Establishing State-of-the-Art CAR T Cell Manufacturing Capabilities in a Not-for-Profit Organization in Sweden

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CCRM Nordic is a newly established not-for-profit organization dedicated to addressing bottlenecks in the translation and commercialization of advanced therapy medicinal products (ATMPs). Based in Gothenburg, Sweden, we support ATMP innovators across the Nordics and Europe. This abstract outlines our ongoing efforts to establish CAR T cell manufacturing workflows that meet the needs of Nordic ATMP developers. We are integrating state-of-the-art, automated, and closed systems into our manufacturing processes to enhance reproducibility while minimizing risks of human error and contamination. Our platforms include modular systems such as the Sepax™ C-Pro, Sefia™ S-2000, Xuri™ Cell Expansion System W25, and G-Rex® system, alongside the functionally integrated CliniMACS Prodigy® system. These systems enable us to meet the demands for scalability, flexibility, and compliance with Good Manufacturing Practice (GMP) during ATMP development. Furthermore, we are establishing quality control (QC) methods to measure various critical quality attributes (CQAs) of CAR T cell products, including safety, viability, quantity, purity, impurity, and potency. Additionally, we are assessing key immunological characteristics of the resulting CAR T cells, such as T cell memory, exhaustion, and CD4/CD8 ratio. These capabilities will provide us with a strong foundation for advancing the development, translation, and commercialization of cellular immunotherapies in the Nordics.

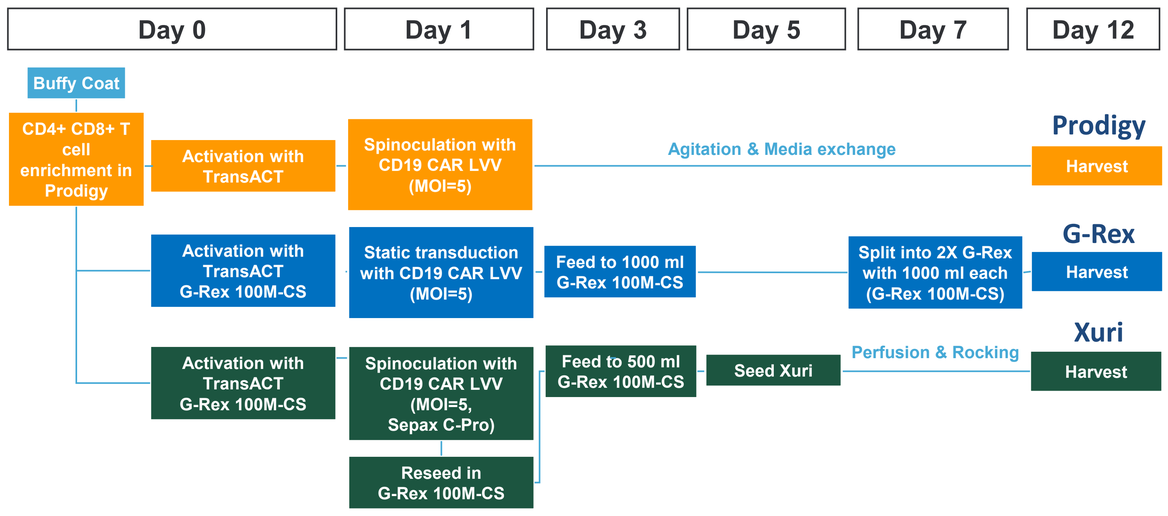

Figure 1. Overview of project workflow. Starting material was obtained from Sahlgrenska University Hospital and enriched in the CliniMACS Prodigy using CD4 and CD8 Microbeads. After seeding the CliniMACS Prodigy, the reapplication bag was disconnected, and remaining cells were used to seed G-Rex 100M-CS vessels. Seeding ranged between 50-100 x 10^6 cells per platform depending on the yield of recovered T cells. Data shown in this poster were derived from two individual donors on three platforms (Prodigy, G-Rex, and Xuri)

