WHITE PAPER: **CDMO SERVICE**

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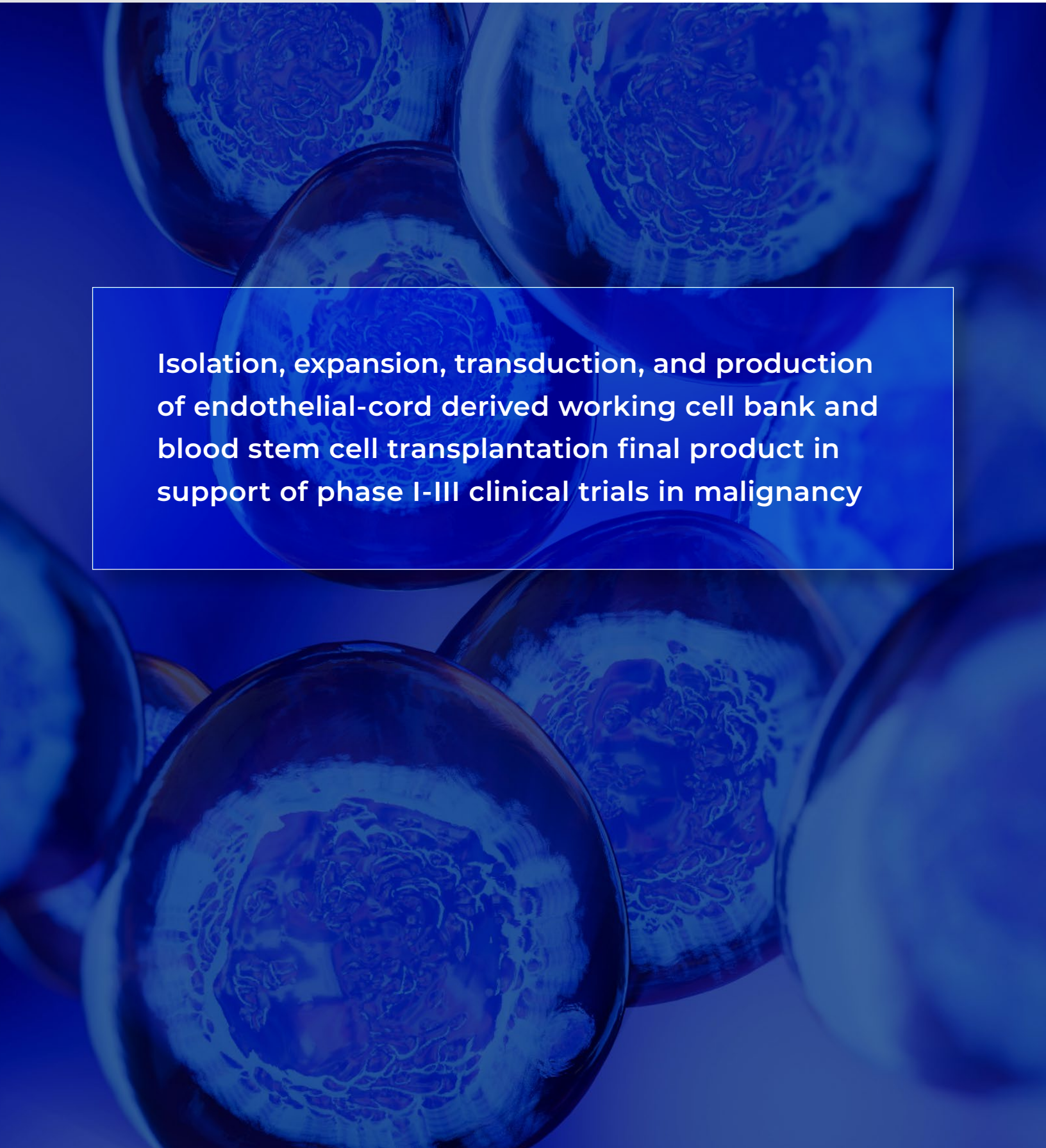

Isolation, expansion, transduction, and production of endothelial-cord derived working cell bank and blood stem cell transplantation final product in support of phase I-III clinical trials in malignancy

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OVERVIEW

Excellos development and manufacturing teams have successfully carried out Current Good Manufacturing Processes (cGMP) production of a retrovirally transduced cell therapeutic, generated from primary umbilical cord tissues in support of Phase I and Phase II clinical trials. There are three stages of production involved in this process.

