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SUPPLEMENTARY INFORMATION

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Biomanufacturing for clinically advanced cell therapies

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Supplementary Information

Content

Supplementary Table 1. Phase II/Phase III/Approval Conversion Rates.

	Pharmaceutical[1]	Cell Therapy[2]
Phase II → Phase III	**26.5% (2482)**	**10.2% (401)**
Phase III → Approval	**48.7% (731)**	**14.3% (42)**

[1]Annual report from BIO entitled "Clinical Development Success Rates 2006-2015 (June 2016)**, pg. 20**.
[2]General search terms of "cell therapy/cellular therapeutic", "autologous", and "allogeneic" were used in order to develop a general clinical landscape of cell therapy products at Phase II and Phase III levels, using a time scope of 1/1/2006 to 12/31/2015. Each individual reported clinical trial was then evaluated as to whether the primary goal was the evaluation of a novel cellular therapy. Thus, any studies including cell therapies as secondary/tangent procedure or already approved therapy (BMT) were not included in our analysis. Trials included in this analysis include both academic-sponsored trials and corporate-sponsored trials, while the BIO report analysis includes only corporate-sponsored trials. Three categories were identified: 1) Cell therapy, 2) Not cell therapy, 3) Tandem cell therapy (roughly equivalent emphasis on cell therapy and additional therapy (ie. drugs)). Phase II Trial #s Cell therapy: 370, Not cell therapy: 945, Tandem: 31; Phase III Trial #s Cell therapy: 36, Not cell therapy: 205, Tandem: 6. Data for cell therapy includes combination trial data. These data were cross-referenced to FDA.gov website for approvals during that period.

See Supplementary Data file for a list of Phase-II and Phase-III clinical trials that include the use of cell therapies.

Supplementary Table 2: Regulatory reference summary for cell therapy manufacturing

	US	EU
Regulatory Agency	Office of Tissues and Advanced Therapies/ Center for Biologics Evaluation and Research (CBER)/ Food and Drug Administration (FDA)	European Medicines Agency (EMA)
Governing Regulations	21 CFR 1271 - Human Cells, Tissues, and Cellular and Tissue-Based Products 21 CFR 600 - Biologic Products: General 21 CFR 610 - General Biological Products Standards	Directive 2001/83/EC - Medicinal Products for Human Use EC 1394/2007 - Advanced Therapy Medicinal Products ("ATMP", ammends 2001/83/EC) Directive 2004/23/EC - Quality and safety for donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells
Good Manufacturing Practice Regulations	21 CFR 210 - Current GMP in Manufacturing, Processing, Packing, or Holding of Drugs, General 21 CFR 211 - Current GMP for Finished Pharmaceuticals	Directive 2003/94/EC - GMP for medicinal products for human use
Key guidances	FDA/CBER - Content and Review of Chemistry, Manufacturing, and Control (CMC) information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs) FDA/CBER - Current Good Tissue Practice (CGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) FDA/CBER - Potency Test for Cellular and Gene Therapy Products	EudraLex - Volume 4 - GMP guidelines
Internationally harmonized guidances	ICH Q6B - Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products ICH Q8 (R2) - Pharmaceutical Development (establishes QbD) ICH Q9 - Quality Risk Management ICH Q10 - Pharmaceutical Quality System	

Note: While manufacture of cell-based products are excluded from some guidances, the principles are generally applicable

Supplementary Table 3: Descriptive summary of quality attributes for cell therapy products

Attribute Category	Description
Identity	One or more cell phenotypes that are expected to provide the targeted efficacy of the CTP, analogous to active pharmaceutical ingredient.
Cellular purity	Closely linked to identity. Cellular impurities include other cell phenotypes, non-viable cells, cell aggregates, or cell debris,that are extraneous and not expected to contribute to efficacy or even could be harmful to the CTP or the patient.
Acellular impurities	Extraneous residual materials from the manufacturing process, such as endotoxin, culture reagents, serum, cytokines, antibiotics, particulate.
Potency	Measure of one or more relevant biological activities that contribute to the targeted efficacy of the CTP, at least related to mechanism of action (MOA) and ideally clinical efficacy.
Cell Number/Viability	Quantity and percentage of viable cells in each dose. Often linked to potency and/or identity
Microbiological Safety	Absence of microbial contaminants including bacteria, fungal, mycoplasma, and viral organisms

Supplementary Table 4: Examples of Cell Therapeutics and Their Corresponding Potency Testing

