# Development of iPSC-derived cytotoxic CD8+ T cells as the basis for innovative off-the-shelf cancer immunotherapies

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T cell immunotherapy with chimeric antigen receptors (CARs) has evolved as part of the standard of care for several hematological malignancies and has transformed the oncology landscape. CAR-T cell products are traditionally generated from autologous, patient-derived αβT cells engineered with a CAR for tumor cell targeting. Generating autologous T cell products for each patient is associated with manufacturing and logistical complexity and high product costs. Furthermore, manufacturing fails in a significant number of cases due to the poor quality and quantity of blood-derived T cells, and restrictions apply in terms of throughput to produce autologous cell products. Consequently, many patients are left without this treatment option, underscoring the need to develop strategies for off-the-shelf T cell products. Immune cells derived from induced pluripotent stem cells (iPSCs) offer the opportunity to manufacture allogeneic T cell products with consistently high quality and scalable quantities. Use of iPSCs as a starting material makes it easier to introduce several genetic modifications (e.g. to enhance both, cell persistence and tumor infiltration) addressing tumor resistance mechanisms for both liquid and solid tumors.

Using a validated GMP iPSC line modified with an NY-ESO-1-specific T cell receptor (TCR) knock-in, we have established a feeder-free differentiation protocol that enables robust production of iPSC-derived αβT cells (iαβT). Flow cytometry and single cell transcriptome analysis ensured a stringent monitoring of all process stages. To demonstrate functional activity of our iαβT, we performed cytotoxicity and cytokine release assays against tumor cell lines presenting the NY-ESO-1 antigen.

Successful knock-in of the NY-ESO-1-TCR-transgene cassette into the iPSC line was confirmed by marker-gene expression. Hematopoietic progenitor cells were induced from knock-in-enriched iPSCs and differentiated into iαβT. During the differentiation process, the T cell markers CD45, CD5 and CD7 were displayed, and cells started to express the TCR. After activation of iαβT, the fraction of NY-ESO-1-TCR-positive cells increased to over 95%. Importantly, iαβT expressed CD8α and CD8β which is crucial for the function of cytotoxic T cells. Transcriptome analysis validated the efficient differentiation from pluripotent cells towards cells with a T cell-specific gene expression profile. Co-culture experiments with NY-ESO-1 antigen presenting tumor cell lines confirmed cytotoxic activity of iαβT and their potential to release cytokines.

Our scalable iαβT differentiation process enables us to generate CD8+ T cells that secrete cytokines and show cytotoxic activity, indicating their potential as a promising cell source for TCR-T or CAR-T cancer immunotherapies.

## Evotec develops fully scalable industrialized GMP manufacturing processes

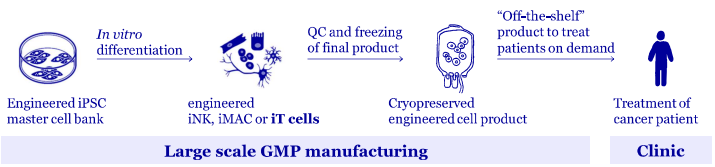


**Figure 1:** Schematic depiction of Evotec's fully scalable GMP manufacturing process.

## Evotec's comprehensive portfolio of iPSC-based cell therapy assets for oncology

| Program | Partner | Protocol development | Pre-clinical research | Pre-clinical development | IND / Phase I |
|---|---|---|---|---|---|
| iNK | | | | | |
| iMAC | | | | | |
| γδ iT | *Pharma partner* | *Undisclosed* | | | |
| αβ iT | | | | | |

**Figure 2:** Evotec's iPSC-based cell therapy pipeline for oncology. Building up a comprehensive portfolio of various iPSC-derived cell types to treat cancer including natural killer cells (iNK), macrophages (iMAC) and αβ and γδ T cells (iT).

Each immune cell type can deliver multiple differentiated cell therapy products.

## Gene-editing of iPSC with T cell receptor genes

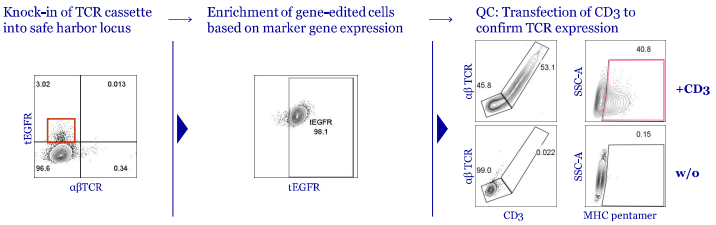


**Figure 3:** An NY-ESO-1-TCR cassette including the marker gene truncated EGFR (tEGFR) was knocked-in a GMP iPSC line and marker gene expression was analyzed before and after enrichment. TCR protein expression was confirmed on iPSC after electroporation of CD3.

## Differentiation of T cells from iPSC with TCR knock-in

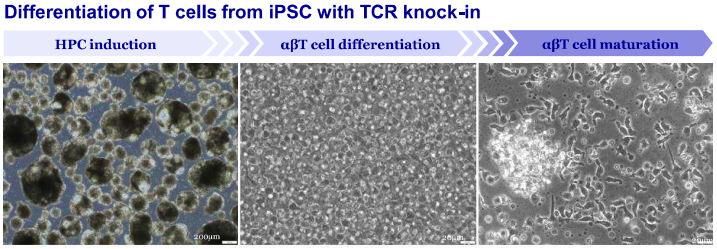


**Figure 4:** Morphology of cells during differentiation process. Evotec has developed a 3D scalable, feeder-free induction process of Hematopoietic Progenitor Cells (HPCs). After enrichment of CD34-positive cells, T cell differentiation is initiated by activation of Notch signaling in a feeder-free process that will be further developed based on Evotec's know-how with other immune cell types.

