Generation of iPSC derived CD19 CAR iNK cells for treatment of autoimmune disorders

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Abstract #6478

Treatment of autoimmune diseases (ADs) relies on broad immunosuppressive agents that are often not curative. Autologous CAR T cell approaches demonstrated disease remission in patients with Systemic lupus erythematosus (SLE). Among cellular abnormalities typical for ADs, NK cells dysfunction was reported. We believe that using iPSC derived CD19 CAR NK cells (iNK cells) to eliminate disease-causing circulating plasma cells could be a novel therapeutic approach for ADs mediated by B cells. Evotec's validated GMP iPSC line was genetically modified by a knock-in of a CD19-CAR and differentiated into iNK cells using a feeder-free 3D differentiation process. Successful knock-in was confirmed by flow cytometry. Expression of the CAR did not negatively impact the differentiation of the iNK cells. The CD19 CAR iNK cell phenotype and scRNA sequencing profiles are comparable to blood-derived NK cells. To evaluate the ability of CD19 CAR iNK cells to selectively eliminate primary B cells, patient-derived B cell chronic lymphatic leukemia (CLL) cells and primary B cells from healthy donors were co-cultured with CAR iNK cells. Compared to wildtype (WT) iNK cells, CD19 CAR iNK showed specific killing of CD19-positive CLL cells and primary B cells. iPSC-derived CD19-CAR NK cells show increased cytotoxicity and specific targeting of CD19 positive primary patient and healthy subject derived B cells, demonstrating their potential suitability for the treatment of ADs such as SLE.

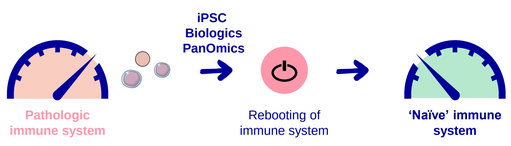
1 Evotec's approach to develop innovative and differentiated medicines:

Inflammation & Immunology (I&I) research focus areas for co-creation

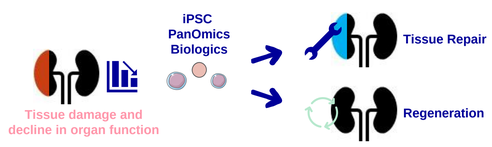
- Our focus is on three defined areas within the I&I space with high medical need and high probability for scientific and commercial success
- Our strategy is modality agnostic, utilizing Evotec's entire technology platforms for drug candidate development
- We are driving development of innovative I&I therapeutics through Evotec's PanOmics-driven drug discovery for precision medicine **(poster # 6312)**
- We believe that the cell therapy will have a major impact in I&I field, and we are building a portfolio of iPSC-derived immune cell product candidates

1 Focus area: Immuno-modulation

2 Focus area: Reset of immune system



3 Focus area: Tissue regeneration



Evotec's key expertise in autoimmune diseases enables development of I&I therapeutics for:

Gastroenterology
- Ulcerative Colitis
- Crohn's Disease
- IBD

Lung fibrosis
- Idiopathic Pulmonary Fibrosis
- Interstitial Lung Disease

Rheumatology
- SLE, Lupus Nephritis
- RA
- Sjögren's Syndrome
- Ab-driven diseases

Kidney fibrosis
- Diabetic Kidney Disease
- Chronic Kidney Disease
- FSGS

Dermatology
- Psoriasis
- Atopic Dermatitis
- Alopecia
- Ab-driven diseases

2 Our scientific starting points to co-create Cell Therapy pipelines with partners in I&I:

	Focus Area	Project	Approach	MoA	Indication space	Target validation	Hit ID-H2L	LO
iPSC cell therapy	Reset of immune system	Target 1	CAR-iαβ T	Reset of pathogenic B cell compartment	Rheumatology Ab-driven diseases	Binder generation		
		CD19	CAR-iNK	Reset of pathogenic B cell compartment	Rheumatology Ab-driven diseases	*In vitro* PoC		
		Target 2	CAR-iNK	Elimination of fibrogenic myofibroblasts	Fibrotic diseases	*In vitro* PoC		
	Tissue regener Ation	Target 2	CAR-iMAC	Elimination of fibrogenic myofibroblasts	Fibrotic diseases	Binder generation		
		–	iMAC	Functional macrophages replacement	hPAP	Project initiation		