CTP	Description	Potency	Indication	Putative MoA	Ref
Prochymal	Allogeneic MSCs	Expression of secreted tumor necrosis factor receptor (sTNFR1)	Graft vs. Host Disease	Immunomodulation	**3**
CD19-CAR-T	Autologous mixture of CD19-CAR+ T cells and residual CD45+ cells	Cytolysis of CD19-expressing cells	ALL	CD107a degranulation in response to CD19+ target cells	1
Ixymyelocel-T	Autologous mixture of CD90+ stromal cells and CD45+ cells	Decreased secretion of pro-inflammatory stimuli, expression of CD206 and CD163, phagocytic activity	Ischemic cardiovascular disease	Promotion of angiogenesis, immune modulation, and tissue repair	**1**
Treg	Autologous Treg	Suppression of T effector cells; Suppression of IFN-γ secretion	Type 1 diabetes (autoimmunity)	Restoration of immune balance between effector and regulatory T cell functions	2
Amorcyte	Autologous CD34+ hematopoietic progenitor cells	Quantity of CD34+ cells, SDF-1 mediated cell migration	Myocardial infarction	Migration of CD34 cells into affected region and prevention of adverse remodeling	3

Supplementary Table 5: Current challenges associated with the integration of microcarriers into MSC manufacturing.

	Benefit	**Challenge**	**Potential Mitigation**
Product Comparability	Reduced labor requirements and ability to implement automated process steps. Stirred bioreactors are well characterized, allowing for changes in scale to be assessed prior to implementation [4]. Reduction in required clean room capacity and equipment (incubators etc.) allowing for facility and operational cost savings.	**Quality:** High risk of changes in MSC product efficacy when moving from existing planar technology to microcarrier-based process.	Development of quality assays of product efficacy.
Product Yield (per bioreactor volume)	Ability to manufacture >10^9 cells to provide >4000 doses per batch compared with <10^8 cells to provide <400 doses per batch in planar culture technology, reducing cost of goods[5].	**Cost of Goods:** Increased MSC number per batch reduces the cost per dose.	Development and optimization of culture medium and process parameters.
Harvest	Reduction in batch pooling and product holding times, improving process scalability and product quality attributes [6].	**Quality:** Increased agitation rate during detachment reduces product quality. **Cost of Goods:** Increased detachment efficiency and MSCs per batch reduces the cost per dose.	Development of MSC detachment protocols prior to implementation at scale [7, 8].
Separation	Improved integration of up- and down-stream unit operations, reducing process time and improving scalability.	**Quality:** Method of separation may affect MSC CQAs. **Scalability:** Process should allow for timely separation, even at large scale.	Development of scale-down models to test product CQAs [9] and downstream processing times early in development [10].
Process Optimization	Homogeneous environment allows for monitoring and control of key process parameters such as dissolved oxygen, pH, nutrients and metabolites[11]. Bioreactor systems allow for flexible modes of operation such as batch, fed-batch or perfusion, allowing for process development activities to improve product quality and yield.	**Quality:** Impact on MSC product CQAs. **Scalability:** Limit to process scale.	Integration of effective process control systems, media optimization and consideration of direct aeration methods [12] in early development.
Purification	Closed system manufacture, reducing	**Quality:** Increase in rate	Integration of

	the risk associated with contamination and failed product lots.	of failed lots due to impurities.	particulate and impurity levels as a screening criteria for development of downstream separation and volume reduction processes.
Attachment		**Sustainability:** Reducing the amount of serum is critical in increasing large-scale sustainability. **Cost of Goods:** Reduced attachment reduces growth rate and number of MSCs per batch.	Development of reduced or serum-free process including methods for microcarrier modification to improve MSC attachment [10].

Supplementary Table 6: Comparison of microencapsulation techniques, materials and their scalability.

Encapsulation Technique	Scalability	Production Rate	Material	Characterization	Ref
Extrusion	Medium to high	Coaxial air flow: 10^2 part./s Electrostatics: 10^5 part./s Vibration: 10^3 part./s JetCutter: 10^4 part./s	Alginate	*In vitro*, rodent, dog, nonhuman primate, human	*13-25*
Emulsion	High	Depends on vessel size	PEG/PEGDA Alginate Agarose	*In vitro*, rodent, nonhuman primate, human *In vitro*, rodent *In vitro*, rodent, dog	*26-35* *36-38*
Microfluidic	Medium	10^4 particles/s	Alginate PEG	*In vitro*, rodent	*39, 40*
Bioprinting	Low to medium	10^5 particles/s	Alginate	*In vitro*	
Surface coat	Low	Depends on coating method	PEG/PEGDA	*In vitro,* rodent, human	*41-43*

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