate yourself about where materials come from and the true impact of that origin

For example, plant origin may not be 'harmless' compared with animal origin when you consider the conditions under which the plant was produced.

DON'T use internal company jargon/terminology in your dossiers - use the language of the regulators

DO get a third party to review your dossier before submitting it

This should preferably be someone from outside of the core project team.

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

EXPERT INSIGHT

Developing and implementing a supply chain management system for cellular therapy programs

David L DiGiusto, Rakib Ouro-Djobo & Uzair Rajput

The past few years have seen explosive growth in the development of cell and gene therapy drug candidates for oncology and genetic diseases. There are currently 17 approved Cell and Gene therapy products as listed by the US FDA (FDA Approved Cellular and Gene Therapy Products). Current and pending approvals of immune cell products in particular (CAR-T, Dendritic, hematopoietic stem cells) have driven substantial demand for increased cell manufacturing technologies and capacity. Additional advancements in iPSC-derived cell-based therapeutics (nerve, bone, skin, cartilage, bladder, cardiac, liver tissue repair and regeneration) are also driving the development of cell manufacturing technologies. The combined growth and demand for increased production capacity has led directly to an increased need for raw materials, facilities and services. The raw material and product supply chain is a critical element of a manufacturing program for cell therapies. The development and implementation of a robust supply chain management system (SCMS) is required for the successful development of any cell therapy platform. An SCMS is the collection of policies, procedures and tools used by manufacturers to define, control and document the flow of materials into and out of manufacturing campaigns. The main purpose of SCMS is to ensure the provision of an uninterrupted supply of clinical materials that meets all regulatory requirements as per the Code of Federal Regulations 21CFR§210,211 Good Manufacturing Practices (GMP). The SCMS must include identification and specifications for raw materials as well as sourcing and qualification of all providers of raw materials and services. It, also, must provide for documentation on procurement, shipping, holding, testing and product distribution with traceability throughout the process and tracking of process intermediates and final drug product. The system must reliably capture and report out supply chain data in a manner that supports continuous cell manufacturing and future process planning and

optimization (e.g., materials mass balance). In this piece we highlight the major components of a successful SCMS and give examples of approaches for supply chain management that help to facilitate control and compliance, reduce risk and ensure the continuity of clinical materials production.

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SUPPLY CHAIN MANAGEMENT SYSTEMS

Source materials development

Tools and techniques developed for pharmaceutical supply chain management can be applied to cell-based therapeutics for the management of much of the raw materials supply chain. However, cell therapies are 'living drugs' which present a few unique supply chain challenges. For example, cell-based therapy products are often manufactured using primary tissue isolates either from the intended patient (autologous) [1–3] or a third party (allogeneic) source [4–6]. Individual primary tissue isolates (e.g., apheresis products) have significant differences in composition and quality and have been shown to be the greatest source of variability in cell manufacturing process with impact on product quality and yield [1,7]. Additionally, autologous cell therapy source materials must be carefully tracked from collection, through manufacturing and back to the correct patient at infusion (so-called 'vein to vein'). Patient-specific supply chain tracking is unique to cell therapies and not addressed in traditional pharmaceutical supply chain models. Finally, certain cell therapy products are infused immediately following manufacturing (without cryopreservation) and thus have very limited 'shelf life'. These products cannot be 'held in inventory' prior to use and require rapid controlled disposition and distribution. Thus, unlike traditional pharmaceutical products, cell therapy supply chain management will include real time tracking and distribution that ensures product integrity and delivery to the intended recipient. Collectively, these challenges must be addressed for each cell therapy product through development of critical quality attributes that ensure the suitability of products for clinical use.

Most cell therapy drug candidates are first identified in an academic laboratory or other research setting and typically employ research grade reagents and supplies. All raw and source materials used in the manufacturing of drugs (cells) intended for clinical use must meet specific quality and safety standards that often exceed those of research reagents. Therefore, a first step in cell product development is the identification of suitable sources of required raw materials and qualified vendors of those materials. A bill of materials (BOM) is a description of all raw materials, supplies, vendors, quality specifications and testing schedules and is used to catalogue the essential components of a manufacturing process. The BOM includes all the information collected for specific materials and vendors and is used as a 'procurement shopping list'.

The initial focus during BOM development is to identify and define the attributes of a safe and reliable source of raw materials (Raw Materials Specifications) and establish GMP compliance in a 'clinical trial phase-specific' fashion throughout product development and clinical testing. A more comprehensive supply list may augment the BOM and encompasses many programs and departmental supply requirements that define the total procurement requirements in support of multiple programs (environmental testing, cleaning, release testing, standards).

The BOM and supply lists are reviewed and approved by the Manufacturing and Quality units to ensure compliance with manufacturing requirements and pre-established quality standards. The BOM is typically used by a supply chain (operations) group to assure material availability and manage required interactions with other planning and finance groups.

As described above, limited control over the quality of a primary tissue harvest makes creating raw material specifications challenging. The current best practice for setting raw material specifications includes the use of healthy donor material during product development and performing confirmatory studies on actual patient products where available [8]. Primary cells as raw materials will typically have a wide acceptance range for raw materials specifications to accommodate the inherent variability of the patient population. Variability can be reduced, for example, by using starting materials from a single or limited source of qualified (allogeneic) healthy donors (blood, biopsy) or decidual tissue (cord blood) and creating banks of cells expanded *in vitro* with retention of desired biological properties (allogeneic CAR-T and MSC References). Source materials may include Master and Working Cell Banks derived from a source tissue as well as cryopreserved cell processing intermediates that must be tracked throughout manufacturing and distribution. Process centric risk assessments drive the extent to which a raw material supply is tested and established to be safe and reliable for manufacturing of a cell product. If research reagents are replaced with more 'qualified' sources (e.g., GMP grade), comparability of biological activity must be verified. Each raw material will require the use of qualified analytical tools for characterization and stability measurements. In addition to basic cell characterization (counts, viability, identity) these third-party materials must be thoroughly characterized for donor suitability (21CFR§1271) prior to use and will always require some form of immune protection when administered.

Non-cell based raw materials may also come from human sources (serum, plasma, platelet lysate) and are subject to strict quality standards associated with blood and tissue donations. While many cell collection (apheresis) and processing steps (platelet collection) can be harmonized using standard operating procedures and similar equipment, lot to lot variability is significant. Periodic supply interruptions (low donor frequency) or non-conformances (infected or otherwise disqualified donations) can threaten the continuity of raw materials. Other raw materials for cell manufacturing include cell culture media and supplements, cell processing buffers and enzymes, growth factors, nucleic acids, viral and non-viral vectors and small molecules.

As products show promise in pilot clinical studies and move towards pivotal trials, supply chain activities turn towards ensuring full compliance with regulatory standards. It is typically at this stage where extensive raw material testing and qualification (e.g., stability) programs are implemented. In many cases, critical raw materials come from vendors who only provide research grade reagents, do not have the ability to produce lots at clinical or commercial scale or are sole source providers. Assay development may be required to qualify raw materials beyond what is offered by the vendor. Few of the required assays are compendial in nature and will often require significant development using knowledge of what raw material attributes determine suitability for intended use. Each of these represents a risk to supply chain reliability and continuity and should be considered as early as possible in supply chain development (See also **Procurement**, below). The knowledge gained during materials selection will be critical to guide subsequent scale-up and product comparability studies.

Procurement

Procurement is a set of business processes encompassing planning, purchasing, inventory control, receiving, and receiving inspection.

Materials procurement must be designed to support manufacturing projections for cell products using a systematic approach. A systematic approach first consists of breaking down the planning process into steps to eventually mitigate risks such as backorders, lot-to-lot variability for biologics and small molecules in addition to shortage of ancillary materials amid manufacturing productions. Second, as described above, capture of metadata from early lots of material used in each manufacturing production will help establish a knowledge base and support trending analysis for supply chain logistics. Metadata includes information on pricing, lead time and availability, reagent grade, as well as vendors' production capacity and history of support for scheduled cell manufacturing productions.

Identification of the most critical components of a supply chain is part of a basic risk analysis. Materials with long lead times as well as materials from sole source vendors are conventionally considered critical, therefore robust controls and monitoring should be implemented. Such items are usually highly priced as well which makes it even more important to exert appropriate controls to eventually lower the Cost of Goods Sold (COGS). Additionally, critical but 'non-GMP grade' materials may be the only source available in early stage clinical studies. They constitute a higher quality risk due to less stringent manufacturing control and documentation and thus a lower degree of compliance with applicable regulatory requirements. Ranking materials based on criticality is part of risk assessment. More critical items should be secured with greater assurance (larger inventory, secondary vendor, supply agreements) and will likely be procured to minimally support several lot-production runs at once. Purchases are made with a clear understanding of lead time and time for lot testing and release as required. At all times procurement must consider component expiration dates to meet demand. Long range planning creates the opportunity to reduce COGS by negotiating pricing with vendors, who often provide price incentives as part of a committed bulk order. Finally, the collected data can now be incorporated into the raw materials forecasting to meet manufacturing demand planning which in turn informs procurement strategies. It is important for the supply chain department to understand their manufacturing processes and stages at which critical materials are required and used during the manufacturing production to prioritize the procurement and storage of those materials. During the planning phase, it is also important to identify materials that are readily available and procured using the just in time approach.

Procurement should be tailored to clinical material production cycles in a way that ensures the availability of the required components at the projected time of manufacturing. Long lead time items can create a level of uncertainty that requires special considerations to ensure order fulfillment. A supply of 6- to 12-month of projected inventory is not uncommon. Lot expiration limits storage lengths and order fulfillment timing can result in just in time deliveries of many materials. Annual estimates for materials need as well as annual financial forecasting is derived from procurement records and subsequently the source of information used to project cost of goods.

Once manufacturing has been initiated, the management of the drug substance and final product from the manufacturing site, throughout storage and ultimately to the clinical center for administration is also a critical aspect of the supply chain management. As described above, many cell therapy products require 'vein to vein' traceability and may have short 'shelf lives'. Therefore, clear definitions of transport procedures, practices and limits are crucial. The development of a SCMS for each 'living medicine' must include development and qualification of methods for transport and delivery of the final product to the clinical center for administration (see **Shipping**, below). As a product works its way towards commercialization, a Sales and Operations Plan (S&OP) is developed that describes the intended procedures and for

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production and distribution of the cell therapy product in order to achieve low cost and maintain a reliable procurement program. Once the S&OP process is well understood it becomes simple to forecast the consumption of materials, which in turn helps establish appropriate levels of inventory at cell processing facility as well as throughout the organization's complete supply chain.

Vendors

Delivering cell and gene therapies is an expensive and highly complex process and strong client-vendor relationships are critical to success. Cell therapy companies benefit from finding vendors who are willing to collaborate extensively and are capable of leveraging other relationships to bring value to the organization. The interactions with the supplier should be ongoing and close attention should be paid on the attentiveness of the supplier. These interactions should occur at all levels of each organization and be supported by the senior management of each party. For example, quality audit, overall site evaluation visits, meetings at cell therapy company offices, etc. These interactions should be viewed as opportunities to cultivate strong relationship with the supply chain company and in the long run will pay off in terms of overall value the supplier brings to the table.

Prioritizing vendors based on the criticality of their products to the process and general availability is also important. Raw materials that are available from multiple qualified sources are important but should be given second priority for review compared to those products obtained from sole source providers. Procurement of specialized or unique materials from single vendors in the industry is perhaps one of the biggest supply chain risks to many operations. Due diligence for vendors often includes, but is not limited to, evaluation of vendor production capacity, raw material grades available (Research, GMP, USP, licensed drug), time for manufacturing, company financial health, in addition to their location in respect to the manufacturing site. When selecting vendors, it is also important to identify vendors who are flexible to work with manufacturer as they are still in the development phase of sourcing new materials. Vendors who are flexible will usual be willing to work with clients to establish a long-term relationship that will support product growth overtime.

A significant contributor to the success of supply chain development is the establishment of strong relationships and well-defined supply and quality agreements between manufacturers and clients. Supply agreements will cover companies' raw materials needs planned throughout the year, in addition to any applicable discount vendors may offer during that period. Quality agreements are equality important and should be in place alongside supply agreements. They contain terms that cover compliance and assurance of quality in production of the raw materials. A quality agreement will ensure that any changes to the manufacturing or sourcing of a raw material or component of a reagent will not be done without notification and/or review by the drug manufacturer (vendor's client). Additional examples of critical supply chain and quality agreement components are given below in Box 1.

It is often difficult for cell therapy manufacturers to determine qualification requirement for a raw material. At this stage in the industry, no single material-grade, manufacturing standard or any standardization of other compliance claims, such as ancillary-grade, clinical-grade, GMP-grade, and animal-component-free exists. Although some regulatory hurdles have been reduced within the last few years, it remains an expensive endeavor to develop and process cell therapy products. With constraints on time and resources, it is essential to find ways to reduce waste. This is where the benefits of the utilization of LEAN methodologies can come into play [9]. Supply chain partners who thoroughly understand LEAN and have implemented LEAN systems can quickly become the 'supplier of choice' for most companies. Such LEAN suppliers

BOX 1

Essential elements of supply and quality agreements

- Definition of goods/services provided and limits as they may apply
- Terms of engagement, resolution of conflicts, remedies for breach of contract or failure to deliver as per contractual agreement
- Commitment of vendor/supplier to strictly adhere to defined Quality Standards
- Adherence to GMP 21 CFR§ 210, 211, 820 (Manufacturing and Quality)
- Compliance with ISO 9001, 13485 (General operations and Quality)
- Vendor/supplier agrees to support customer (client) requirements for GMP compliance (supply quality, traceability, documentation of process/production) as required for client drug development
- Agreement to allow customer audits, inspections
- Establishing and auditing of third party suppliers by vendor and/or customer
- Notification of customer of product or document changes, planned deviations, unplanned deviations, FDA audits of production, testing, release of products
- Handling of non-conformance, CAPA and customer complaints
- Indemnification of customer from legal action taken against company
- Terms for termination of agreement and process
- Non-compete statement where client (customer) is material in and/or sponsors the development of a custom product

often bring value by streamlining the supply chain while continuously looking to eliminate waste from the entire value stream of cell therapy.

LEAN Thinking in procurement is essential. Due to extensive growth within the cell therapy industry, many labs and startup companies may not have the resources to perform all business processes with individual suppliers as required by their Quality Management System. Therefore, companies with limited bandwidth should attempt to minimize the number of suppliers, placing the burden of compliance on their suppliers. In this way, it may be beneficial to find a third-party service company who can act as a procurement agent, managing the value stream of many components, compounding or compiling many units to one and/or providing the individual components, when necessary, to the cell therapy company. With only one company to manage, and fewer products to inventory, the cell therapy company reduces the need for further bandwidth. Barcoding incoming supplies would be another aspect of LEAN techniques. With the massive number of items needed to process a batch of cell products, reading individual components and transcribing the traceability information even into a computer can be time consuming and should avoided when possible.

Receiving inspection

In order to ensure that a supplier complies with the requirements of the Raw Material Specification (RMS) for cell therapy raw materials, it is paramount that specifications are well understood throughout the value stream. Ideally speaking, every component, ranging from pipette tubes to sera and reagents, should have well defined specification sheets, communicated to the supplier, and verified through signature process to ensure that there are no mistakes. An additional benefit of using specification sheets is that they allow the 'warehousing' function at cell therapy companies to evaluate the supplies upon receipt in their receiving inspection processes. The Receiving Inspection Process is an important element within an established Quality

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Management Systems for Cell Therapy companies. This safeguards regulatory needs as well as administering the highest standards of patient safety, all of which require assurances that the material is appropriate for use and has strong traceability.

All products arriving at a manufacturing site are typically inspected for container integrity, temperature, identity and alignment with procurement records. Materials are held, documents certifying materials (certificates of analysis, origin, conformance) are collected and entered into a data capture and management system (see below). Sampling of lots of materials for release testing may also be performed at this time. Materials not meeting any of the technical, procurement or shipping requirements will be rejected and must be held in a manner to prevent mix-ups with accepted or in process components. Receiving inspections processes are the first line of control of materials in the value stream and thus are of critical importance to be managed against a well-defined operating plan.

Inventory management

Inventory management is critical to the success of well-functioning cell therapy supply chain. Many organizations fail to understand the importance of inventory management, treating it as a second level priority activity. Inventory Management services can be implemented to ensure proper materials forecasting, first in first out (FIFO) consumption of goods and just in time reordering of supplies as they are consumed. The inventory management system must account for any expiring components that might jeopardize the quality and continuity of the supply. This matter of expiration is one of the issues that requires established business reviews between supplier and cell therapy companies to ensure that monthly usage is tracked, and inventory levels are adjusted accordingly. Eliminating the waste of unused but expired goods is also critical step in order to minimize the overall cost of goods sold (COGS).

At the terminal end of the supply chain, the focus on health and safety are directed towards patients' safety. All products must be managed throughout production and testing and during shipping to the patient to prevent mix-ups resulting in the delivery of the wrong product or dose to the wrong patient. Bar coding systems are often used to label and track products and product labeling standards such as ISBT 128 have been developed to address these requirements. See also **Shipping**, below.

It is worth mentioning here that supply chain management must also include a plan for the impact of raw materials on the environment; or on the health of the manufacturing staff and end users (patients). Safety measures are typically directed towards storage of the raw materials in the proper temperature and space and in the correct packaging to prevent spills. Additionally, testing may be required for animal derived sources of materials (e.g., serum, albumin, transferrin, platelet lysates) for the presence of adventitious (infectious) agents and acute toxicity for concentrated forms of other reagents (e.g., retinoic acid, solvents, concentrated acids and bases used to pH media and buffers). A complete and thorough review of the BOM for any product should be performed with environmental health and safety (EHS) specialist to ensure the proper storage and handling of all materials.

Cell therapy manufacturing requires the use of a considerable number of biologics, small molecules, growth factors and a variety of types and sizes of custom plastics with attendant physical and chemical hazards and risks. Final products are well tested for safety prior to use in humans. However, it is extremely important to discuss the impact of exposure to raw and waste materials on the health and safety of at-risk staff (shipping, handling, testing and manufacturing staff) as well as impact of material use and disposal on the environment. These discussions should happen during planning and forecasting with an internal health and safety officer or department prior to initiating their sourcing. Doing so, a proper waste management plan will be

crafted in accordance with local cities rules and regulations to potentially mitigate public health risks in cities where media disposal rules and regulations are minimal and have yet to catch up with these new trends.

At the time of writing of this article the world is coping with the C0VID-19 viral pandemic. Lives are impacted in many ways but one of the more subtle disruptions is the impact on supply chains throughout a wide variety of industries and commercial sales. These events emphasize that disaster planning and recovery should be a consideration for supply chain continuity in cell therapy.

Shipping

The transportation of raw materials to manufacturing sites and products from manufacturing sites to clinics requires demonstrable control over the transportation process and documentation of the history of shipped material. Working with vendors and transportation providers who understand and can support these shipping requirements is critical. Tracking shipment location and temperature are critical parameters part of the value stream of cell therapy. Delays in transportation due to weather, customs, agricultural inspection and other reasons can adversely affect the quality or availability of critical supplies. The shipment of raw materials and drug products with temperature sensitivity (-20°C, 4°C) must be recorded to ensure maintenance of material quality.

Several mechanisms exist to monitor and report on shipping conditions and even real time location of products. For example, data recorders (e.g., TempTale®) can be placed inside the shippers to record the temperatures which can be transmitted to the virtual (cloud-based) storage locations in real time or downloaded at end of shipping cycle to ensure that the cell products did not experience out of specification temperatures. GPS tracking systems such as CryoPortal®/Smartpak II® system and cloud-based data recording (e.g., SenseAnywhere™) track samples from pickup through delivery to the end user and provide real time monitoring of location with 24/7/365 traceability.

Validation of shipping systems is also required to ensure compliance with industrial standards such as Good Distribution Practices (GDP). Guidelines for shipping and handling of drug products or active pharmaceutical ingredients (API) have been developed in the EU (Article 1(33) of Directive 2001/83/EC) and by WHO (World Health Organization – WHO Technical Report Series, No. 957, 2010) and are currently under development in the US. must include ability of the shipping system to maintain temperature and protect the cell therapy product physically. In cases where validation must be performed, some form of verification techniques must be employed. To this end, several other standards are also available that can be followed, e.g., ISTA and ASTM.

ELECTRONIC DATA CAPTURE & MANAGEMENT

Supply chain data capture, management and analysis are critical activities designed to ensure operational efficiencies and regulatory compliance during product manufacturing and distribution. Many small companies and academic institutions approach this task initially with spreadsheets and text documents but quickly realize the limits of these stand-alone data sources. A more reliable approach is to develop (or purchase) software that can integrate the planning, procurement, management and finances of supply chain activities. Enterprise Resource Planning (ERP), Manufacturing Execution Systems (MES) and Inventory Control software are widely available (e.g., TrackCel) but the integration of these packages with each other can be challenging, somewhat time consuming and expensive. Inter-platform data transfer presents challenges in both defining relationships between disparate data sets and verifying the integrity of the data upon transfer between systems. Achieving a robust system for inter-platform data management requires significant time and investment in 'systems

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engineering' and infrastructure when licenses or technologies to allow systems to interface are not always available from the software vendors.

An alternative approach is to build a system on a platform technology that can be used to integrate disparate enterprise system and product-specific materials management data. An example would be to implement a configurable Laboratory Information Management System (LIMS) that is designed to accommodate supply chain but also integrates with other financial and facilities planning software. We have previously described the development and implantation of such a system to aid in the operations of an academic cell therapy laboratory [10]. The time and effort required for implementing each system will vary and it is prudent to begin the assessment of supply chain software needs through a gap analysis in which all of the above parameters can be mapped out into a process flow diagram to determine the flow of materials, data collection points, interrelations between data sets and expected reporting requirements. Production demand can be overlaid on the process flow diagrams to determine cadence of ordering and supply consumption and allow for establishing materials management efficiencies using Lean manufacturing principles. Creating a supply chain data management plan from such an exercise will enhance the likelihood of a successful system implementation and establish responsibilities, timelines and cost of implementation.

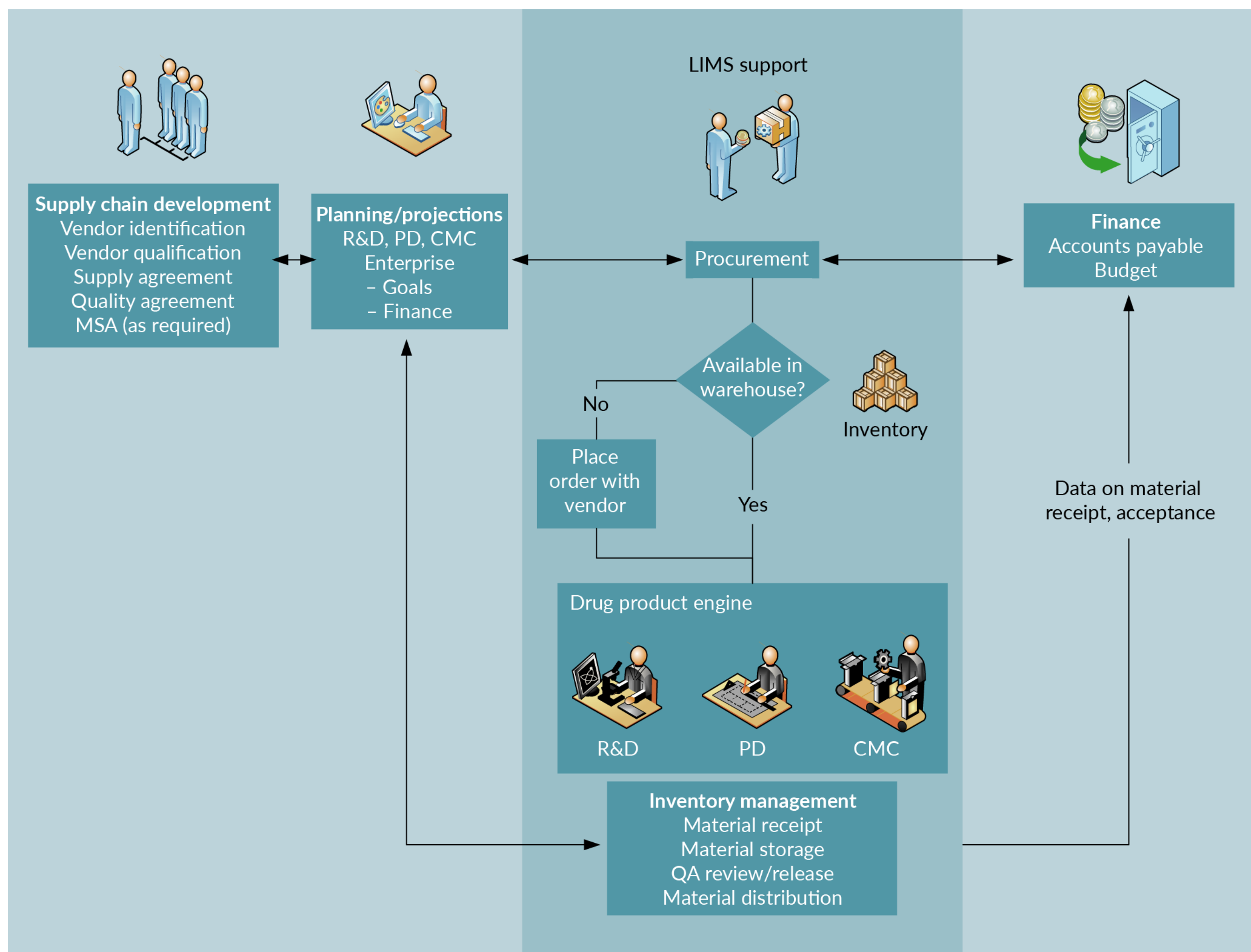
The development of a supply chain data management system should ideally include stakeholders from business entities supporting drug manufacturing operations. Typically, representation from finance, facilities, manufacturing, operations and quality systems is required to completely map out the supply chain management work streams. Each entity will define critical data capture and reporting needs related to their respective functional areas and help shape the structure and utilization of the data collection, analysis and reporting tools. The integration of supply chain management data with other enterprise data enhances the coordination of the activities of various stakeholders in the supply chain. Careful forecasting of demand, logistics, costs and time for implementation are typical drivers of decision making in this area. The involvement of 3rd party logistics providers (3PL) in the supply chain will add another level of complexity both in the design of the data capture and management system as well as integration across stakeholder platforms. Figure 1 shows critical elements and information flow between various stakeholders of a typical supply chain data ecosystem within an organization.

Quality Systems personnel can control the BOM and supplier lists, implement changes and add or eliminate items in a regulated fashion. Procurement can then place orders based on this controlled materials sourcing dataset which will substantially reduce the risk of use of non-qualified materials or vendors. A qualified vendor and material list also allow procurement to mitigate backorders or other order fulfilment delays by having pre-approved alternatives on record. Linking enterprise systems for procurement (ERP) to materials receipt and management allows for traceability of orders and facilitates payments of materials received and accepted. MES or LIMS systems can (and should) be designed to support (optimize) warehouse utilization and quarantine/release logistics once inventory is received and materials are assigned to ambient or cold chain storage. The information related to the BOM and specifications can also be used to plan, procure, receive, store, qualify and distribute materials. Trending of material use rates, failure rates, delivery delays and other supply chain metadata will support continuous process improvement and operational efficiencies in the manufacturing plant.

Throughout production, the assignment of BOM items to each campaign is recorded to serve as the record of raw materials usage in each batch. The data is also used to determine inventory levels in real time and to facilitate product reporting requirements in the event of a raw material recall. The information on usage will also be used to guide lean manufacturing practices and just in time supply procurement. During the creation of the drug product, manufacturing process

FIGURE 1

Elements of a supply chain data ecosystem.



intermediates and drug substance are critical materials in the supply chain must be tracked as would any other production material. Analytical and storage data must be captured as does significant amounts of product metadata (manufacturing date, batch release, storage conditions and lot disposition) to facilitate product distribution and recall.

Data integrity

FDA expects that all data collected as part of a batch record (including materials) be complete, consistent and accurate (21 CFR§211). Records keeping traditionally has been achieved using paper records but the movement towards electronic records is becoming more common. Electronic record keeping and signature requirements are specified in 21 CFR§11 and in an FDA guidance document (Data Integrity and Compliance With Drug cGMP Questions and Answers – Guidance for Industry). FDA recommends that firms employing electronic records should implement meaningful and effective strategies to manage their data integrity risks based on their process understanding and knowledge management of technologies and business models (see also, ICH guidance for industry Q9 Quality Risk Management).

There are standard practices and procedures that can be undertaken to ensure data integrity. Controls should be in place to ensure that

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the data is complete, entered at the time of performance and protected from adulteration using secure access practices. Any required changes to data records should be performed in a documented fashion with Quality Systems oversite, review and control. The safety and integrity of the data should be ensured through qualified backup procedures with periodic audits to ensure the integrity and completeness of the backup. Where available, original paper records can also be used to verify an electronic version of the same information. Mechanisms such as controlled login and traceability of entries and changes also help to ensure the integrity of electronic data sets. Systems should also be in place to detect omissions and other errors if and when they occur (e.g., incomplete records, lack of units for measurements, etc.). Metadata (that is information that puts all data into context) is also part of electronic records and must be recorded and maintained with the same level of integrity as raw data. All data must be verified through a secure (non-corruptible) audit trail containing information on times, dates and other information that allows for a complete reconstruction of events recorded in the data set.