## Phenotypic analysis of iPSC and induced Hematopoietic Progenitor Cells

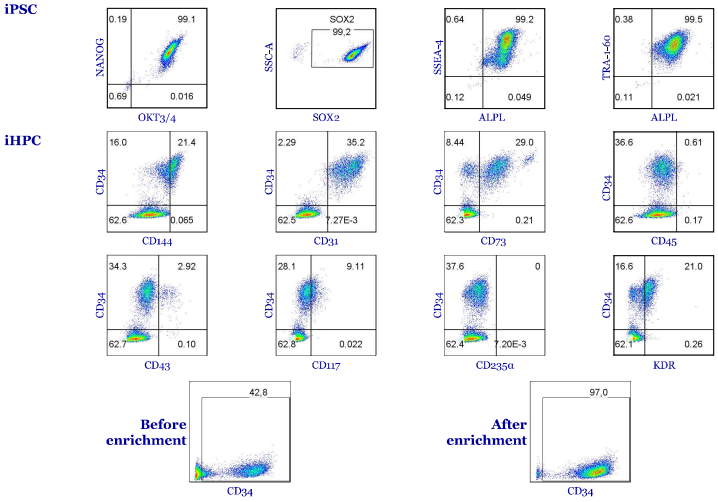


**Figure 5:** Quality control of iPSC line and induced Hematopoietic Progenitor Cells (iHPC) by flow cytometry. iPSC showed a high expression of pluripotency markers. 14 days after induction, a population of CD34-positive iHPC could be detected. HPCs were enriched by MACS based on CD34 expression.

## Differentiation of iαβT cells expressing TCRs and CD8αβ heterodimers

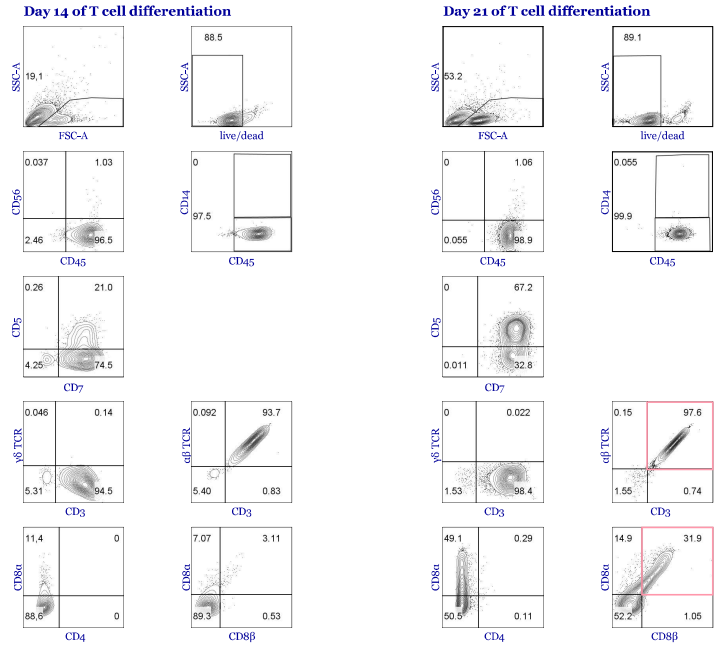


**Figure 6:** Phenotypic analysis of iPSC-derived αβT cells by flow cytometry. Cells were analyzed at different time points during the differentiation process (day 14 and 21 of T cell differentiation are shown). Cells expressed the hematopoietic lineage marker CD45. Absence of NK cells (CD56) and monocytes/macrophages (CD14) was confirmed. The expression of T cell surface proteins CD5 and CD7 as well as αβTCRs and CD8 increased over time. No expression of γδTCRs was detected.

## iαβT cells show functional characteristics of cytotoxic T cells

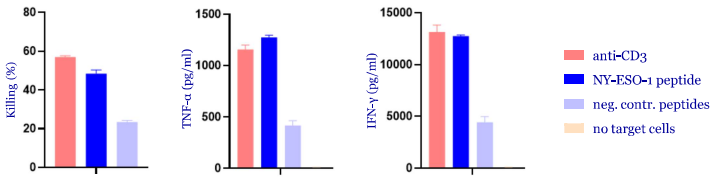


**Figure 7:** Functional characterization of iαβT cell. iαβT cells were cocultured with a tumor cell line loaded with the NY-ESO-1 peptide or negative control peptides. Anti-CD3 antibodies were used as a positive control. Cytotoxic activity and the release of cytokines (TNF-α and IFN-γ) was analyzed.

## Summary

- Evotec is developing a scalable GMP-compatible iαβT cell differentiation process
- iPSC-derived αβT cells express T cell markers including αβTCRs and CD8αβ heterodimers
- iαβT cells show specific cytotoxic activity and secrete cytokines like TNF-α and IFN-γ
- Evotec's iαβT cells are a promising cell source for TCR-T or CAR-T cancer immunotherapies