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Distributive Manufacturing of CD19 CAR-T Cells Using CliniMACS Prodigy: Feasibility and Real-World Experience from India

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Therapy with chimeric antigen receptor-modified T cells (CAR-T) is an established and accepted standard of care for relapsed refractory B cell malignancies (1, 2). Access to CAR-T cell therapy is prohibitively expensive, has significant logistic challenges with centralized manufacturing, and remains difficult to access even in developed countries (3). In a resource-constrained environment and a health care delivery system with a very high out-of-pocket expense, such as in India (4), for high-end therapy, access to CAR-T cells is for all practical purposes, not a considered option. The currently established industry and profit-driven commercial process of centralized manufacturing of the CAR-T cells is financially unviable and is unlikely to make this technology accessible to most of our patients. An alternative to centralized industry-driven manufacturing is a decentralized, distributive or point of care manufacturing process at academic / tertiary care centers using a closed GMP compliant CliniMACS Prodigy (Miltenyi Biotec, Germany) system. Early phase clinical trials with this approach have been completed in USA and Europe (5). The costing structures in India and countries with similar economies (low and upper middle-income countries – L/UMIC) are however different, with a relatively higher purchasing power parity and a lower cost of skilled manpower which would make this approach an attractive option to consider.

Requirements for successful decentralized CAR-T cell manufacturing process:

Background setting: It would be preferable to do this in a center that already has an established allogeneic hematopoietic cell transplant (allo HCT) program. A number of the required elements such as clean rooms, equipment's (apheresis machine), blood banks and laboratory capacity to deal with needs of cell therapy will already be in place. More importantly, skilled and trained personnel to handle all these requirements from doctors, nurses, intensivists, allied health care givers, technicians and social workers needed for such a program would already be in place. To start without this level of expertise would pose some challenges, at every stage, in establishing a cell therapy program such as one needed for CAR-T cell manufacturing and administration.

Basic infrastructure and equipment requirements: One of the significant advantages of decentralized manufacturing system with a closed system such as the CliniMacs Prodigy system is the lack of requirement of a very high end GMP facility, which is not only expensive to set up but even more expensive to maintain and will not be viable unless there is a significant workload to justify the cost. A CliniMacs Prodigy can be housed in a Class 100,000 facility which is the equivalent of a clean laboratory room without any additional expenses for maintaining this space. It would be preferable that this clean room is air conditioned and closed to the external environment with restricted access and movement. However, the initial cost of the machine along with a multi-parameter flow cytometer would be required to as basic equipment's. Funding for all elements should be secured prior to attempting to start this, either with institutional support to establish a cell therapy program or via comprehensive grants to cover a fairly long incubation period from setting up to regulatory approvals for clinical applications.

Regulatory approvals: It is important to work with regulators within one's country to understand the requirements and comply with it appropriately. In many L/UMICs, the regulators would themselves

be in the process of learning through the process and requirements of cell therapies and hence is likely to take a longer time. It is important to keep regular communications open and where required work with them to take this forward.

Our experience in attempting to establish a decentralized CAR-T cell manufacturing program:

Our department has one of the largest allo HCT programs in India. Our program started in 1986 and we currently do around 250 allo HCTs / year. We had a Class 10,000 facility for cell processing, and we already had expertise with ex-vivo stem cell graft manipulations. We had to acquire a CliniMacs Prodigy system and high end multiparameter to complement the already available equipment for our CAR-T cell program, this was partly funded by grants and institutional support to help set up an advanced cell therapy program. Initial assistance to set up the equipment and certified training was provided by Miltenyi-Biotec along with training in setting up the dedicated flowcytometer for this program. Equipment and expertise with handling and processing real time polymerase chain reaction (RQ-PCR) is critical for the testing the final product before it can be released for clinical use.

Approval process: After getting regulatory approvals from Institutional Review Board (IRB) and an Institutional Biosafety Committee (IBSC) we had to approach central government agencies to import the vectors and for pre-clinical work and validation manufacturing runs. For this we had to submit a request to the 'Review Committee on Genetic Manipulation' (RCGM), Department of Biotechnology, India with our IRB and IBSC approvals. It is only after getting RCGM approval can we get a license to import any vector or genetically manipulated cell product. After we got this approval, we could import the vector and start our pre-clinical validation runs.

Pre-clinical validation manufacturing runs: We initiated our preclinical validation runs as part of an ongoing research project and process to get regulatory body approval for prospective clinical trials. Two (unprimed) acute lymphoblastic leukemia patients who were in remission and one (primed) healthy volunteer donor were enrolled and underwent apheresis after getting written and informed consent. The CliniMACS Prodigy, an automated system for manufacturing CAR-T cells was used, using a T cell transduction protocol with a single-use tubing set (TS520). This platform allows magnetic microbeads (CD4 and CD8) based cell separation process followed by activation using a proprietary polymeric nano-matrix with CD3/CD28 antibodies. Cells were transduced with anti CD19 lentiviral vector (LTG1563; CD19 CAR construct with 4-1BB co-stimulatory domain and TNFRSF19 transmembrane domain) with a multiplicity of infection (MOI) of 16 and further expanded under stable culture conditions with automated media exchange for a period varying from 9 to 12 days (6). The steps are summarized in Figure 1 and the process is completely closed and automated with skilled and trained personnel to handle trouble shooting at critical stages.