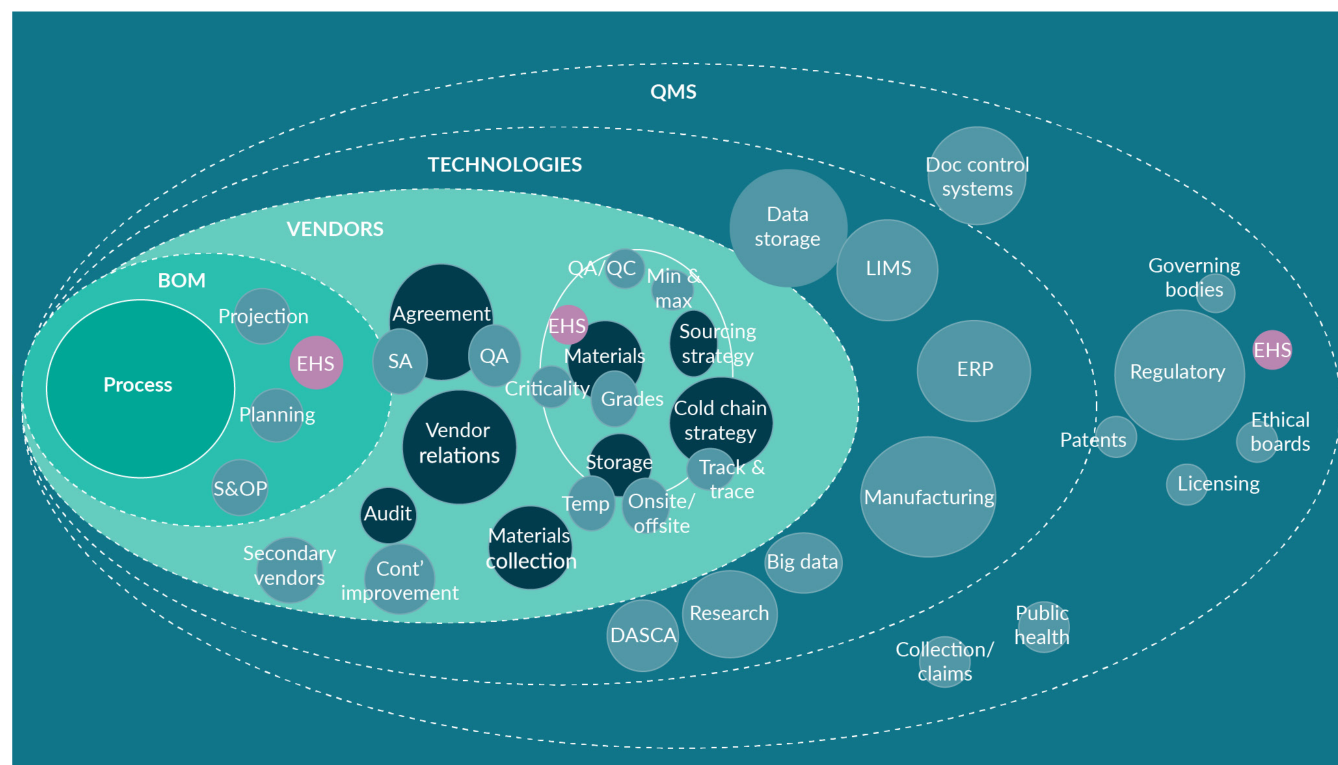
Data collected and stored in an electronic system should be backed up using a reliable method that represents a 'true copy' of the original record. Backups can be in electronic or original paper format but must be verified to be accurate and complete. The design of systems and software used to store supply chain and production data are typically validated by the vendors at the time of creation but implementation of each system for each installation and process will also require some level of validation. These services should be planned ahead of the implementation and use of data capture and storage systems.

CONCLUSIONS

The development of cell therapy supply chain management system is both complex and essential to the successful development of a cell

FIGURE 2

Process centric spheres of consideration during supply chain development.



therapy product. Demonstrable specification of and control over all source materials from the time collection/procurement, throughout manufacturing, shipping and delivery of final formulated cell product to the patient is critical for the safe and effective use of these products. The supply chain is built around a manufacturing process, sourced against a BOM using qualified vendors, procured and tracked using ERP and other data management technologies and overseen by the QMS policies and procedures of the organization. Figure 2 outlines the universe of disciplines, technologies and stakeholders involved in supply chain development.

The take home points of this article can be summarized as follows:

- The development of a supply chain management system is a complex, time consuming task that is essential to the success of a cell-based drug development program;
- Cell therapy products are living drugs and have unique supply chain requirements both for raw materials as well as final product;
- Supply chain needs will largely be driven by production and quality requirements but contributions from all disciplines of the company is required to develop a cohesive supply chain system that serves all compliance and corporate operational needs;
- The development of a supply chain management system is a complicated process and should be initiated early in the product lifecycle;
- Sufficient resources and systems should be identified to ensure the continuous availability of safe and effective clinical materials.

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David L DiGiusto
Semma Therapeutics, Cambridge, Massachusetts, USA

Rakib Ouro-Djobo
Semma Therapeutics, Cambridge, Massachusetts, USA

Uzair Rajput
Instant Systems, Norfolk, Virginia, USA

AUTHORSHIP & CONFLICT OF INTEREST

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

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

COMMENTARY

The role of raw materials in the manufacturing of cell and gene therapy medicinal products: practices and challenges

José Caraballo

The biopharmaceutical industry has increased the number of cell and gene therapy medicinal products under development. The complexity of the new technologies to enable these therapies and the regulatory expectations have also increased. Within this context, the role of raw materials in the manufacture of CGT medicinal products is examined. This commentary assesses the controls applied to raw materials and evaluates challenges and best practices ensuring product quality. The fundamentals of good manufacturing practices still apply. Well characterized materials from reliable suppliers are key elements to maintain a dependable supply of products for our patients.

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RAW MATERIALS ARE IN HIGH DEMAND

The development and manufacturing of medicinal products using gene and cell therapy (CGT) technologies is increasing year after year. More than 2,000 clinical trials have been conducted worldwide between 2004 and the first half of 2019 [1]. In the United State alone, the FDA anticipates more than 200 investigational new drug applications per year starting

in 2020. By 2025, FDA predicts that the agency will be approving 10 to 20 cell and gene therapy products a year [2]. The increase of CGT medicinal products will create higher demands for new technology, skilled workforce, and materials.

There are multiple definitions to refer to materials (e.g., substances, active substances, non-active substances or excipients, source cells, starting materials, ancillary materials, raw materials, cell banks, components, etc.). An important distinction is made between starting materials and raw materials. Starting materials are those used in the manufacturing of Advanced Therapy Medicinal Products (ATMP) and intended to be part of the final product. This definition would include different types of starting materials depending on which ATMP is intended to be manufactured (i.e., cell therapy, gene therapy, or an engineered tissue product). European Union (EU) regulations refer to materials that are not starting materials as ancillary materials, while FDA may consider these other raw materials, or components. It is important to designate all materials with the correct category consistent with regulatory agency definitions. The general term "raw material" is used in this article to refer to raw materials, substances, starting material, ancillary materials, and excipients used to realize the production of ATMPs. Specific terms will be used if necessary to distinguish between different types of materials.

PRINCIPLES OF GOOD MANUFACTURING PRACTICES (GMP) REMAIN UNCHANGED

Despite the fact that new technologies are used for the manufacturing of novel medicinal products, the basic principles for GMP apply. GMP for raw materials consists of applying well-known quality system elements such as following written and approved procedures, receipt and storage of materials, establishment of methods to verify material identity, sampling and testing for material attributes against approved specifications, use of approved components, rejection of non-conforming components, evaluation and qualification of suppliers, and material qualification activities. Training, risk management, change control, discrepancy management, and corrective and preventive actions (CAPA) completes the essential GMPs for raw materials. In addition, raw materials undergo additional controls to ensure effective inventory controls, prevention of material cross contamination, and full monitoring and control of the material supply chain.

The degree of control measures depends in part on the understanding of the product quality risks related to raw materials. By knowing the material critical quality attributes, material characteristics (impurities, identity, and potency), material origin, and methods of production, we can evaluate which aspects need to be controlled to mitigate potential impact to product quality. Regulatory agencies also add requirements to the list of controls we must apply to ensure a safe and compliant supply of materials.

Specific requirements for materials to be used in the manufacturing of CGT products are further defined in existing EU and US regulations (a summary of relevant regulatory guidance documents can be found in Appendix A of reference [3]), such as EU Directive 2001/83/EC, Ph. Eur. 5.2.12, and USP <1043>. These cover some of the unique type of materials such as somatic cells, viral vectors, growth promoters, and other biological entities. Table 1 provides examples of requirements applicable to CGT to be filed in the EU to obtain approvals for marketing authorizations [4,5]. These examples illustrate the specificity of requirements to ensure materials are well characterized, controlled, and fit for intended use. Each CGT medicinal product can have unique material requirements based on the type of product, manufacturing process, and the interaction of the different materials during manufacturing and during use by the patient.

Changes to raw materials are to be expected during the development of CGTs. These changes need to be evaluated for impact to

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TABLE 1

Example of EU filing requirements for materials used in CGT medicinal products.

Requirement	Gene therapy	Cell therapy
Traceability of material (from manufacturing to use)	X	X
List all active substances*. Active substances shall consists of nucleic acid sequence(s) or genetically modified microorganisms or viruses	X	
List of starting materials. For virus or viral vectors, the starting materials shall be the components from which viral vector is obtained. For plasmids, non-viral vectors and genetically modified microorganisms, the starting material shall be the components used to generate the producing cells	X	
Cell characteristics prior and after genetic modifications	X	
Genetic sequence information on all parental strains	X	
Description of process related impurities	X	
Information on donation, procurement, and testing of human cells (starting materials)		X
For xenogeneic cell-based products, information on source of animals, acceptance criteria, and controls to prevent infections in the source		X
For cell-based products derived from genetically modified animals, information on specific cell characteristics, including methods of creation and characterization of transgenic animals		X
Testing regimen for any additional substance used (scaffolds, matrices, devices, biomaterials, bio molecules, etc.)		X
Characterization of reference standards for active substances		X
Consideration for toxicology testing of active substances and excipients	X	

*Active substances are defined in EU directives [5] as "Any substance or mixture of substances intended to be used in the manufacture of a medicinal product and that, when used in its production, becomes an active ingredient of that product intended to exert a pharmacological, immunological or metabolic action with a view to restoring, correcting or modifying physiological functions or to make a medical diagnosis".

the final medicinal product. Raw material changes at latter stages of the product life cycle can be expensive and time consuming and should be avoided if possible. The use of GMP or commercial grade materials as early as possible in the development process will reduce the change burden and impact.

A control strategy for raw materials will depend on the criticality of the different characteristics of each material. A risk-based approach for raw materials consists of collecting knowledge about the raw material, performing a risk assessment, evaluating material attributes and risks, and implementing a mitigation plan [6]. The level of control to be applied should be relative to the criticality. In this approach, we end up characterizing the materials, assessing controls to mitigate risks, and evaluating the process capability through the assessment process.

An alternate approach to a materials control strategy would be the application of Quality by Design (QBD) principles when developing CGTs. Under QBD principles, raw materials are pre-selected based on their consistency and well-defined manufacturing process. As a result, the source of variability from materials is well understood, facilitating the design for process capability. Designing for process capability (instead of assessing process capability) becomes the most robust approach in a raw material control strategy.

Additional practices for effective raw material control are described in the Table 2.

CHALLENGES & OPPORTUNITIES

Ensuring a reliable supply of quality raw materials can present special challenges for CGT medicinal products.

The source of some materials are unique (e.g., complex biological materials, viral vectors, special components), or the materials may be in a very early developmental stage. The supplier and the developer may not have enough experience with the materials and will need to collaborate to define and manage the critical parameters of interest.

TABLE 2

Best practices for the effective control of raw materials

Best practice	Description
Understand raw material variability	Characterize raw materials to understand lot-to-lot variability. Use this knowledge to improve control strategies
Select materials from capable suppliers	Supplier selection should include supplier's capability to produce GMP grade materials in different quantities to match manufacturer needs and future product demands
Use GMP materials early in the development process	Consider the use of GMP or compendia grade materials as early as possible in clinical stages to minimize comparability challenges when moving from clinical to commercial stages
Understand and monitor material source	Monitor material attributes back to the source and proactively assess impact of changes that can have a global impact in supply or quality. Global impact may include multiple products, companies, or processes
Partner with suppliers	Have supply and quality agreements in place to ensure robust collaboration and performance
Avoid sole sourced or single sourced materials if possible	Qualify all uniquely sourced or single sourced materials and suppliers. Consider additional suppliers early in the development process to avoid uniquely sourced materials and to assess process robustness when using alternate material sources

There is usually a lack of robust analytical methods to enable the adequate characterization of raw materials. This lack of methods will hinder the prompt generation of data related to material attributes and will affect the ability to gain knowledge to define parameters and understand their criticality. Significant effort should be invested early in the development program to create appropriate analytical methods to conduct early characterization studies.

The scale of some CGT processes can offer opportunities for automation. Reducing variability via the adoption of automation and standardization will increase our ability to ensure consistency and process robustness.

Single use technologies (SUT) and disposable components are frequently used in the manufacture of CGT products for their flexibility and modularity. These systems present new challenges during manufacturing in terms of closure integrity, the potential for process leaks, and system integrity to prevent ingress of particles or bioburden. It also introduces the need to assess how leachable and extractable (LE) components may affect the manufacturing process, cell cultures, and final levels of impurities. SUTs needs to be qualified for closure integrity and clearance of LE components below safety levels.

Raw materials for CGT include the use of cells (human cells, animal cells, genetically modified cells, etc.). Cell sources need to be fully documented and protected from any cross contamination with other cells or by adventitious agents. Cell banking systems need to be in place, including risk mitigation measures to prevent losing cultures during unexpected events.

Some cell therapy products and raw materials have specific storage conditions and may be unstable after a short period. In these cases, speed to deliver products to the patients while assuring that product conditions are maintained within specifications is a critical product feature. The right logistics, foolproof traceability, and optimum product storage conditions are essential to preserve product quality.

CONCLUSION

Raw materials play a critical role in the manufacturing and control of cell and gene therapy medicinal products. The types of materials needed to manufacture CGTs have increased in complexity; however, the basic GMP principles to control materials and ensure quality still apply. Well characterized materials from reliable suppliers in robust processes are key elements to ensure the dependable supply of products for our patients.

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AFFILIATION

José Caraballo
Bayer U.S.
800 Dwight Way
Berkeley, California 94701 USA

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

INTERVIEW

Raw material qualification and optimization: the Janssen approach

LILI BELCASTRO is a Scientist at Janssen and is responsible for the qualification of raw materials used in Janssen's cell and gene therapy products. Dr. Belcastro has over 10 years of experience in preclinical and clinical cancer biology, cell and gene therapy product development, and analytical method development. Prior to joining Janssen in 2017, she was finishing her PhD in cancer biology in a joint program with the University of the Sciences and The Wistar Institute in Philadelphia. Prior to joining industry, Dr Belcastro led pediatric, preclinical, in vivo testing programs at the Children's Hospital of Philadelphia for small molecule inhibitors, oncolytic viruses, antibody-drug conjugates, and radiopharmaceuticals. Dr Belcastro holds two bachelor's degrees from Temple University and the University of the Sciences.

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Q What are you working on at the moment?

LB: I work in the Material Sciences department at Janssen, which qualifies, sets the specifications for, and evaluates the raw materials used in our drug substance and drug product manufacturing processes. Janssen's biotherapeutics division develops a broad range of therapeutics and modalities including monoclonal antibodies, duobodies, proteins, vaccines, cell and gene therapies. My focus in Material Sciences has been on the raw

materials used specifically in cell and gene therapy manufacturing. This includes plasmids, ancillary materials, viral vectors and excipients used in the gene therapy and cell therapy manufacturing processes.

Q What are the key considerations for you in terms of raw material qualification – specifically regarding Janssen's CAR T cell therapy products?

LB: For CAR T manufacturing, in addition to routine safety testing, we have performed a robust qualification for each material as part of our overall control strategy. A number of the materials were novel to Janssen and are proprietary to their manufacturers, so we've worked on building our relationships with the manufacturers and suppliers. Through these relationships they provide as much information and data as they can, while maintaining appropriate confidentiality.

Lot-to-lot variability is of particular concern when it comes to cell therapy raw materials – human cells are inherently variable by nature, as is disease progress. By understanding and controlling our process and raw material variability, our goal is to reduce variability and achieve consistency in the end product as much as possible.

Q What about on the AAV gene therapy side?

LB: Our approach to AAV gene therapy products is similar to our approach to our cell therapy portfolio. We work with a number of suppliers or CMOs in this space, who may use proprietary processes, methods and materials for manufacturing plasmids and vectors. Depending on which model we build our relationship upon, we work with them to ensure they are following an appropriate level of qualification and routine testing approach that is in alignment with our internal practices and policies. Additionally, we ensure that they are using high-quality, compendial, and GMP grade materials.

Q To what extent can a large company like Janssen adapt or repurpose qualification methods and processes from other areas? And on the other hand, when is a more cell and gene therapy specific approach required?

LB: With the addition of cell and gene therapies to our portfolio there were many new and novel raw materials introduced to us, such as in the CAR T manufacturing process as I mentioned above.

For the plasmid and lentiviral vector manufacturing processes, we were able to adopt processes we use for some of our traditional monoclonal qualification processes, working with CMOs to ensure they have the highest quality raw materials that we also source for

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"Lot-to-lot variability is of particular concern when it comes to cell therapy raw materials – human cells are inherently variable by nature, as is disease progress. By understanding and controlling our process and raw material variability, our goal is to reduce variability and achieve consistency in the end product as much as possible."

in-network use. So especially when it comes to materials with which we are familiar, we are able to leverage some of our internal qualification approaches.

In my view, the CAR T raw materials have been the most challenging in terms of qualification and routine release testing strategies when dealing with proprietary formulations or materials. In addition, the raw materials are often biological in origin, so traditional compendial-type testing is not available. We're developing novel methods and testing new attributes, which are new not only to Janssen, but to the industry as a whole. It's been a challenge, but an exciting one.

Q Diving deeper into the challenges presented by the current lack of specific guidance for the qualification of plasmids, what's the best approach in the absence of clear guidance? And what can be done to remedy this situation?

LB: This is a gap for the entire cell and gene therapy community – I know that a lot of my industry colleagues are running into the same issues.

In the USA, there is guidance available for plasmids that are used as drug products, but there is no specific guidance for plasmids used upstream in cell and gene therapy bioprocesses – for example, their use in lentiviral or AAV vector production. The USP Chapter <1043> does consider plasmid as an ancillary raw material. However, the guidelines in Europe refer to plasmids as starting materials when used in cell and gene therapy requiring qualification activities that are similar to how a master cell bank would be qualified for a monoclonal antibody.

The difficulty is in aligning what each regulatory agency requires. When we first started developing our plasmid qualification strategy, the approach was to scour every bit of guidance or pharmacopeial chapter from any of the regulatory agencies for mention of plasmids. The aim was to compile all methods and attributes that are recommended for plasmids, then set acceptance criteria for a particular attribute based on a harmonization of all guidances.

Ideally, it would be helpful to have a chapter similar to the USP gene therapy chapter <1047>, which provides a table with all the specifications for plasmids for use as drug products. A similar chapter for plasmids used in cell and gene therapy would be a great asset for the entire community.

Q What are your top tips on approaching raw materials optimization in the cell and gene therapy space?

LB: My advice would be to choose raw materials based on your process needs, and your knowledge of what those materials and their attributes are. Consider how they apply to your critical process parameters.

There are a lot of unknown variables when you first start these processes. Collecting as much data as possible through early qualification activities – for example, assessing lot to lot variability – allows the user to obtain a baseline of variability for a particular material attribute. If any issue should occur during the cell manufacturing process, the raw materials can be ruled out based on adequate knowledge of the material.

Another approach is to group similar materials together when developing a strategy. For instance, if you're working with T cells and have two types of antibodies for cell selection, or two types of media, these could be qualified similarly. Likewise, grouping materials based on USP <1043> tier classification allows the user to tackle qualification in an organized manner. Then as the process evolves and further develops, and new information and data is gained, the qualification strategies can be adjusted to more specifically pertain to each individual material.

Q How does Janssen mitigate risk in its raw material supply chain?

LB: The raw materials needed for cell and gene therapy are often proprietary, off the shelf products that may be supplied by very few manufacturers, or even a single manufacturer. This presents a major risk in terms of supply chain.

Our risk mitigation approach for these types of materials is to work closely with manufacturers and to provide them with a demand forecast as far in advance as possible, to try and ensure our supply chain is kept consistent. We stringently check variability and safety, and use the highest grade, purest materials available to reduce quality and safety risks.

> "We stringently check variability and safety, and use the highest grade, purest materials available to reduce quality and safety risks."

We also work diligently with our suppliers. Essentially, it comes down to good relationships: working together, helping each other, and always keeping the patient in mind. This way we can ensure drug products are produced as safely and as quickly as

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possible, as ultimately, our materials enter a process that ends with them being injected into a patient.

Q Turning to enabling technologies, where would technological innovation make the greatest difference in your day-to-day work? What are the key gaps in your toolbox?

LB: A platform identity test method for proprietary materials would make a great difference. It is very difficult to properly identify a material if you don't know the formulation. You're essentially working in the dark, but you still need to have an identity assay as it's an FDA requirement.

A rapid, non-destructive analytical instrument that would be utilized to generate a unique signature for a given material would be ideal. We could then use this to measure materials on a routine quality release basis; a non-destructive spectrometer type instrument that is very robust and dependable. In addition, it would reduce overall cost of goods. The raw materials used in cell and gene therapy manufacturing are often very small in volume and very expensive, and a certain number of vials will already be destroyed for testing. A non-destructive method for identity would help reduce some of those costs.

Q Finally, what will be your key priorities in your role over the next two years?

LB: Key priorities over the next two years will continue to be focused on cell and gene therapy raw materials and strategies for their qualification and control, with a focus on industry collaboration on best practices.

AFFILIATION

Lili Belcastro
Scientist, Janssen Inc., PA, USA

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CELL & GENE THERAPY INSIGHTS

RAW AND STARTING MATERIALS:
TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

INTERVIEW

Optimizing and standardizing apheresis: critical next steps for the cell therapy industry

JOSEPH M ROIG got his BSc in Chemistry in 1980 (University of Barcelona) and started his professional career at Coulter (Beckman-Coulter nowadays), where he worked in cell counting and flow cytometry. He joined COBE BCT (currently Terumo BCT) in 1991 to help the company start operations in Spain, moving to the US to continue working for the same company in 2000. During the years that he worked for COBE/Terumo BCT he was involved with all existing apheresis variants (donor collections, leukapheresis, therapeutic apheresis) as well as cell processing. He became part of the Terumo BCT Scientific Support group in 2012 and the Medical Affairs group in 2016, the year he got a Masters in Transfusion Medicine and Advanced Cell Therapies (EMTACT) by the Autonomous University of Barcelona. He is currently working as an autonomous cell collections/cell therapy consultant for the industry.

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Q What are you working on right now?

JMR: I am working with two different cell therapy companies. One is in the very early stages of development, in the preclinical phase. In that case, I'm helping them put together

> "The cell therapy industry really needs to pay more attention to the different characteristics of the patient and how these characteristics actually affect cell collection..."

the things they're going to need for their future success and the implementation of standardization – namely a database, which will include all the data they will need in the future, and a leukapheresis manual. These are the key first steps. (The leukapheresis manual already includes concepts heading towards standardization).

The other company I'm working with is at a more advanced stage – in late Phase 1 clinical trials and with multiple clinical trial sites. They need help with the standardization of their actual collections as well, so there's data to be analyzed there. I'm already working on trying to provide more consistency with their collections.

The type of cells I'm working with right now range from CD34$^+$ cells for gene therapy, to tumor infiltrating lymphocytes, to lymphocytes for allogeneic CAR-T cell manufacturing.

Q You are newly established as an independent apheresis consultant – can you frame the chief reasons why the cell therapy industry needs help in this particular area?

JMR: The one big reason is the cell therapy industry has been complaining about the lack of consistency of the final cell therapy product, but at the same time, only paying attention to what they consider to be 'manufacturing' – steps such as transfection, or gene editing, or expansion, or selection. All of these steps have been well taken care of by cell therapy companies, but in general, they have been kind of forgetting about the very first step in the manufacturing process, which is collection of the raw materials – in this case, the cells they are going to use to later on and manipulate in different ways.

I would say this is particularly the case with those cell therapies that come from the patient him or herself – autologous cell therapy products. These are the ones the cell therapy industry really needs to pay more attention to. I have had opportunity to see many leukapheresis manuals from these companies, including companies with products on the market – and they basically ignore the nature of the patient, the disease, and co-morbidities.

I'll give you an example. There was a company a while ago that I was working for that was proposing a treatment for renal cell carcinoma, and they were ignoring the patients' co-morbidities such as blood viscosity, blood alterations, and cell pre-counts. The result was not good. I'll not name the company, but they were failing their products more than 40% of the time because they didn't have the right cell dose.

The cell therapy industry really needs to pay more attention to the different characteristics of the patient and how these characteristics actually affect cell collection, unfortunately in a negative way.

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> "...every individual cell therapy company is developing a leukapheresis manual completely independently ... The first key, strategic step will necessarily involve cell and gene therapy companies sitting down together and coming up with some sort of master leukapheresis manual, and by this I mean a basic leukapheresis manual that can be easily customized."

Blood viscosity, for example, can significantly decrease the collection efficiency of whatever apheresis device you're using. At the end of the day, apheresis machines were designed by engineers who had to make a number of assumptions about the characteristics of blood. However, a lot of patients do not meet those assumptions or characteristics, and it's with collections from those individuals that the cell therapy industry really need help.

I have also never observed a single leukapheresis manual that did not state that the endpoint of the apheresis collection should be defined as a set volume of blood. However, they often don't like the results, and the reason for this is they are ignoring the fact that different individuals, and particularly patients with diseases, present to the apheresis center with very different cell pre-counts. If you always process the same volume of blood in a 'one size fits all' manner, the final cell yields are going to differ greatly from patient to patient, sometimes to a degree where the apheresis product is not going to meet your expectations.

So there is work that needs to be done to make these yields more predictable.

Q Pressure on apheresis sites and calls for standardization from the same – around apheresis protocols, for instance – have been steadily increasing of late. How do you reflect upon this issue and what for you are the key strategic steps for all stakeholders to take in this regard?

JMR: I think the initial steps at least need to be taken by the cell therapy industry. There is certainly pressure coming from apheresis and clinical trial sites now concerning standardization. I speak with them and they tell me that every time a new cell therapy company comes to them proposing participation in a particular clinical trial, or to offer their product commercially, they come with their own leukapheresis manual. And all of them are very different. It's very obvious that at the moment, every individual cell therapy company is developing a leukapheresis manual completely independently, and no effort whatsoever has been made to harmonize as yet.

Therefore, I would say that the first key, strategic step will necessarily involve cell and gene therapy companies sitting down together and coming up with some sort of master leukapheresis manual, and by this I mean a basic leukapheresis manual that can be easily customized. Different companies might require X, Y or Z number of cells per kilo, which is totally normal – they may need a greater or lesser amount depending on their bioprocess efficiency, for example. But somebody has to start with the idea of creating some sort of template manual – or rather, a set of templates: one for each different type of cell.

Cell therapy companies simply should stop developing their own leukapheresis manuals without having a conversation with other, similar industries and coming up with some sort of template that with minimal changes could be presented to the hospitals and the apheresis collection sites.

Q Does the need for standardization apply equally to all types of cell/gene therapy manufacturing processes? Why?

JMR: I would say no. There are cell therapy treatments that are highly dose dependent – engineered stem cell therapies or dendritic cell-based cancer vaccines, for example. With these technologies, it's critical that the patient receives a minimum dose of modified cells in order for the treatment to be a success. In these cases, standardization is very important and highly relevant. I would say that companies developing highly dose-dependent therapies will not succeed if they don't consider making efforts towards standardization such as the ones we've discussed.

However, there are other types – CAR and TCR T cell therapies, for instance – where there isn't agreement on what would be the minimal dose of cells necessary for successful treatment. This is due to the fact that those cells expand following infusion into the patient. In those cases, even though it is always desirable to be talking about a well-defined number of cells, it is somewhat secondary and so standardization is less relevant.

Q What kind of cost would the implementation of standardization in cell collections have for either cell therapy companies or hospitals performing these collections?

JMR: I think this is an extremely relevant question, because there is a total misconception within the cell therapy industry that any efforts around the implementation of standardization are going to be expensive for them.

The basic pillars of implementing a standardization that belongs to the industry are in fact already in place. The very first step towards implementing standardization in cell

> "...companies developing highly dose-dependent therapies will not succeed if they don't consider making efforts towards standardization..."

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collections is to have patient information – cell pre-counts, lymphocyte pre-counts, sometimes we require flow cytometry, we require cell blood counts, etc, etc. All of these are things that are already in place – they are just not used, or not requested. Again, mentioning no names, but there's a particular company that even though they have very easy access to all of this information, choose to ignore it. Instead, they run procedures based on the number of liters of blood per patient, thus totally ignoring the patient as an individual.

> "Once your collection efficiency is where it needs to be, step two is to create and implement a prediction algorithm..."