1. Cord tissue collection, primary cell isolation, characterization by Fluorescent Activated Cell Sorting (FACS) analysis, *ex vivo* expansion in tissue culture flasks, lentiviral transduction, and cryopreservation.
2. Larger-scale expansion of thawed cells in hollow-fiber perfusion bioreactors and cryopreservation for the establishment of working cell banks (WBCs) for subsequent expansion and production of the clinical final product.
3. Post-thaw processing, bioreactor seeding and large-scale expansion in the hollow-fiber bioreactors for production of the final product to be used in Phase I and Phase II clinical trials. The WBCs that were established are also being used to manufacture phase III final products for ongoing Phase III pivotal trials.

All processes, beginning from primary isolation of cells from cord tissue through final product for clinical use, are carried out under cGMP conditions in ISO 7 cleanrooms. This process includes:

- Environmental monitoring
- Qualified materials management
- Vendor qualification
- Quality Assurance (QA) and Quality Control (QC) oversight
- A fully documented staff training process
- Execution using formal batch production records enabling the preparation and use for production of manufacturing intermediate cell banks (WBCs)
- Final product for clinical use.

The following provides a brief overview of the cell banking and final production as executed by Excellos staff.

Note: the operations were performed at San Diego Blood Bank (SDBB) prior to Excellos spin-out; however, the same personnel, systems, infrastructure, and documents were transferred to Excellos as a condition of the separation.

BACKGROUND

When cancer patients undergo chemotherapy, treatment-related toxicities occur that can be dose-limiting or life-threatening to the patient. Mitigation of this issue is still a major unmet medical need to assure better treatment outcomes for cancer patients. A specific example of this is the development of cell-based approaches to mitigate treatment-related tissue and organ toxicities with enhanced regenerative responses in hematological malignancy patients who have undergone myeloablation for bone marrow transplants (BMT). The final cell product was manufactured by Excellos team members as a Contract Development and Manufacturing Company (CDMO) service for the client and was used to carry out Phase I-II clinical trials.

