Gene edited iPSC-derived macrophages (iMACs) show increased phagocytosis in pre-clinical tumor models

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A critical checkpoint in the tumor microenvironment (TME) of solid tumors is the CD47-SIRPα axis that acts as a "don't eat me" signal and prevents macrophages from phagocytosing CD47-expressing tumors. Several agents aiming to block this checkpoint have entered early clinical trials in recent years, including anti-CD47 (αCD47) and anti-SIRPα monoclonal antibodies. Combining these checkpoint inhibitors with adoptively transferred immune cells targeting tumors could potentially increase clinical efficacy. Recently, autologous macrophages have gained increasing attention for cancer treatment due to their potential to infiltrate into the immunosuppressive tumor microenvironment (TME) and their unique immunomodulatory characteristics. While early clinical results are encouraging, producing autologous macrophage cell products for clinical and commercial applications is challenging due to limited patient material, intricate genetic manipulations, and manufacturing complexity. Induced pluripotent stem cell (iPSC)-derived macrophages (iMACs) offer the opportunity to overcome many of these challenges and allow the production of allogenic cell products with consistent high quality. Additionally, the use of iPSCs as starting material enables the straightforward introduction of genetic modifications to further optimize the iMAC cell product and limit the need for combination therapies. A promising modification is the knock-out (KO) of SIRPα in iMACs to generate a potent cell therapy product resistant to phagocytosis inhibition by CD47-expressing tumor cells.

We introduced a KO of the SIRPα gene into a fully characterized GMP iPSC line and differentiated these gene edited iPSCs to iMACs with Evotec's 3D differentiation protocol. SIRPα–KO iMACs were loaded with tumor-targeting monoclonal antibodies before co-culture with CD47-expressing tumor cells and evaluated for their antibody-dependent cellular phagocytosis (ADCP) capacity in comparison to antibody-loaded wildtype (WT) iMACs.

Evotec develops industrialized GMP manufacturing processes with integrated iPSC gene editing platform for precise gene editing

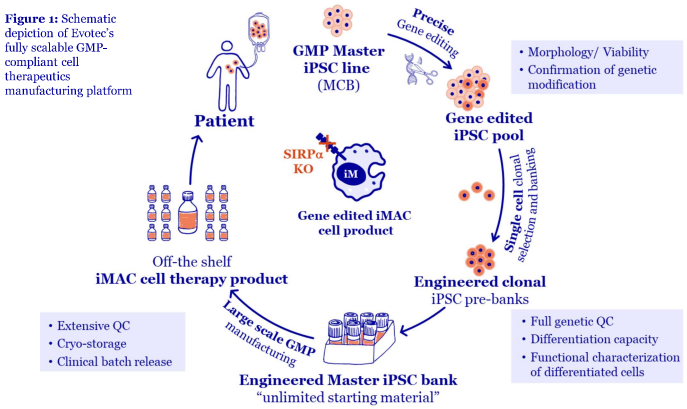

Figure 1: Schematic depiction of Evotec's fully scalable GMP-compliant cell therapeutics manufacturing platform

Evotec's robust and scalable 3D iMAC differentiation process

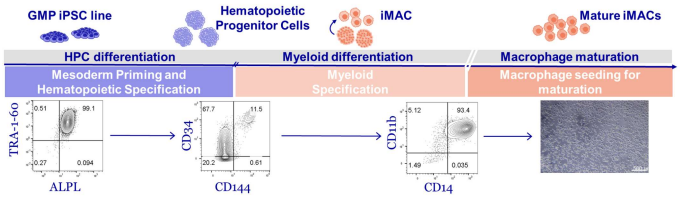

Figure 2: Scheme depicting the outline of a differentiation towards iMACs. TRA-1-60$^+$/ALPL$^+$ iPSC are used for cluster formation and through mesoderm priming and hematopoietic specification CD34$^+$ hematopoietic progenitor cells are generated. Via further myeloid specification CD11b$^+$/CD14$^+$ iMACs are generated, and the cells can be further matured.

