Point-of-care manufacturing of FOLR1-directed CAR T cells for the treatment of ovarian cancer

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Introduction

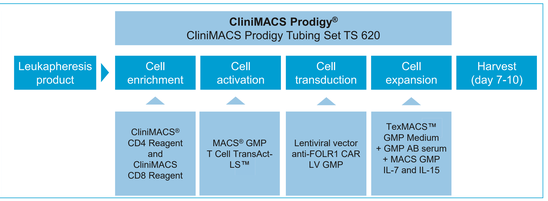
Treatment options for ovarian cancer patients are limited, and a high unmet clinical need remains for targeted and long-lasting, efficient drugs. Adoptive cell transfer therapies have made tremendous progress in the past years. Genetically engineered T cells expressing a chimeric antigen receptor (CAR) are promising drugs that can be directed towards a defined target and have shown efficient, as well as persisting anti-tumor responses in different indications. Recently, we have reported the preclinical evaluation of a novel FOLR1-targeting CAR directed against ovarian cancer and potentially other FOLR1-expressing tumors. T cell therapy development is advancing swiftly, but producing a functional and robust cell product in the necessary quantities remains challenging. Available processes for the preparation of CAR T cells are laborious and include numerous manual handling steps, which increase the risk of errors and may impose safety risks. There is a need to further develop reliable and automated manufacturing processes to produce high-quality cellular products for immunotherapy. This study aimed to develop an automated, closed, and good manufacturing practice (GMP)-compliant process for FOLR1-specific CAR T cells using the CliniMACS Prodigy® T Cell Transduction – Large Scale (TCT-LS) process.

Methods

1 Experimental Setup and workflow

By using the CliniMACS Prodigy® Platform we could establish an automated and GMP-compliant process in a closed system, which meets the increasing regulatory requirements for cell-based therapeutics and, additionally, reduces hands-on time. We optimized culture conditions to reduce the process time to 7 days. However, cultivation time can be extended to yield even higher CAR T cell numbers. Starting from a cryopreserved leukapheresis, $CD4^+$ and $CD8^+$ cells were enriched using CliniMACS® Reagents. Following the enrichment, T cells were activated with MACS® GMP T Cell TransAct™-LS. The next day, cells were transduced with the GMP-grade lentiviral vector carrying the FOLR1-directed CAR construct. Cells were then cultivated in TexMACS™ GMP Medium supplemented with human AB serum, IL-7, and IL-15 cytokines. Cells were harvested between day 7 to day 10. The automated CliniMACS Prodigy® Platform enables point-of-care (POC) manufacturing of FOLR1 directed CAR T cells (Figure 1).

Figure 1



Cell samples were taken at several timepoints in the process for quality control purposes. Cells were analysed via flow cytometry using the MACSQuant® Analyzer. For that purpose, several antibody panels were designed to analyse the cellular composition of the cell product, the transduction efficiency of the lentiviral vector, the T cell phenotype, and activation markers as well as several other functional T cell attributes (Table 1). Together, these panels enable a comprehensive characterization of the T cell population during the entire process.

Table 1

Flow cytometric readout of In-Process-Controls

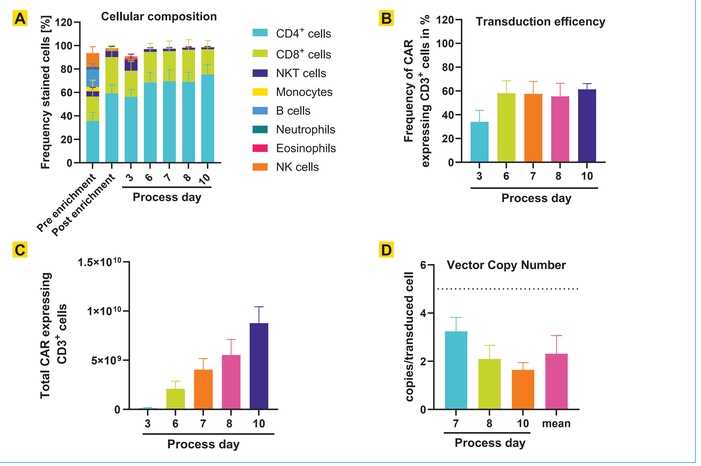
Channel	Fluorochrome	Cellular Composition	Transduction efficiency	Differentiation	Proliferative ability	Exhaustion	Activation	Staining Control
V1	VioBlue	CD45	CD45	CD45RA	CD27	CD223	CD69	-
V2	VioGreen	CD4	CD4	CD4	CD4	CD4	CD4	CD4
B1	FITC	CD3	CD3	CD3	CD3	CD3	CD3	CD3
B2	PE	CD16 / CD56	CAR DR + Anti-Biotin	CAR DR + Anti-Biotin	CAR DR + Anti-Biotin	CAR DR + Anti-Biotin	CAR DR + Anti-Biotin	CAR DR + Anti-Biotin
B3		7-AAD	7-AAD	7-AAD	7-AAD	7-AAD	7-AAD	7-AAD
B4	PE-Vio770	CD19	-	CD62L	CD279	CD279	CD25	-
R1	APC	CD14	CD14	CD45RO	CD127	CD366	CD137	-
R2	APC-Vio770	CD8	CD8	CD8	CD8	CD8	CD8	CD8

Results

1 Automated manufacturing with the CliniMACS Prodigy® yields high CAR T cell numbers after 7 days

Cell samples were stained with several antibody panels, the first focussed on the cellular composition (Figure 2A). The TCT-LS on the CliniMACS Prodigy® yields over 95 % T cells after 6 days. By directly staining the CAR construct we can infer the transduction efficiency of the lentiviral transduction with the anti-FOLR1 CAR vector (Figure 2B). We achieved a stable transduction efficiency of ~60 % and reached 4 x 10^9 CAR T cells after 7 days and over 8 x 10^9 CAR T cells after 10 days (Figure 2C). Vector copy number (VCN) analysis shows a consistent VCN lower than five in all processes (Figure 2D). Cells harvested on day 7 or day 10 show no significant difference in their cellular composition or transduction efficiency and the VCN is always below the threshold.