So the cost is really low, because it's just making good use of patient information that is already available, but that is sometimes not communicated. Take red cell distribution width, for example – a very interesting parameter relevant to apheresis, which is useful for telling you whether a patient has more than one red cell population. This is a parameter that is part of the standard cell blood count, but most hospitals do not communicate this information to the cell therapy industry. A further parameter that would be very low in terms of cost is any surrogate measurement of blood viscosity. There are several such measurements, but all of them are super cheap and easy to obtain.

It's just a matter of talking, of saying 'hey, we know you have this patient information – could you please share it with us so we can use it to customize apheresis collection to individual patient characteristics?' Then once this generally freely available information has been obtained, the actual implementation cost should be equally low, in the big picture of things.

Q Managing variation in starting materials is increasingly a critical point of focus for cell therapy developers and manufacturers – can you highlight any strategic or practical steps that may be taken at the material procurement stage that can help alleviate this particular issue?

JMR: Collection efficiency suffers when the apheresis machine has not been appropriately programmed or used with the optimal settings, so this is the first step. It's probably the most complex, although it is not tough to do – it just requires some of the readily available patient information we discussed earlier. This data allows for adjustment of the settings in the apheresis machine, with the goal being to optimize the collection efficiency. Returning to the example of renal cell carcinoma patients, they usually have a high blood viscosity because of the presence of C-reactive protein (CRP) – that's relatively easy to compensate for in the apheresis machine.

Once your collection efficiency is where it needs to be, step two is to create and implement a prediction algorithm – another key element in the standardization of cell collections. There are different types of prediction algorithm, but they are all based on the efficiency of the apheresis device and on the cell pre-count of the patient.

For example, you might be collecting CD34+ cells. You may have one patient with 40 cells per microliter, but another patient has 200 cells per microliter. If you keep processing the same volume of blood from each patient, the final yields are obviously going to be drastically different. But using a prediction algorithm will help you calculate the specific amount of blood you need to process from any given patient to give you the yield you want.

So to summarize, the practical steps would be:

1. Identify the co-morbidities of the patient;
2. Use that information to programme the apheresis device appropriately to achieve an optimal collection efficiency;
3. Take advantage of your more consistent collection efficiency to create a prediction algorithm that will tell the apheresis center exactly how much blood needs to be processed per individual patient to achieve the desired cell yield.

Q Where is technology-/tools-related innovation most needed in this area? What new advance(s) could have the greatest positive impact, for you?

JMR: As I've mentioned, obtaining the appropriate patient data is crucial. However, I have been personally involved with leading University teaching hospitals with great reputations that clearly have logistical issues getting flow cytometry results in a timely fashion. You have situations where the patient's sample needs to travel across campus to the flow cytometry lab and by the time you get the result back at the apheresis center, the patient has already been in the machine for 3 hours, rendering the pre-counts useless.

So I would say the one piece of technology – and to my knowledge, it does actually already exist, although I don't know exactly how reliable it is – would be a table-top, point of care device that basically does a similar job to flow cytometry, but not like a full-blown flow cytometer. It allows you to use a whole blood sample, so you don't need a centrifuge, and typically gets you the result in 30 minutes right there in the apheresis unit itself. I imagine the cost of such a device would be low compared to the alternatives, and it would certainly be a very useful technology to implement because it would get around this logistical issue that many hospitals face.

I believe everything else is already in place, technologically speaking.

AFFILIATIONS

Joseph M Roig
Autonomous Cell Collections/Cell Therapy Consultant

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CELL & GENE THERAPY INSIGHTS

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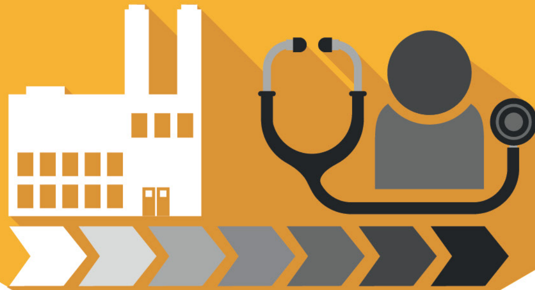

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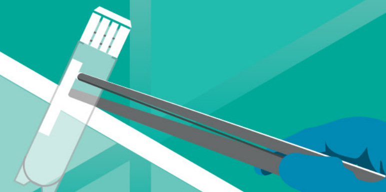

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN CHANNEL: Global cell & gene therapy supply chain strategies at commercial scale

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Volume 6, Issue 2

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Enabling clinical development of cell and gene therapies on a global basis

Brandon Fletcher, Tamie Joeckel, Martin Lachs & Olivier Saulin

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CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: GLOBAL COMMERCIAL STRATEGIES

PODCAST INTERVIEW with:
Brandon Fletcher, Cell and Gene Therapy Principal;
Tamie Joeckel, Cell & Gene Therapy Business Lead;
Martin Lachs, Vice President, Project Management, Oncology & Cell Therapeutics;
Olivier Saulin, Cell & Gene Therapy Principal at ICON, plc

Enabling clinical development of cell and gene therapies on a global basis

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Q Could you provide an introduction to your roles and activities at ICON?

> “...every new study will probably have ... risks that we never thought of, so we try to be reactive and build processes in order to mitigate those risk as much as possible.”

TJ: As the Business Lead for the ICON Cell and Gene Therapy Group. I assist with the overall strategy for our cell and gene therapy focus, and help manage and identify strategic alliances that we have with specialized service providers. I'm also involved in connecting sponsors to the correct ICON resources for the opportunities that we pursue.

ML: As VP of Project Management I run the Oncology and Cell Therapeutics Group here at ICON Clinical Research, and I work with Olivier, Tamie and Brandon in executing strategy and making sure that we run a tight ship and have a strong, forward-looking operational strategy.

BF: I'm a biochemist and cancer immunologist by training. My role as a cell and gene therapy principal is to provide scientific-based strategic and thought leadership, and to support education and training throughout all of our cell and gene therapy projects and teams.

OS: I'm a biochemist cell and gene therapy expert in the oncology group. I also have a role as program manager; I oversee operations of clinical programs with adoptive cell therapy for two clients. In the last 5 years I've built significant hands-on expertise in cell and gene therapy clinical development, and I work with Brandon to support our operation and clients in executing projects.

Q When it comes to designing and running trials, what are the biggest operational issues to consider, and how can they be addressed?

ML: I'll start with design. With any good design in any clinical trial, having the endpoint in mind is going to be a critical factor: the target product profile (TPP). In cell and gene therapy, when we talk about design, the difficulty is in making sure that scientific enquiry is balanced by practical execution – and this overlaps with the operational component of it.

Whether you're considering the end-to-end chain of custody of a living therapy, or the basics of how hospitals can set themselves up in order to actually run effective clinical trials, the design has to be built in line with operational reality. This is one of the biggest challenges in an ever-emerging field, where more complex and innovative designs are being brought to bear all the time. And of course, there's the regulatory component to consider too.

Q What are the main issues surrounding data generated from trials?

BF: This is the gorilla in the room, indeed. In the cell and gene therapy world, data management continues to be an ongoing challenge; it is magnified by diminishing site resources and increasing competition for them, leading to overburden and overburn at the site level. Not to

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mention the overwhelming amounts of non-traditional data that are generated. In response, one of the key strategies we have adopted is to use predictability in our favor.

It is imperative to understand and manage the fact that cell and gene therapies simply do not correspond with standard metrics. They must be planned, resourced and managed differently. To mitigate the negative impact of the blinded approach to data, we created and use a technology to vet out these unique expectations for strategic planning, both for us and our sites. For example, if you were to visualize the standard metric on a non-cell and gene therapy trial – such as recruitment, which could be in parallel with the Site Initiation Visits (SIVs), or data accumulation, which could be paralleled with on-site monitoring – then those graphs would be quite predictable. However, when compared to the metrics graphed for cell and gene therapies, they're drastically different. Thus, data intelligence has become the foundation of our planning, communications and resourcing in order to help us better manage high volumes and unpredictability.

OS: Due to the nature of these living therapies and the unique safety requirements of these processes, the trial sponsor usually requests a lot of data deliverables and data cuts throughout the life of the study. This can be for independent review – for example, a committee to review radiology data, or the Data Safety Monitoring Board (DSMB) to review AE/SAE (Serious Adverse Event). We also have the Biological License Application (BLA) submissions, which are a very important milestone in a study. These are important timepoints at which to review the efficacy and safety data and as a company, we have been involved in multiple BLA submissions. We have observed the intensity of those data transfers, which often require 100% of the data to be clean. We also need to ensure that the transfer is compliant to the FDA-accepted CDISC standards.

Submitting this data involves a lot of coordination between our clinical, data management, statistics, and medical writing teams. This is a real challenge because these products need quick approvals and very frequent reviews of their safety.

ML: You could summarize these issues by saying that very often cell and gene therapy studies are like clinical trials on steroids. They may involve some of the elements you expect in regular oncology clinical trials, but they're magnitudes greater in terms of intensity, and the speed at which you need to make decisions.

What strategies can help to mitigate predictability challenges?

BF: The importance of proper and thorough education and training in the realm of cell and gene therapies cannot be stressed enough. This is a complex area, and even the heavily seasoned clinical care and clinical research desks have often never worked with cell and gene therapies. Going a step further, a good portion of those on the development and sponsor side have very little experience in the field. It's still quite new to the mainstream,

> "...data management continues to be an ongoing challenge; it is magnified by diminishing site resources and increasing competition for them..."

> "Our training academy is a repository of our best practices and tools that we have developed to execute the trials."

and has been mostly managed through the National Cancer Institute (NCI) and other top tier centers. Very few of us can say we have a lot of experience in this.

Therefore, taking the time to understand the science and technical modalities behind these therapies is critical for aligned and effective drug management and development. A deep and thorough understanding of what you're working with, along with the need for flexibility and what that may mean operationally, is key to mitigating predictability challenges. One of our primary ways to manage this is through our grassroots, proprietary 6-month training program called the Cell and Gene Therapy Academy. We have roughly 300 dedicated cell and gene therapy colleagues enrolled, and this serves as the backbone of our knowledge base.

As mentioned earlier, using predictability in our favor here is key, and it's what we try to build our strategies around. In the case of data management on a site level, we have developed strategies along with predictive modelling to support proactive planning and effective communications for efficient data management to help reduce site overburden and overburn, which is a big problem in this field. The technology creates an evidence-based prediction of the site's anticipated data volume, to properly assess the data needs which lie ahead. This helps us ensure that the project is prepared and resourced appropriately, and provides tangible evidence for resource redirection.

Q What are the unique challenges posed by manufacturing and developing cell and gene therapies?

ML: We've already touched on the issue of predictability. You've also got very large numbers of individual stakeholders, more than you would expect in any other kind of oncology development program.

Another challenge is that no two technologies are necessarily alike. For example, you can't assume that autologous studies are the same as allogeneic studies, or that the needs within them are the same. There are lots of commonalities, of course. You're still talking about a living therapy. But when it comes to processes such as apheresis, there are different nuances which have to be taken into account.

There's also the notion of access. For example, familiarity with administering cell and gene therapies both in the marketed products space and also in the trial space is limited to accredited sites, for the most part. This means sites themselves have to have the appropriate infrastructure in place. We're able to help with that as part of our role, but currently there are a finite number of sites that have the capability and capacity to undertake this kind of drug development.

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Very often we are working in uncharted territory as new technologies, vectors and gene editing aspects come to light. Because some of the translational models are not highly predictive, we don't necessarily know what the outcomes will be. Therefore, we are in a constant phase of stopping and starting. Manufacturing is a major issue in this space as it can be limited, and it is also subject to numerous halts owing to emerging safety data, as well as to ensuring maintenance of purity of cells.

There's a whole raft of challenges in the development space – from logistics, to manufacturing, to patient safety, to the limitation on facilities that can actually administer these drugs. The unique challenges are expressed through limitations on who can actually execute studies of this nature, because of the highly coordinated and complex interactions.

Q What patient recruitment issues have you faced, in particular for oncology and the rare diseases?

ML: Most of the development thus far, and the products that are marketed currently, are in the hematology-oncology space, specifically lymphoma and leukemia.

When we started doing these trials about 5 years ago, it was very easy to find willing patients because the results were looking to be very hopeful; and they continue to be in terms of response rates and durability. But now, everyone has jumped on to this bandwagon for perfectly good technical and scientific reasons – hematology-oncology indications have been shown to be the most straight forward indications to target. This means the space has become quite congested in a way, and as we are increasingly moving these treatments closer to the frontline, there are alternative and cheaper treatments knocking at the door which are more accessible to a broader number of physicians and hospital institutions. This competition, not just from the cell and gene space but from other therapies such as immunotherapies and so on, is putting pressures on enrolment capabilities and capacities.

> "Whether you're considering the end-to-end chain of custody of a living therapy, or the basics of how hospitals can set themselves up in order to actually run effective clinical trials, the design has to be built in line with operational reality."

There's also a burgeoning interest in solid tumors, but it's more difficult to actually develop or find targets with which you can demonstrate the same level of response and durability as we've had in the lymphomas and leukemias. Once you get into other indication areas that are not oncology – rare diseases, CNS, etc. – you're in an area where physicians may not be as familiar with the particular challenges and requirements for cell and gene therapy studies. This places

> “We learned the hard way never to assume anything when you’re dealing with a new therapy, especially related to cancer.”

you in novel territory in terms of educating sites, and actually setting those sites up with the capability to access and treat patients themselves.

TJ: The implications of rare disease patient recruitment are obvious: we’re looking at an extremely small patient population. Not only rare, but in many cases ultra-rare. It’s really important to understand where these patients are located. Often there are pockets of patients found geographically, so being able to identify the sites that treat those patients is key to build awareness of the trial.

For example, when we were starting a study on severe combined immune-deficiency (SCID), we worked very closely with patient advocacy groups. These groups are already proving support services to these patients and know where these families are. We learned that there are pockets of communities inside the United States where there’s a relatively high occurrence of that disease state. Advocacy groups can also help us build awareness within the community about the availability of the trial.

Q Once a client gets past their first in human trials, what are the next big hurdles they face?

ML: Scalability would be number one when operating a first in human study with a small number of patients, and being able to manufacture enough product for the treatment of a small number of patients. Let’s remember that for autologous treatments the patient is effectively the main source of the drug. In allogeneic studies, that’s a different story again. Scalability is an issue in terms of manufacture, in terms of organizing the logistics of getting the therapy to the patient, and something that comes with engaging with specialist organizations and specialist platforms that allow workflow management to actually get the patients through the trials.

Many development companies with these innovative and ingenious ideas are smaller, and they may be venture capital funded or be in collaboration with larger pharma. Cost is a very sensitive issue as you scale up for a larger patient population, and then you start rubbing up against the competitive space as well.

TJ: The lack of standardization is a big issue in this space, which is exactly why ICON formed a dedicated center of excellence for cell and gene therapies. We understood very early on that these trials had to be delivered differently. We have learned along with our sponsors, and we’ve also learned from our own mistakes. Those experiences have become best practices that are now the foundation of how we execute these trials. Our training academy is a repository of our best practices and tools that we have developed to execute the trials. Additionally, every protocol has unique requirements and each site has its own standard operating procedures (SOPs), and these have to be integrated into our required workflows.

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The lack of standards, combined with the sheer number of stakeholders required in these trials, dictates the need for very detailed training and defined workflows. This becomes even more important as sponsors move from early phase to Phase 2 and 3, and on to commercialization. As the number of patients and number of sites grow, it only exacerbates the issues and challenges we face.

We're doing a lot of work with standards. We work with the International Society for Cellular Therapy (ISCT), the Alliance for Regenerative Medicine (ARM), and standards coordinating bodies. The field is constantly evolving and there's still so much work to be done. Not to mention the reimbursement hurdles from a commercialization perspective that can impact patient access.

Q How do these challenges evolve when you then consider not just scalability, but running multinational trials? What would you say are the most important considerations?

ML: Regulations are heterogeneous. What goes on in Japan is different from what goes on in the US or European countries, or Australia or China, which are all big centers for cell and gene therapy development. Navigating this heterogeneous regulatory field is something we have a lot of expertise in, both through experience and the ability to database the information.

The other component is moving materials, which are also subject to regulatory and safety considerations, across borders. For example if you look at allogeneic cell therapy and developments in specific areas like gamma delta T cells, you might have to source tissue from which you extract cells, which are then going to be the source of some of the components for your therapy. How do you move those across borders? You're not talking about just the cells themselves, you're actually talking about tissue – possibly from plastic surgeons, for example. Again, coordinating those activities, having a strong knowledge base about how that can be affected, is something that a CRO has a big part to play in.

TJ: As Martin says, these are living therapies. Therefore, you have a chain of condition that has to be monitored, because there's temperature sensitivity. Therapies are shipped in liquid nitrogen dry vapor shippers, and you have to monitor those for temperature excursions. The chain of custody has numerous hands-offs, and end-to-end traceability and trackability has to be maintained and documented between all of the stakeholders involved.

Then there's the unpredictability of customs clearance. If you're moving these therapies between countries, you can ship something 20 times via a specialty courier and have it pass through customs just fine. But then there may be a time there's a particular person at the border that holds up the shipment because of insufficient documentation,

> "Cost is a very sensitive issue as you scale up for a larger patient population, and then you start rubbing up against the competitive space as well."

or some other issue, and at that point you've got to make sure you can mitigate the risk of temperature excursions. It's an extremely complicated process – it's not a case of just putting it on a plane and assuming it's going to reach the destination.

Q How is trial design evolving to meet the needs of the cell and gene therapy space, and what are the biggest innovations you are seeing in this area?

OS: One of the most innovative trials we worked on recently involved evaluating patients based on the presence of cancer specific antigens in solid tumor, regardless of the tumor type. The study tested the effect of drugs in a variety of cancer histologies, which allowed us to test a lot of different tumor types, which then leads more rapidly to results on which specific tumor type to concentrate on.

This type of design is reducing patient exposure to these drugs and allows us to have quicker results than if we're doing one study for each Phase 1, 2, 3. I think we will see more and more of this type of adaptive design in the future, and that's going to really help with the specific challenges of this space – for example, looking at CAR-T in multiple solid tumor.

ML: In common with much of oncology development and beyond, because you're not talking about mass manufacturing, we're seeing that trials are becoming more registration-focused at an earlier phase. There's a move towards more adaptive designs, such as basket type trials, umbrella trials and platform trials. We're trying to combine as many possible treatment groups within the framework of a single trial.

Long-term follow up studies are becoming increasingly important. Subjects and patients who are receiving these treatments are effectively becoming genetically modified organisms, and we're still in a position where we have to follow through with patients for a very long time after they are treated. Designing long-term follow up protocols to allow us to accrue data for an extended period of time is also challenging.

Q Looking beyond trial design, what would you consider to be the hot topics and biggest hurdles cell and gene therapy development is facing, and how can CROs in particular be involved in addressing them?

> "…we are focused on building solid risk assessment categorization tools, or risk management plans…"

TJ: As we move from an autologous 'one and done' dose therapy to an allogeneic therapy that may be multiple doses, these patients are going to be discharged and going back into their own communities. ICON has been a leader in this area: we acquired a home health services group and bringing the trials to the patients within their communities is something we're

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> “The chain of custody has numerous hands-offs, and end-to-end traceability and trackability has to be maintained and documented between all of the stakeholders involved.”

working to anticipate. A lot of these patients are very sick, and the four or five-hour long drive to a site to be administered a dose may not be practical for them. We're really looking at how we can support our sponsors in doing this not only in the clinical trial phase, but potentially in the commercialization phase.

ML: Cost is probably the largest tangible challenge if you look into the commercialization of what are otherwise extraordinarily exciting technologies. Not just the cost of doing trials, which is not trivial, but also the cost of the product once you get close to commercialization. We've seen from the two marketed products out there at the moment, Yescarta® and Kymriah®, that they don't come cheap. When you factor in the additional costs of hospitals administering treatment and general care for the patient, they have an even higher price. A lot of development is targeted towards managing that cost downwards, whether it's through allogeneic therapies, which arguably should be a lot cheaper, or in other ways. There are other approaches to patient treatment that are a lot cheaper. If they can be proven to be as effective – which may or may not happen – that is going to be the threat for the further development of cell and gene therapy.

Realistically, we can work to support companies as we do because we have our own pricing, market access, and commercialization groups who are experts at looking at things like net present value and doing full evaluations on potential. They take into account patient groups that would benefit at an international level, and also look at the market as a whole in terms of what is out there, what has got the greatest chance of success, and what that means for payers in terms of the long-term benefit for patients. Bringing that level of expertise in helps drug developers visualize where they may be going with their particular assets.

Q What have been your most educational mistakes when supporting the development of therapies in the cell and gene therapy space?

BF: Looking back, we've certainly had many blind spots along the way! In the beginning, we kept trying to frame this area and create boundaries with clear definitions, so that we could frame our own work. For instance, in our work with CAR-T: this area of cell and gene therapy is itself definitely unique, but as soon as we got our heads around this concept, developed our systems and processes and deployed our troops, the field changed. New scientific modalities such as TCRs, tumor infiltrating lymphocytes (TILs), marrow infiltrating lymphocytes, and multiple tumor associated antigens, *et al.*, emerged. We reframed again, adding the allogeneic realm. Then we reframed it all again for solid tumors. This process of tearing down and

rebuilding shed light on how easy it is to take a limited view, and the importance of keeping our minds extremely open.

In clinical research we're programmed to standardize processes, but with cell and gene therapies, we had to look to the learning and listening aspects, and the challenging of our processes, because there are no standards. And it's too soon to make standards just yet because it's moving at lightspeed.

We had to plough our way through the dark and there were a lot of educational mistakes. A lot of that was trying to put boundaries around this area, which is massive – we just didn't know it at that time. Trying to create process and standardizations within something that is exploding is a very difficult thing to do. We learned the hard way never to assume anything when you're dealing with a new therapy, especially related to cancer. And we learned not to assume that all cell and gene therapies are created equal, or would bring similar challenges or similar results.

OS: I agree. With cell and gene therapy trials, we have built a culture of lessons learned and tried to learn from every error or mistake in order to apply and refine our processes. We do this for every study and we are focused on building solid risk assessment categorization tools, or risk management plans, to ensure that we mitigate the risk related to manufacturing processes changes, limited capacity, logistical issues, and the unexpected volumes of data. We also know that every new study will probably have other risks that we never thought of, so we try to be reactive and build processes in order to mitigate those risk as much as possible.

Q Looking to the future, what do you predict as the key challenges and opportunities for the cell and gene therapy sector within the next 5 to 10 years?

BF: We have already spoken on the challenges of expanding access to these therapies, and this further challenges CROs and sponsors to lead the guidance and support for community centers to manage these safely and accurately. A strong focus will be on streamlining logistics and reducing cost to truly support the opportunity of access.

In oncology, the field still awaits a clear demonstration of clinical efficacy of cell and gene therapies in solid tumors. This challenge is becoming a defining issue in cellular immunology as the new decade begins. Solid tumors make up almost 80% of all cancers, and the key challenge is creating and managing therapies which can manipulate and/or withstand the inhospitable environments of these tumors, known as the tumor microenvironment (TME). In the future we'll see a focus on bringing these therapies to solid tumors in a meaningful and effective way.

Persistence of these therapies, whether they be hematologic, oncologic, or rare diseases, is also a key driver. The primary challenge is understanding and combatting the mechanisms that act against our cells, including these therapeutic cells, and decrease their ability to persist once they're active against their target. This is an area where we've not had a breakthrough yet, and I think that's going to be one of the major focuses moving forward.

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Most importantly, from a broader perspective, our knowledge of disease, the genome, and tissues still far outweighs our ability to respond from a medical technology perspective. We're going to be playing catch-up for quite some time. In the cell and gene therapy world, the emergence and elevation of gene editing technologies is key. We have the ability to modify genes *in vivo*, and the potential here is mindboggling. We also now have the ability to recreate our starting materials from, for example, master induced pluripotent stem cells. This is extremely exciting. As gene therapies and genetically modified cell therapies leverage these emerging gene editing technologies, coupled with ever more optimal material resources, the future of cell and gene therapies is more than just promising – it's tangible.

ML: Brandon has summed it up beautifully: there is going to be expansion. In the same way that we have moved to mark 1 and 2 of immunotherapies, whether it's through antibodies or immune checkpoint inhibitors, I think the potential here is vast. This is not limited to oncology, hematology, or solid tumors. I think we will see a raft of indication spaces for rare diseases, ophthalmology, and even CNS.

There's a huge mountain to climb and we're not even at base camp yet, but we're going to have increasingly accelerated progress. The knowledge base is vast – it's how you tailor the application to address that knowledge base that is the real challenge. In a way, it's a different kind of translational medicine.

TJ: A few years ago, Dr Tim Cripe of the FDA said that cell and gene therapy represented the most exciting new therapeutic breakthrough he had ever seen. I think it truly is, and it's going to continue. When I joined ICON, I really appreciated how we internalize what an honor it is to work on these trials, and the importance of the hope that we are able to bring to patients. I expect to see the space continue to grow and flourish.

BIOS

Brandon Fletcher

Brandon Fletcher is a Biochemist and Cancer Immunologist with over 27 years of research experience; 23 in hematology-oncology and four in infectious disease. She has held roles in broad immune-oncology and cell and gene therapy research within academia and industry. Brandon is a collaborator with NCI's origination of cancer CGTs and co-founded a global immune-oncology research training and support organization.

Tamie Joeckel

Tamie Joeckel started her career with Arthur Andersen & Co (now Accenture) as an ERP manufacturing and distribution systems consultant, and has over 20 years of experience in specialty biologics working in both clinical and commercialization sectors. A former senior executive at one of the largest drug distributors, she specialized in distribution, patient hub services and reimbursement support for newly approved therapies for oncology and rare diseases. For the last 6 years, Tamie has worked with cell and gene therapies as a global logistics and ecosystem expert. At ICON, she supports the Cell and Gene Therapy Solutions Group with ongoing strategy and innovation.

Martin Lachs

With over 28 years' experience in clinical development, Martin has worked across a number of therapeutic areas whilst specializing in oncology. Martin heads up ICON's Oncology and Cell Therapeutics Project Management Group, lending operational and indication expertise across a group of over 260

international project management staff globally, dedicated to oncology and cell therapy drug development. He has worked in developing key oncology site networks in the US and the UK and in 2020 was a member of a clinical trial review panel for University of Sydney affiliated hospitals. Martin has produced a number of position papers in the Pharmaceutical Press as well as hosting / presenting at numerous international events at scientific and pharma industry meetings. Recently the key focus of his publications has been thought leadership related to cell therapies in oncology, e.g., CAR T.

Olivier Saulin

Olivier Saulin has over 18 years of research experience in oncology and rare disease and holds a MS in Bio-chemistry. His expertise includes extensive roles in DM and project management for both sponsors and CROs; including large CAR-T programs in Phase 1 to Phase 3 trials. He has also worked on GMO studies involving gene therapy (AAV vector) and cell therapy products (CAR-t/ lentivirus). Olivier is the EU cell and gene therapy expert for ICON supporting CGT teams. He co-developed the adoptive cell therapy training academy for the company and authors the internal cell and gene therapy newsletter.

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Transforming Oncology Trials in Cell and Gene Therapy

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INTERVIEW

Best practice for raw materials management on a global basis

STEPHANE LAMEYNARDIE has a Pharm D from the University of Montpellier with specialization in Industrial and Biomedical Pharmaceutical Sciences. He has worked in the industry for the last 20 years in various domains ranging from QA and GMP manufacturing to Project Management and Development, and across both small and large organizations.

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Q What are you working on right now?

SL: The technical development team is working on several programs, in both cell therapy and gene therapy. As we don't have an IND submitted or approved yet for our current main project, I can't say too much about it, but we are certainly very active from a development perspective, building upon our first approved products – Kymriah® in cell therapy and Zolgensma® in gene therapy.

Q Of course, Novartis is unique in having successfully reached commercial scale with both a cell therapy and a gene therapy. How do you reflect today upon that journey and the challenges you faced?

SL: Traditionally, when you are in the early stages of R&D – working in the lab with a cell therapy, for example – you don't tend to focus too much on the quality of the raw materials you're using. You need a culture medium, say, and you simply use whatever works best. When I started working in cell and gene therapy 6 years ago, we didn't really have the same awareness as we do today that we were using products and materials that would one day move all the way to clinical and GMP manufacturing. Of course, you reach the stage where you do need to select the right suppliers and the right materials to move from research grade to GMP grade, because at a given point the agencies expect to see that in the dossier you're submitting. You also to bear in mind that specifications for each of these materials will have to be established.

I became lead of raw materials work streams as there was not many candidates to do it within the project team and the importance of it was obvious to everyone. I began working in very close collaboration with the process and pharmaceutical development team, keeping everyone appraised of the raw materials available on the market and making sure the right ones were implemented in the process, and at the earliest possible stage. For example, you might have 15 or 20 culture media available that all do the same job, basically, but perhaps only 1 or 2 will be usable in a GMP environment. Close collaboration with Regulatory Affairs is key as you can understand from an early stage what will be expected for the filing.

It was not an easy task. In some areas, there's no way you can replace a given raw material because it's so specific or even unique. In such cases, it becomes a matter of collecting and building as much information as possible on that material, sometimes working closely with the supplier on your regulatory approach – for instance, on how you will explain to the agency what kind of controls you have put in place for a very specific raw material that can perhaps not be produced at a GMP grade. You need to show agencies that you have been thoughtful on safety and control measures regarding these raw materials.

Q Can you tell us more about the processes/strategies Novartis has in place to mitigate risk in the critical raw materials supply chain, especially in the commercial product manufacture setting?