CQA Parameters	Attributes	Methods
Safety	Endotoxin	Recombinant Factor C
	Sterility	Bact/Alert or qPCR
	Mycoplasma	Digital PCR
	Residual Replication Competent Lentivirus (RCL)	Digital PCR
	Vector Copy Number (VCN)	Digital PCR
Viability	% Viable cells	Cell counter, Flow cytometry
Quantity	Total viable cell numbers	Cell counter
	Total CAR-T cell numbers	Flow cytometry
	Fold expansion (FE)	Calculated
Purity	% CD3+ cells (T cells)	Flow cytometry
	% CAR+ cells (transduction efficiency)	Flow cytometry
Impurity	% Other blood cells (B, NK, NKT, etc.)	Flow cytometry
	Residual microbeads	N/A
Potency and T cell immunological characteristics	T cell memory	Flow cytometry
	T cell exhaustion	Flow cytometry
	CD4/CD8 ratio	Flow cytometry
	Cytokine release	ELISA
	Cytotoxicity	Flow cytometry

Table 1. Critical quality attributes of CAR T cell products. We are setting up quality control methods to measure various critical quality attributes covering the safety, viability, quantity, purity, impurity, and potency parameters of the CAR T cell products.

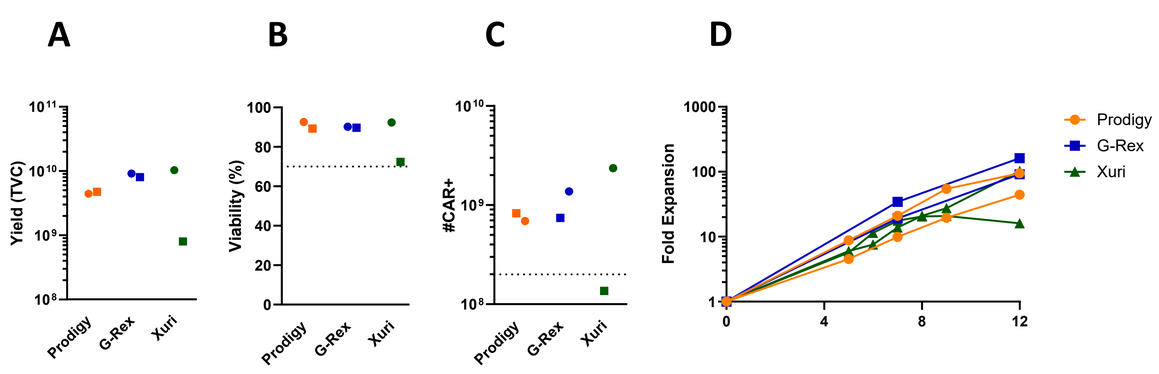

Figure 2. Viability and quantity of the CAR T cells produced on Prodigy, G-Rex, and Xuri platforms.
A. Total viable cells (TVC) at harvest on day 12. Circle: donor 1. Square: donor 2.
B. Percentages of viable cells at harvest on day 12. Dotted line shows a suggested acceptance criteria at 70% viability. Circle: donor 1. Square: donor 2.
C. Enumeration of viable CD19 CAR+ cells for all three platforms at harvest. Dotted line represents an adult CAR T cell dose based on 2E6 CAR T cells per kg for a 100 kg patient.
D. Fold expansion during CAR T cell manufacturing processes on all three platforms. Each line represents an individual process.