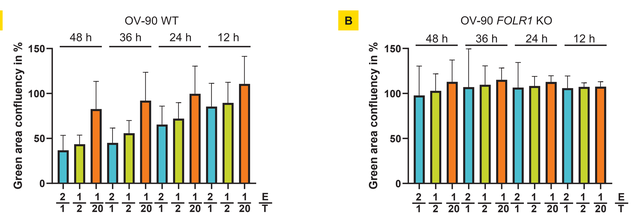
Figure 2



2 FOLR1-directed CAR T cells show target-specific cytotoxic activity against ovarian cancer cells *in vitro*

To characterize the target-specific cytotoxic activity of FOLR1-directed CAR T cells, they were co-cultured with ovarian cancer cells that either expressed FOLR1 (OV-90 WT; Figure 3A) or were FOLR1-deficient (OV-90 *FOLR1* KO; Figure 3B) and co-expressed GFP. Cells were co-cultured in different ratios and the change in GFP signal was detected using the Incucyte® Live-Cell Analysis System. The change of green area confluency over time (Figure 3) shows a target-specific and dose-dependent toxicity of FOLR1-directed CAR T cells. We could not observe a significant difference in cytolytic activity between the CAR T cell products harvested at different time points

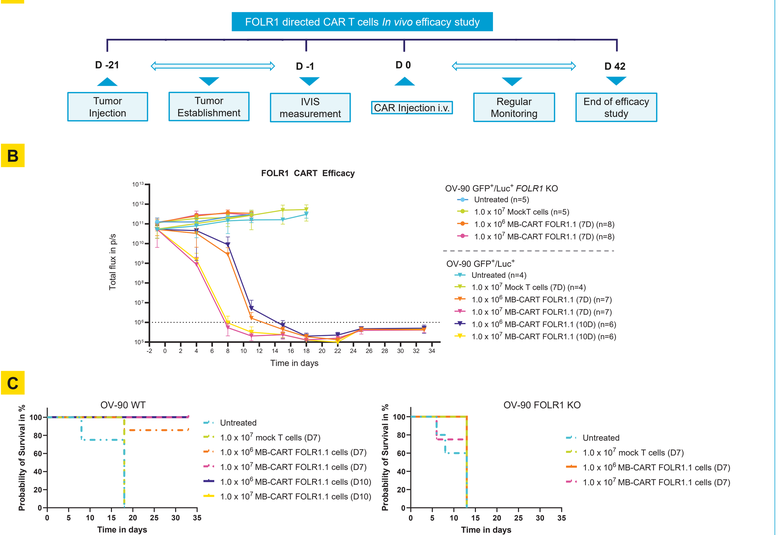
Figure 3



3 FOLR1-directed CAR T cells are functional and specific *in vivo*

The anti-tumor efficacy of FOLR1-directed CAR T cells from 7 day and 10 day manufacturing processes were compared using a murine ovarian cancer xenograft model. NSG mice were injected s.c. with 8.0 x 10^6 OV-90 GFP^+/Luc^+ tumor cells or with 8.0 x 10^6 OV-90 *FOLR1* KO GFP^+/Luc^+ tumor cells. Single doses of 1.0 x 10^6 and 1.0 x 10^7 CAR T cells/mouse were used in order to determine the therapeutic effect on CAR T cells *in vivo* (Figure 4A).
After 21 days of tumor establishment a single dose of CAR T cells was injected i.v.. Two control groups were included: An untreated group and a Mock T cell group at a dose of 1.0 x 10^7 T cells. T cells were generated using cellular material from the same donor.
The mice were closely monitored in regards to behavior, fur, activity, eyes, bodyweight, and tumor burden. The tumor burden of the mice was tracked over the time of the study and is plotted in Figure 4 B. The mice injected with higher doses of 1.0 x 10^7 CAR T cells were tumor free after 8 days and the mice treated with the lower doses after 14 days.
At the same time neither the mice with OV90 *FOLR1* KO tumor cells, nor any of the control groups exhibited loss of tumor. This suggests a target-specific activity of the FOLR1-directed CAR T cells. This is also reflected in the time it takes each group to reach the animal study endpoint, as shown in Figure 4 C.

Figure 4



Conclusion

In conclusion:

- We established a robust, rapid, and flexible process with high yields on the CliniMACS Prodigy® platform.
- This process is well suited for automated and decentralized POC manufacturing of anti-FOLR1 CAR T cells.
- This Process is also well established for manufacturing of other CAR T cell products.
- The anti-FOLR1 CAR T cells exhibit target-specific and dose-dependent cytolytic activity *in vitro*.
- In an *in vivo* xenograft model anti-FOLR1 CAR T cells showed efficient and specific tumor erradication.