SL: For some of the most critical raw materials, it's identifying a second and even a third source. This work should be done before reaching the market because after that, if it's a critical raw material, it can have quite some impact on the file that was submitted – you will need to provide evidence of comparability and so on. It's important to think about this while you are in the clinical phase because there, changes can be a bit easier to make. Of course, when you identify a second source you also have to prove this second source of material has no negative impact on the final product. Again, doing this before the commercial phase is ideal. I've seen examples where a product was marketed and they then had to change one of the critical materials… I don't want to say 'nightmare', but it's certainly complex.

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> "What we try to do as much as possible with those materials that are used across the whole company is to put in place some form of supply construct that can also apply to the CMO, allowing us to benefit from better pricing ... We also tend to use off-the-shelf products, avoiding custom products wherever possible, which makes life simpler for the CMO."

It's also key to establish a strong collaboration with the supplier. It is not too long ago that suppliers generally just sold research material or culture media material without giving much consideration to if and how they could be brought up to GMP grade. Today, I see that there's really been evolution in this regard, because the suppliers have been asked by numerous different cell and gene therapy companies that they need to provide this specific quality material, with this particular kind of documentation package, and this specific kind of testing, and so on. I don't have full broad knowledge of all the material suppliers, but certainly the ones I've been working with recently have picked up on this trend very early, and have shown that they have a good understanding of what we require (and will require) from an early stage of development. For example, if you go to Merck Millipore, the full regulatory package is even available online for some of their products, which makes our lives extremely easy.

With some suppliers, specific development was possible in terms of volume/weight packaging in order to reduce handling during processing in the cleanrooms. Moving from culture media bottles to bags, for example.

There's nothing standardized in this regard at the moment. It's really based on the individual supplier company's willingness to move in this direction. I would say they are all tending to do so, but at different speeds.

Q Can you share any examples of how you have sought to troubleshoot potential material sourcing bottlenecks?

SL: We had to switch human serum albumin (HSA) supplier for two different projects. The product was perfectly fine and doing its job – the issue arose because the supplier deemed that our projects were competitive to one of their own. So we had to switch HSA during the development stage of one project, but the other case was actually with a commercialized product. The switch went quite easily for the former product, largely because we already had a back-up plan in place – we had tested another albumin supplier. For the marketed product, it was also feasible but just a little trickier, because you have a product that's fully approved and so again, you have to demonstrate comparability between both types of HSA.

The lesson here is that the more you think about these things early on and in advance, the more you can ensure that a given supplier will follow you all the way through, reducing materials supply chain risk considerably. HSA is a good example because you have plenty of suppliers to choose from, as well as options in terms of the actual material itself – you can use either human albumin or recombinant albumin. In my project, we assessed several suppliers of both human and recombinant albumen in advance. This allowed us to understand which product was doing the best job – all of them worked adequately, but one or two were a bit better than the others for our purposes – but also helped prevent disruption caused by future sourcing issues.

Of course, you cannot do this with all materials. For instance, some cytokines can be really specific in terms of available format, and you simply don't have much (if any) choice. It's really on a case-by-case basis for each material.

Q What 'best practices' would you highlight in working with CMOs that relate to optimizing raw material quality and continuity of supply?

SL: The first thing to do before you go to a CMO is to really get to know your own process. You will then be in a position to assess the potential impact on it from changes in materials.

In our particular case, the CMO we work with is really following the instructions we've provided – in other words, the process we transferred to them. They are not doing any optimization. However, we are open to discuss their feedback and experience on specific steps of the process.

What we try to do as much as possible with those materials that are used across the whole company is to put in place some form of supply construct that can also apply to the CMO, allowing us to benefit from better pricing. So we tend to include the CMO in our own overall volume calculation for a given material, even though those materials may be supplied directly to the CMO. We also tend to use off-the-shelf products, avoiding custom products wherever possible, which makes life simpler for the CMO. All of this is done in collaboration with our Sourcing/Purchasing department to seek harmonization of raw materials across the company.

When you perform the tech transfer, you provide your list of raw materials to the CMO. Our CMO is not in the same country as us, so they have to assess if these materials are available in their country and if they aren't, to find an equivalent. This is done in a gap analysis performed by the CMO. We then need to figure out whether this is essentially the same product with a different name, or something completely different. This assessment is carried out between the two parties. Usually this approach works fine but we have had some cases in the past where, for example, the material was developed in the US with some

> "...where possible, it's beneficial to select materials that are available in Europe, the US, and Japan..."

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medical device or consumables that are specific to the US, but that were not available in Europe because they did not have CE mark authorization. So we had to find an equivalent material, because the US suppliers were not willing to start the registration process to allow their product to be made available in Europe. That's a key thing to bear in mind: where possible, it's beneficial to select materials that are available in Europe, the US, and Japan. (Japan can be very tricky in this regard, because the market there is a little more closed – you really have to assess what would be available locally).

> "…you firstly need to asses which raw materials you will be using, including which ones can be switched later to GMP materials…"

Consumables can present similar issues. For example, GE Healthcare had some culture bags for the Wave bioreactor that were very specific and (for a period of time) were not available everywhere in the world.

To summarize: during development, you firstly need to asses which raw materials you will be using, including which ones can be switched later to GMP materials or even better, to registered medicinal products. This will not be possible in every case, but it's important to have this mindset from an early stage – certainly by the time you begin development for clinical manufacture. It is also optimal to favor off-the-shelf, GMP-grade products where possible – GMP-grade culture medium or cytokines, for examples – and to make sure your supplier has the capacity to sustain supply in the future. This type of assessment will be very much on a case-by-case basis: some projects require liters and liters of culture medium, for example, while others do not. It's up to you to look into this thoroughly. This includes gauging whether it will be preferable to have your media delivered in a bag rather than a bottle for ease of handling, for instance. I would strongly advocate spending a bit more time assessing all of this from the beginning, thus avoiding later changes wherever possible.

In addition, the CMO has to establish material specifications and in-coming ID testing. Depending on the clinical phase you are in, a close collaboration with the CMO is needed in order to agree upon the different tests to be performed without overdoing it. Minimum requirements need to be defined and implemented for each of the materials depending on its criticality to the process. Syringes will not be considered in the same way as cytokines, for example. Registered medicinal products would be managed differently than research grade material. Therefore, the CMO has to have a clear critical assessment and ranking of raw materials in order to define the appropriate level of testing. Support from the customer may be needed for some complex materials such as culture media. ID testing on these can be difficult to establish.

Q Are there any further 'do's and don'ts' for working with raw material suppliers in this space that you can share?

SL: I would add that it's really important to have a dedicated raw materials lead within a cell or gene therapy development project team/unit. It's not that I want to

advocate having raw materials leads for the sake of it! It really does make sense to have someone who is dedicated to understanding all the myriad requirements to work very closely with the regulatory team, because they will be writing the file, they will be asking, "do you have this certificate of analysis (COA)? Do you have this list of materials? How is this material manufactured exactly?" and so on.

So it's really important to have someone who has the overview, who is focused more or less exclusively on building this raw materials list and collecting all the information. And believe me, it really does take some time to do this, because while it will be very easy to obtain the information and documentation from some suppliers or materials, it will be more difficult with others.

When you work with complex products like culture medium, that are an assembly of many, many ingredients, the virus safety assessment can be extensive. You have to look very closely into what exactly is of chemical origin, what is of biological origin, and then how those ingredients of biological origin are manufactured. For example, we had a culture media which contained insulin. The supplier of the insulin had two different ways of producing it – one method involved using porcine enzyme, the other did not use material of animal origin. So you really need to dig into those details and understand how your materials are produced, because again, once the file goes to the authorities, it will make your life a lot easier. If you go to Japan and you have bovine serum albumin in use for your product, the authorities there will ask you all kinds of questions, and they will want to see the traceability of the individual cow that provided this albumin! And not just that individual cow but going back a number of generations in order to really have a good understanding that there is no contamination risk by BSE, for instance. (It is also worth adding that using non-animal component ingredients or raw materials is generally preferable, of course).

Collecting all of this information: viral safety, COA, certificate of compliance, certificate of origin, GMP, testing for things like sterility (and is it all being done against US, European or Japanese Pharmacopoeia?) – I think having someone who has a really good overview of everything and who can provide something robust to the authorities later on is of great value.

Q What would you pick out as the single greatest need in terms of raw materials evolution or innovation for the cell and gene therapy space today?

SL: It would be to have some form of harmonization of standards across the various regulatory agencies for what is expected in terms of raw materials quality and testing, as similar raw materials are used across multiple cell therapy projects.

Several manufacturers are registering Drug Master Files in the US for their products. However, there is no such thing in Europe, for example. Even the basic terminology used in the US and EU differs: 'ancillary materials' versus 'raw materials', for example.

If one regulator says a material needs to be GMP-grade, it would be nice if this was a little better defined. You can get some materials that are ISO 9001 or 13485 (for medical devices) certified, for instance, but that's not the same level as something that is fully GMP compliant.

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If there were a clearer definition of exactly what is expected by the agencies, and that could go across all the different materials suppliers, that would be extremely helpful. Each supplier has its own interpretation and implementation of GMP. Even though there is some guidance, harmonized regulations for the manufacturing of raw materials for cell therapies still need to be clearly established. As a result, no official certification is available for the manufacturing of GMP grade raw materials. At the end of the day, it's to avoid having to deal with the different levels of GMP compliance that exist: some suppliers will push it as far as possible, others will do the minimum. A harmonized standard that everyone works to would be hugely beneficial.

The same goes for starting materials, such as blood-derived materials (apheresis). Simplification of mutual recognition of accreditation across countries and organization would be helpful there.

Cell and gene therapy has become global and it would be good if organizations at the national level had the same mindset from a regulatory point of view.

Q Finally, what are your chief goals and priorities for the 12–24 months ahead?

SL: We are expecting an IND 'safe to proceed' in the relatively near future, so the next step for me will be to put everything in place to run the clinical program. That's the main goal, because we have advanced past the raw materials selection process. It's nearly time to see how our product does in patients, and we're really looking forward to that!

AFFILIATION

Stephane Lameynardie
Novartis, Switzerland

AUTHORSHIP & CONFLICT OF INTEREST

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EXPERT INSIGHT

Supply chain challenges and issues facing the autologous cell manufacturing industry

Kan Wang, Ben Wang, Aaron D Levine & Chip White

We discuss three supply chain challenges and issues facing the autologous cell manufacturing industry that may impact patient outcome and supply chain performance in a commercial setting. These are (i) the potential value of giving priority to the sickest patients with respect to the start of manufacturing their therapy, (ii) determining the optimal manufacturing capacity and reagent replenishment policy for a single cell manufacturing facility, given demand forecasts and patient service levels, and (iii) the resilience of reconfigurable supply chain networks for supply chains with geographically distributed manufacturing capacity.

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Autologous cell therapy, a new form of personalized medicine, is an emerging therapeutic method where a patient's own cells are used as medical treatment. In one prominent example, T cells are bioengineered to express chimeric antigen receptors (CARs) that identify, attach to, and subsequently kill tumor cells. These CAR-T cell therapies have proven highly effective for treating patients with life-threatening blood cancers. These successes, along with a wave of research and investment, suggest that autologous cell therapy is poised to play a key role in a new era of cancer care and to benefit

patients with a range of other medical conditions as well.

The resulting emerging industry and supplier base are generating new and important supply chain design and operations challenges. The NSF Engineering Research Center for Cell Manufacturing Technologies (CMaT) Center, headquartered at the Georgia Institute of Technology, is a translational research center dedicated to enabling the robust, scalable, low-cost biomanufacturing of high-quality therapeutic cells in order to bring affordable, curative therapies against incurable chronic diseases to as many individuals as possible. CMaT is currently supporting simulation-based studies, led by the authors, that aim to reduce total manufacturing and logistics cost and risks, improve patient benefits (with focus on improving patient safety, reducing mortality rate, and increasing access), and incorporate patient and regulatory perspectives. Details of several of these studies are described in [1].

A variety of supply chain challenges and issues facing the autologous cell manufacturing industry have received attention in the industry literature. A partial list of these challenges and issues includes: cost of goods [2,3], a variety of risks including raw and starting materials, quality, variability, demand surge, reagent supplier disruption [2,4–8], the need for flexible facility growth plan for increased capacity for future commercialization [2,9], procurement [10], the identification of Critical Quality Attributes [6], data management [11,12], upstream and downstream bio preservation [3], reagent inventory control [13], supply chain network design, i.e., centralized manufacturing capacity versus distributed [13], product consistency, automation, and distributed manufacturing capacity [13], firm structures in the industry, i.e., whether to outsource or depend on internal capabilities, the 'make or buy' decision, mergers and acquisitions [13–15], regulatory considerations [16], and workforce considerations [17]. We now elaborate on a few of these challenges and issues and address three in more detail – priority queuing, capacity planning, and reconfigurable supply chain network resilience – that in our opinion are emerging challenges and issues that have been insufficiently examined.

With regard to the cost of goods and other costs, cost is a major barrier to the broad accessibility of these immunotherapies [18]. Novartis' novel CAR-T cell therapy Kymriah has a list price of $373,000 or $475,000, depending on the type of cancer. Yescarta, a cell therapy produced by Kite Pharma (recently acquired by Gilead), has a list price of $373,000. These list prices are only a portion of the total cost of treatment, which can easily exceed $750,000. As a result, cost reduction is a key interest across the industry.

With regard to reagent availability risk, emerging industries typically have emerging supplier bases, which may have only a single supplier for a key good or service. As a result, a shutdown of a major supplier can be highly disruptive. One such disruption, which resulted from sterility issues, recently caused the unavailability of a key cell therapy reagent, bringing therapy production to a halt for 2 months for some manufacturers. In one of our 'what If' studies, due to the above cost considerations, we reduced the number of bioreactors and reagent inventory in our single facility simulation model to a minimum, producing a lean therapy production facility. We then introduced a 2-month reagent disruption. Once the delivery of reagent was resumed and a backorder surge was experienced, the facility was not able to return to normal operations until the number of bioreactors and the amount of reagent in inventory were increased, which provided the facility with the resilience that it needed but at an added cost. Examples of how increasing the number of suppliers for key reagents and how multi-facility (distributed) manufacturing supply chain networks can mitigate reagent availability risk can be found in [1]. We remark that the short shelf-life of the reagents and the in-house manufacturing of buffers and medias with even shorter shelf-lives exacerbate the impact of a supplier disruption.

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A key question in designing a supply chain network in this industry is whether to have a centralized manufacturing network with a single manufacturing facility or a distributed manufacturing network with multiple, geographically distributed manufacturing facilities. Centralized networks, compared to distributed networks, have less demanding regulatory requirements, greater potential for economies of scale, greater consistency in operations, less total capital expenditure, but slower fulfillment times (which may reduce access for some patients). Further, if goods and/or specimens can be transshipped and manufacturing capacity is easily relocatable (e.g., bioreactors, 3D printers) in the distributed network, then a dynamic form of resilience is possible, reducing total resilience capital expenses and allowing facilities to run lean when possible and be resilient when necessary. Dynamic resilience is an emerging and incompletely understood feature of reconfigurable supply chain networks. Automation can potentially help mitigate the difficulty in ensuring uniformity of operations at multiple facilities, although the fast clock speed of technology innovation in this industry represents a barrier to achieving uniformity of operations. Determining the best network design, which our software can support, is application specific and influenced by regulatory requirements, patient demand, and other factors.

The emerging need to optimize reagent inventory, given the inherently high cost of cell therapies, is extensively discussed in [13], and this need is inextricably linked to demand forecasting. Demand forecasting for a new product is invariably a challenge; this challenge for cell manufacturing therapies is heightened by uncertainties surrounding the future regulatory environment and the fast pace of innovation. Using our software, we are now able to determine the optimal manufacturing capacity level and an optimal reagent replenishment policy, given therapy demand and a patient service level (PSL). We describe therapy demand as the probability distribution over demand and PSL as the probability α, where the probability that one or more patients will have to wait for their therapy production to begin is less than or equal to α (ideally, a small probability). We leave scenario development and the number of scenarios to consider up to the user, where a scenario is the pair (therapy demand, PSL). Our foundational analysis is currently based on a stylized model that considers multiple reagents but does not consider the possibility of supply disruptions. Simulation models can be more granular in order to consider supply chain disruptions for different materials with different suppliers and lead times and to provide a more realistic view of how costs accrue throughout the supply chain.

Another challenge that we anticipate emerging in the near future is the specimen queuing discipline at a cell manufacturing facility. Typically, therapy manufacturing begins in the order that patient specimens arrive at the facility, i.e., the first-in-first-out queuing discipline. However, patients can be in various states of health when their specimens arrive. We are currently investigating the impact of another queuing discipline, the priority queue, where a small number of the sickest patients are given priority with respect to the start of their therapy manufacturing, i.e., go to the front of the line. Initial results indicate that the priority queue can significantly reduce the average mortality rate with an indiscernible impact on the mortality rates of patients who are not considered priority cases. These initial results are based on sparse data, are dependent on the percentage of patients that are given priority status, and hence can only be considered tentative. However, the priority queue shows promise as a way of improving overall patient outcomes.

TRANSLATION INSIGHT

Reducing the cost of resilience of reconfigurable supply chain networks for supply chains with geographically distributed manufacturing capacity, determining the optimal manufacturing capacity level and an optimal reagent

replenishment policy for a single cell manufacturing facility, given a demand forecast and a patient service level, and reducing the average mortality rate by giving priority to the sickest patients with respect to the start of their therapy manufacturing are all intended to improve the societal and commercial value of cell manufacturing and supply chain networks.

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AFFILIATIONS

Kan Wang
Georgia Tech Manufacturing Institute, Georgia Institute of Technology, Atlanta, GA 30332, USA

Ben Wang
Georgia Tech Manufacturing Institute, Georgia Institute of Technology, Atlanta, GA 30332, USA
and
H. Milton Stewart School of Industrial and Systems Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA
and
School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA

Aaron D Levine
School of Public Policy, Georgia Institute of Technology, Atlanta, GA 30332, USA

Chip White
H. Milton Stewart School of Industrial and Systems Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA

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CELL & GENE THERAPY INSIGHTS

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EXPERT INSIGHT

Durable and curative responses in heart disease: promises and practicalities

Gabor Foldes & Vasiliki E Kalodimou

Stem cell research led to many therapies becoming available to treat or repair injured or diseased tissues in a range of diseases, from cancers to spinal cord injuries. For cardiovascular therapy, the unique capacity of pluripotent stem cells to replicate and provide large numbers of replacement cells in culture for transplantation purposes may give an advantage over the use of adult stem cells. Mechanisms of their retention are in correlation with route and method of cell delivery, a build-up of cell constructs with materials as well as delivered doses and optimal timing. Yet, their cell potency in vivo is a critical element which warrants a better description. Reliable biomarker identification will advance our understanding of how long the replacement cells will continue to function and their mechanism of action; leading to accelerated novel therapies where cell therapy products show a better homing, integration, quantity, or overall quality upon transplantation.

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"I think my pre-industry experience is me building LEGO houses and wishing people would go through them."– Brenda Brathwaite, an American game designer

INTRODUCTION

Cosmas and Damian were two physicians and reputedly twin brothers, who practiced their profession in the Roman province of Syria over 2000 years ago. Based on a story

attractive for many artists and clinicians over hundreds of years, they miraculously transplanted the black leg of a recently dead Ethiopian man onto the white body of the patient with a "cancerous" leg [1]. With a somewhat unchanged aim for dramatic and durable cures, there is a growing number of regenerative medicine and advanced therapy trials underway worldwide (currently around 1,000). Whilst most of these works are focusing on oncology, over 70 of these early studies are on cardiovascular disease, the second most important patient group in the ranking. Both clinical trials and preclinical research projects in cell and gene therapy represent a marked increase in number year-on-year and this includes a significant number progressing to Phase 3, the later stage of clinical development [2].

CARDIAC THERAPY WARRANTS A DIFFERENT APPROACH

Modern cardiology has improved in terms of secondary prevention and post-myocardial infarction maintenance, drastically reducing complications and driving patients to an almost normal life. Despite substantial improvements in survival with current device and pharmaceutical-based therapies [3], coronary artery disease remains a common cause of death and the rising prevalence of chronic cardiovascular disease is of major concern [4]. It seems that novel techniques cannot reverse cardiovascular disease and use of transplantation or assisted devices during the chronic phase is further limited due to organ shortage, high costs, and complex postoperative management. Targeted cell replacement therapy has emerged as a promising future approach to regenerate functional myocardium or vascular system. The approach we would follow should, however, be largely different from the cell and gene therapies successfully trialed already for blood-based malignancies like leukemia. Complex changes upon injury and repair of the heart affect 3D structures, vascular system and multicellular composition of the organ, not only cells. Furthermore, multiorgan dysfunction related to heart failure and low perfusion deteriorates other organs like kidney and the peripheral vasculature. Thus, infusion of cell suspension may not be enough to show benefits in this highly structural disease. Not surprisingly, infusion or injection of hematopoietic stem cells failed to produce cardiac cells and did not lead to biologically relevant benefits in cardiac disease [5]. There are several hurdles which may limit the translation of tissue-engineered therapies to rebuild these complex cardiac structures. As summarized recently by Eugene Braunwald [6], this includes not only the selections of cells, recipients and donors but also the methods of cell delivery and construction of matrices to provide cells with scaffolds.

THE FATE OF THE TRANSPLANTED CELLS REQUIRES CONTROL & BETTER UNDERSTANDING

Predictive biomarkers for patients with cardiovascular risk factors may guide assessing the timing and optimal doses for cell therapy candidates. Similarly to high levels of circulating biomarkers predicting an unfavorable effect in hematopoietic cell therapy, the potential clinical utility of the baseline biochemical signatures in cardiovascular cell therapy may also improve the selection of patients, personalize cell sources, or enable a prediction of individual responses to cell therapy [7]. However, our understanding of cell fate after transplantation is limited. In addition to issues with tissue engineering, approaches to repair cardiovascular tissue can be challenging even due to the complexity of living cardiovascular cells. This means an inherent variability of the cellular starting material, their complex mechanisms of action or just the poor definition of the product's Critical Quality Attributes. Most of our preclinical information comes from *in vitro* and xenotransplant assessments, but we have little idea of how human cells function *in vivo*.

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In fact, the challenges for cardiac cell therapy are therefore not profoundly different from those already reported in CAR T therapy for solid tumors [8,9]. Cardiovascular cells also become metabolically "exhausted", and thereby less proliferative, with poor expansion capacity and inefficient trafficking [10]. This can negatively influence manufacturing capabilities and full activation of the product. Additionally, the value of adding an exhausted cell may be questionable for patients with comorbidities where endogenous regeneration is also limited, increasing the probability of off-targets and related side effects. So how do we know that we have the right cell quality, like stable and surviving cell products? To answer this, we need a variety of biomarkers to quantitate expansion (proliferation of cardiac cells), persistence (cell retention and angiogenesis), the phenotype of transplanted cardiovascular cells (i.e. trans-differentiation via endothelial-to-mesenchymal transition and residual stem cells), and a correlation between implant (of hiPSC derivatives) behavior and cardiovascular effects. Currently, there are no biomarkers associated with these changes or tracking their effects i.e. with the prediction of therapeutic outcome. Indirect assessment currently comes from multimodal imaging platforms, RNA sequencing and immunohistology, however, we would still need to develop assays to measure the concentrations, compare functional cells with less functional ones and thereby predict cellular product potency. Post-infusion potency assays, such as those for patients with diabetes where an implanted pancreatic cell responds to glucose only days after infusion, are not yet available in cardiovascular disease. Given that these attributes are monitored over a long-term follow-up, these readouts are critical even if treatment is successful or curative. Furthermore, these aspects may represent a multilevel translational barrier for non-clinical to clinical use, patient stratification, and may limit the chance of success. Preclinical studies showed good regenerative potential for second-generation cell therapy [11] products such as both mesenchymal stem cells (MSC) and hPSC-derivatives in damaged or dysfunctional tissues. MSCs have been investigated in clinical trials (68 in total, according to www.clinicaltrials.gov) for cardiac repair. Yet, we have a limited understanding of how largely diverse populations of MSC from different sources may work upon transplantation. At this point, all we have are cell properties poorly defined simply by surface markers [12]. Functional properties of (not off-the-shelf) MSC products are dependent on modes of isolation, manufacturing, cues from the cell culture microenvironment and storage. Indeed, the attractiveness of MSC in cardiac therapy relies on their release of cytokines and less known paracrine-mediated mechanism via immunomodulation, angiogenesis, cell viability and pro-survival effects [13].

TRANSLATION INSIGHT: NEW SOLUTIONS OVER THE EXISTING ONES?

We would require strong early signals to prove the competitive advantages of these new technologies over the current pharmacological and device therapies. Given that new cardiovascular cell products do not behave like traditional therapies, modern design is required for early-phase trials. Despite the pressure from regulatory agencies, traditional dose-finding studies with clear dose-response or dose-toxicity relationships may not be feasible and affordable [14]. We need an early therapeutic game plan to select patients who would benefit most from the new treatments in comparison with available therapies. Majority of the cardiovascular cell trials are in the early phases. Although no guidelines can guarantee the desired outcome of these novel therapies, the introduction of cellular standards would establish a uniform level of practice to support patient safety. Standards for cellular therapies have been available since the 1980s and can be used to improve cellular therapy programs even for non-hematopoietic cells or treating non-hematological diseases (www.aabb.org, http://www.factwebsite.org). These could be well applied to donor selection, cell sourcing, product processing, storage, clinical

administration, and are intended to be used throughout product development and first clinical trials for cardiovascular disease. Either for acute injuries after infarction or chronic dysfunction like heart failure and peripheral artery disease, long-term follow-up on safety as well as disease subset analysis after administration is needed. Would we be able to choose human embryonic stem cells, induced pluripotent stem cells or mesenchymal stem cells for these studies? For either cell type, this should include an autologous approach, where the patient's own cardiovascular or non-cardiovascular cells are chosen and used later after a heart attack or other injury. Alternatively, point-of-care cells and constructs can be transplanted from a universal donor right upon injury. In the current animal models, the time between the injury of the heart and the application of cells affects the degree to which regeneration takes place, and this has real implications for the patient who is rushed, unprepared, to the emergency room in the wake of a heart attack. For the future, the scientific community must address these questions based on new research and whether patient's cells could be harvested, reprogrammed or efficiently expanded for use. Can a patient at risk donate their cells in advance, to minimize the preparation needed for the cell administration? Can these stem cells be genetically "programmed" to migrate directly to the site of injury and to synthesize immediately the proteins necessary for the cardiac regeneration process? As patients now would be staggered in early stages of trials, selecting the best-suited cells, tailoring patient population and understanding how cells match the recipient will be the key to their translation from preclinical to routine clinical use.

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AFFILIATIONS

Gabor Foldes
Author for correspondence
National Heart and Lung Institute, Imperial Centre for Experimental and Translational Medicine, Imperial College London, Du Cane Road, London W12 0NN, UK
and
Heart and Vascular Center, Semmelweis University Budapest, H1122, Hungary
g.foldes@imperial.ac.uk

Dr Vasiliki E Kalodimou
Director at Flow Cytometry - Research & Regenerative Medicine Department, IASO Maternity & Research Hospital, Athens, Greece
and
CBB Director & Processing Facility Director at MedStem Services, Athens Greece

AUTHORSHIP & CONFLICT OF INTEREST

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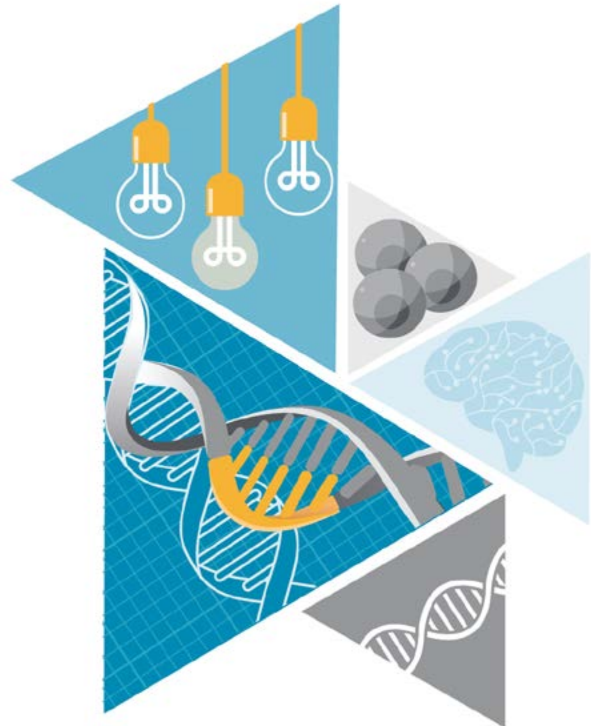
CELL & GENE THERAPY INSIGHTS



Innovation Insights

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INTERVIEW

ATMP innovation in liver disease

ANIL DHAWAN graduated in medicine in 1986 and after completing his residency in Pediatrics in India, joined King's College Hospital, London as a trainee in Paediatric Hepatology in 1992. In March 1995, he was appointed visiting professor and consultant in pediatric gastroenterology at the University of Nebraska Medical Center, Omaha, NE, and USA. He relocated to King's College Hospital, London in August 1996 as a consultant Paediatric Hepatologist. He was appointed Professor of Paediatric Hepatology at Kings College London in 2005. He became the Director of the Paediatric Liver Center and the Head of the Paediatrics department of King's College Hospital, London in 2009. Professor Dhawan's basic science research interest is liver cell transplantation. His lab was the first to transplant human liver cells in the UK for liver based metabolic disorders and first in the world to transplant human hepatocytes in alginate beads to treat acute liver failure in children. His other research interests are the outcome of children after liver transplantation, immunosuppression, metabolic liver disease and acute liver failure. He has published over 300 research papers and edited four books in pediatric hepatology and human hepatocyte transplantation. He has been a board member of ESPGHAN, ILTS, BSPGHAN and CTRMS and is the chair of the membership committee of ILTS. He is on the editorial board of the *Journal of Hepatology*, *Pediatric Research*, *Pediatric Transplantation* and *Experimental and Clinical Hepatology*. Professor Dhawan is proud of developing basic science laboratories for research in liver disease (Mowat Labs) and the Slorech International learning hub. He has served as the President of the Cell Transplantation and Regenerative Medicine Society.

