Automated Point-of-Care Manufacturing of ΔNPM1 TCR-Engineered T Cells: a novel approach for treatment of Acute Myeloid Leukemia

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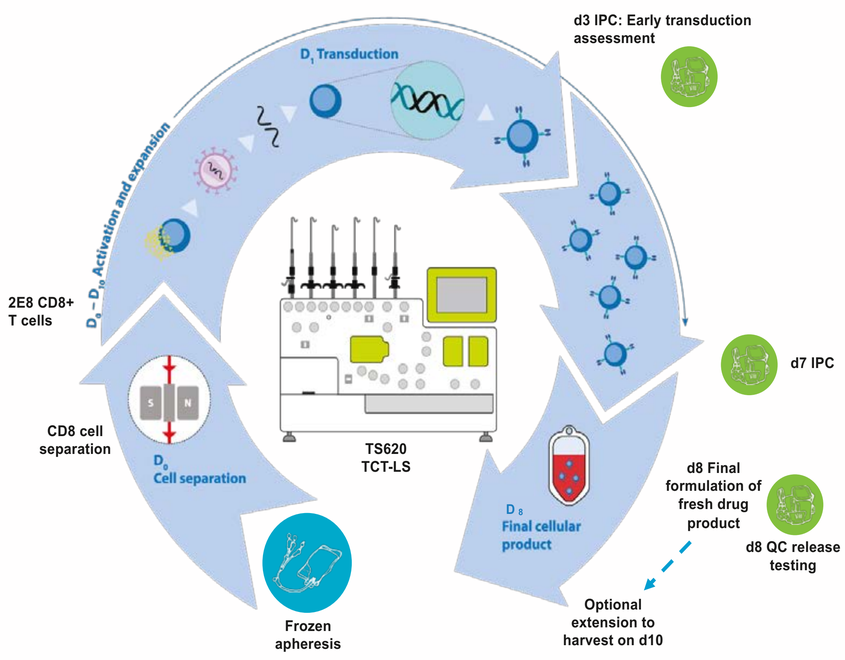
Introduction

Acute myeloid leukemia (AML) is a heterogeneous and highly complex malignancy with poor prognosis on relapse despite many recent advances in chemotherapy and small molecule drug protocols. A recently discovered T cell receptor (TCR) targeting a common mutant nucleophosmin 1 (ΔNPM1) neoepitope presents an attractive opportunity to design a novel cancer antigen targeted cellular therapy. We have recently developed a rapid (8-day), closed and GMP-compliant, automated TCR-T cell manufacturing process using the CliniMACS Prodigy® to enable the generation of a MB-dNPM1-TCR.1 drug product in a point-of-care setting.

Methods

1 Manufacturing of ΔNPM1-specific TCR modified CD8+ T cells

Fig. 1

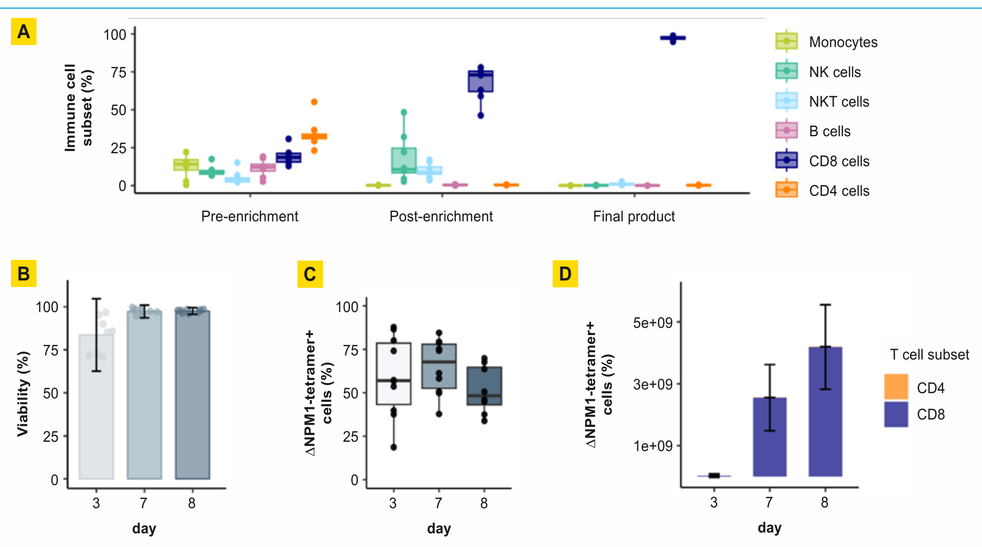


Starting from cryopreserved leukapheresis, 2E8 CD8-positive T cells from healthy donors were magnetically enriched and taken into culture in a CliniMACS Tubing Set 620 in TexMACS GMP medium supplemented with 12.5µg/mL MACS GMP Recombinant Human IL-7 and IL-15. The T cells were immediately activated with MACS GMP TransAct, transduced one day later with lentiviral vector expressing an HLA-A*02:01 restricted recombinant ΔNPM1-specific TCR and were expanded prior to formulation with CliniMACS Formulation Solution on day 8 of the process. In process controls (IPC) were collected on days 3, 7 and 8 and analyzed by flow cytometry using the MACSQuant Analyzer.