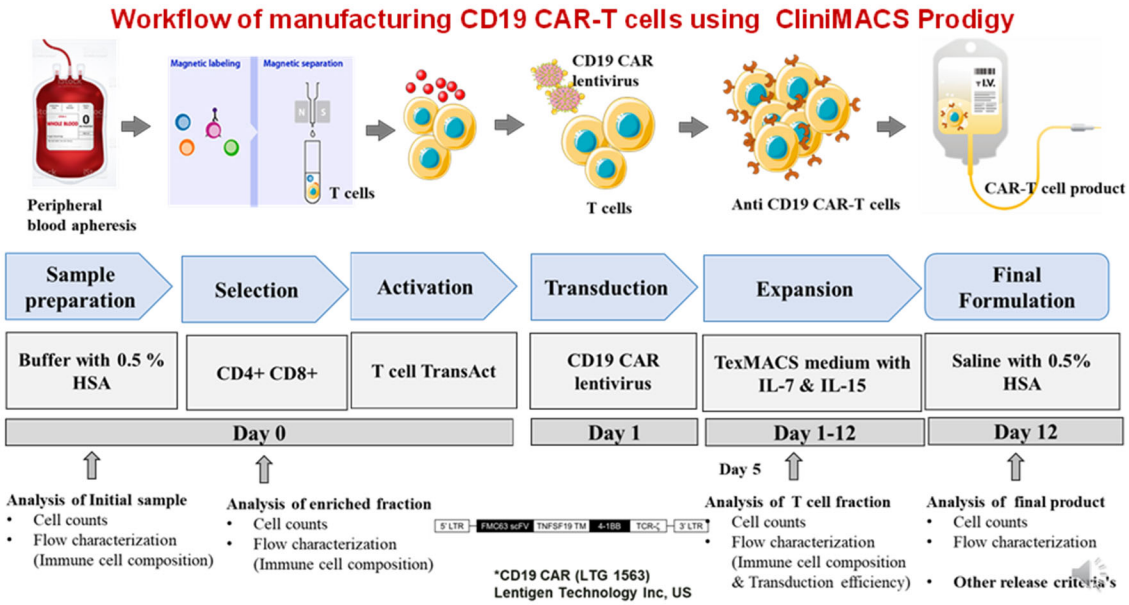


Figure 1: Overview of steps for manufacturing CAR-T cells using CliniMACS prodigy

CAR-T Release Criteria Assays: Aliquot of the culture from final product day 9/12 was used to evaluate the release criteria assays. Release criteria tests were performed based on an FDA Center for Biologics and Evaluation of Research mandated schedule. Aliquots of media from the T cell cultures was plated on bacterial and fungal growth media for sterility tests; mycoplasma detection was conducted on media aliquots using the PCR method and endotoxin levels was determined by LAL method. DNA was isolated from CAR-T cells and the vector copy number was determined by qPCR using GAG DNA sequence and replication competent lentivirus evaluated using VSVG PCR (Lentigen Corporation, Gaithersburg, Maryland, USA). The TaqMan based qPCR assays were caried out on an ABI 7500 fast real time PCR detection system (Applied Biosystems, Massachusetts, USA).

The pass criteria were defined as follows (7):

- Trypan Blue/ Flow cytometry 7AAD Viability: ≥70%
- Percentage CD3+ of CD45+ viable cells: ≥90%
- Percentage CD19+ of viable cells: ≤3%
- Transduction efficiency (percentage CAR+ of viable CD3+ cells): ≥15%
- Endotoxin: <5EU/ml
- Sterility: No bacterial and fungal growth
- Mycoplasma PCR-negative
- Replication-competent lentivirus (RCL): PCR-Negative
- Vector copy number ≤ 5 / CAR-T cell

Characterization of CAR-T cells: a detailed cellular phenotype of the expanded cells was done on the final product. The final product was predominantly T cells with CD3+ cells (97% to 99%) and CD3+ CD56- T cells (95.3 to 99.7%). The median transduction efficiency was 48.8 % (range 46.7% to 66 %) with the median viability of 97%. The average vector copy number determined by QPCR was 2.2 ± 0.4 per CAR-T cell and the replication competent lentivirus (VSVG) was not detected in any of the CAR-T products which further confers the safety and efficacy of our CAR-T cell manufacturing process using CliniMACS prodigy. In addition, the sterility assessment with mycoplasma PCR and bacterial growth was negative in the CAR-T products along with the endotoxin level (<0.05EU/ml).