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Q What are your working on right now?

AD: For the last 20 years, my lab has been working on providing hepatocyte transplantation as an option to avoid or postpone liver transplantation in children with either liver-based metabolic disorders, or acute liver failure.

Regarding liver-based metabolic disorders, the original hypothesis behind our approach was that the liver has lots of redundancy in terms of enzymes – in other words, that we don't need all the enzymes that are produced and just a fraction of them are enough to correct a defect by changing the phenotype from homozygous to heterozygous. With that background, I think I was lucky to be at Kings College Hospital, where parallel treatment was offered for auxiliary liver transplant.

Auxiliary liver transplant is a technique for patients with liver-based metabolic disorders that are not sclerotic. In these patients, you can replace part of the liver – the right or left lobe – and that replaced lobe will then provide the missing gene product (enzyme) and treat the symptom. For example, in Crigler-Najjar syndrome, urea cycle defect, or organic acidemia, there is a single gene defect, which causes either systemic problems, or (in the case of Crigler-Najjar) severe neurological problems connected with encephalopathy. So we tried this technique in several patients with very promising results – all of them did extremely well: their symptoms disappeared and they went back to a normal diet, where diet was a restriction, or jaundice was cleared in the case of the Crigler-Najjar patients.

The main problem has been that this is a surgically demanding technique and patients do subsequently require lifelong immunosuppression medication. Consequently, patients have struggled to see the advantage of auxiliary liver transplant over traditional full liver transplant.

The rationale for auxiliary liver transplant was that if you keep the patient's liver, then that could become a target for gene therapy in future. If you remove a patient's entire liver, you take away that possibility of future benefit. It was to preserve that option, and make the surgery less invasive, that we started the hepatocyte transplantation programme at Kings. The programme was based upon my experiences and collaborations with Ira Fox at the University of Nebraska and Stephen Strom at the University of Pittsburgh.

The logic of cell transplantation is that the hepatocyte is a functional unit of the liver: it provides detoxification and synthetic function. In conditions like Crigler-Najjar syndrome, it will metabolize bilirubin into conjugated bilirubin; in the case of factor VII deficiency, it will produce factor VII; and in the case of urea cycle defect, it will detoxify ammonia. So we offered treatment to patients with these conditions. However, we found that after a year, the effect of the cell function that was initially observed was waning, and people were going back to traditional therapy.

> "I do think that ultimately, the answer will be provided by emerging approaches such as induced pluripotent stem cell-derived hepatocytes."

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> "The [cell] source we currently use is human and it's those cells isolated from the liver that are not used for liver transplantation. I think that's a problem because increasingly ... surgeons are ...using all of the organ for transplantation. So cells from donor livers that are not used for transplantation are getting increasingly scarce."

Q Can you tell us more about key bottlenecks and obstacles to success that you have encountered and how you have sought to address them?

AD: Firstly, there is cell source. The source we currently use is human and it's those cells isolated from the liver that are not used for liver transplantation. I think that's a problem because increasingly, the surgical techniques are getting better – surgeons are getting bolder, and they're using all of the organ for transplantation. So cells from donor livers that are not used for transplantation are getting increasingly scarce. We therefore have to innovate, improve and improvise in sourcing cells from the human liver.

We're approaching this challenge in a number of ways. For example, a good donor liver can currently be used for two patients: one lobe will be given to an adult patient, and the second, smaller lobe to a child. However, our surgical colleagues have provided us with a modified technique where they remove segment four and segment one of the liver, providing us with about 100 grams of tissue – yielding about 800 million to a billion cells – that can be used to treat an additional patient. We published on this surgical advance enabling three patients to benefit from a single liver a while ago.

A further potential source is those individuals for whom you cannot obtain donor permission while their heart is still technically beating. A rapid harvesting technique was developed so these patients' organs could be preserved even when the heart stops – what is known today as donation after cardiac death (DCD). When it started, not many people believed in or were comfortable using livers obtained through DCD. But we did some work in our laboratory and were able to prove that liver cells isolated from DCD were of good quality and had good function, and we also proved these cells could be successfully transplanted into patients. We published that work some time back, in *Liver Transplantation*.

Around the same time, the fatty liver debate started to come to the fore as a result of the increasing incidence of obesity worldwide. Obviously, this also impacts the donor population. We began working on improving the quality of cells isolated from fatty livers, conducting a prospective study where we had the same fatty liver divided into two. One part we treated with

N-acetylcysteine, while the other was a control arm. We showed that the donor liver treated with N-acetylcysteine had improved cell yield and better function in terms of albumin production. So we were then able to start using cells from fatty livers.

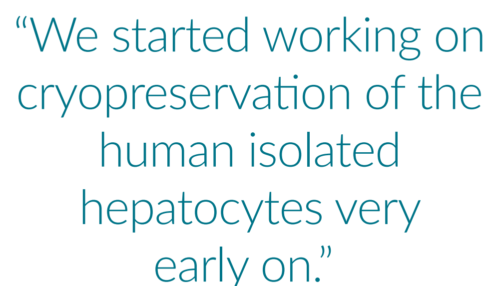
The next source we investigated was the new-born liver. Unfortunately, some new-born babies are lost either due to massive brain injuries or having a congenital malformation which makes their survival impossible long-term. Some families have been very, very generous and have volunteered to let us use those new-born babies' livers, and we've found that those liver cells were very good quality. They maintained a very good function, and we were able to use them for cell transplantation. That paper was published 2 years ago in by Lee CA from my lab. (*Liver Transplantation*, 2018)

So all of this work has been focused on increasing the quality and amount of human donor cells available for our work. However, I do think that ultimately, the answer will be provided by emerging approaches such as induced pluripotent stem cell (iPSC)-derived hepatocytes. Our lab is not currently working on iPS cells because we don't have the bandwidth to do so, but we're collaborating with others who are, and we are testing their cells to make sure they're of good quality, that they maintain function, and that they don't turn into anything undesirable – in short, that they are suitable for the GMP production of stem cell-based hepatocytes for human use.

The second key issue or bottleneck is more of a financial one: the availability of GMP facilities where the livers and cells are processed. Because activity in this space has been limited to date, the cost of production is relatively high. And due to the fact that it's a very academic field at the moment, there is also a lack of people available who are suitably trained. These factors combine to ensure the field has perhaps not advanced at the pace it should have. However, I do believe that continued scientific progress will help to resolve these kinds of issues.

The next area is the availability of the cells at the right time. You may have a patient today, but you don't have a donor liver – how will you treat them? Conversely, you may have a donor liver, but there's no patient waiting – so what to do with the cells?

We started working on cryopreservation of the human isolated hepatocytes very early on. We improvised a cryoprotectant solution, and came up with a modified, optimized cryopreservation protocol that we have used subsequently published (in *Cell Transplantation* a decade ago). As a result, we now have cell stock cryopreserved for more than 7 years that still has good function and quality, and we have been successfully transplanting cryopreserved hepatocytes into our patients. We do lose a lot of cells in cryopreservation, and that is a problem, but hopefully with additional cryopreservation techniques we'll be able to minimize that loss. Unfortunately, not many people are working on this particular issue of cryopreservation, but there are a few: ours is one group, there is also a group in Germany at the Martin Luther University of Halle-Wittenberg, and then there are pharma companies like Promethera, based in Brussels.



Immune function is a further barrier. When we put the cells into humans, they enter via the

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bloodstream. The innate immune system immediately recognizes these cells as foreign, and the resulting activation of the complement system leads to the destruction of a lot of them. To minimize this cell loss, we have sought to improvise techniques. People have used N-acetylcysteine, and we have tested Alpha-1 antitrypsin as an anti-inflammatory molecule. We found in our *ex vivo* model that using Alpha 1 antitrypsin reduced cell loss and also improved cell functionality. Our idea is to take this Alpha-1 antitrypsin molecule to clinical trial so that we can use it to improve cell delivery to the liver where these cells are then going to be engrafted.

"Animal models show that if you submit the liver to radiation and then transplant the cells, engraftment is much higher."

While I'm on this subject of immunity, we also have difficulty in monitoring rejection. In the whole organ, it's very easy to diagnose rejection. With liver transplant, you can measure liver enzymes and do a liver biopsy to prove whether a patient has a rejection or not. But with cell transplantation, it has proven to be extremely difficult for us to tell if and when a patient is rejecting cells. The current markers used to predict rejection are not very good. Unfortunately, I think it may be rejection-mediated cell loss that ultimately leads to the loss of cell function after around a year that I mentioned earlier.

However, there are cell-free DNA technologies coming through, which may be of benefit to us, allowing us to detect a rise in cell free-DNA that will tell us if the transplanted cells are dying. We are not working a great deal on that area ourselves, but I believe there is a group at MIT/Harvard who are in the field of islet transplantation.

Finally, we come to the issue of how to ensure that the maximum number of cells are engrafted. There are certain ways to do this. One is to damage the existing liver – with radiation, for example. Animal models show that if you submit the liver to radiation and then transplant the cells, engraftment is much higher. That's the model that was used by the Pittsburgh group and they now have clinical data in human patients, which got published in *Journal of Hepatology* last year. However, again, the results were not long-lasting. Another group from Karolinska opted to remove the left lobe of the liver and transplant the cells into the right lobe, hoping a stimulus coming from the hepatectomy would increase engraftment. Unfortunately, though, while this approach was again borne out in animal studies, it was not very successful in clinical application.

So improving engraftment continues to be a challenge, but there is work ongoing. The Pittsburgh group currently has a larger trial underway with the NIH where they are looking into improving engraftment after radiation.

Q Can you go deeper on the most promising cutting-edge approaches as you see them – to help alleviate the immunological issues you've mentioned, for instance?

AD: As we've established, it's the immune loss of cells that is the major issue here. The other problem is the longevity of the liver cells. In that area, people have worked on

"ATMPs [are] spreading rapidly into key therapeutic areas ... Consequently, ATMPs are absolutely core for the Kings College Hospital Research and Innovation Division..."

immortalizing the cells on the one hand. On the other, some have looked into inserting a suicide gene to trigger apoptosis in the event that the cells become cancerous, for example.

Some researchers are taking the approach of seeking to ensure the cells are not recognized by the immune system. Others are investigating the use of T regulatory cells that can regulate the immune system, or mesenchymal stromal cells that when transplanted can reduce the risk of cell loss. CTLA-4 modified cells have been assessed in pig models and have shown function in terms of immune tolerance. So I would say there are several possibilities out there, but currently, there is no clinical data to support them.

Nanoparticle coating is another approach that has been proposed and studied in animals, though not in humans. It has not been widely investigated as yet, because of questions over how much nanoparticle coated material could be transferred, but I don't think that will be a big problem moving forward.

Finally, I would highlight *in vivo* and *ex vivo* gene therapy approaches. *In vivo* gene therapy is easier in a way – you can utilize your vector to deliver the gene into liver cells *in situ*. But increasingly, people are looking at *ex vivo* approaches where you take the patient's own cells that are defective, correct them *ex vivo*, and then re-transplant them. With such autologous cell therapy approaches you don't need immune suppression, which is a major advantage. There is work ongoing in that area – using gene editing to modify the cells, for instance – and I think that will play a key role in the future of liver cell transplantation.

There are challenges to be overcome for allogeneic *ex vivo* gene therapy. As we've touched upon, you will have to contend with compliment mediated activation of the patient's immune system. Other issues relate to the specific cell source: the emerging approaches will be based on either iPSC-derived or embryonic stem cell (ESC)-derived technologies. The problem with iPSCs at the moment is nobody has been able to produce really functional and stable iPSC-derived hepatocytes, and then there is always the risk of off-target mutation/malignant transformation. Cost is a further barrier, because all the growth factors used in iPSC-based technology are extremely expensive. There are groups working on using modified growth factors in a bid to make the technology more affordable, but currently, you are looking at a cost of a few hundred thousand pounds per patient. So I think the cost effectiveness of these approaches needs to be improved or at least tested.

Turning to ESCs, while they offer an advantage in terms of their greater potential to differentiate, they do not come from such a well-defined source as iPSCs and they may consequently be more difficult to direct. Additionally, the United States' restrictions on the use of embryonal stem cells has hampered the progress of the field. (However, there are groups in France and Thailand working on it).

Finally, there are other groups just beginning to look at certain other molecules that can be applied *ex vivo* to improve or augment the function of the gene, but I think this is very far-fetched at the moment.

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Q Changing tack, can you tell us more about how King's College Hospital is set up to foster innovation – for example, in terms of novel and emerging biotherapeutics such as ATMPs?

AD: ATMPs obviously represent one of the most significant areas of development in medical science over the past decade. We see this continuing with ATMPs spreading rapidly into key therapeutic areas, including hematology, CNS and cardiology.

Consequently, ATMPs are absolutely core for the Kings College Hospital Research and Innovation Division that I direct. So we have a setup to take either one of two fundamental approaches: we can produce our own ATMPs though in-house capabilities to generate liver cells, islets, mesenchymal stromal cells and viral vectors; we can also conduct clinical studies with products that biopharma companies have produced themselves – CAR T cell therapies, for example.

I would say that Kings College Hospital research and innovation has placed ATMPs right at the top of its agenda.

Q How do you see the dynamic between industry and academia continuing to develop in order to provide the best possible platform for the advancement of novel ATMPs towards and through the clinic?

AD: I think it's extremely important that academic institutions work with pharma very closely. Academia generates the initial ideas, but they have neither the bandwidth nor the resources in terms of lab capacity, personnel and money to really move them forward. Biotechnology companies are needed to take these ideas on to clinical application because the extensive regulatory requirements and the cost of clinical trials are formidable barriers to academia progressing ATMPs into and through the clinic.

So I think pharma has a big role to play. Obviously, there is benefit in this for them, but there is benefit for mankind also. CAR T cell therapy provides a good example of pharma driving technology taken from the academic centers and made it a reality for patients. I believe we will see the same thing happening with hepatocytes, islet cells and other ATMPs in the future.

By way of an example, let me return to the first question and provide some more detail on our work in acute liver failure.

The story of Prometheus from Greek mythology speaks to the extraordinary regenerative powers of the liver. If you have acute liver failure or sudden onset of a short-lived illness – such as might be caused by a very high dose of paracetamol or a virus, for instance – if you can support the patient through the immediate danger, then they will generally get better because their own liver will regenerate.

With this in mind, our lab was the first in the world to come up with a therapy called allogeneic encapsulated human hepatocytes. The paper has just come out in the *Journal of Hepatology* (March 2020).

This technique involves the encapsulation of human hepatocytes in alginate – an algae-derived product, which is bioinert and doesn't cause an immune reaction. It also acts as a

semipermeable membrane, allowing the free entry of ammonia and exit of urea and factor 7, but not allowing lymphocytes to come in and attack the liver cells. We put these cells into the peritoneal cavity (which is by necessity a very easy and non-invasive technique) where they then function, producing molecules that promote regeneration of the liver. Our data shows 50% of patients survived against 90% predicted mortality. The next step is to take this approach into a further clinical trial with a different peptide, which we are hoping will further improve the quality and functionality of the implanted cells.

So this is an example of a 100% academic bench-to-first in human clinical trial project. Now, if you were to ask me where we can take this approach, ultimately, I would say the real dream is to be able to develop a product that can be cryopreserved whilst still maintaining cell function. If we could succeed in this, you can imagine that we could use drones to take cryopreserved, off-the-shelf cells to almost any location in the world, where local doctors would simply need to thaw the product in warm water before injecting it into the peritoneal cavity of patients suffering from acute liver failure.

But in order to realize this vision, we need pharma support. We need a big company to show interest and take it to that level. It will not be possible for us to do that kind of work. We have proof of concept, but to prove it at large-scale, I do think we need that collaboration.

AFFILIATION

Anil Dhawan
Kings College Hospital, London, UK

AUTHORSHIP & CONFLICT OF INTEREST

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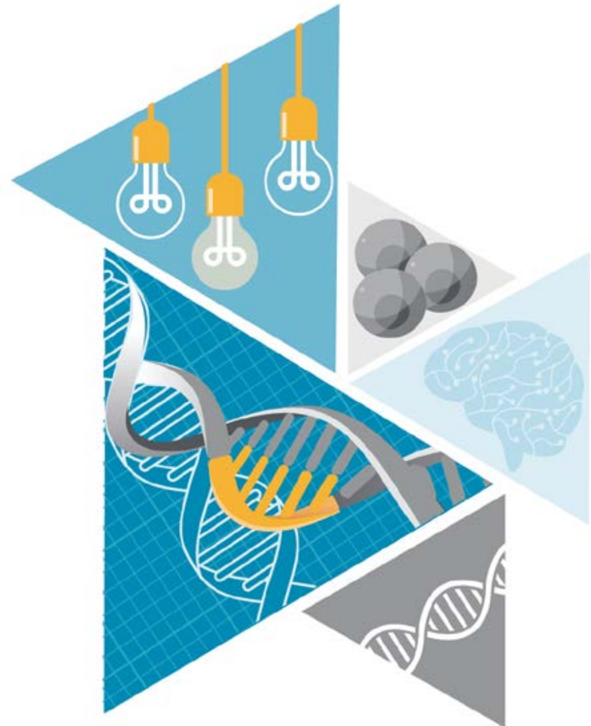
CELL & GENE THERAPY INSIGHTS



Innovation Insights

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INTERVIEW

Current and future directions for dendritic cell vaccines

GERARD BOS was born in Wageningen, The Netherlands. After Medical School and a PhD program on immunology (both at the medical school in Maastricht) Gerard Bos started training in internal medicine in Maastricht and The Hague. After training for internal medicine he was trained as a Hematologist followed with a fellowship at the Daniel den Hoed Cancer Centre, Rotterdam, for 3 years (head Professor Dr B Lowenberg). Thereafter he obtained a fellowship (Clinical Research Award by the Dutch Cancer Foundation) to perform a 2-year research program on tumor-immunology (Leiden, Brussels and Utrecht). He started in Maastricht 2000, as internist-hematologist. His extra focus is on M. myeloma and chronic myeloid leukemia. Preclinical research has a focus on cell therapy: development of cancer vaccines, the role of natural killer cells in the fight against cancer and immune reconstitution after bone marrow transplantation. Under this topic special attention is present for a possible role for vitamin C in immune reconstitution. In 2013, he was appointed a professor of medical education and tumor-immunology. In 2016, he was appointed a professor of immunology of cancer. Since 2015 he has been CEO of CiMaas, a biotech company with a focus on immunotherapy.

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Q Tell us about CiMaas and your current projects

GB: CiMaas is a start-up company from the University of Maastricht in The Netherlands, where we worked for 15–20 years on immunotherapy in general. One of the things that came out of that academic work was research relating to the activation of dendritic cells (DC) in order to get good antigen presenting cells. We observed that we could improve the maturation cocktail.

You have to mature a dendritic cell to make it a correct antigen presenting cell, and you can do this in several ways. While investigating these methods, we found out that interleukin (IL)-12 is an extremely important molecule to active immune cells, and T cells especially. We then worked on improving the IL-12 produced by dendritic cells, and that led to a patent that we believe is superior for a number of reasons to all other dendritic cells that have been assessed in clinical trials to date.

So we are now looking to test this approach in a clinical trial. We're starting in lung cancer and are currently talking to the authorities about what is the best way to do the Phase 1 followed by a Phase 2 clinical trial. We expect that we will be able to dose our first patients at the end of this year, providing all the regulatory aspects go smoothly.

We've chosen not to go to a CMO – we've built our own GMP facility close to Maastricht with this study in mind – again, we are just waiting for regulatory approval in order to be able to use that cleanroom facility to make the product ourselves.

Another key decision was to study our dendritic cell vaccine in clinical application not as a monotherapy, but only in patients who are also being treated with a checkpoint inhibitor. Checkpoint inhibitors are more or less the standard of care in several cancer indications now, including some patients with lung cancer. However, there is unmet medical need, because the value of checkpoint inhibitors is still limited: only a relatively small subset of patients really respond to them, and additionally, checkpoint inhibitors as a first line treatment don't necessarily offer a very long progression-free survival benefit – less than a year in metastatic lung cancer, for example – meaning that treatment is far from optimal.

One popular theory behind the limitations of checkpoint inhibitors is that a lot of patients' tumors do not provide adequate antigens, meaning the immune cells do not know what to respond to. That's a problem we try to solve by giving a vaccine to a protein, WT1. In effect, we want to see whether the combination of a vaccine and a checkpoint is better than checkpoint alone.

Q What are the key challenges you're facing in the clinical translation of this technology?

GB: There are several, but number one is always money. Clinical trials are pretty expensive, at least for people like us

> "...the value of checkpoint inhibitors is still limited: only a relatively small subset of patients really respond to them..."

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"Where should you inject these dendritic cells – intravenously? Subcutaneously? Can you inject them intranodally? There still isn't any sort of agreement on the best option. Linked to this question of where the cell should be injected to get the best immune response is the question of how many cells you need. With this uncertainty comes risk."

who are used to working with scientific grant money. Validation of scientific work is a big step. Both the need to produce the cells in a GMP environment and organizing the trials are costly – we need a couple of million Euros to do a Phase 1/2 study. So finding investors is a big focus for us at the moment, because while we do have the money available for this initial study, the aim will of course be to continue with a larger Phase 2 study as quickly as we can.

Unfortunately, there is also a lingering negative perception of dendritic cell vaccines due to the fact they haven't been really successful in the clinic thus far. Provenge remains available on the market, but it has enjoyed limited success due to its relatively low efficacy. Again, though, we really feel that the combination of a dendritic cell vaccine with a checkpoint inhibitor can help overcome that particular issue and open the field up again.

Q How do you reflect upon the ups and downs for DC vaccines since Provenge started its pivotal clinical trial journey, and can you go into more depth on what gives you cause for new optimism today?

GB: I think the answer to that is simply 'more knowledge'. More knowledge on what a dendritic cell actually is, and on what the best dendritic cell might be. If you look at what those earlier programmes defined as a dendritic cell, it was different to what would be considered as an optimal dendritic cell today. And as I mentioned earlier, our scientific papers demonstrate that the amount of IL-12 produced by the dendritic cells is crucial – the more IL-12, the better the T cell response. That's relatively new knowledge. In the past, people were happy that their cells were producing any IL-12 at all, but our cells produce it on a totally different scale to those earlier approaches. That might also be true for other cytokines, such as IL-15.

So I really think it's our basic knowledge, that everyone is doing, not only we of course, a lot of people in the world study dendritic cells. And that will lead to a better chance to get a result.

It's the same as anything else – as CART, for example: it's the science that moves the field forward and spawns new generations, and we are fortunate to work in an area that has always attracted a healthy amount of academic investigation. That basic and translational science has

"In general, I think the NK cell approach is a good one, although that is based on relatively little data. There has been little evidence to date in solid tumors, but some good evidence in leukemia."

really developed over the past decade – you could certainly argue that the early approaches that reached late-stage clinical trials and in the case of Provenge, the market itself, were just too early.

Of course, there are others who aren't keen on patient-specific therapeutics, period. That's another reason why the autologous DC vaccine field has moved forward in the clinic relatively slowly, and why other companies, such as DCprime in The Netherlands, are trying to work with dendritic cell banks to see if they can solve these patient-centric cell therapy concerns. But this concept is for leukemia only, as far as I know, and not for other diseases and indications. It is not a platform like our DC.

Q What are your thoughts on the allogeneic DC vaccine field and its prospects?

GB: It's a completely different concept, I think. Because the cell is allogeneic, it's liable to be destroyed by the patient's immune system so if you bring in antigens via a donor DC cell, you might profit from cross-priming. It has little to do with presenting peptides in an autologous class 1 or class 2 system, as our platform seeks to do. But let's certainly hope they are successful in leukemia.

Q What challenges do you foresee for your approach moving forward, and how will CiMaas seek to address them?

GB: One of the key issues in dendritic cell vaccination is still the route of administration. Where should you inject these dendritic cells – intravenously? Subcutaneously? Can you inject them intranodally? There still isn't any sort of agreement on the best option. Linked to this question of where the cell should be injected to get the best immune response is the question of how many cells you need.

With this uncertainty comes risk. You might have great cells but the wrong system to get them into the patient and to the right location for presenting antigens to the immune cells (which we consider to be the lymph node).

Some people are pursuing intranodal delivery, but that's not easy and also leads to the destruction of the lymph node. Will that lead to optimal presentation, though? We still don't know. And so we're still working, and will continue to work, on whether you can make comparisons of the routes of administration. People have already demonstrated good success via different routes, so we do know where to start in this regard.

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Q CiMaas also has an NK cell therapy candidate in development – what can you tell us about that?

GB: Our scientific and clinical interest in NK cells comes from the fact that as hematologists and transplant doctors, we worked in mice models on haploidentical transplantation – like many others, we were motivated to solve this problem of having to find HLA-matched donors for bone marrow transplantation by finding a universal donor for everybody. So we investigated whether it might be feasible because at that time, there was very little evidence that haploidentical transplant would be feasible in patients, due to the mismatch and graft-versus-host disease.

We developed a model in breast cancer and it turned out that about 50% of the mice were cured by this haploidentical transplant. Around that time, a very famous paper came from Italy (Ruggeri *et al.*) where they found that in haploidentical transplantations where you have a mismatch, you can have activity of donor NK cells that is different from that with HLA-identical transplantations, where the donor NK cells will be non-respondent to HLA identical cells. In other words, the mismatch will lead to better NK cell activity. We then went back to our mouse model for breast cancer and observed to our surprise that the cure of these mice was completely dependent on NK cells, not on T cells as we and most others in the field of bone marrow transplantation (where T cells do induce the graft versus leukemia activity) had expected.

We looked to see if the same might be true for myeloma *in vitro* and *in vivo* models – whether the NK cells could kill myeloma cells. We found that they could *in vitro*, and we even observed a small clinical benefit in a mouse model for myeloma.

That secured our interest, but you need a lot of NK cells to treat patients. However, we then met some people in the United States who were to later form the company, CytoSen. They had a patent on NK cell proliferation by using tumor cells that are transduced with IL-21, and they made particles out of that so that you don't have to use the tumor cells, because K562 as you probably know is a tumor cell line. We were lucky to collaborate firstly with the academic group and later with CytoSen. We discussed and agreed upon a license agreement allowing us to use the particle from them in myeloma and breast cancer in Europe, but while we were waiting for the final signature on the agreement, Kiadis took over CytoSen, and they did not want us to have a sub-license agreement anymore. Suddenly, we had no commercial approach to use the particles, which was of course a big disappointment for us.

CiMaas is now working on its own methodology to make sufficient NK cells in order to be able to develop and commercialize an NK cell approach. But still, as a clinician, I think the patient is more important than money in a company, so in the meantime, we continue working on NK cells (and myeloma especially) in the clinic. We did a haplo bone marrow transplantation trial to see whether NK mismatched donor transplant can cure

> "...we really feel that the combination of a dendritic cell vaccine with a checkpoint inhibitor can ... open the field up again."

"...we can now start with WT1 as tumor antigen ... If we pass the safety hurdle, I think the challenge will be to do as many Phase 2 trials as possible."

patients. We found it could not, but we did make some interesting observations, which lead us to believe that NK cells do really work in patients with multiple myeloma. For example, one patient got progressive disease after the transplant, but 3–6 months after the transplant, the NK cells from the donor came up from the bone marrow and at that time point, the disease disappeared. There was a clear coincidence between getting the donor NK cells into the blood and into the bone marrow, and a response to the disease. So we do feel there is some argument apart from preclinical work that donor NK cells can be active in myeloma. We're currently applying for an academic-level grant to conduct a trial with haplo transplantations and NK cells.

So that was a challenging pathway, and it's still challenging. We unfortunately learned the hard way that life is not all about science and patients, but even more so about patents and freedom to operate.

In general, I think the NK cell approach is a good one, although that is based on relatively little data. There has been little evidence to date in solid tumors, but some good evidence in leukemia – for instance, a group from Houston demonstrated that there is probably better survival with transplant-plus-NK cells. There is also data for response in leukemia with donor NK cells without a transplant, but then you still have to continue the treatment with something else, because NK cells will probably not cure the patient by themselves. (There needs to be more follow-up done around these studies to know for sure). Perhaps NK cells' greatest utility will be as a bridge to another treatment?

Many people work with iPS-derived NK cells and to create NK cell banks, but our approach is to try to do it in combination with the donor transplantation, as explained above. The reason why we are not in favor of cell lines is because you then risk introducing the host vs donor NK cell issue. We think it is optimal to firstly try this approach in combination with the transplant, as was proven successful by Dr Ciurea in Houston in leukemia. If it doesn't work in that context, I don't think it will work via donor NK cells on their own, based upon the current data – again, unless you intend to use the donor NK cells as a bridge to another treatment.