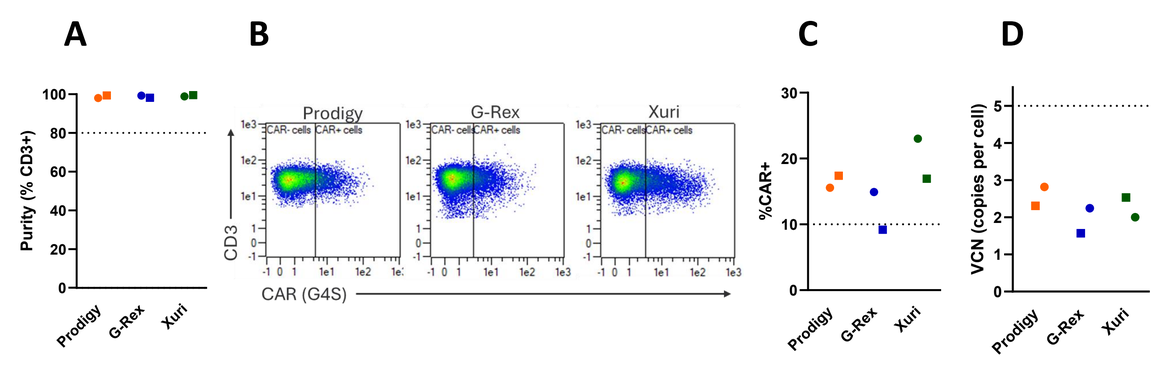

Figure 3. Purity and impurity of the CAR T cells produced on Prodigy, G-Rex, and Xuri platforms.
A. Percentages of CD3+ cells of all viable CD45+ cells at harvest on day 12. Dotted line shows a suggested acceptance criteria at 80% purity. Circle: donor 1. Square: donor 2.
B. Flow plots showing CAR expression among CD3+ cells at harvest on day 12 from all three platforms using an antibody targeting the G4S-linker.
C. Percentages of CD19 CAR+ cells of all CD3+ cells. Dotted line shows a suggested acceptance criteria at 10%. Circle: donor 1. Square: donor 2.
D. Vector copy number (VCN) per cells as determined by digital PCR on cells harvested on day 12. Dotted line shows a recommended threshold for VCN value. Circle: donor 1. Square: donor 2.

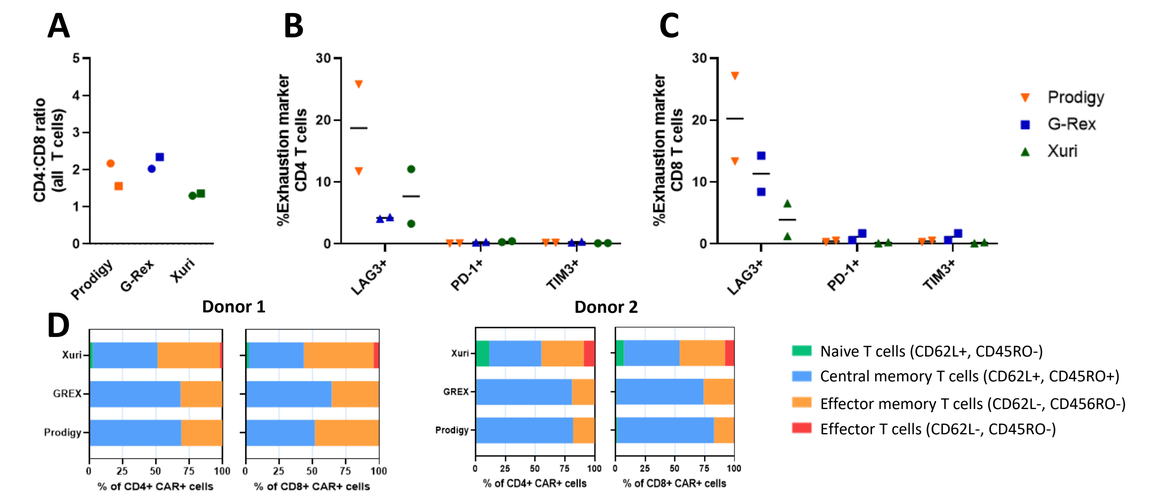

Figure 4. Immunological characteristics of the T cells produced on Prodigy, G-Rex, and Xuri platforms.
A. CD4:CD8 ratio of T cells from all three platforms at harvest on day 12. Circle: donor 1. Square: donor 2.
B. Expression of exhaustion markers LAG3, PD-1 and TIM3 among CD4 T cells at harvest.
C. Expression of exhaustion markers LAG3, PD-1 and TIM3 among CD8 T cells at harvest.
D. Bar graphs showing proportions of naive, central memory, effector memory, and effector T cell phenotypes at harvest from all three platforms derived from two individual donors.