Results

2 Automated manufacturing reproducibly yields appropriate doses of ΔNPM1 TCR-engineered T cells

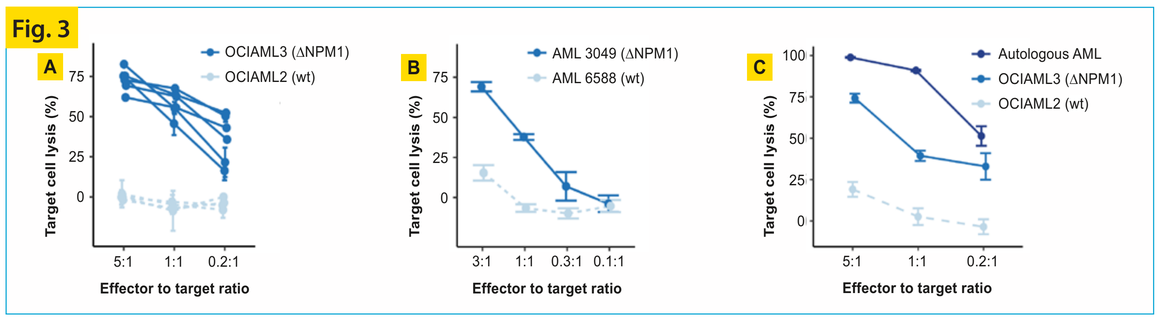
Fig. 2



ΔNPM1-engineered T cells were manufactured from CD8 cells of healthy donors using the T Cell Transduction – Large Scale (TCT-LS) process (n = 10; 10 runs) (A) Flow cytometric analysis of the cellular composition pre- and post-enrichment and of the final product. The viability after 7-AAD staining (B), the transduction efficiency (C) and the yield of ΔNPM1+ CD8 and CD4 cells (D) were analyzed by flow cytometry analysis on days 3, 7, and 8.

3 ΔNPM1 TCR-engineered T cells are potent and kill primary AML cells

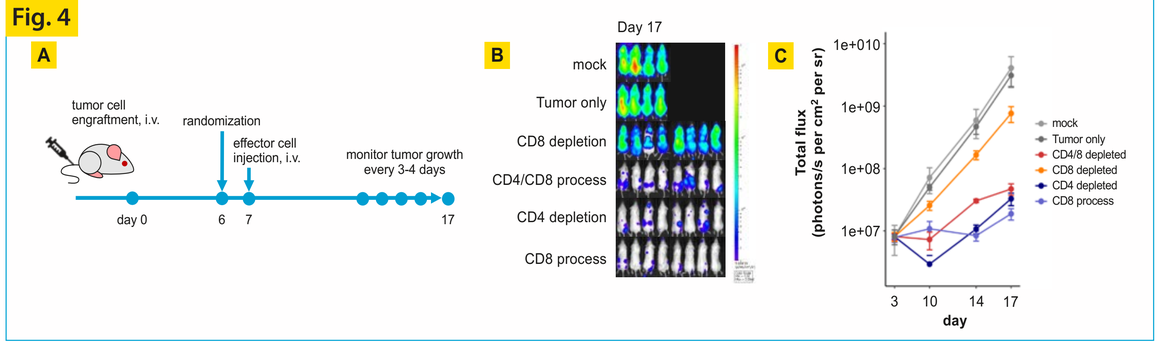
Fig. 3



Following harvest after TCT-LS manufacturing, cytotoxicity was assessed by co-culturing ΔNPM1-engineered CD8 T cells at the indicated effector-to-target cell ratios (A-C; mean ± SEM). Target cell lines used were (A) ΔNPM1- (OCI-AML3) or wtNPM1 (OCI-AML2)-expressing cells and (B) primary AML samples. In an additional small-scale experiment (C), we also verified that the planned approach yielded functional and potent ΔNPM1-specific TCR-T cells when starting with frozen material from AML patients. Potency of patient-derived T cells was assessed by co-culture with ΔNPM1-positive (OCI-AML3), wtNPM1 (OCI-AML2), or autologous AML cells. Target cell lysis was determined by flow after 24 h (mean ± SEM).