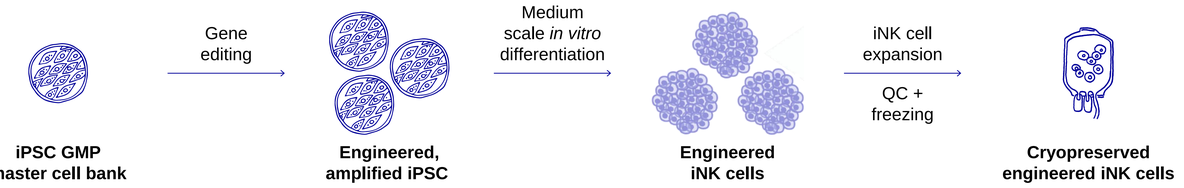
3 End-to-end process for iPSC-based therapeutics @Evotec:

Exploratory ✓	Pre-clinical research ✓	Pre-clinical development ✓	Clinical ✓
• iPSC differentiation • Gene editing • *In vitro* PoC	• GMP-compatibility • Upscaling • *In vivo* PoC	• CMC • Pre-clinical safety • Regulatory/IND	• Clinical supply • Patient stratification
Dedicated team of >100 scientists with industry-leading expertise	Validated GMP iPSC line and GMP-compatible differentiation protocols	GMP-compatible gene editing technology for flexible selection of genetic modifications based on indication/strategy	GMP-compatible bioreactor format for upscaling and production of clinical material

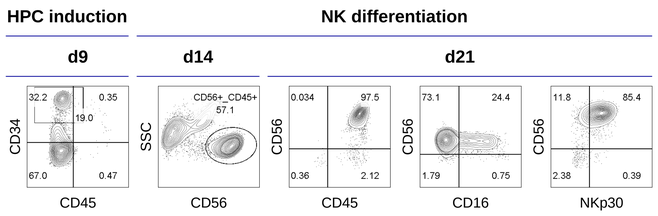
In-house solution for GMP gene editing and clinical manufacturing

4 iPSC can be efficiently differentiated into iNK and CD19 CAR iNK with a comparable phenotype to blood-derived NK cells:

Manufacturing workflow of iPSC-derived NK cell product

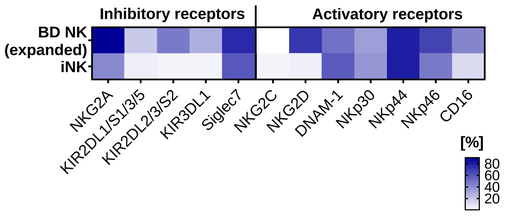


Homogenous population of NK produced from GMP iPSC line



iNK cells were differentiated in a bioreactor and the induction of hematopoietic (CD34, CD45) and NK marker (CD56, CD16, NKp30) expression was monitored by flow cytometry.

iNK cells are phenotypically comparable to blood-derived NK cells

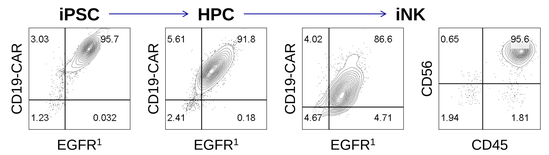


Analysis of various NK inhibitory & activating receptors by flow cytometry showed a similar expression pattern between iNK cells and healthy donor blood-derived NK cells (BD-NK).