SIRPα KO iPSCs show the expected WT iPSC morphology and phenotype and can be efficiently differentiated into SIRPα-deficient iMACs

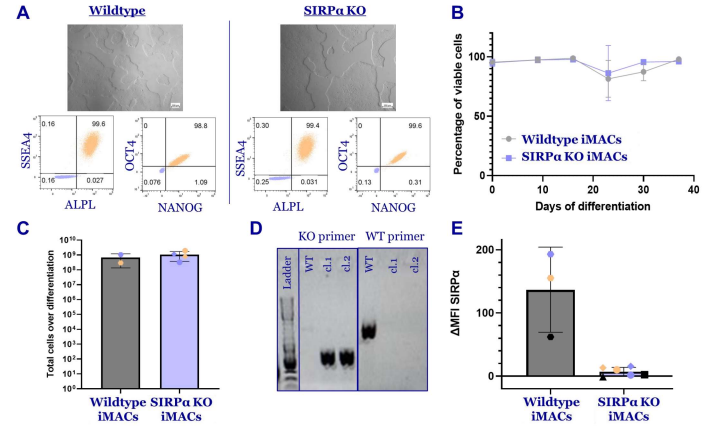

Figure 3: (A) Morphology of WT and SIRPα KO iPSCs as well as expression of pluripotency markers SSEA4, ALPL, NANOG and OCT4. Purple = unstained, orange = stained sample. **(B)** Viability over n=3 iMAC differentiations. **(C)** Total cell number of iMACs produced in two differentiation runs of the same scale. Symbols represent wildtype or specific clones. Matching colors represent individual differentiations, n=2. **(D)** PCR from samples from wildtype iMACs (WT) and two different SIRPα KO clones (cl1 + cl2). Primers were either binding in wildtype SIRPα sequence (WT primers) or sequence with deletion of several exons (KO primers). **(E)** SIRPα expression in wildtype and SIRPα KO iMACs measured by flow cytometry. ΔMFI calculated by median intensity of fluorescence of isotype and stained sample. Symbols represent wildtype (●) or individual clones (●■◆▲). Matching colors represent individual differentiations, n=3.

SIRPα KO iMACs retain typical phenotype comparable to WT iMACs

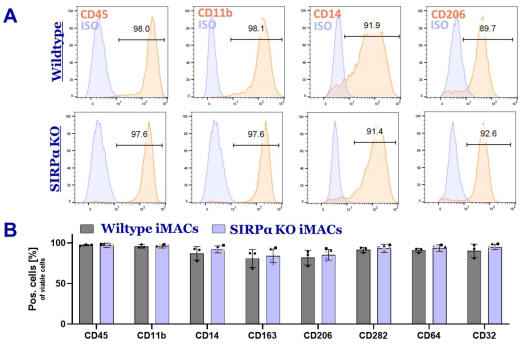

Figure 4: (A) Example of expression of CD45, CD11b, CD14 and CD206 on wildtype and SIRPα KO iMACs. Isotype in purple (ISO) and stained sample in orange. **(B)** Phenotype of wildtype and SIRPα KO iMACs. Both show high expression of CD45, CD11b, CD14, CD163, CD206, CD282, CD64 and CD32 with no significant differences between them over n=3 independent experiments. Symbols represent wildtype (●) or individual clones (●■◆▲). Matching colors represent individual differentiations.

SIRPα KO iMACs loaded with anti-CD20 therapeutic antibody show increased phagocytosis of tumor cells compared to WT iMACs