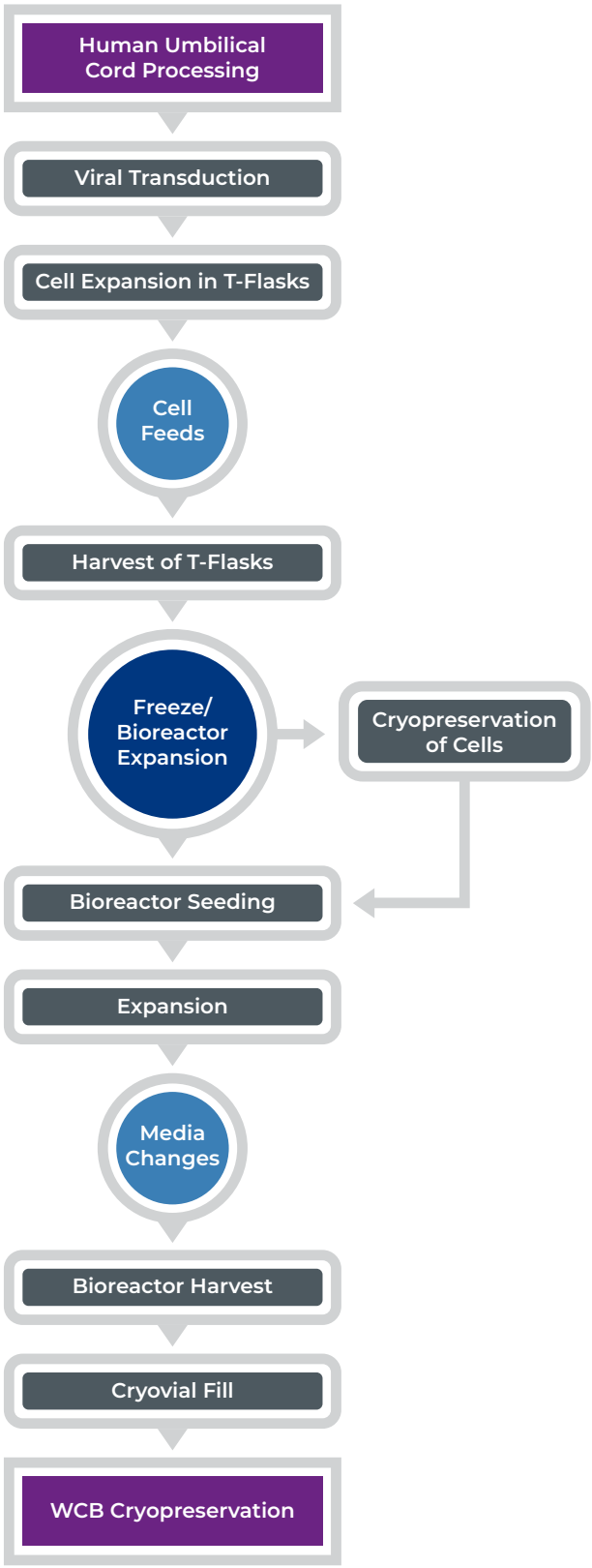
RATIONALE

The client developed a cord-tissue-derived cell therapy for lymphoma cancer patients undergoing high-dose chemotherapy and autologous bone marrow transplant (BMT). The cell therapy is generated by the isolation of primary umbilical cord cells that are expanded in tissue culture flasks, lentiviral transduced, cryo-preserved and used for large-scale expansion to produce clinical product that is administered systemically. This therapy is intended to facilitate tissue repair, regeneration, and recovery from radiation and/or chemotherapy-induced tissue and organ damage. Doing so reduces treatment-related morbidity and improves quality of life for BMT cancer patients. The client partnered with Excellos for process development, tech transfer, and cGMP manufacturing of this product in support of their Phase I and Phase II IND studies and was granted FDA Regenerative Medicine Advance Therapy (RMAT) status for their clinical trial therapy.

TISSUE COLLECTION AND PRIMARY CELL ISOLATION (Figure 1)

Cord segments, aseptically collected from consenting mothers, serve as the starting material for primary cell isolation. Cells are harvested using aseptic techniques under cGMP in ISO 5 biosafety cabinets, located in ISO 7 cleanroom environments, by trained manufacturing staff. Harvested cells are then expanded *ex vivo* in tissue culture flasks in tri-gas incubators with identity and purity confirmed by FACs analysis. All steps are carried out under cGMP conditions with appropriate environmental monitoring, staff training, QA oversight, materials management, and facilities management.

Figure 1.
Process Flow: Generation of Working Cell Bank (WCB)



LENTIVIRAL TRANSDUCTION (Figure 1)

After initial isolation, expansion, and confirmation of cell identity by FACs, lentiviral transduction of the *ex vivo* expanded cells is performed in an HVAC-dedicated ISO 7 cleanroom and an ISO 5 biosafety cabinet, to reduce the risk of area and cross-product contamination. Viral transduction efficiency is confirmed by multiplicity of infection (MOI) analysis, and aliquots of transduced cells are immediately expanded in customized media or cryopreserved for later processing.

EX VIVO EXPANSION AND CRYOPRESERVATION (Figure 1)

The lentiviral-transduced cells are expanded in customized media using hollow-fiber perfusion bioreactors over 9-11 days. Manufacturing staff monitor glucose and lactate levels and perform media exchanges during the expansion phase. When cells reach the required density, they are harvested and cryopreserved in vapor-phase LN2 storage. The cryopreserved WCBs serve as manufacturing intermediates for final product production. All processes are carried out under cGMP and the intermediate material undergoes a release process with full QA oversight prior to use in subsequent process steps (refer to Table 1 regarding analyses performed during process).