The lymphocyte subset characterization showed an increase in central memory T cells compared to other phenotypes. Although effector memory T cells were known to have higher cytotoxic activity, it was reported that the central memory cells T cells have a longer persistence after adoptive transfer and can differentiate into effector cells and support anti-tumor activity in vivo (8). Table 1 summarizes the data generated in our pre-validation clinical runs.

Table 1: summary of results of pre-clinical validation runs

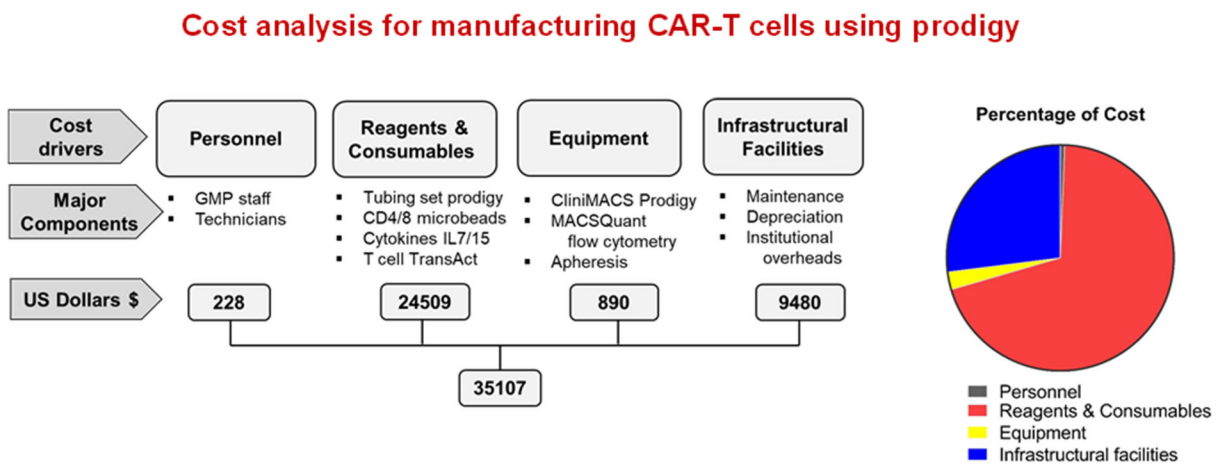
No	Details/ Assay	Method	Results		
			Validation Run 1	Validation Run 2	Validation Run 3
1	Donor	NA	ALL under remission (unprimed)	ALL under remission (unprimed)	Healthy donor (primed)*
2	No of days	NA	12	9	9
3	Viability	▪ 7AAD Staining ▪ Trypan Blue Staining	▪ 99 % ▪ 98 %	▪ 97 % ▪ 98 %	▪ 96 % ▪ 97 %
4	Transduction Efficiency	Flow Cytometry - CAR reagent	46.7 %	66 %	48.8 %
5	Product Phenotype at harvest	Flow Cytometry ▪ CD3+ cells ▪ CD3+ CD56- T cells (viable CD45+ cells)	▪ 99 % ▪ 95.3 %	▪ 97 % ▪ 97 %	▪ 99.4 % ▪ 99.5 %
6	Sterility	Microbial Culture	No Growth	No Growth	No Growth
7	Gram Stain	Gram's Method	Negative	Negative	Negative
8	Endotoxin	Endosafe PTS	< 0.05 EU/ml	< 0.05 EU/ml	< 0.05 EU/ml
9	Mycoplasma Contamination	PCR	Negative	Negative	Negative
10	Vector Copy Number	RQ-PCR	2.3 Copies per cell	2.6 Copies per cell	1.8 Copies per cell
11	VSVG	RQ-PCR	Undetectable	Undetectable	Undetectable

*Primed – mobilization with G-CSF

Costing

Our preclinical validation data highlights the feasibility, reproducibility, and safety of the process for use in the planned future clinical trials. Further, we have also evaluated the activity-based, bottom-up micro-costing method for costing the major cost drivers from the perspective of the health care providers (excluding the cost of acquisition of the vector). Based on this the cost per patient would be approximately US$ 35,100. We believe that this costing is not only feasible but will significantly improve access to CAR-T cell therapy for our patients in India. Figure 2 summarizes the costing structure of this manufacturing process at our center.

Figure 2:



As one can see from the break-up of the costs in figure 2 the major drivers of cost are the costs of the reagents and consumables. The cost of the vector which is not factored into this costing will also be an important variable. However, within this automated manufacturing process there is a significant amount of redundancy, and it should be possible to further optimize the use of some of the expensive reagents to further reduce the cost of manufacturing.

Conclusion: Through this process of pre-clinical validation, we have demonstrated the feasibility of decentralized manufacturing process of CAR-T cells at a cost that makes it a feasible option for patients in India. We have currently submitted our data to the regulators in India to start a Phase 1 clinical trial.

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