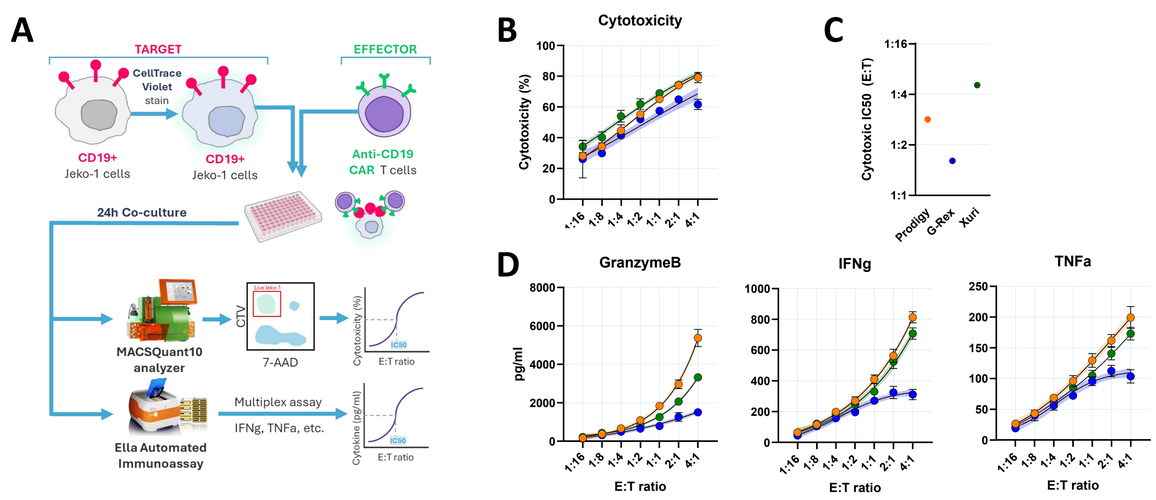

Figure 5. Potency of the CAR T cells produced on Prodigy, G-Rex, and Xuri platforms.
A. Experiment design of the cytotoxicity and cytokine release assays for CD19 CAR T cells.
B. Cytotoxicity of CAR T cells from all three platforms. Data represents the mean + SD of four technical replicates per E:T ratio. A Sigmoidal 4PL curve is fitted to the data (bottom restriction = 0, top restriction = 100).
C. Cytotoxicity IC50 expressed as E:T ratio.
D. Concentration of GranzymeB, IFNg, and TNFa in supernatants after co-culture of CAR T and CD19+ Jeko-1 cells.

In conclusion, we have:

- **Successfully established automated and closed CAR T cell manufacturing workflows using both modular systems (e.g., Sepax™ C-Pro, Sefia™ S-2000, Xuri™ W25, G-Rex®) and functionally integrated system, e.g., CliniMACS Prodigy®.**
- **Developed a full suite of Quality Control (QC) methods to measure the safety, viability, quantity, purity, impurity, and potency of CAR T cells.**
- **Evaluated key immunological characteristics of the resulting CAR T cells, including T cell memory, exhaustion, and CD4/CD8 ratio.**

Our scalable and flexible platforms can support the ongoing development and future commercialization of CAR T as well as other cellular immunotherapies in the Nordics.

Supported by VINNOVA · GU Ventures · AstraZeneca · cytiva · GETINGE · GoCo Health Innovation City · TaKaRa · BICO · VERIGRAFT · CCRM