POST-CRYOPRESERVATION EXPANSION AND FINAL PRODUCT PRODUCTION AND RELEASE (Figure 2)

Final product production is initiated using the cryopreserved WBCs, which are thawed, evaluated for viability and yield, and used to seed a series of hollow-fiber bioreactors for large-scale expansion using an expansion procedure similar to that carried out for the manufacturing intermediate production process. When the appropriate cell density has been reached, cells are harvested, analyzed for safety (i.e., endotoxin, mycoplasma, sterility), and cryopreserved as final product. The final product specifications are tested using in-house and outsourced assays and released by the QA team for use in clinical settings (Table 1).

Figure 2.
Process Flow: Generation of Final Product

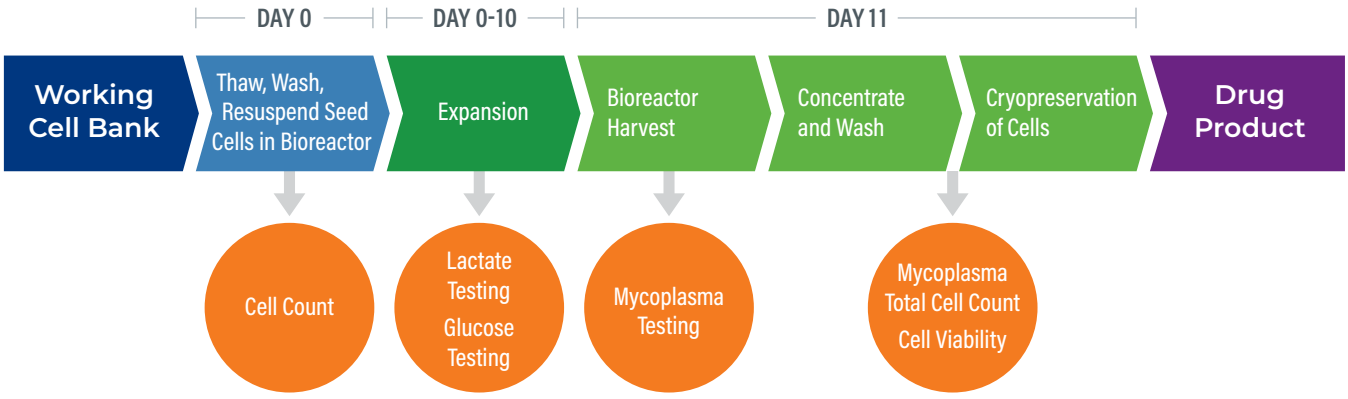


Table 1.
Analyses Performed on Working Cell Bank and Final Product

Stage	Test	In-House / Outsourced
Working Cell Bank	Safety (Sterility, Gram Stain Endotoxin, Mycoplasma)	Outsourced
	Potency	
	Replication competent retrovirus (RCR) adventitious virus testing	
	Flow Cytometry — FACS	In-house
	Total Cell Count	
	Cell Viability	
Final Product	Safety (Sterility, Gram Stain Endotoxin, Mycoplasma)	Outsourced
	mRNA expression of E4RF1	
	Flow Cytometry — FACS	In-house
	%CD45-CD31+ Cells	
	Total Cell Count	
	Cell Viability	

DISCUSSION

The process of cGMP manufacturing the final product has some general similarities to CAR-based therapies production in terms of *ex vivo* expansion and viral transduction, but differs substantially in terms of primary cell isolations, expansion and characterization of an adherent cell type, banking of a cGMP-compliant working cell bank for later final product generation manufacturing campaigns, and the types of potency and identity assays carried out for CAR-T products. Excellos worked closely with the Client during all aspects of technical transfer and in house production.

Excellos routinely performs T cell subset isolations using the Miltenyi MACs systems and Excellos staff have experience with non-adherent cell expansion systems. The production of the Client's final product has a higher level of complexity than typically seen for CAR-T manufacturing and this experience demonstrates that Excellos is well positioned to carry out CAR-based therapies manufacturing for both T and NK cell types.