Q Can you summarize your key priorities and goals for your work at CiMaas over the foreseeable future?

GB: Firstly, to get our dendritic cell vaccine clinical trial up and running, and to proceed past the Phase 1, ensuring that the combination of our cells and a checkpoint inhibitor is safe.

Secondly, because our dendritic cells more or less represent a platform, we can now start with WT1 as tumor antigen. We start with lung cancer, but WT1 is actually on a lot of

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tumor cells, including leukemia and colon carcinoma. If we pass the safety hurdle, I think the challenge will be to do as many Phase 2 trials as possible. Of course, we need co-partners to develop because for a small company like CiMaas, that would mean a very big investment. We will hopefully be able to share the results of that Phase 1 study very soon.

AFFILIATION

Gerard Bos
CEO, CiMaas, The Netherlands

AUTHORSHIP & CONFLICT OF INTEREST

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Regulatory Insights

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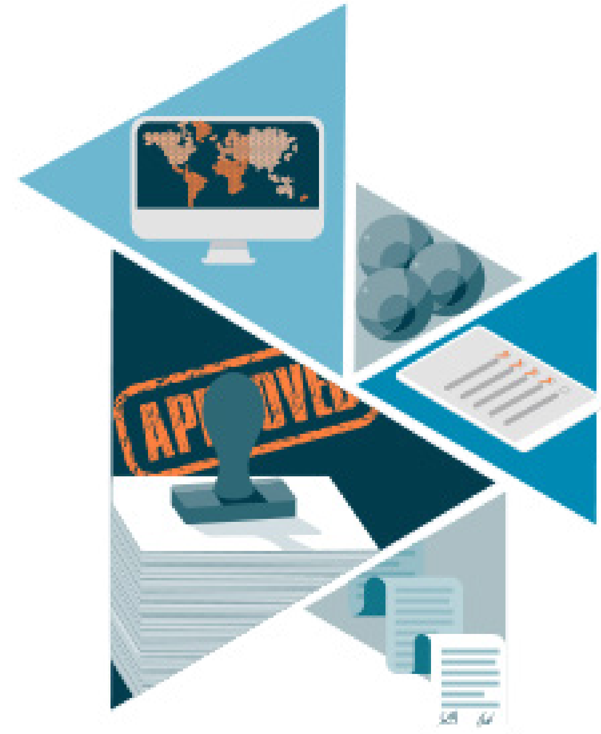

Regulatory Trends

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EDITORIAL

Curing ultra-rare diseases while ensuring access, equity and affordability

R. Jude Samulski & Laura Hameed

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CELL & GENE THERAPY INSIGHTS

Regulatory Insights

Curing ultra-rare diseases while ensuring access, equity and affordability

EDITORIAL

R. Jude Samulski & Laura Hameed (pictured)

By definition, rare diseases are rare. Yet, while individual diseases may be rare and only affect a small population, the total number of people living with a rare disease is quite large. Surprisingly, if you amalgamate all the people living with the more than 7,000 known rare diseases, the population would be larger than the third most populous country in the world. And, one out of two patients diagnosed with rare disease is a child. (Global Genes, 2016) This presents a worldwide humanitarian challenge worthy of those we aim

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to help. And, to solve for it, we need to find the right process, honest prioritization and a regulatory policy that is customized to fit the needs of this greatly underserved population.

History tells us that when industry and government – with popular support – collaborate, innovations in science coupled with a right-sized public policy can save lives. A shining example was the Orphan Drug Act (ODA) of 1983, which catalyzed the orphan drug industry such that it has had a powerful impact on the rare disease market and dramatically changed outcomes and potential for many suffering from the impacts of rare diseases. Laws such as ODA and subsequent revisional iterations created a welcome focus on rare diseases that were previously not researched at the same rate as many of the diseases impacting a larger population. The Evaluate Pharma Orphan Drug Report (2019) notes that by 2024, the orphan drug market will make up one-fifth of all prescription sales. This shows us that when resources are marshalled and an appropriate framework and incentives are created and implemented, cures get developed and lives can be saved.

Over the past few decades the rare disease community has grown astronomically in sophistication, capability and approach through the dogged efforts by families, physicians, researchers, and advocacy organizations. Due to advances in scientific understanding and a strong network of scientific and philanthropic support, parents have exchanged hopelessness for hopefulness, and they are determined to find new ways to bridge the innovation gap and save the lives of their children and others who are relegated to a shortened lifetime of pain and isolation.

Yet, with all this focus and progress, a vast majority of rare diseases still have no known cure and there continues to be a significantly underserved market – namely children with ultra-rare degenerative genetic diseases. It is incumbent upon us all to ensure these children are not left behind when cures are in reach.

Simply put, it is time for us to refocus, deliver, and accelerate hope for those who need it most.

With the real promise of gene therapy and other novel curative solutions well suited to address life-threatening genetic disorders, which make up a disproportionate number of ultra-rare diseases, we are on the verge of being able to dramatically change outcomes. There is now great optimism for delivering curative treatments that have previously lagged behind for ultra-small patient populations that have struggled to secure the kind of R&D investment that larger-population orphan diseases have been able to attract. However, to successfully prioritize and accelerate treatments for ultra-rare diseases that lack realistic commercial viability but have clinically viable curative solutions on the horizon, we need a new regulatory framework and novel development models to bring cures to the kids who will continue to suffer and ultimately die without them. And, we need to advance them with urgency.

The good news is that the conversation related to concerns over lagging treatments for many ultra-rare diseases has already started. In a 2017 NPR article, then FDA Commissioner Scott Gottlieb, MD, indicated the Agency was open to rethinking aspects of the Orphan Drug Act and pointed out that there continue to be many rare diseases without treatments, even under the current incentive system. He stated that "You have to ask why various uses of drugs are not getting studied." (Tribble, 2017) Additionally, the NIH and FDA have begun to hold meetings to discuss a workable approach, and the Director of the Center for Biologics Evaluation and Research, Peter Marks, PhD, has begun to boldly advocate for a sui generis regulatory framework for ultra-rare diseases. These are bold statements coming from the highest regulatory levels, bringing this important issue and need for a new framework for programs that are scientifically possible, but not realistically commercially viable, to the forefront of health care research and funding discussions. Additional forums with contributions from FNIH, NIH, large Pharma and key academic colleagues have primed the pump to provide a clear "road map" to the many parents,

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patients, foundations, and organizations committed to achieving this objective. It is in essence the "moon shot" of the molecular medicine revolution with more than enough human fuel to get us to our destination.

The issues are indeed complex, but there are three fundamental tenets that should guide a new development and regulatory framework for treatments of ultra-rare diseases:

- Expanding access through a regulatory framework that recognizes the specific challenges related to commercializing products for ultra-rare small population diseases;
- Advancing equity by ensuring that all children who might benefit from a treatment have access to a treatment;
- Increasing affordability by using a nonprofit model to advance safe and effective cures with cost, access to manufacturing, and regulatory process adjustments.

So, in order to get there, a new regulatory framework has much to consider when seeking the best way to accelerate and ensure access to cures for ultra-rare diseases:

STANDARDIZE & CREATE A ROADMAP

The science and development process behind these cures are highly complex. Patient groups, foundations and universities have taken on the bulk of the effort to advance basic R&D for ultra-rare diseases. But, this fragmented process raises challenges related to quality, access and equity. We believe that a road map needs to be developed that is simpler and that uses a standardized approach, so the process is not new every time. With industry and regulatory support, this roadmap could include streamlined protocols, better CMC and regulatory transparency, and off-the-shelf process improvements (where possible) to be made available in the public domain or for nonprofit use for researchers and patient advocacy organizations to support better outcomes, improve safety and accelerate cures. While there are many unique aspects for each disease indication, parents and patient organizations should not have to reinvent the wheel every time, as there are many places within the development process where a packaged approach and targeted expertise would assist in advancing a therapeutic more quickly. And, when this roadmap is used, it should be expected that the investment philanthropic and corporate responsibility interests made would benefit the broader community through shared learning and transparently sourced data.

NONPROFIT DEVELOPMENT MODEL

It is a challenge for the pharma/biotech industry to advance cures for ultra-rare diseases in a traditional commercial model that has typical ROI expectations and other fiduciary responsibilities dependent upon commercial production volume and sales. And in some cases, if a cure is advanced through a commercial model with a marginally commercially viable product, the broader healthcare community and patients suffer because the cost of cures may be so high that equity, affordability and access become real concerns. The Columbus Children's Foundation nonprofit model and others like it are designed to lower costs and speed advancement for programs that otherwise may lag. We effectively ensure that the programs we support have clinical/regulatory expertise and access to low cost manufacturing and other materials. We believe industry needs to align on this model that recognizes that some of the ultra-small population programs simply are not well suited for a commercial enterprise. A nonprofit model may be a better approach to ensure innovation doesn't lag and that we move with urgency to advance programs to ensure children with ultra-rare diseases are not left behind when cures are in reach.

CURES, NOT PRODUCTS: A MINDSET SHIFT

Perhaps we need to even change the way we view and talk about innovative treatments for ultra-rare diseases. Rather than viewing treatments as products to be sold, maybe we should view them as therapeutics to be delivered. This shift in mindset could lead us down a more sensible and right-sized regulatory pathway that is appropriate for an ultra-small patient population that aims to cure kids, not commercialize a product. We need a pathway that provides accountability and integrity, but without the costly post-registration and other burdens the commercial path requires which are not necessary for a noncommercial path. CMC requirements could be adjusted, because in many cases, the small clinical-grade vector batch is enough to serve all patients for generations with a particular ultra-rare diseases. Further, regulators could consider open INDs as a legitimate strategy for such diseases rather than an aberration or one-off approach. And, just maybe, we could design a process to allow clinical trials to progress to some sort of new regulatory pathway for non-commercial registration with appropriate financial incentives, resulting in lower costs and accelerated availability for a subset of ultra-rare diseases.

TREATING ALL KIDS WHO WOULD BENEFIT

While we advocate for consideration of a new framework for ultra-rare disease therapies that are not commercially viable, there also needs to be consideration for clearing away barriers that restrict access to clinical trials within the commercial market. Genetic diseases move fast, often claiming the lives of children and devastating their families during the development of a commercial product. Time equals lives. The current clinical trial process in commercial enterprises can lead to families being left heartbroken simply because their children do not fit into a specific prescribed cohort or the development timeline is so long that cures do not come in time. There are even too many instances where families have sacrificed everything to secure funding and R&D for a potential cure that is then taken on by a company, only to find the company doesn't advance the treatment or their own children are excluded from the study due to efficacy and end point requirements of a particular trial. This devastating story happens over and over. We must find a better way to either allow companies risk protection for having a parallel open clinical trial to serve children outside the study cohort without being penalized through regulatory liability or we should consider a secondary pathway using the nonprofit model to ensure access to these potentially life-saving cures for kids who would benefit from a potential curative solution.

The conversation has started. The need is tremendous. The children with ultra-rare diseases and their families don't have time to wait and they don't have time for inaction. A new framework is required to tackle complex issues, harness technology, and accelerate hope for those who need it most. And, humanity demands our action. With clear signals from the FDA and NIH addressing the issues head on, we only need to assemble the field generals and resources that will execute a well mapped out plan to remove these genetic diseases one by one off the list similar to how the vaccine community has impacted once dreaded diseases that infected the entire population as a whole (e.g. small pox, polio) and continue to do so (HIV, measles, flu, ebola virus). The question is whether the biotech community focused on rare diseases is up to the task and ready to look in the mirror. We believe we are.

AFFILIATIONS

R. Jude Samulski
Chairman of Columbus Children's Foundation

Laura Hameed
Executive Director, Columbus Children's Foundation

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Ensuring viral vector and gene therapy commercial readiness

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CURRENT STATE & FUTURE PERSPECTIVES IN GENE THERAPY

The field of viral vector-driven gene therapy, incorporating both direct *in vivo* and *ex vivo* cell-based approaches, has enjoyed tremendous growth in recent years.

The key breakthrough of the first CAR-T cell therapies achieving market authorization worldwide (Novartis's Kymriah® and Kite Pharma's Yescarta®) has paved the way for further advances in the past 24 months, including the first AAV vector-driven *in vivo* gene therapy to receive US FDA approval (Spark Therapeutics' Luxturna®).

In terms of disease indications, oncology continues to dominate with approximately 62% of all cell and gene therapy products targeted to cancer. However, orphan monogenic diseases are also well represented in the *in vivo* gene therapy space in particular, and increasingly, so too are larger patient population indications in key therapeutic areas such as cardiovascular and infectious diseases.

The gene therapy clinical pipeline has grown in step with rising valuations placed on individual gene therapy companies. It is now just over 2.5 years since the announcement of Gilead Sciences' landmark acquisition of Kite Pharma for $11.9 billion and the trend continues unabated.

Of course, the speed with which gene therapy is driving towards a commercially successful future does present challenges, not least in the area of viral vector manufacture.

When considering the manufacture of any biological, whether it's a monoclonal antibody or a gene therapy,

there are certain key areas for consideration: ensuring raw materials, equipment and consumables are all compatible with GMP production is crucial; scalability of equipment and of each individual process step is equally important; and robustness and reproducibility are prerequisites for a commercially viable bioprocess.

However, the speed of evolution in gene therapy in general, combined with the fact that many product candidates are now on expedited development pathways (e.g., Breakthough Designation and RMAT in the USA; PRiME in the European Union) means that the bioprocess/CMC development window is effectively shrinking. In addition to this need for acceleration, gene therapy developers and manufacturers must contend with the reality that gene therapy bioprocesses are still derived to a large extent from the academic world, presenting problems both upstream and downstream in terms of quality, reproducibility and scalability. Not only that, but much of the current manufacturing equipment continues to be repurposed from the monoclonal antibody field. Although the effective repurposing of these tools is ongoing and novel solutions tailored to the specific needs of gene therapy production are emerging, this remains a work in progress.

Finally, gene therapy manufacture remains a comparatively highly complex field. There are multiple vector types, with multiple serotypes of these vectors (of AAV, in particular) used in the manufacture of products. Moreover, viral vector production currently involves the use of multiple cell lines. This lack of standardization stands in stark contrast to the more mature monoclonal antibody field, which has coalesced solely around CHO cells and a relatively very well defined, bioreactor-based upstream and downstream process.

This article will explore three vitally important areas in which strategic and technical manufacturing innovation is required to support commercialization:

- Scalability and reproducibility
- Testing and quality
- Regulatory requirements for manufacturing sites

Success in these three primary areas will be critical if we are to get past the current imbalance between supply and demand and effectively deliver on the enormous potential of gene therapy.

SCALABILITY & REPRODUCIBILITY

We can divide any viral vector bioprocess using mammalian cells into three main phases: upstream production, midstream processing, and downstream purification.

Upstream production typically starts with a cell thaw and expansion step, using either adherent or suspension culture. This is followed by a transition to the production phase, where cells are either infected or transfected to generate the viral vector.

Once the vector material is in the media, one proceeds to midstream processing, which begins with a vector material harvesting step. It may be necessary to conduct a lysis process to extract the virus from the cell, but equally, the virus can already be lytic and already present in the media, or indeed, it can be a combination of the two – it depends on the both the virus in question and on the particular program.

One may minimize the amount of host cell protein present through Benzonase® treatment before removing cell debris using stratification. Again, depending on the program and on the scale of production, it may be necessary to also do a concentration step at this point as well as a biofiltration through an appropriate buffer.

The next step is downstream purification where the main goal is to remove contaminants and obtain the pure product. That material is then concentrated prior to final formulation, final filtration and ultimately, fill-finish.

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Of course, it is necessary to factor in testing of the entire process, including the amount of material available for this purpose. During upstream production, the volumes are large enough to mean there is no issue with testing but as you proceed down the production scheme, volumes become smaller and the material becomes increasingly precious – it's important when devising a sampling and testing plan to keep this in mind.

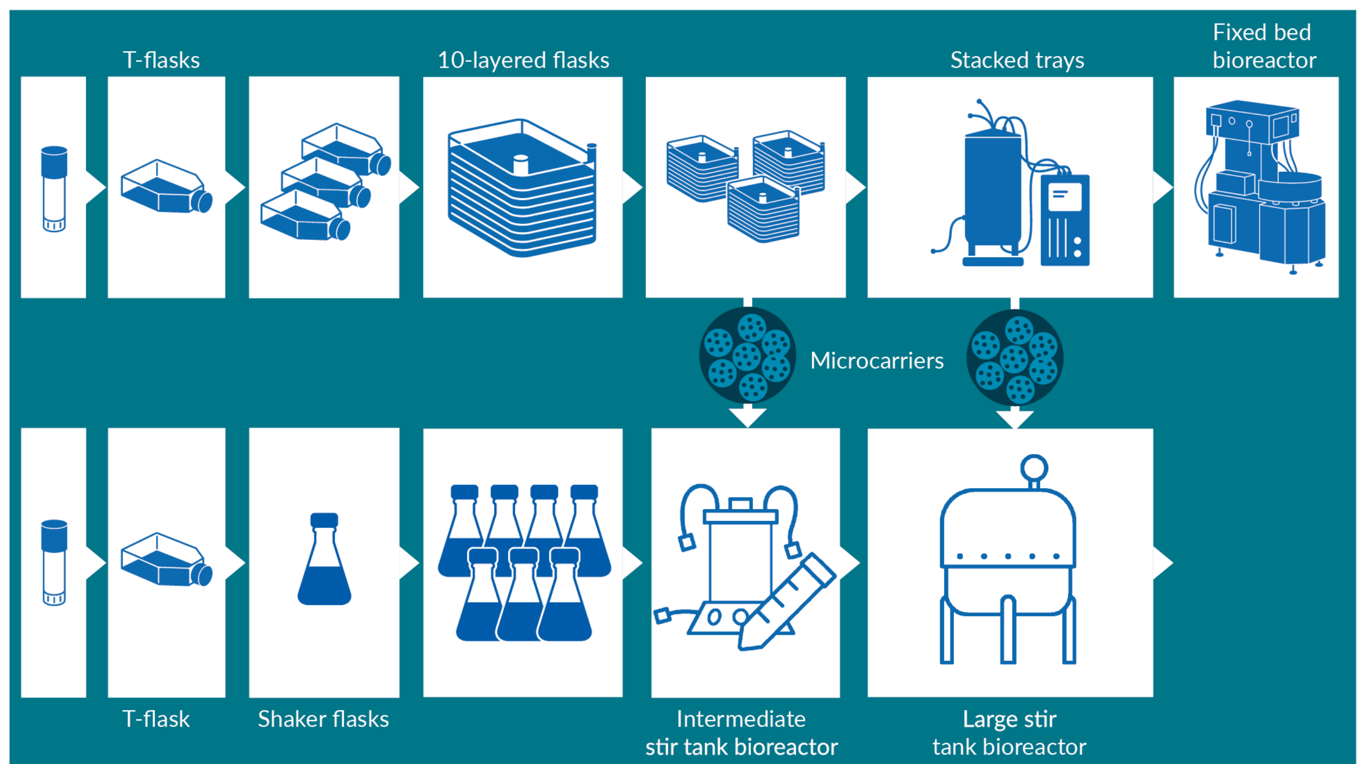
This type of process usually works well at small scale but as demand increases, so too does the need to produce more material. One approach is to scale-out: carrying out the same defined unit operation multiple times. This is a good idea in principle because it is known that the unit operation works. Taking scale-up of cell expansion as an example, one might begin with T-flasks, then proceed to a slightly larger platform that is still in the flask format – layered flasks. At that point, one would scale-out by using multiple layered flasks. The same thing might apply with suspension cells, with the initial transition into shaker or spinner flasks followed by scale-out to multiple flasks. However, this approach only works well up to a certain limit, at which point a major bottleneck is encountered in the form of the amount of space and the number of manipulations required. Clean room space is limited – there is only room for a certain number of incubators. The amount of labor required is a further limiting factor.

Scale-out is therefore only viable for small- to mid-scale production. Beyond this scale, it is necessary to think about a different approach, i.e., scale-up. Scale-up uses the same concept of larger surface area as scale-out but requires a greater focus on the unit operations and then on the process itself.

For example, for scale-up for adherent cells, the same principle applies as for scale-out: one begins with T-flasks before moving on to a 10-layered flask stack. One can then

FIGURE 1

More scale up – beyond traditional approaches.



Shake Flask images: Corning Life Sciences; Stacked Trays and Fixed-Bed Bioreactors: Pall Corporation.

proceed to a larger surface area again – 36-layer HyperStack® – and then do multiples of these. Coming to suspension cells, one might begin again with shaker or spinner flasks but then proceed to a small bioreactor (e.g., a rocking-bed or small disposable bioreactor) before transitioning once more to a mid-scale stir tank bioreactor.

This is a slightly improved approach in that it offers greater bandwidth. Nonetheless, one will still eventually encounter the same bottleneck of having insufficient space to conduct all the unit operations. Scale-up is therefore a viable approach only for mid-scale to perhaps the beginnings of large-scale production.

Novel technologies are emerging with the potential to enable genuinely large-scale production. These tools involve a scale-up approach but go beyond the traditional methods. For example, whereas in the past one might have gone from 10-layer cell stacks to HyperStack®, which would then be limited to around 20 or 30 per batch, it is now possible to go to a fixed-bed bioreactor offering a small footprint but a very large surface area of up to 500m².

Similarly, on the suspension cells front, huge strides have been made recently in bioreactor innovation. Devices are now available in a wide range of sizes – from 50 liters up to 2,000 liters – with footprints compatible with cleanroom capacities. This innovation is not particularly new – large bioreactors have long been used in the monoclonal antibodies field - but for the first time, demand for viral vectors has reached the level where their employment has become a necessity in gene therapy.

To summarize, there have been strong advances in upstream bioprocessing productivity over recent years. Today, the challenge is increasingly about optimizing midstream and downstream bioprocessing.

With regard to midstream processing, one must be cognizant of the larger volumes of viral vector material generated upstream: aware of the contaminants generated, for example, and the need to scale-up the clarification step accordingly. This requires in-depth knowledge of the flow rate used, the pressure, and the shear. Multiple filter trays – and multiple types of tray – may be required. These scale-related considerations all need to be factored in by the process development group.

The same applies to downstream purification. At small-scale, lower contaminant load means only a relatively simple purification scheme is required. However, it is important to remember that a more concentrated product comes with more concentrated contaminants. Volume is critical for downstream processing. One must factor in the type of media used during production – the protein composition of the chosen media plays a critical role in the binding of virus product to the matrices.

Typical virus size and properties, and elements such as pressure and shear must be considered in identifying the best chromatography and/or the purification scheme to use for a given application. Chromatography is generally recommended as a means of purification because of its robustness and scalability, which is of course crucial with larger volumes coming from upstream and midstream.

There are a number of variables and options available at this stage: One, two or three purification steps may be required to generate the material needed at the desired level of purity, depending on the eventual use of that material; bind/elute may be focused either on the product itself, or on the contaminants while the product flows through. These are critical development decisions impacting the robustness of the process.

Once the final product has been obtained, it's important to understand the nature of that material in order to avoid massive losses at the final filtration step. (Again, depending on need and program, this may be followed by final formulation).

As demand for viral vector has increased rapidly over recent years, certain realities have emerged. Optimization steps are clearly required in order to arrive at a more robust, scalable process. Additionally, it is important to think outside the box when exploring methods to increase productivity, not to

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simply rely upon mass production. For example, harnessing novel fixed-bed bioreactors will help scale-up adherent cultures, potentially removing the need to transition or adapt your process to a suspension culture system, which can be challenging. Alternatively, using microcarriers in suspension might be considered, as indeed could the transition to completely serum-free suspension cells.

To conclude, while viral vector upstream bioprocessing has perhaps been the subject of the majority of focus and innovation to date, it is very important not to forget the midstream and downstream processes – to ensure that the whole is scalable, not just one or two component parts.

It is also worth noting that producing greater volumes of vector is not the only answer to meeting increasing demand. Further innovation and improvement in gene delivery and targeting should result in reduced quantities of vector being required in future. Ongoing efforts to optimize transduction efficiencies and/or delivery systems will hopefully lead to the same result. Finally, a number of companies are currently seeking to improve media, buffers and other components in order to better support cell growth and virus production.

TESTING & QUALITY

Characterization and safety testing of the viral vectors used in gene therapy products essentially follows the same basic tenets as for all biologics, including identity, impurity, potency, and freedom from residuals of the production process: these are the fundamentals for assuring product safety and quality.

Identity focuses on demonstrating that the viral vector and its construct is what it is supposed to be, and that it remains so throughout the process.

Titer can be either biological activity, tissue culture infectious dose (e.g., TCID50) or it could be particle enumeration by qPCR.

Potency essentially describes how well the gene therapy product or the viral vector works. It can be based on a variety of different test methods, all of which relate to the mechanism of action or the expression of the transgene.

Purity is verification that the product is free from impurities and adventitious agents. The gene therapy vector is identified and possible contaminants such as related vectors or replication-competent vectors must be confirmed as absent.

Residuals are process intermediates and other holdovers from the process, such as host DNA, protein and enzymes that might be used in production.

As already established, the gene therapy manufacturing process is both complex and varied. There is no one-size-fits-all approach to viral vector production, and the exact same is true for characterization of a viral vector. Different vectors have unique characteristics. However, regardless of the specific approach used, safety and identity profile must be addressed for all components of the process. These include the cells that are used to produce the virus, the viral seed, helper virus or plasmids that are used for transfection or transduction of the cells, and the raw materials that go into the manufacturing process. All must be tested to ensure patient safety and to reduce risk in the manufacturing process as part of the overall quality of the product.

Throughout manufacturing, samples are taken for testing and as previously discussed, the amount of material available for this purpose varies throughout the process. At times, sample volume availability is quite low – a common challenge faced by manufacturers and companies going into a testing program. It is important that viral vector manufacturers as well as testing labs take this into consideration, particularly when designing methods and when setting up the sampling program. A well-designed sampling plan devised with this downstream testing in mind should be established during process development.

Testing of the cell banks used for production is a key component of the overall testing strategy. The cells form the foundation of any manufacturing program. It is worth noting that cell bank testing may take most of the

total testing time available, and this should be accounted for upfront. There tends to be a greater focus on the vector or the downstream product testing, and it is often assumed that the cell characterization will simply occur - it can often be an unwelcome surprise just how much time it actually takes.

The following is a general outline for cell bank testing that would apply to any species:

1. Cells should be evaluated for sterility to demonstrate they are free from any bacterial or fungal contaminants;
2. Cells must be demonstrated to be free of mycoplasma through mycoplasma testing (of which there are now several methods available, including traditional culture-based methods as well as more recent nucleic acid-based tests);
3. Cells must be demonstrated to be free from adventitious viruses, including non-vector retroviruses and non-vector viruses (both broad spectrum and species-specific methods are available);
4. The identity of the cells must be confirmed – by at least one method in the USA, and two methods in Europe. (These could be either genotypic or phenotypic methods for identification of the cell).

There is now a constant flow of novel technologies coming to the field that enable faster testing and release for cell manufacturing.

Viral vector testing requirements depend to a large extent upon the vector in question. While all viral vectors must be tested again to confirm identity, titer, and to demonstrate purity, there may be additional safety considerations and concerns depending on the vector type and its application.

In the case of AAV, for example, the vector is also the final gene therapy product that will be put directly into patients. Testing is therefore more stringent to assure patient safety, particularly as it pertains to purity, freedom from residuals that might be part of the manufacturing and purification processes, and also freedom from recombinant replication-component virus in the final product. In general, AAV is considered relatively safe in this particular realm – it is a non-pathogenic and non-integrating virus. However, we must be able to demonstrate its safety.

By contrast, retroviral and the closely related lentiviral vectors are often not the final product, but rather are a raw material used in the transfection of cells – for CAR T cell therapy, for example. The focus of testing in this case is more on the characterization and safety profile of the viral vector. There are also additional testing considerations and requirements for the final cell therapy product, such as the pro-viral or transgene analysis. Again, though, identity, titer, purity and potency are all part of the testing scheme for retroviral vector batches.

As with cell testing, this space is benefitting from a steady stream of novel technologies that are enabling more agile and rapid testing and release of viral vectors. Additionally, as evidenced by recent guidance documents, the regulatory bodies are also building their understanding of the capability of these faster testing methods.

It is encouraging that both manufacturers and regulators are giving serious consideration to these technologies as viable alternatives to conventional methods. This will allow faster approaches to quality testing, which will be vital to the success of the many cell and gene therapy products with designated expedited development or accelerated approval pathways. It is strongly recommended that developers and manufacturers talk to their testing labs and also the regulatory agencies early in the process of planning the characterization and safety testing scheme. This is in order to understand the full range of rapid methods available, and to allow for consideration of alternative methods - either in parallel with or in place of conventional methods – that may ultimately help reduce time to commercialization.

Overall, testing should be thought of as a continuous process. As one progresses

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through the clinical development phases, the complex nature of the product as well as of the safety testing itself requires a variety of testing strategies for viral vector systems. These can be challenging to design, especially when trying to meet regulatory expectations. However, to reiterate, characterization and safety testing of viral vectors and gene therapy products is basically the same as for all biologics. If one designs or employs testing methods to assure the identity, purity, potency, and freedom from adventitious agents and residuals in the production process, then one should find greater ease on the path to commercialization.

REGULATORY REQUIREMENTS FOR MANUFACTURING SITES: A CASE STUDY

Millipore Sigma's BioReliance® Manufacturing facility in Carlsbad recently dealt with the need to very rapidly get ready for inspections for commercial products as part of their BLA or European registration.