Successful generation of CD19 CAR knock-in iNK cells

CD19 CAR and NK marker expression during iNK differentiation

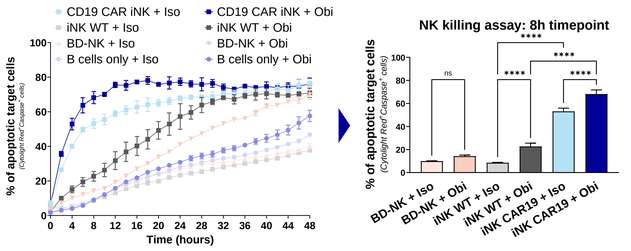
- A cassette expressing CD19 CAR-P2A-tEGFR was inserted into a dedicated safe harbor locus in our GMP iPSC line using RNA-guided nucleases and cellular HDR
- Post engineering, cells were enriched by FACS. Sorted cells were expanded and re-sorted to achieve a >98% CAR expressing bulk population.
- CD19 CAR expression was maintained throughout iNK cell differentiation



1 truncated EGFR was used as a marker gene

5 CD19 CAR iNK cells show strong activation and CAR-directed cytotoxicity against Systemic Lupus Erythematosus (SLE) patient derived B cells:

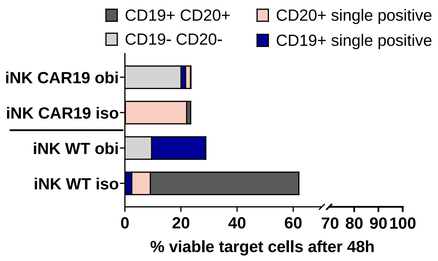
CD19 CAR iNK cells show strong cytotoxicity towards B cells from SLE patients in co-culture assay



NK killing assay with primary B cells isolated from SLE patients as target cells

- iNK without a CAR (WT), CD19 CAR iNK or healthy donor blood-derived NK cells (BD-NK) were used as effector cells and co-cultured in a 1:1 (E:T) ratio with primary B cells from SLE patients + 10µg/ml anti-CD20 antibody (Obinutuzumab (Obi)) or isotype control (Iso) (n=2)
- The % of dead B cells was quantified by IncuCyte live-cell imaging over a period of 48h
- CD19 CAR iNK cells showed strong cytotoxicity towards SLE B cells. In comparison iNK WT and BD-NK cells showed slower killing kinetics and a lower frequency of dead cells after 48h
- Obinutuzumab could further potentiate B cell killing of all three NK populations, with cytotoxicity of CD19 CAR iNK cells remaining to be the strongest

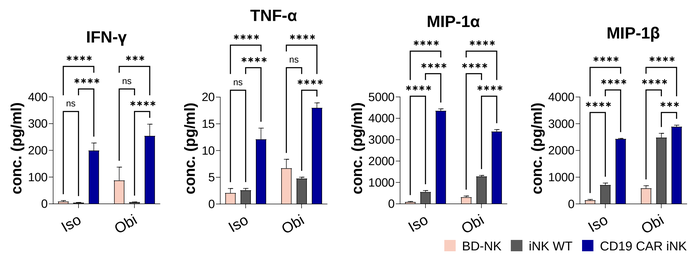
CD19-targeted cytotoxicity of CD19 CAR iNK cells



Phenotype of remaining viable B cells after 48h of co-culture

- CD19 CAR iNK showed targeted depletion of CD19+ B cells, with most spared viable cells being CD20 single positive. In contrast iNK WT + Obinutuzumab (Obi) showed CD20 targeted depletion of B cells.
- The combination of CD19 CAR iNK + Obinutuzumab had an additive effect, depleting double positive as well as CD20 & CD19 single positive cells

Increased inflammatory cytokine release in CD19 CAR iNK co-cultures



Quantification of cytokines in the medium after 48h

Co-cultures with CD19 CAR iNK showed significant higher concentrations of cytokines IFN-γ, TNF-α, MIP-1-α & MIP-1β @48h compared to those of iNK WT or BD-NK cells, indicating an increased NK cell activation

Conclusion

- Evotec is developing industrialized GMP manufacturing processes for gene-edited CD19 CAR iNK for cell therapy as a true off-the-shelf and on-demand treatment option for autoimmune disorders.
- The CD19 CAR iNK cells show CD19-dependent cytotoxicity and targeted depletion of primary CD19+ B cells from SLE patients
- CD19 CAR iNK allogeneic cell therapy has the potential to treat relapsing or refractory SLE patients, prospectively also targeting pathogenic tissue-resident B cells including CD20-negative autoantibody producing plasmablasts, supporting sustained remission

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