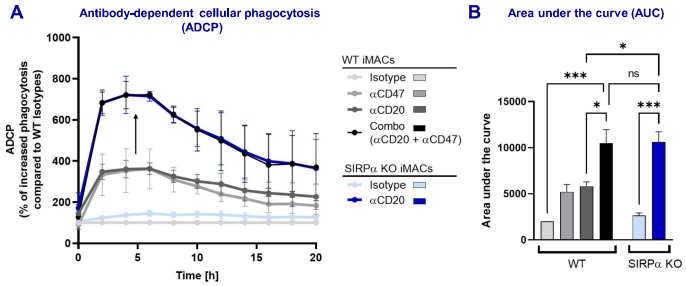

Figure 5: (A) Antibody-dependent cellular phagocytosis of RAJI cells by WT and SIRPα KO iMACs. RAJI cells were labelled with the pH sensitive dye pHrodo (red) and co-cultured for 20 hours with WT or SIRPα KO iMACs at a ratio of 5:1 (iMAC:RAJI) in the presence of different treatments (Isotype controls, αCD20, αCD47 or αCD20 + αCD47 (combo)). The tumor cell uptake by macrophages was then monitored by Incucyte through the measure of the red fluorescence intensity. Data represent an average of three independent experiments and are expressed as means ± SEM **(B)** The area under the curve was measured in **(A)** for all three independent experiments and plotted as a mean ± SEM in a bar graph to compare the overall significant difference between groups. *ns* = not significant ; *<0.05 ; ***<0.001 ; **(C)** CD47 protein expression by RAJI tumor cells measured by flow cytometry. Light grey = Isotype control, dark grey = aCD47 antibody.

Tumor killing capacity of SIRPα KO iMACs loaded with anti-CD20 therapeutic antibody is comparable to WT iMACs in the presence of anti-CD47 antibody

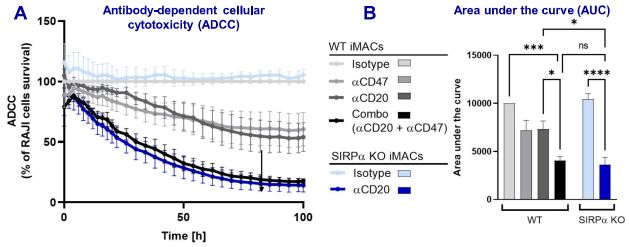

Figure 6: (A) Antibody-dependent cellular cytotoxicity against RAJI cells promoted by WT and SIRPα KO iMACs. GFP positive RAJI cells co-cultured for 100 hours with WT or SIRPα KO iMACs at an E:T ratio of 5:1 in the presence of different treatments (Isotypes, αCD20, αCD47 or αCD20 + αCD47 (combo)). The tumor cell killing by macrophages was then monitored by Incucyte through the measure of the green fluorescence intensity. Data represent an average of three independent experiments and are expressed as means ± SEM **(B)** The area under the curve was measured in **(A)** for all three independent experiments and plotted as a mean ± SEM in a bar graph to compare the overall significant difference between groups. *ns* = not significant ; *<0.05 ; ***<0.001 ; ****<0.0001

Summary

- Evotec develops industrialized GMP manufacturing processes for iMAC cell therapeutics starting with genetically engineered iPSC GMP master cell banks for optimized functionality.
- Evotec's fully scalable, GMP-compatible 3D iMAC differentiation process delivers highly pure, genetically modified iMACs that lack SIRPα expression rendering them resistant to CD47-dependent inhibition of phagocytosis.
- In the presence of a therapeutic antibody for tumor targeting, SIRPα KO iMACs showed increased phagocytosis that was comparable to WT iMACs treated with anti-CD47 blocking antibody.
- This novel allogenic off-the-shelf iMAC cell product candidate overcomes the need for a treatment combination with anti-CD47 or anti-SIRPα checkpoint inhibitors and will serve as the basis to develop innovative treatments for solid tumors.

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

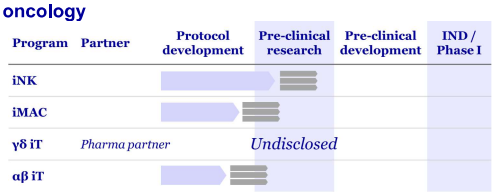

Figure 7: Evotec's iPSC-based cell therapy pipeline for oncology. Building up a comprehensive portfolio of various iPSC-derived cell types to treat cancer incl. natural killer cells (iNK), Macrophages (iMACs) and αβ and γδ T cells (iT). Each immune cell type can deliver multiple differentiated cell therapy products.

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