Immune-shielded and safe iPSC-derived cardiomyocytes for heart repair

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Evotec's end-to-end process for iPSC-based therapeutics

From iPSCs to patients – Evotec's know-how and expertise

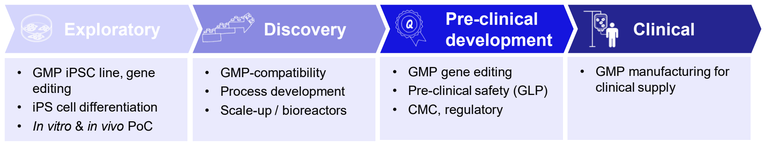


Figure 1: Platform overview

Off-the-shelf cell therapeutic product to remuscularize the failing heart

Pure iPSC-derived cardiomyocytes (iCM) with enhanced safety and durability

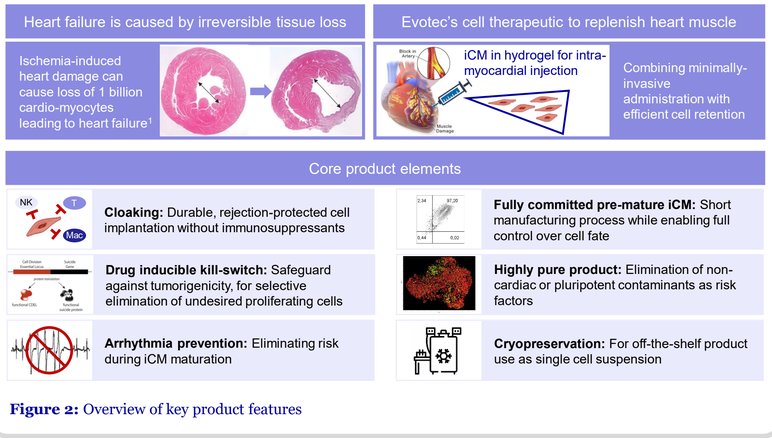


Figure 2: Overview of key product features

Summary

- We develop iPSC-derived cardiomyocytes (iCM) for functional replacement of irreversibly lost tissue in failing hearts
- Our product is genetically engineered to evade immune cell-mediated rejection ("cloaking"), combined with a drug-inducible kill-switch and an anti-arrhythmia strategy
- Our scalable and GMP-compatible 3D differentiation process of highly pure cardiomyocytes runs in GMP-compatible bioreactors and includes predictive in-process QC
- We successfully implemented *in silico* modelling to stabilize cell yield after mid-stage of iCM differentiation
- We evaluated 2 cloaking strategies, i.e. HLA-I/II KO and iACT cloaking[2,3] and could demonstrate equally high differentiation capacity of engineered vs. wild type iPSC into iCM
- While iACT cloaking efficiently protects iCM against NK cell killing, it has only moderate effectiveness against T cells, which contrasts with the high T cell antagonism of an HLA-I/II KO
- Evotec develops a proprietary cloaking based on HLA-I/II KO and an additional innovative anti-NK cell strategy, which further increases the immune-shielding properties of our iCM

Scalable and GMP-compatible iPS cell expansion process in bioreactors

Highly homogeneous iPSC-cluster population and increased cell yield after expansion

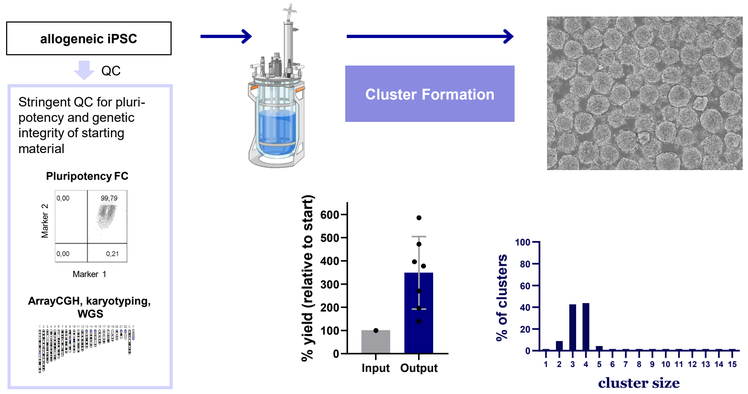


Figure 3: Outline of iPSC mass production with cell yield before and after expansion and cluster size distribution at the end of cluster formation.[4]

Robust and scalable 3D iCM differentiation process in bioreactors

Informed by predictive in-process controls and optimized based on *in silico* modelling