Traditionally, this site has worked with about 12–15 audits a year. Most of these audits were with biotech company clients. However, in the past 2–3 years, an increasing number of pharmaceutical companies have become involved in gene therapy and have visited the site. There has also been an increase in the number of visits from the regulatory agencies over this period. Carlsbad is in the state of California, which means the site had be licensed by the state even for the initial Phase 1 products. However, the FDA and EMA didn't actually visit for inspections until the site was ready to manufacture a commercial product. At the time in question, three clients had either announced, or were expecting to announce shortly, a BLA submission.

Preparation involved not only reviewing the quality systems but the different processes involved for each of these three products, which included different types of viral vector. The initial expectation was for an 18-month window to prepare, and 6 separate workstreams were identified for further development.

However, the development of all three products accelerated, which resulted in the need to accelerate the site preparation program as well. The initial 18-month timeframe was reduced to 12 months.

The FDA visited in June of 2019, with the EMA visiting in September of the same year. Further visits from the regulators are expected, not only relating to the aforementioned three clients, but for other clients as they move towards future commercialization.

There are a few key learnings to share from this overall experience. It is strongly recommended to manufacturers to expect only around 12 months' preparation time to achieve a state of commercial readiness. The longer the better, of course, but it is important to realize that Fast Track and PRiME designations will often accelerate development considerably. Bear in mind the requirement to fit in Process Performance Qualification (PPQ) runs, too.

One very positive impression received is that the regulatory bodies appreciate this is an emerging technology area – that everyone is in this together, and it's important to work together in this spirit to ensure that patients can access these potentially life-saving therapies.

CONCLUSION

In the field of viral vector-based gene therapy where demand is far outstripping supply, there is a need within industry to scale-up rather than just scale-out. This is true whether the manufacturing is in-house or outsourced to a CDMO.

Clearly, initial viral vector process development is more scale-out than scale up, but in order to meet demand, it is certainly preferable for the manufacturer and the client to intensify their processes.

It has been established that there is a pressing need not only for scalability and

reproducibility, but in conjunction with that, for appropriate testing to adequately measure the potency and safety of the product.

Finally, for those manufacturing facilities that are going to have an approved product, it is very important to approach preparations in a strategic manner – to develop workstreams to ensure all of the requirements are met.

As an example, the Millipore Sigma facility at Carlsbad was able to bring in perspectives not only from elsewhere in the company, but also ex-FDA personnel who conducted mock audits along with all the regulatory oversight activities around the PPQ runs. It is obviously key to complete all these activities in time for regulatory inspections for a commercial product.

This article has been created from a transcript of a webinar held by Merck featuring presentations by Dave Backer, Elie Hanania & Marian L McKee.

To view the webinar, please click here.

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When considering the manufacture of any biological, whether it's a monoclonal antibody or a gene therapy,

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there are certain key areas for consideration: ensuring raw materials, equipment and consumables are all compatible with GMP production is crucial; scalability of equipment and of each individual process step is equally important; and robustness and reproducibility are prerequisites for a commercially viable bioprocess.

However, the speed of evolution in gene therapy in general, combined with the fact that many product candidates are now on expedited development pathways (e.g., Breakthough Designation and RMAT in the USA; PRiME in the European Union) means that the bioprocess/CMC development window is effectively shrinking. In addition to this need for acceleration, gene therapy developers and manufacturers must contend with the reality that gene therapy bioprocesses are still derived to a large extent from the academic world, presenting problems both upstream and downstream in terms of quality, reproducibility and scalability. Not only that, but much of the current manufacturing equipment continues to be repurposed from the monoclonal antibody field. Although the effective repurposing of these tools is ongoing and novel solutions tailored to the specific needs of gene therapy production are emerging, this remains a work in progress.

Finally, gene therapy manufacture remains a comparatively highly complex field. There are multiple vector types, with multiple serotypes of these vectors (of AAV, in particular) used in the manufacture of products. Moreover, viral vector production currently involves the use of multiple cell lines. This lack of standardization stands in stark contrast to the more mature monoclonal antibody field, which has coalesced solely around CHO cells and a relatively very well defined, bioreactor-based upstream and downstream process.

This article will explore three vitally important areas in which strategic and technical manufacturing innovation is required to support commercialization:

- Scalability and reproducibility
- Testing and quality
- Regulatory requirements for manufacturing sites

Success in these three primary areas will be critical if we are to get past the current imbalance between supply and demand and effectively deliver on the enormous potential of gene therapy.

SCALABILITY & REPRODUCIBILITY

We can divide any viral vector bioprocess using mammalian cells into three main phases: upstream production, midstream processing, and downstream purification.

Upstream production typically starts with a cell thaw and expansion step, using either adherent or suspension culture. This is followed by a transition to the production phase, where cells are either infected or transfected to generate the viral vector.

Once the vector material is in the media, one proceeds to midstream processing, which begins with a vector material harvesting step. It may be necessary to conduct a lysis process to extract the virus from the cell, but equally, the virus can already be lytic and already present in the media, or indeed, it can be a combination of the two – it depends on the both the virus in question and on the particular program.

One may minimize the amount of host cell protein present through Benzonase® treatment before removing cell debris using stratification. Again, depending on the program and on the scale of production, it may be necessary to also do a concentration step at this point as well as a biofiltration through an appropriate buffer.

The next step is downstream purification where the main goal is to remove contaminants and obtain the pure product. That material is then concentrated prior to final formulation, final filtration and ultimately, fill-finish.

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Of course, it is necessary to factor in testing of the entire process, including the amount of material available for this purpose. During upstream production, the volumes are large enough to mean there is no issue with testing but as you proceed down the production scheme, volumes become smaller and the material becomes increasingly precious – it's important when devising a sampling and testing plan to keep this in mind.

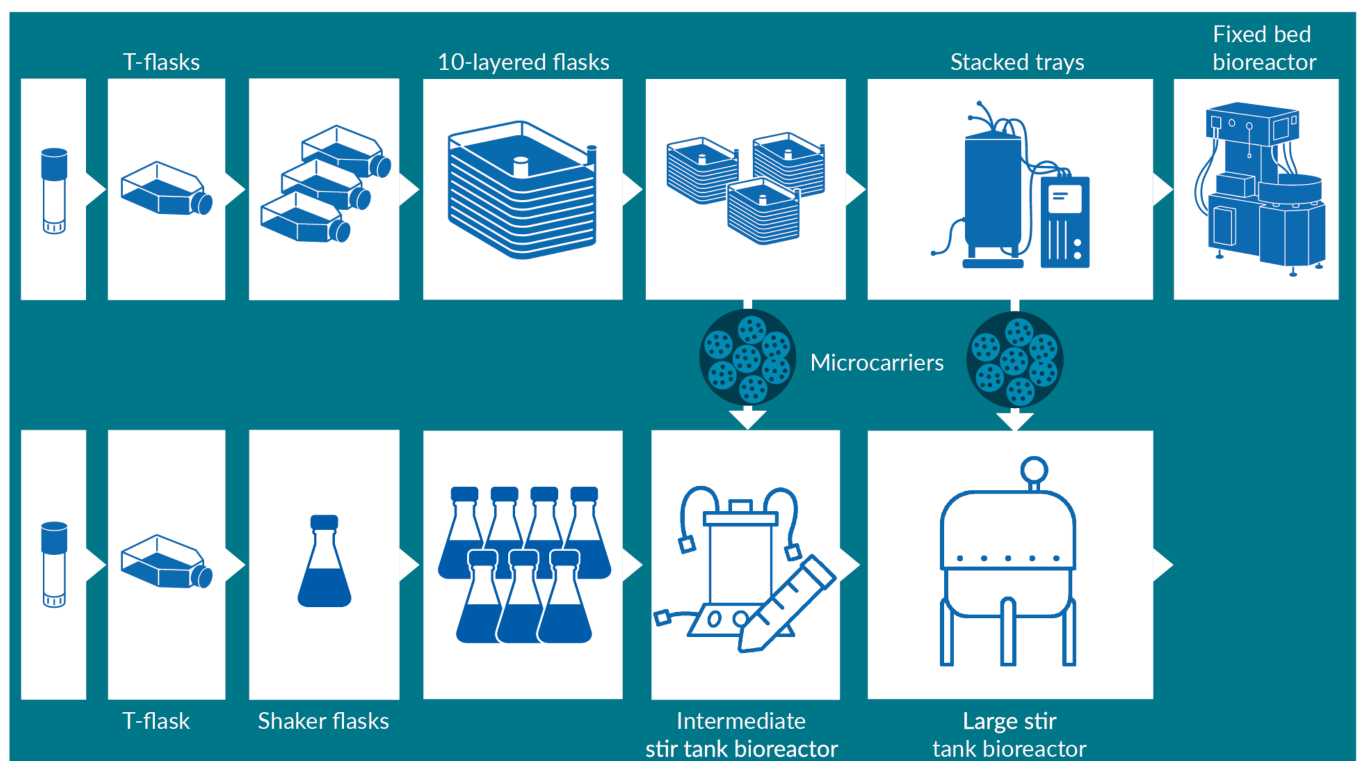
This type of process usually works well at small scale but as demand increases, so too does the need to produce more material. One approach is to scale-out: carrying out the same defined unit operation multiple times. This is a good idea in principle because it is known that the unit operation works. Taking scale-up of cell expansion as an example, one might begin with T-flasks, then proceed to a slightly larger platform that is still in the flask format – layered flasks. At that point, one would scale-out by using multiple layered flasks. The same thing might apply with suspension cells, with the initial transition into shaker or spinner flasks followed by scale-out to multiple flasks. However, this approach only works well up to a certain limit, at which point a major bottleneck is encountered in the form of the amount of space and the number of manipulations required. Clean room space is limited – there is only room for a certain number of incubators. The amount of labor required is a further limiting factor.

Scale-out is therefore only viable for small-to mid-scale production. Beyond this scale, it is necessary to think about a different approach, i.e., scale-up. Scale-up uses the same concept of larger surface area as scale-out but requires a greater focus on the unit operations and then on the process itself.

For example, for scale-up for adherent cells, the same principle applies as for scale-out: one begins with T-flasks before moving on to a 10-layered flask stack. One can then

FIGURE 1

More scale up – beyond traditional approaches.



Shake Flask images: Corning Life Sciences; Stacked Trays and Fixed-Bed Bioreactors: Pall Corporation.

proceed to a larger surface area again – 36-layer HyperStack® – and then do multiples of these. Coming to suspension cells, one might begin again with shaker or spinner flasks but then proceed to a small bioreactor (e.g., a rocking-bed or small disposable bioreactor) before transitioning once more to a mid-scale stir tank bioreactor.

This is a slightly improved approach in that it offers greater bandwidth. Nonetheless, one will still eventually encounter the same bottleneck of having insufficient space to conduct all the unit operations. Scale-up is therefore a viable approach only for mid-scale to perhaps the beginnings of large-scale production.

Novel technologies are emerging with the potential to enable genuinely large-scale production. These tools involve a scale-up approach but go beyond the traditional methods. For example, whereas in the past one might have gone from 10-layer cell stacks to HyperStack®, which would then be limited to around 20 or 30 per batch, it is now possible to go to a fixed-bed bioreactor offering a small footprint but a very large surface area of up to 500m².

Similarly, on the suspension cells front, huge strides have been made recently in bioreactor innovation. Devices are now available in a wide range of sizes – from 50 liters up to 2,000 liters – with footprints compatible with cleanroom capacities. This innovation is not particularly new – large bioreactors have long been used in the monoclonal antibodies field - but for the first time, demand for viral vectors has reached the level where their employment has become a necessity in gene therapy.

To summarize, there have been strong advances in upstream bioprocessing productivity over recent years. Today, the challenge is increasingly about optimizing midstream and downstream bioprocessing.

With regard to midstream processing, one must be cognizant of the larger volumes of viral vector material generated upstream: aware of the contaminants generated, for example, and the need to scale-up the clarification step accordingly. This requires in-depth knowledge of the flow rate used, the pressure, and the shear. Multiple filter trays – and multiple types of tray – may be required. These scale-related considerations all need to be factored in by the process development group.

The same applies to downstream purification. At small-scale, lower contaminant load means only a relatively simple purification scheme is required. However, it is important to remember that a more concentrated product comes with more concentrated contaminants. Volume is critical for downstream processing. One must factor in the type of media used during production – the protein composition of the chosen media plays a critical role in the binding of virus product to the matrices.

Typical virus size and properties, and elements such as pressure and shear must be considered in identifying the best chromatography and/or the purification scheme to use for a given application. Chromatography is generally recommended as a means of purification because of its robustness and scalability, which is of course crucial with larger volumes coming from upstream and midstream.

There are a number of variables and options available at this stage: One, two or three purification steps may be required to generate the material needed at the desired level of purity, depending on the eventual use of that material; bind/elute may be focused either on the product itself, or on the contaminants while the product flows through. These are critical development decisions impacting the robustness of the process.

Once the final product has been obtained, it's important to understand the nature of that material in order to avoid massive losses at the final filtration step. (Again, depending on need and program, this may be followed by final formulation).

As demand for viral vector has increased rapidly over recent years, certain realities have emerged. Optimization steps are clearly required in order to arrive at a more robust, scalable process. Additionally, it is important to think outside the box when exploring methods to increase productivity, not to

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simply rely upon mass production. For example, harnessing novel fixed-bed bioreactors will help scale-up adherent cultures, potentially removing the need to transition or adapt your process to a suspension culture system, which can be challenging. Alternatively, using microcarriers in suspension might be considered, as indeed could the transition to completely serum-free suspension cells.

To conclude, while viral vector upstream bioprocessing has perhaps been the subject of the majority of focus and innovation to date, it is very important not to forget the midstream and downstream processes – to ensure that the whole is scalable, not just one or two component parts.

It is also worth noting that producing greater volumes of vector is not the only answer to meeting increasing demand. Further innovation and improvement in gene delivery and targeting should result in reduced quantities of vector being required in future. Ongoing efforts to optimize transduction efficiencies and/or delivery systems will hopefully lead to the same result. Finally, a number of companies are currently seeking to improve media, buffers and other components in order to better support cell growth and virus production.

TESTING & QUALITY

Characterization and safety testing of the viral vectors used in gene therapy products essentially follows the same basic tenets as for all biologics, including identity, impurity, potency, and freedom from residuals of the production process: these are the fundamentals for assuring product safety and quality.

Identity focuses on demonstrating that the viral vector and its construct is what it is supposed to be, and that it remains so throughout the process.

Titer can be either biological activity, tissue culture infectious dose (e.g., TCID50) or it could be particle enumeration by qPCR.

Potency essentially describes how well the gene therapy product or the viral vector works. It can be based on a variety of different test methods, all of which relate to the mechanism of action or the expression of the transgene.

Purity is verification that the product is free from impurities and adventitious agents. The gene therapy vector is identified and possible contaminants such as related vectors or replication-competent vectors must be confirmed as absent.

Residuals are process intermediates and other holdovers from the process, such as host DNA, protein and enzymes that might be used in production.

As already established, the gene therapy manufacturing process is both complex and varied. There is no one-size-fits-all approach to viral vector production, and the exact same is true for characterization of a viral vector. Different vectors have unique characteristics. However, regardless of the specific approach used, safety and identity profile must be addressed for all components of the process. These include the cells that are used to produce the virus, the viral seed, helper virus or plasmids that are used for transfection or transduction of the cells, and the raw materials that go into the manufacturing process. All must be tested to ensure patient safety and to reduce risk in the manufacturing process as part of the overall quality of the product.

Throughout manufacturing, samples are taken for testing and as previously discussed, the amount of material available for this purpose varies throughout the process. At times, sample volume availability is quite low – a common challenge faced by manufacturers and companies going into a testing program. It is important that viral vector manufacturers as well as testing labs take this into consideration, particularly when designing methods and when setting up the sampling program. A well-designed sampling plan devised with this downstream testing in mind should be established during process development.

Testing of the cell banks used for production is a key component of the overall testing strategy. The cells form the foundation of any manufacturing program. It is worth noting that cell bank testing may take most of the

total testing time available, and this should be accounted for upfront. There tends to be a greater focus on the vector or the downstream product testing, and it is often assumed that the cell characterization will simply occur - it can often be an unwelcome surprise just how much time it actually takes.

The following is a general outline for cell bank testing that would apply to any species:

1. Cells should be evaluated for sterility to demonstrate they are free from any bacterial or fungal contaminants;

2. Cells must be demonstrated to be free of mycoplasma through mycoplasma testing (of which there are now several methods available, including traditional culture-based methods as well as more recent nucleic acid-based tests);

3. Cells must be demonstrated to be free from adventitious viruses, including non-vector retroviruses and non-vector viruses (both broad spectrum and species-specific methods are available);

4. The identity of the cells must be confirmed – by at least one method in the USA, and two methods in Europe. (These could be either genotypic or phenotypic methods for identification of the cell).

There is now a constant flow of novel technologies coming to the field that enable faster testing and release for cell manufacturing.

Viral vector testing requirements depend to a large extent upon the vector in question. While all viral vectors must be tested again to confirm identity, titer, and to demonstrate purity, there may be additional safety considerations and concerns depending on the vector type and its application.

In the case of AAV, for example, the vector is also the final gene therapy product that will be put directly into patients. Testing is therefore more stringent to assure patient safety, particularly as it pertains to purity, freedom from residuals that might be part of the manufacturing and purification processes, and also freedom from recombinant replication-component virus in the final product. In general, AAV is considered relatively safe in this particular realm – it is a non-pathogenic and non-integrating virus. However, we must be able to demonstrate its safety.

By contrast, retroviral and the closely related lentiviral vectors are often not the final product, but rather are a raw material used in the transfection of cells – for CAR T cell therapy, for example. The focus of testing in this case is more on the characterization and safety profile of the viral vector. There are also additional testing considerations and requirements for the final cell therapy product, such as the pro-viral or transgene analysis. Again, though, identity, titer, purity and potency are all part of the testing scheme for retroviral vector batches.

As with cell testing, this space is benefitting from a steady stream of novel technologies that are enabling more agile and rapid testing and release of viral vectors. Additionally, as evidenced by recent guidance documents, the regulatory bodies are also building their understanding of the capability of these faster testing methods.

It is encouraging that both manufacturers and regulators are giving serious consideration to these technologies as viable alternatives to conventional methods. This will allow faster approaches to quality testing, which will be vital to the success of the many cell and gene therapy products with designated expedited development or accelerated approval pathways. It is strongly recommended that developers and manufacturers talk to their testing labs and also the regulatory agencies early in the process of planning the characterization and safety testing scheme. This is in order to understand the full range of rapid methods available, and to allow for consideration of alternative methods - either in parallel with or in place of conventional methods – that may ultimately help reduce time to commercialization.

Overall, testing should be thought of as a continuous process. As one progresses

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through the clinical development phases, the complex nature of the product as well as of the safety testing itself requires a variety of testing strategies for viral vector systems. These can be challenging to design, especially when trying to meet regulatory expectations. However, to reiterate, characterization and safety testing of viral vectors and gene therapy products is basically the same as for all biologics. If one designs or employs testing methods to assure the identity, purity, potency, and freedom from adventitious agents and residuals in the production process, then one should find greater ease on the path to commercialization.

REGULATORY REQUIREMENTS FOR MANUFACTURING SITES: A CASE STUDY

Merck's BioReliance® Manufacturing facility in Carlsbad recently dealt with the need to very rapidly get ready for inspections for commercial products as part of their BLA or European registration.

Traditionally, this site has worked with about 12–15 audits a year. Most of these audits were with biotech company clients. However, in the past 2–3 years, an increasing number of pharmaceutical companies have become involved in gene therapy and have visited the site. There has also been an increase in the number of visits from the regulatory agencies over this period. Carlsbad is in the state of California, which means the site had be licensed by the state even for the initial Phase 1 products. However, the FDA and EMA didn't actually visit for inspections until the site was ready to manufacture a commercial product. At the time in question, three clients had either announced, or were expecting to announce shortly, a BLA submission.

Preparation involved not only reviewing the quality systems but the different processes involved for each of these three products, which included different types of viral vector. The initial expectation was for an 18-month window to prepare, and 6 separate workstreams were identified for further development.

However, the development of all three products accelerated, which resulted in the need to accelerate the site preparation program as well. The initial 18-month timeframe was reduced to 12 months.

The FDA visited in June of 2019, with the EMA visiting in September of the same year. Further visits from the regulators are expected, not only relating to the aforementioned three clients, but for other clients as they move towards future commercialization.

There are a few key learnings to share from this overall experience. It is strongly recommended to manufacturers to expect only around 12 months' preparation time to achieve a state of commercial readiness. The longer the better, of course, but it is important to realize that Fast Track and PRiME designations will often accelerate development considerably. Bear in mind the requirement to fit in Process Performance Qualification (PPQ) runs, too.

One very positive impression received is that the regulatory bodies appreciate this is an emerging technology area – that everyone is in this together, and it's important to work together in this spirit to ensure that patients can access these potentially life-saving therapies.

CONCLUSION

In the field of viral vector-based gene therapy where demand is far outstripping supply, there is a need within industry to scale-up rather than just scale-out. This is true whether the manufacturing is in-house or outsourced to a CDMO.

Clearly, initial viral vector process development is more scale-out than scale up, but in order to meet demand, it is certainly preferable for the manufacturer and the client to intensify their processes.

It has been established that there is a pressing need not only for scalability and

reproducibility, but in conjunction with that, for appropriate testing to adequately measure the potency and safety of the product.

Finally, for those manufacturing facilities that are going to have an approved product, it is very important to approach preparations in a strategic manner – to develop workstreams to ensure all of the requirements are met.

As an example, the Merck facility at Carlsbad was able to bring in perspectives not only from elsewhere in the company, but also ex-FDA personnel who conducted mock audits along with all the regulatory oversight activities around the PPQ runs. It is obviously key to complete all these activities in time for regulatory inspections for a commercial product.

This article has been created from a transcript of a webinar held by Merck featuring presentations by Dave Backer, Elie Hanania & Marian L McKee.

To view the webinar, please click here.

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

COMMERCIAL INSIGHT: JAN 2020

Commercial Insight: cell & gene therapy

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

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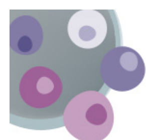


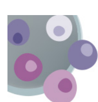
CELL THERAPY – Mark Curtis. Director, Manufacturing Partnerships, AVROBIO

Vancouver-Based Aspect Biosystems announced a series A round of $26 million this past month, showing that investors have a real and growing appetite for 3D printing platforms and future applications that will benefit from complex, 3D, multi-cellular aggregates, including disease modelling and drug screening. Artisan Biotechnologies, a stealth biotech based in Denver, made its debut announcing a deal with Takeda for development of next generation cell therapies. While little is known about the terms of the deal, or Artisan's platform, any tech combining synthetic biology, gene editing, and machine learning should produce some interesting designer cells! On the CDMO front, which continues to see an incredible amount of investment and M&A activity, Deerfield Investment made some waves with the announcement that it would be investing $1.1 billion to build a 680,000 square foot cell and gene therapy development and manufacturing ecosystem. The facility will be located at a former GSK site in King of Prussia and house as many as 100 GMP suites once fully built-out.

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

The start of 2020 sees success at both ends of the clinical development cycle, with the roll-out of Bluebird bio's ZYNTEGLO™ treatment for beta-thalassaemia in Germany using a value-based pricing model, and the start of Phase 1/ 2 for AvroBio's lentiviral therapy for Gaucher disease. Both treatments use *ex vivo* transduction of $CD34^+$ stem cells as the basis for treatment, and therefore need clinical centres with all the necessary local processes and skills available to harvest, transduce and reinfuse stem cells. Manufacturing a reliable product remains a challenge for many companies working on gene therapy treatments; it is therefore interesting to see Hitachi Chemical announcing a new cell and gene therapy manufacturing facility in New Jersey, further expanding its global footprint in the space of contract manufacturing.

Clinical Regulatory



CELULARITY TO INITIATE OFF-THE-SHELF NK CELL THERAPY FOR GLIOBLASTOMA MULTIFORME

Celularity's CYNK-001 has received clearance from the US Food and Drug Administration (FDA) for its Investigational New Drug (IND) Application in patients with glioblastoma multiforme (GBM), the most aggressive tumor of the central nervous system.

Celularity is a clinical-stage company developing allogeneic cellular therapies from human placentas. CYNK-001 is a cryopreserved allogeneic, off-the-shelf NK cell therapy being developed from placental hematopoietic stem cells. Preclinical data has demonstrated that a single dose of CYNK-001 was well-tolerated and showed enhanced *in vivo* anti-tumor activity in animal models of GBM. It is currently being investigated as a treatment for acute myeloid leukemia, multiple myeloma, and as a potential treatment option for various solid tumors.

The IND clearance has enabled the company to clinically investigate CYNK-001 in patients with GBM and the trial is expected to be the first clinical trial in the US to investigate intratumoral administration of an allogeneic NK cell therapy. The study is expected to evaluate the safety, feasibility, and tolerability of multiple doses of CYNK-001 in subjects with relapsed GBM.

Dr Robert Hariri, CEO of Celularity commented:

"The FDA clearance of our IND validates the versatility of our allogeneic, off-the-shelf, placental-derived NK cell therapy platform to generate novel clinical candidates against a broad range of devastating cancers. This IND represents a significant step toward a potential immunotherapy option that is more accessible and tolerable to patients with glioblastoma multiforme."

IN VIVO GENOME EDITING IN STEM CELL DNA PROVIDES HOPE FOR DMD THERAPY

Harvard university researchers in collaboration with Sarepta Therapeutics are developing *in vivo* genome editing technology to correct Duchenne Muscular Dystrophy (DMD) in mice.

DMD, one of the most common inherited genetic diseases, is a fatal genetic neuromuscular disorder affecting an estimated one in approximately every 3,500–5,000 males born worldwide. It is a progressive, X-linked degenerative

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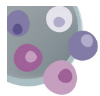
disorder caused by the absence of dystrophin. Dystrophin protein levels are affected due to out-of-frame mutations in the dystrophin gene.

The research at Harvard led by Dr Amy Wagers aims to use in-vivo genome editing, in mouse models of DMD, to fully and precisely restore the function of the dystrophin protein, which is crucial for proper muscular growth and development. Unlike other similar strategies tested, this approach aims to fully correct the genetic template for dystrophin at its source, in the DNA of stem cells (satellite cells) that create and regenerate muscle cells. Approaches validated by this work if successful could pave the way to an eventual therapeutic strategy to reverse DMD in humans.

The new approach is pursued in collaboration with Sarepta Therapeutics, under a multi-year collaboration agreement coordinated by Harvard's Office of Technology Development. Under the terms of the agreement between Harvard and Sarepta, the company will have the exclusive option to license any arising intellectual property for the purpose of developing products to prevent and treat human disease.

Dr Wagers commented:

"In skeletal muscle, muscle fibers are terminally post-mitotic, meaning they cannot divide, and they cannot reproduce themselves. If you lose muscle fibers, the only way to produce new muscle is from stem cells, specifically the satellite cells. The satellite cells are self-renewing, self-repairing, and ready to spring into action to create new muscle fibers. So we expect that a satellite cell with the corrected DMD gene would quite quickly and continuously propagate the edited gene throughout the muscle tissue."



CELYAD DOSES FIRST PATIENT IN ITS NKG2D-BASED CAR-T THERAPY TRIAL

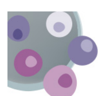
Celyad has dosed the first patient in its Phase 1 CYCLE-1 trial. The trial is designed to use next-generation, NKG2D-based CAR-T therapy, CYAD-02, in patients with relapsed/refractory acute myeloid leukemia (r/r AML).

CYAD-02 engineers an all-in-one vector approach in patient's T-cells to express both (i) the NKG2D chimeric antigen receptor (CAR), a receptor expressed on natural killer cells that binds to eight stress-induced ligands expressed on tumor cells, and (ii) short hairpin RNA (shRNA) SMARTvector technology licensed from Horizon Discovery to knockdown the expression of NKG2D ligands MICA and MICB on the CAR-T cells. In preclinical models, shRNA-mediated knockdown of MICA and MICB expression on NKG2D CAR-T cells has shown enhanced *in vitro* expansion, as well as enhanced *in vivo* engraftment and persistence, of the CAR-T cells, as compared to first-generation NKG2D-based CAR-T cells.

The CYCLE-1 trial is a dose-escalation trial and will evaluate the safety and clinical activity of a single infusion of CYAD-02 produced with the OptimAb manufacturing process following preconditioning chemotherapy cyclophosphamide and fludarabine, or CyFlu, in patients with r/r AML and myelodysplastic syndromes (MDS).

Frédéric Lehmann, VP of Clinical Development & Medical Affairs at Celyad commented:

"Dosing the first patient with CYAD-02 marks another major milestone to systematically advance our pipeline of proprietary autologous product candidates in our relapsed/refractory acute myeloid leukemia program. We look forward to investigating this next-generation approach which combines our NKG2D receptor, shRNA technology and OptimAb manufacturing process. Enrollment in the CYCLE-1 trial will continue over the coming months and we expect to report preliminary data from the study during the second half of 2020."



EMA VALIDATES KITE'S MARKETING APPLICATION FOR ITS SECOND CAR-T THERAPY

The European Medicines Agency (EMA) has fully validated Kite's Marketing Authorization Application (MAA) for KTE-X19, an investigational CAR-T cell therapy for treating adult patients with relapsed or refractory mantle cell lymphoma (MCL), a rare form of non-Hodgkin lymphoma (NHL) that arises from cells originating in the "mantle zone" of the lymph node.