4 ΔNPM1 TCR-engineered T cells show efficacy *in vivo*

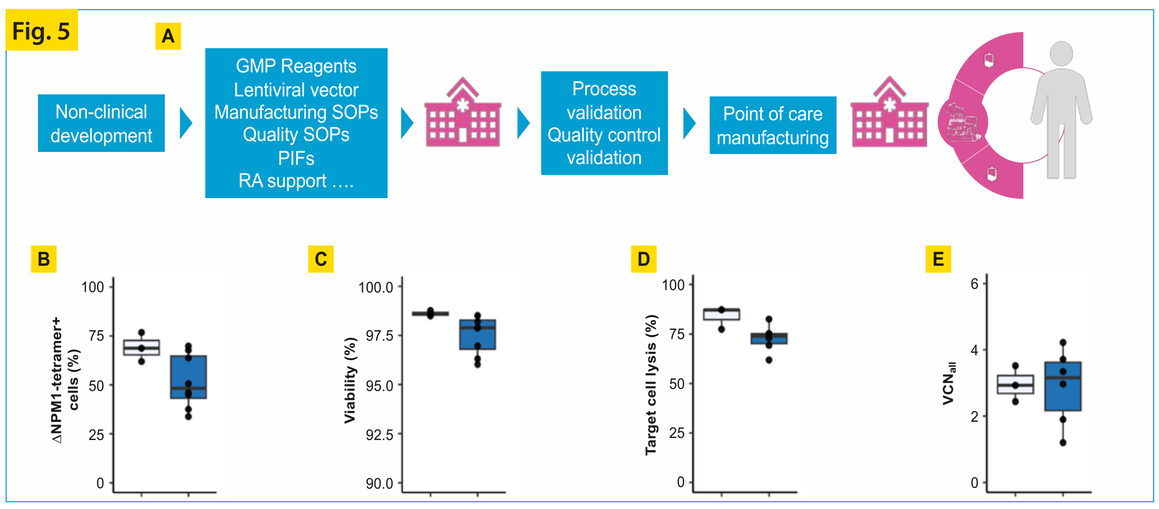
Fig. 4



An AML xenograft model was used to assess and compare the cytolytic potential of ΔNPM1 TCR-engineered T cells manufactured in a CD8 TCT-LS process vs. a conventional CD4/CD8 process including controls depleted of either CD4 or CD8 T cells. (A) Graphical scheme of the *in vivo* experiment workflow. (B and C) NSG mice were injected i.v. with 1E6 OCI-AML3Luc, randomized after 6 days based on tumor engraftment, and injected i.v. at day 7 with either mock (untransduced) T cells or 5E6 ΔNPM1 TCR-engineered cells derived from different manufacturing processes including or excluding CD8 and CD4 T cells. Tumor burden (mean ± SEM) was measured over time using an *in vivo* tumor imaging system (IVIS).

5 Successful transfer of manufacturing "blueprints" to the point of care manufacturing site

Fig. 5



After optimization of the manufacturing process, the protocols and associated quality control assays, so-called "blueprints", were transferred to the Leiden University Hospital manufacturing site for validation of the processes for clinical manufacturing at the point-of-care. Robustness and reproducibility of manufacturing were confirmed in a side-by-side comparison of the development and manufacturing sites (n = 10 Miltenyi Biotec vs. n = 3 LUMC). As expected, the robust manufacturing process demonstrated comparable performance in all relevant IPC and QC assays yielding >5E9 CD8+ΔNPM1+ T cells with >98% viability. (A) transduction efficiency, (B) viability, and (C) cytotoxicity as well as (D) numbers of integrated lentiviral genomes were assessed for ΔNPM1 TCR-engineered CD8 T cells manufactured at two different sites by different teams on varying devices. In all boxplots, median is shown by central line, boxes represent the IQR, and whiskers show distribution up to 1.5 times the IQR from the box.

Conclusions

We show that across independent donors, devices, manufacturing sites and operators, this process consistently yielded therapeutic doses of potent TCR-engineered CD8 T cells while maintaining the product's high viability, purity and favorable early memory phenotype, which is known to support *in vivo* persistence and efficacy. This novel state-of-the-art manufacturing process has been implemented in a newly opened phase I/II clinical trial (NCT06424340) to treat patients with relapsed or refractory NPM1-mutated AML.

- Robust manufacturing of a novel AML neoantigen-specific, TCR-modified CD8+ T cell product established
- Efficient transfer of manufacturing "blueprints" to the point-of-care
- Comparability of the manufacturing process has been demonstrated
- A phase I/II clinical trial (NCT06424340) to treat patients with relapsed or refractory NPM1-mutated AML is open for recruitment at the LUMC.

Yonezawa Ogusuku IE, *et al.*. Automated manufacture of ΔNPM1 TCR-engineered T cells for AML therapy. Mol Ther Methods Clin Dev. 2024;32:101224.

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