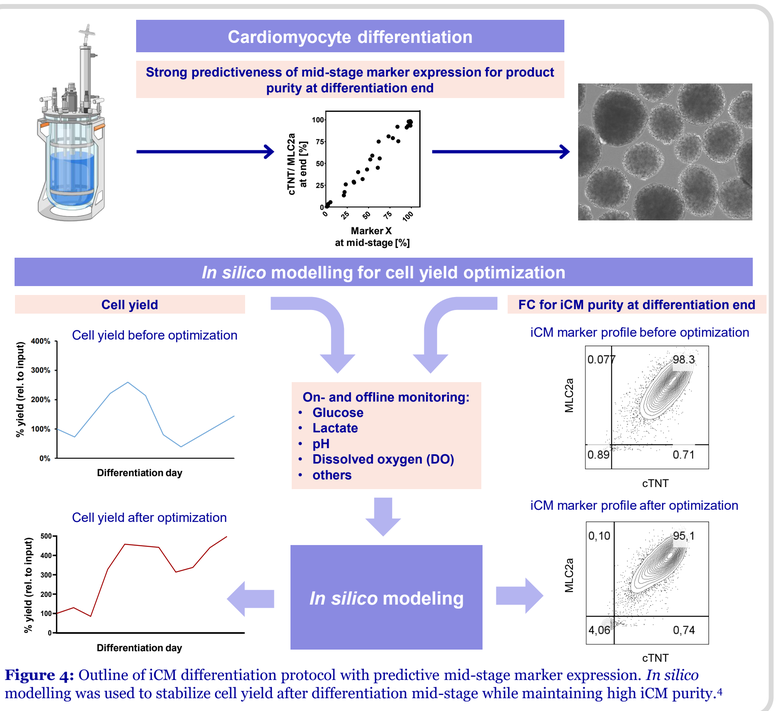


Figure 4: Outline of iCM differentiation protocol with predictive mid-stage marker expression. *In silico* modelling was used to stabilize cell yield after differentiation mid-stage while maintaining high iCM purity.[4]

Engineering of iPSC for immune-shielded ("cloaked") cardiomyocytes

Evotec evaluated two genetically engineered cloaking strategies for long-term graft acceptance

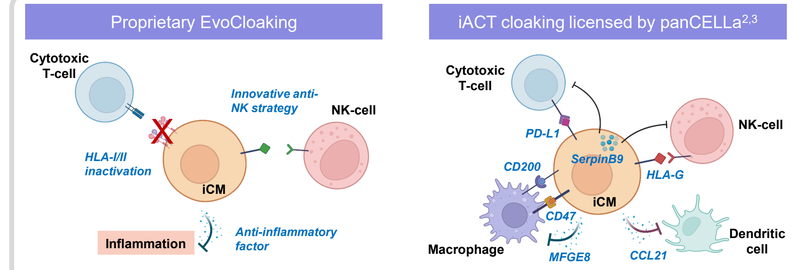
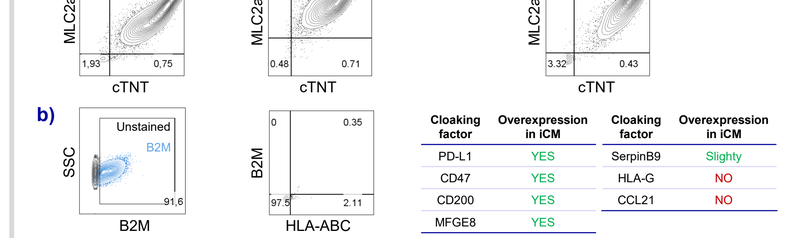


Figure 5: Representation of 2 cloaking strategies investigated. iPSC were genetically engineered to inactivate HLA-I/II and/or overexpress cloaking factors as indicated prior to their differentiation into iCM.[4]

Cloaking-engineered iPSC differentiate into cardiomyocytes with a high purity comparable to WT cells

Confirmed HLA KO and sustained expression of most cloaking factors in cardiomyocytes



Cloaking factor	Overexpression in iCM	Cloaking factor	Overexpression in iCM
PD-L1	YES	SerpinB9	Slighty
CD47	YES	HLA-G	NO
CD200	YES	CCL21	NO
MFGE8	YES		

Figure 6: FC profiles of differentiated iCM. a) High purity of pre-mature cardiomyocytes demonstrated by MLC2a/cTNT double stain. b) Summary of surface expression (FC) and secretion (ELISA) of cloaking factors.

Cloaked iCM reduce T cell and NK cell cytokines and cytotoxicity

HLA-I/II inactivation protects against T cells but raises NK "missing-self-response"; iACT iCM show moderate protection against T cells and high protection against NK cells

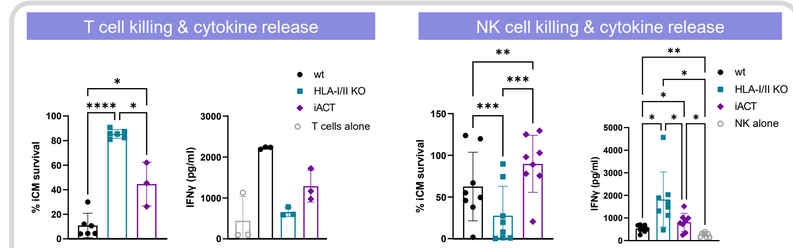


Figure 7: Wild type (WT), HLA-I/II KO and iACT cloaked iCM were co-cultured with primed T cells or expanded NK cells of human donors. iCM survival and IFNγ release from immune cells was measured after 6-24 hours.

[1] Laflamme MA, Murry CE. Regenerating the heart. Nat Biotechnol. 2005 Jul;23(7):845-56; [2] Harding J et al. Induction of long-term allogeneic cell acceptance and formation of immune privileged tissue in immunocompetent hosts. bioRxiv. 2019; [3] Lanza R, Russell DW, Nagy A. Engineering universal cells that evade immune detection. Nat Rev Immunol. 2019 Dec;19(12):723-733; [4] Image generated with BioRender (www.biorender.com)

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