The MAA is supported by data from the Phase 2 ZUMA-2 trial, which demonstrated an overall response rate of 93 percent, including 67 percent with complete response, following a single infusion of KTE-X19 (median follow-up of 12.3 months). In the safety analysis, Grade 3 or higher cytokine release syndrome (CRS) and neurologic events were seen in 15% and 31% of patients, respectively. No Grade 5 CRS or neurologic events occurred. Detailed findings from this trial were recently presented during an oral session at the 61st American Society of Hematology (ASH) Annual Meeting & Exposition in Orlando.

Kite has submitted a Biologics License Application (BLA) for KTE-X19 to the FDA in December 2019 for the treatment of adult patients with r/r MCL. KTE-X19 has been granted Breakthrough Therapy Designation (BTD) by the FDA and Priority Medicines (PRIME) by the EMA for relapsed or refractory MCL.

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

Dr Ken Takeshita, Kite's Global Head of Clinical Development commented:

"Relapse rates in mantle cell lymphoma remain overwhelmingly high and there is a significant need for new therapies that may improve patients' prognosis. The EMA validation of our marketing application brings us closer to delivering on the promise of our industry-leading cell therapy development program, with the hope that we can bring KTE-X19 to appropriate patients in Europe as quickly as possible."

BLUEBIRD BIO LAUNCHES ZYNTEGLO™ IN GERMANY

bluebird bio has announced the launch of its gene therapy ZYNTEGLO™ in Germany for patients 12 years and older with transfusion-dependent beta-thalassemia (TDT) who do not have β^0/β^0 genotype. This is the first time ZYNTEGLO is commercially available.

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

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

bluebird's gene therapy provides hope for a category of TDT patients above 12 years, those who do not have a β^0/β^0 genotype for whom hematopoietic stem cell transplantation is appropriate but a human leukocyte

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antigen (HLA)-matched related HSC donor is not available.

Due to the highly technical and specialized nature of administering gene therapy in rare diseases, bluebird bio is working with institutions that have expertise in stem cell transplant as well as in treating patients with TDT to create qualified treatment centers that will administer ZYNTEGLO. bluebird bio has established a collaboration with University Hospital of Heidelberg as the first qualified treatment center in Germany.

In addition, bluebird has entered into value-based payment agreements with multiple statutory health insurances in Germany to help ensure patients and their healthcare providers have access to ZYNTEGLO and that payers only pay if the therapy delivers on its promise. bluebird's proposed innovative model is limited to five payments made in equal instalments. An initial payment is made at the time of infusion. The four additional annual payments are only made if no transfusions for TDT are required for the patient.

Expert Pick

The world of gene therapy is increasingly likely to see alternative approaches to reimbursement, including annuity and outcomes-based models, for treatments that are notoriously expensive to develop and manufacture, and where the final market can be very small, resulting in a high cost of therapy. Bluebird bio's ZYNTEGLO™ (autologous CD34+ cells encoding βA-T87Q-globin gene) gene therapy for beta-thalassaemia follows this trend, with approval in Germany using a value-based pricing model. Simply put, this means that the company will only be reimbursed following successful treatment, in this case defined as transfusion independence (TI) for the patient. In completed or ongoing studies of ZYNTEGLO, rates of TI are in the range of 75–80%, so the company will likely not be reimbursed for around 20% of patients treated. At a likely cost of $1–2 million per successful treatment, the company will cross its fingers that there is no fall-off in efficacy as the product is rolled out to different centers and countries.

– Richard Philipson

FDA CLEARS AVROBIO'S IND APPLICATION FOR GAUCHER DISEASE GENE THERAPY

The US Food and Drug Administration (FDA) has granted clearance to AVROBIO's investigational lentiviral-based gene therapy, AVR-RD-02, for the treatment of Gaucher disease. This follows receipt of FDA orphan drug designation status for AVR-RD-02, and now clears AVROBIO to expand its ongoing Phase 1/2 clinical trial in Gaucher disease to the United States, supported by the Company's proprietary plato™ gene therapy platform.

Gaucher disease is caused by an inherited deficiency of the enzyme glucocerebrosidase and causes the build-up of the fatty substance glucosylceramide in numerous tissues and

organs. AVR-RD-02 targets the faulty gene via a modification of the patient's own hematopoietic stem cells. A one-time treatment, it is delivered via infusion and expected to sustain a long term supply of the endogenous enzyme. It is hoped that the treatment will be able to replace the current enzyme replacement course of treatment.

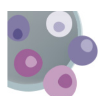
The company believes AVR-RD-02 could slow, halt or potentially reverse symptoms throughout the entire body and brain, such as GBA-related Parkinson's disease which occurs more frequently in people with Gaucher disease type 1.

AVROBIO is now actively recruiting patients for its Phase 1/2 clinical trial of AVR-RD-02 in Australia and Canada, with additional sites planned in the United States. The study aims to evaluate the safety and efficacy of the therapy in patients with Type 1 Gaucher disease. It is intended to recruit 8 to 16 patients between the ages of 16 and 35 with Gaucher disease type 1, including both those who are treatment-naïve and those who are stable on enzyme replacement therapy. AVROBIO's gene therapy platform, plato, has now been cleared by regulators in Canada, Australia and the United States for use in the AVR-RD-02 clinical program.

Ones to Watch

The progression of AvroBio's lentiviral gene therapy for Gaucher disease marks an important milestone for the treatment, which utilizes plato™, the company's proprietary gene therapy platform. The platform encompasses key elements in the company's gene therapy treatment paradigm that include the lentiviral vector, the chemotherapeutic conditioning regimen, cryopreservation of CD34+ cells and the closed manufacturing process for producing the final product. The Phase 1/ 2 study will enrol patients aged 16–35 years with confirmed type 1 Gaucher disease who are either maintained on enzyme replacement therapy (ERT) or treatment naïve. It will be interesting to see how many patients currently maintained on ERT will be willing to undertake the bone marrow harvest and chemotherapy that is required prior to re-infusion of gene modified CD34+ cells.

– Richard Philipson



GAMIDA CELL COMPLETES PATIENT ENROLMENT IN ITS PHASE 3 TRIAL OF OMIDUBICEL

Israeli biotech Gamida Cell has completed patient enrolment in its Phase 3 study of the company's lead clinical program, omidubicel, in patients with high-risk hematologic malignancies.

The investigational cell therapy has the potential to serve as a universal bone marrow donor source for patients with hematologic malignancies who are in need of a

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bone marrow transplant. Topline data from the study are expected in the first half of 2020. If proven successful, the company intends to file its first BLA in the second half of 2020.

More than 40% of eligible patients in the US do not receive a bone marrow transplant for various reasons and even for patients who do receive a transplant, the procedure is not always effective and can lead to serious complications that dramatically affect quality of life. Omidubicel is intended to address the current limitations of bone marrow transplant by providing a therapeutic dose of stem cells while preserving the cells' functional therapeutic characteristics.

The Phase 3 study is designed to evaluate the safety and efficacy of omidubicel compared to standard umbilical cord blood in patients with high-risk hematologic malignancies who need a bone marrow transplant and do not have an available matched donor. The primary endpoint is time to neutrophil engraftment. The study includes approximately 120 patients aged 12–65 with acute lymphoblastic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome or lymphoma. The study is taking place at over 50 clinical centers in the US, Latin America, Europe and Asia.

Omidubicel (formerly known as NiCord), is the first bone marrow transplant product to receive Breakthrough Therapy Designation from the FDA and it has also received Orphan Drug Designation in the US and EU. In a Phase 1/2 clinical study, omidubicel demonstrated rapid and durable time to engraftment and was generally well tolerated. Omidubicel is also being evaluated in a Phase 1/2 clinical study in patients with severe aplastic anemia. The aplastic anemia investigational new drug application is currently filed with the FDA under the brand name CordIn®, which is the same investigational development candidate as omidubicel.

DECIBEL THERAPEUTICS TO FOCUS ON REGENERATIVE MEDICINE FOR THE INNER EAR

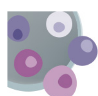
Decibel Therapeutics, a development-stage biotechnology company developing novel therapeutics for hearing loss and balance disorders, has announced a new strategic research focus on regenerative medicine approaches for the inner ear. To support the new research focus, the company is restructuring its employee base and discontinuing some early-stage discovery programs.

The first program in Decibel's regeneration portfolio aims to restore balance function using an AAV-based gene therapy (DB-201), which utilizes a cell-specific promoter to selectively deliver a regeneration-promoting gene to target cells. In collaboration with Regeneron Pharmaceuticals, Decibel will initially evaluate DB-201 as a treatment for bilateral vestibulopathy, a debilitating condition that significantly impairs balance, mobility, and stability of vision. Ultimately, this program may have applicability in a broad range of age-related balance disorders. There are currently no approved medicines to restore balance. Decibel expects to initiate IND-enabling experiments for this program in the first half of 2020.

Decibel is also pursuing novel targets for the regeneration of critical cells in both the vestibule and cochlea of the inner ear; these targets may be addressable by gene therapy or other therapeutic modalities. As a key component of that program, Decibel has also announced an exclusive worldwide option agreement with The Rockefeller University, which has discovered a novel series of small-molecule LATS inhibitors. LATS kinases are a core component of the Hippo signaling pathway, which plays a key role in

regulating both tissue regeneration and the proliferation of cells in the inner ear that are crucial to hearing and balance. The agreement gives Decibel an exclusive option to license this series of compounds across all therapeutic areas. Decibel will work with Prof. James Hudspeth of Rockefeller University, a world-renowned neuroscientist.

In parallel with its new research focus on regenerative strategies, Decibel will continue to advance key priority preclinical and clinical programs. DB-020, the company's clinical-stage candidate designed to prevent hearing damage in people receiving cisplatin chemotherapy, is in an ongoing Phase 1b trial. Decibel will also continue to progress DB-OTO, a gene therapy for the treatment of genetic congenital deafness, which is being developed in partnership with Regeneron Pharmaceuticals. The DB-OTO program aims to restore hearing in people born with profound hearing loss due to a mutation in the otoferlin gene and is expected to progress to clinical trials in 2021.



LARGE-SCALE CELL AND GENE THERAPY CDMO TO LAUNCH IN PENNSYLVANIA

The Discovery Labs and Deerfield Management Company have formed The Center for Breakthrough Medicines, a Contract Development and Manufacturing Organization (CDMO) and specialty investment company, to alleviate the critical lack of capacity that is preventing patients from accessing critically needed cell and gene therapies. The CDMO is occupying over 40 percent of The Discovery Labs' 1.6 million square foot biotech, healthcare and life sciences campus in King of Prussia, PA.

The CDMO provides preclinical through commercial manufacturing of cell and gene therapies and component raw materials. It offers process development, plasmid DNA, viral vectors, cell banking, cell processing, and support testing capabilities all under one roof. The immense $1.1 billion facility will provide instant capacity as the largest known single source for accelerating the delivery and affordability of lifesaving and life-changing therapies from the bench to the patient's bedside.

The company has initiated a substantial hiring effort targeting the best and brightest of the life sciences community including, experts in CGMP manufacturing. The company expects to hire over 2,000 team members within the next 30 months.

In addition to the cell and gene therapy manufacturing facility, The Discovery Labs is establishing THE COLONY which will provide custom built discovery labs, breakthrough funding, sponsored research agreements, housing and relocation for the world's leading iconic experts in cell and gene therapy. THE COLONY will seek to work hand in hand with scientists from both academic and pharmaceutical institutions to unlock and expedite ground-breaking therapies. THE COLONY seeks to unlock institutional barriers prohibiting the world's greatest scientists from moving at a pace necessary in today's ever-changing therapeutic revolution. THE COLONY will partner with the institutions where the scientists currently work by providing equity, license fees, and revenue sharing.

The addition of this end-to-end manufacturing capability is expected to significantly enhance the offerings of The Discovery Labs in an area that has become one of the largest life sciences hubs in the world. Renovations are underway to construct a total of 86 plasmid, viral vector production, universal cell processing, cGMP testing, process development and cell banking suites. The viral vector and cell processing suites will be fully compliant with both FDA and EMA standards.

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GENPREX RECEIVES FDA'S FAST TRACK DESIGNATION FOR LUNG CANCER GENE THERAPY

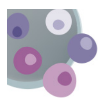
Genprex, a clinical-stage gene therapy company focusing on delivering tumor suppressor genes to cancer cells, has received the FDA's Fast Track Designation for its Oncoprex™ immunogene therapy in combination with AstraZeneca's EGFR inhibitor osimertinib for the treatment of non-small cell lung cancer (NSCLC) patients with EFGR mutations that progressed after treatment with osimertinib alone. Oncoprex is comprised of the TUSC2 (Tumor Suppressor Candidate 2) gene complexed with a lipid nanoparticle. TUSC2 is the active agent in Oncoprex.

Genprex has treated more than 50 lung cancer patients with Oncoprex in Phase 1 and 2 clinical trials. The company believes the data from these trials are encouraging in terms of safety and efficacy.

Fast Track Designation is granted to drugs that have the potential to address unmet medical needs for a serious or life-threatening disease or condition. This provision is intended to facilitate development and expedite review of drugs to treat serious and life-threatening conditions so that an approved product can reach the market expeditiously.

The initial disease indication for Oncoprex is NSCLC, the most common form of lung cancer. Genprex is preparing to initiate a Phase 1/2 clinical trial evaluating Oncoprex in combination with osimertinib, as well as a new Phase 1 clinical trial evaluating Oncoprex in combination with a checkpoint inhibitor.

Licensing agreements & collaborations



SORRENTO AND CELULARITY TO INITIATE NK CELL THERAPY FOR CORONAVIRUS INFECTION

Sorrento Therapeutics has entered into a clinical and manufacturing collaboration with Celularity to expand the therapeutic use of Celularity's CYNK-001, an allogeneic, off-the-shelf, placental-derived Natural Killer (NK) cell therapy, to the treatment and prevention of coronavirus infections.

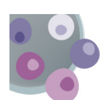
NK cells derived from the placenta have proven to be well tolerated, safe

and versatile, allowing potential uses across a range of organs and tissues. NK cell therapy is currently being investigated as a treatment for various liquid and solid tumors, but also has the demonstrated potential to be effective against virally infected cells.

Under the collaboration, Sorrento and Celularity would assess CYNK-001 as a potential novel therapy for the treatment and prevention of coronaviruses, focusing in particular on the newly emerged 2019 Novel Coronavirus (2019-nCoV). In addition, Sorrento would utilize current existing capacity in its "state-of-the-art" cGMP cell therapy manufacturing facilities in San Diego, California, to supplement Celularity's new cGMP facility in Florham Park, New Jersey. The combined capacity would support the rapid scale-up and sustained production of the novel cell therapy.

Sorrento is already in contact with leading scientists and local Chinese experts to discuss the clinical validation and logistics requirements to fast-track CYNK-001 cell therapy available in China for this particularly urgent indication.

Sorrento owns 25% of Celularity and the companies have a longstanding relationship. Both companies have expressed their interest in starting clinical development efforts for anti-coronavirus allogeneic NK cell therapy immediately.



ARTISAN BIO COLLABORATES WITH TAKEDA TO DEVELOP NEXT-GEN CELL THERAPIES

Artisan Bio, a cell therapy engineering company, has entered a global research and collaboration agreement with Takeda Pharmaceuticals for the discovery, development and commercialization of novel cell therapy products.

Under the terms of the agreement, Artisan Bio intends to deploy its data analysis STAR platform and synthetic biology expertise to construct customized and precisely engineered cell therapies. Artisan will lead discovery efforts, including gene editing, and Takeda will be responsible for developing, manufacturing, and commercializing the resulting cell therapy products.

Artisan's vision is to design, build, and deliver cells and precision engineering processes that advance cellular therapies across a broad range of human health indications. The company's designer cell engineering and STAR platform enables partners to more rapidly and cost effectively generate safer and more efficacious cell therapies. By engaging in strategic collaborations with innovative partners, Artisan seeks to deliver customizable cell engineering solutions that meet the complexities associated with next-generation cell therapies. Artisan has offices in Denver, Colorado and Copenhagen, Denmark.

ASC THERAPEUTICS AND VIGENE COLLABORATE FOR GENE THERAPY DEVELOPMENT AND MANUFACTURING

ASC Therapeutics, a privately-held gene therapy company developing transformative gene-based medicines for serious diseases, has announced that it has entered into a long-term strategic manufacturing partnership with Vigene Biosciences, a Maryland-based Contract Develop and Manufacturing Organization (CDMO).

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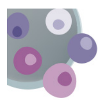
ASC is using AAV-based gene therapy, CRISPR-Cas9 and proprietary gene editing platforms to develop transformative gene-based medicines. Vigene will provide ASC with access to GMP manufacturing including viral vectors and plasmid DNA for its hemophilia A gene therapy clinical program, as well as a manufacturing platform for future gene therapy programs.

Dr Ruhong Jiang, ASC Founder & CEO commented:

"The genetic platform technology developed by ASC is going to change the way serious diseases are treated in the future. We have seen remarkable potency data in our Hemophilia A IND-enabling gene therapy studies. We are proud to welcome Vigene, a global leader in gene therapy process development and GMP manufacturing with a proven track record and expertise in the field of viral vector manufacturing, to become an integral part of ASC long-term plan. Our partnership provides ASC access to Vigene's world-class team with expertise for both plasmid DNA and viral vector manufacturing as well as high-caliber QC and QA teams."

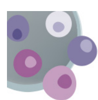


NCARDIA JOIN HANDS WITH BLUEROCK FOR MANUFACTURING IPSC-DERIVED CARDIOMYOCYTES

Ncardia and BlueRock Therapeutics has signed an agreement covering process development technologies for the manufacture of induced pluripotent stem cell (iPSC)-derived cardiomyocytes. Under the terms of the agreement, Bluerock gains access to Ncardia's large-scale production processes and intellectual property for the production of iPSC-derived cardiomyocytes for therapeutic use.

There are hundreds of millions of people worldwide that suffer from degenerative cardiovascular disease where the root cause is the loss of healthy heart muscle cells, and where medical treatment options are limited. BlueRock's authentic cellular therapy is a novel approach that has the potential to transform the lives of patients, but will require the manufacture of our cell therapies at unprecedented scale. The Ncardia team has developed key technologies related to this scale-up challenge, and we are pleased to work with them as we advance BlueRock's novel CELL+GENE platform towards the clinic and those patients in need", said Emile Nuwaysir, President and CEO, BlueRock Therapeutics.

BlueRock Therapeutics is a wholly owned and independently operated subsidiary of Bayer AG. BlueRock's CELL+GENE™ platform harnesses the power of cells for new medicines across neurology, cardiology and immunology indications. BlueRock Therapeutics' cell differentiation technology recapitulates the cell's developmental biology to produce authentic cell therapies, which are further engineered for additional function. Utilizing these cell therapies to replace damaged or degenerated tissue brings the potential to restore or regenerate lost function. BlueRock's culture is defined by scientific innovation, highest ethical standards and an urgency to bring transformative treatments to all who would benefit. For more information, visit www.bluerocktx.com.



ALLOGENE PARTNERS WITH SPRINGWORKS THERAPEUTICS FOR ANTI-BCMA CAR-T THERAPY

Allogene Therapeutics, a clinical-stage biotechnology company developing allogeneic CAR-T cell (AlloCAR T™) therapies for cancer has partnered with SpringWorks Therapeutics

to evaluate its investigational anti-B-cell maturation antigen (BCMA) AlloCAR T therapy (ALLO-715) in combination with SpringWorks' investigational gamma secretase inhibitor (GSI), nirogacestat, in patients with relapsed or refractory multiple myeloma.

Gamma secretase inhibition prevents the cleavage and shedding of BCMA from the surface of myeloma cells. In preclinical models, nirogacestat has been shown to increase the cell surface density of BCMA and reduce levels of soluble BCMA, thereby enhancing the activity of BCMA-targeted therapies. In addition, emerging clinical data suggest that a GSI may increase antitumor efficacy of BCMA-targeted autologous CAR-T therapy in patients with relapsed and refractory multiple myeloma.

Under the terms of the agreement, Allogene will sponsor and conduct the Phase 1 trial to evaluate the safety, tolerability and preliminary efficacy of the combination therapy. Allogene and SpringWorks will form a joint development committee to oversee the clinical study, which is expected to commence in the second half of 2020 pending discussions with regulators.

SpringWorks is currently enrolling patients in a global Phase 3 clinical trial to evaluate nirogacestat in adults with progressing desmoid tumors.

ALLO-715 utilizes TALEN® gene-editing technology pioneered and owned by Cellectis. Allogene has an exclusive license to the Cellectis technology for allogeneic products directed at the BCMA target. Allogene holds global development and commercial rights for this investigational candidate.

Saqib Islam, CEO of SpringWorks Therapeutics commented:

"Gamma secretase inhibition has emerged as a clinically validated mechanism to potentiate BCMA therapies and we believe that nirogacestat has the potential to become a cornerstone of BCMA combination therapy for patients with multiple myeloma. We are delighted to partner with Allogene, a pioneer in the allogenic cell therapy field, to further explore nirogacestat in combination with an 'off-the-shelf' CAR-T therapy for these patients where the need for treatment options remains great."

Finance

EMENDO BIOTHERAPEUTICS RAISES $61 MILLION TO ADVANCE GENOME EDITING THERAPEUTICS

Emendo Biotherapeutics, a biopharmaceutical company developing next-generation gene editing therapeutics using synthetic biology has announced a Series B investment totaling $61 million

The finance round was led by Japan-based biopharma AnGes, reflecting its strategic

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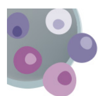
interest in partnering with Emendo on the development of specific indications.

Emendo Biotherapeutics is pioneering OMNI, a next-generation allele-specific gene editing platform that uses synthetic biology to expand what is possible in genome-editing. In 2019, Emendo granted an option to Takeda to use the OMNI nuclease gene editing program for two research and development targets. Emendo received an undisclosed investment from Takeda Ventures that was converted in the Series B.

Emendo's OMNI technology enables precision gene editing while maintaining high efficiencies, uniquely addressing dominant indications such as Severe Congenital Neutropenia (SCN), caused by mutations in the neutrophil elastase gene ELANE. Dominant indications represent the vast majority of genetic diseases which until now have been untreatable.

Dr David Baram, President & CEO of Emendo commented:

"This financing provides a strong foundation from which we can accelerate our proprietary OMNI gene editing platform towards a broad clinical pipeline for addressing devastating untreatable diseases. We are grateful for such strong support from so many high-quality investors and strategic partners including AnGes, OrbiMed Advisors, OrbiMed Israel Partners and Takeda Ventures who share our vision to translate this powerful science into transformative medicines."



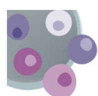
ASPECT BIOSYSTEMS RAISES US $20 MILLION IN SERIES A FINANCING

Aspect Biosystems, a biotechnology company pioneering microfluidic 3D bioprinting of human tissues, has announced that it has raised US $20 million in a Series A financing round. Radical Ventures led the round with participation from existing and new investors, including Pangaea Ventures, Pallasite Ventures, and Rhino Ventures. Funds raised will be used to advance multiple tissue therapeutic programs, expand its technology platform capabilities, and expand its workforce.

Aspect's proprietary technology platform enables the creation of living human tissues with unprecedented control, flexibility, and precision. Aspect's technology is thought to have the potential to shape every aspect of human health by enabling the creation of human tissues for medical research, therapeutic discovery, and regenerative medicine.

Tamer Mohamed, CEO of Aspect Biosystems commented:

"We are thrilled to close this important institutional financing round with a group of world-class investors who believe in our bold vision. This funding speaks to the power of our technology and strategy in addressing multiple applications in therapeutic discovery and regenerative medicine and will allow us to accelerate internal innovation and expand our global partnerships. With our technology platform, interdisciplinary team of scientists and engineers, and leading collaborators, we are developing a new wave of solutions that have the potential to transform how we heal injury and disease."



JASPER THERAPEUTICS SECURES $50 MILLION IN SERIES A FINANCING

Jasper Therapeutics, a biotechnology company focusing on hematopoietic cell transplant therapies, has announced the expansion of its Series A financing with an additional investment of $14.1 million. The current financing round was led by Roche Venture

Fund and the company has raised a total of more than $50 million to date.

Proceeds from the funding will be used to advance and expand the study of the company's lead clinical asset, JSP191. JSP191 is a humanized antibody in clinical development as a conditioning agent that clears hematopoietic stem cells from bone marrow. It targets CD117, a receptor for stem cell factor (SCF) that is expressed on the surface of hematopoietic stem and progenitor cells. The interaction of SCF and CD117 is required for stem cells to survive.

JSP191 blocks SCF from binding to CD117 and disrupts critical survival signals, causing the stem cells to undergo cell death and creating an empty space in the bone marrow for donor or gene-corrected transplanted stem cells to engraft.on hematopoietic stem cells. The treatment is designed to replace toxic chemotherapy and radiation therapy as conditioning regimens to prepare patients for curative stem cell and gene therapy. JSP191 is the only antibody of its kind in clinical development as a single conditioning agent for people undergoing curative hematopoietic cell transplantation.

Preclinical data has demonstrated its potential in safely depleting normal and diseased hematopoietic stem cells in animal models. This creates the space needed for transplanted normal donor or gene-corrected hematopoietic stem cells to successfully engraft in the host bone marrow. To date, JSP191 has been evaluated in more than 80 healthy volunteers and patients.

JSP191 is currently being evaluated in a Phase 1/2 dose-escalation study as a conditioning agent to enable stem cell engraftment in patients with severe combined immunodeficiency (SCID) who received a prior stem cell transplant that resulted in poor outcome. Jasper plans to expand the Phase 1/2 clinical study to include patients with AML and MDS receiving hematopoietic cell transplant. The development of JSP191 is supported by a collaboration with the California Institute for Regenerative Medicine.

Expert Pick

NOVEL CONDITIONING REGIMEN FOR TRANSPLANT OR GENE THERAPY

Patients undergoing hematopoietic stem cell transplant for the treatment of cancer or receiving ex vivo gene therapies for rare genetic disorders, must undergo conditioning prior to being treated to remove the patient's stem cells and make room for incoming stem cells. The current standard of care utilizes small molecule compounds which kill cells in the bone marrow compartment indiscriminately. While effective, the collateral damage caused by pharmaceutical agents cause a number of different adverse events. Novel approaches, like the anti-CD117 antibody being developing by Jasper Therapeutics, promise to deliver fewer off target effects and make conditioning more approachable for transplant and rare disease applications. Jasper already has data in humans (presented at ASH in December) showing that its conditioning agent, JSP191, was well-tolerated and safely cleared stem cells in SCID patients receiving a donor stem cell transplant.

– Mark Curtis

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Ones to Watch

ASPECT BIOSYSTEMS

After 3D printing technology was adapted to print living cells it became possible to create 3D cellular aggregates out of various cell types to mimic *in vivo* environments. This technology has been directed towards disease modelling, drug screening, and cell therapy drug products. Aspect is one of the few companies globally with a highly advanced and robust platform for 3D printing of living cells. The technology and its applications haven taken time to mature, and Aspect is well positioned to be a leader in the space with a strong team and a series of partnerships to drive development.

– Mark Curtis

Movers & Shakers

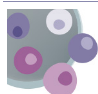
GENEMEDICINE APPOINTS PROF. ROBERT S. LANGER TO ITS SCIENTIFIC ADVISORY BOARD

GeneMedicine, a developer of oncolytic adenovirus platform for treating intractable cancers has appointed Prof. Robert S Langer, the David H. Koch Institute Professor at MIT to its Scientific Advisory Board.

Dr Langer is a world-renowned scientist and entrepreneur and is widely regarded for his decades of accomplishments and contributions to medicine and biotechnology. His pioneering work in the field of drug delivery systems has been widely recognized. Dr Langer has authored more than 1,500 scientific papers and has over 1,350 issued and pending patents worldwide. His patents

have been licensed or sublicensed to over 400 pharmaceutical, chemical, biotechnology, and medical device companies.

Dr Langer served as a member of the US FDA's Science Board, the FDA's highest advisory board, from 1995 to 2002 and as its Chairman from 1999 to 2002. He received his Bachelor's Degree from Cornell University in 1970 and his Sc.D. from MIT in 1974, both in chemical engineering. He has received numerous honorary doctorates from prestigious academic institutions worldwide, including Harvard University and Yale University.



MICHAEL COOKE JOINS IFM THERAPEUTICS AS CSO

IFM Therapeutics, a privately held biopharmaceutical company developing therapies that modulate novel targets in the innate immune system, has appointed Dr Michael Cooke, as its CSO. In his role, Dr Cooke will work with IFM's leadership and board to guide overall scientific and research strategy, partnerships and capabilities.

Dr Cooke joins IFM from Magenta Therapeutics where he most recently served as CSO, leading an organization responsible for developing curative medicines for hematologic malignancy, genetic diseases and autoimmune disease through advances in bone marrow transplantation.

Prior to that, Dr Cooke was a founding scientist at the Genomics Institute of the Novartis Research Foundation, where he held several positions including Executive Director of Immunology. He facilitated the development of a portfolio of therapeutic discovery programs. Before joining Novartis, he served as Director of Functional Genomics at SyStemix in the area of hematopoietic stem cell biology.

Dr Cooke obtained PhD in Biochemistry from the University of Washington and completed postdoctoral research on the molecular basis of B cell tolerance with Dr Christopher Goodnow at Stanford University. He is a co-author on over 60 peer-reviewed publications and is a co-inventor on over 20 patents and patent applications.

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

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