iPSC-derived Natural Killer cells (iNK cells), front-runner program of EVOcells Oncology platform: from inception to translational validation using B-CLL patient samples

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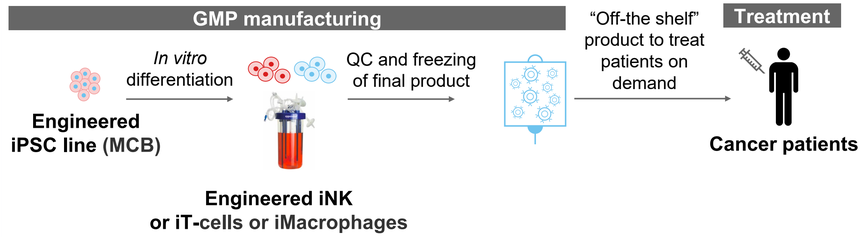
Abstract: 2749

EVOcells Oncology platform: cellular immunotherapies delivered "off-the-shelf" for the treatment of a large number of patients



- Streamlined manufacturing process to overcome hurdles of current autologous cell therapies
- Fully scalable cell therapy products for clinical use in large patient populations
- Tailored genetic editing to deliver cell therapies as precision cancer medicines

EVOcells Oncology programs: taking advantage of iPSC technology to produce a broad range of allogeneic immune cell types

Program/Project	R&D source	Exploratory	Pre-clinical research	Pre-clinical development	IND / Phase I
iNK	Internal				
γδ iT-cells	Internal				
αβ iT-cells	Internal				
iMacrophages	Internal				

Comprehensive arsenal of assays to characterize iPSC-derived immune effector cells

Generation of iPSC-based immune effector cells

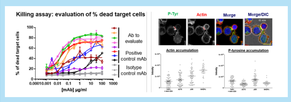
iPSC-derived cells without engineering

Genetic engineering of iPSC-derived cells to enhance clinical efficacy

- Improve homing to tumor site
- Increase persistence
- Boost function

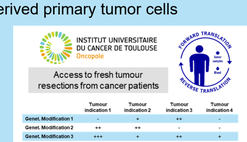
Functional characterization using established assays

***In vitro* functional assays**
Benchmarking with human blood-derived effector cells

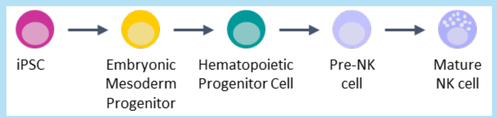


***In vivo* efficacy**
Highly immuno-compromised mice (e.g. NSG) with xenograft of human tumour cell lines and adoptive transfer of iPSC immune effector cells

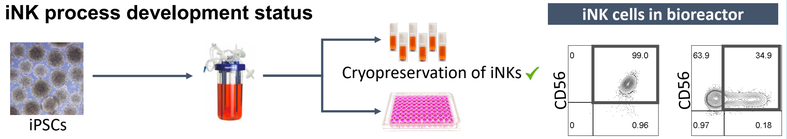
***Ex vivo* - Translational**
Translational assays using patient-derived primary tumor cells

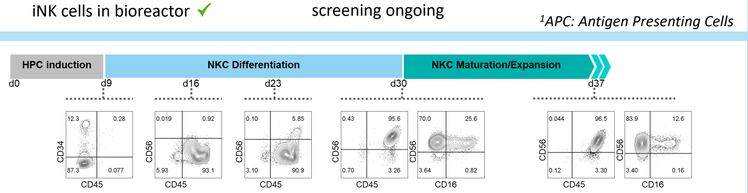


Development of a robust feeder-free 3D differentiation protocol to produce iNK cells



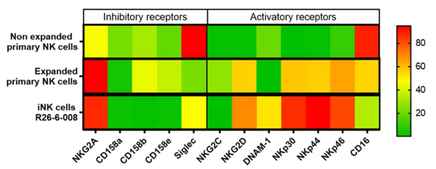
iNK process development status



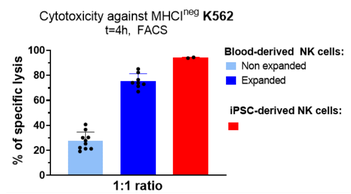


iNK cells display expected phenotypic profile and are fully functional

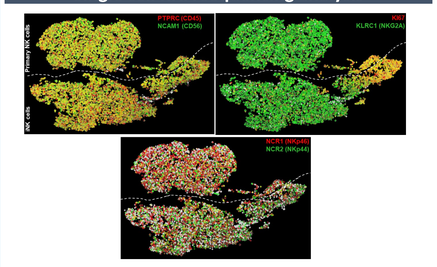
Phenotypic analysis by multiparametric flow cytometry



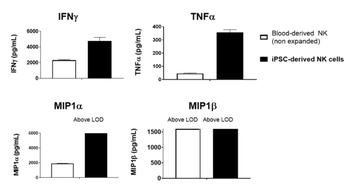
Killing potency of K562 tumor cells



Single cell RNA sequencing analysis

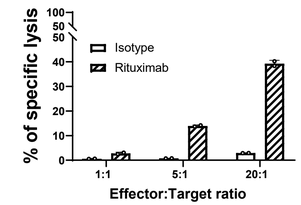


Cytokines release after co-culture with K562 tumor cells



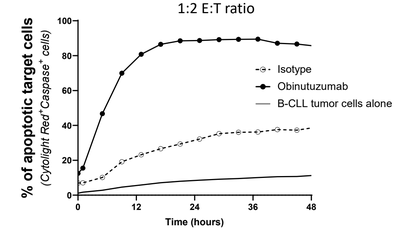
Translational validation of iNK functionality with freshly isolated CLL patient tumor cells

ADCC against CLL-patient tumor cells
t = 4 h, FACS



Methods: *Evaluation of patient PBMC death by FACS (viability dye) after 4 hours of co-culture at various E:T ratios*

CLL-patient #6 tumor B cell apoptosis
1:2 E:T ratio

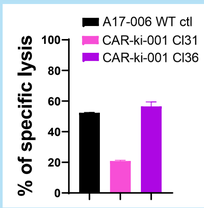


Methods: *Evaluation of apoptosis of CLL-patient-derived tumor B cells co-cultured with iNK cells ± biologics at 10µg/mL (Caspase 3/7 activity measured by Incucyte)*

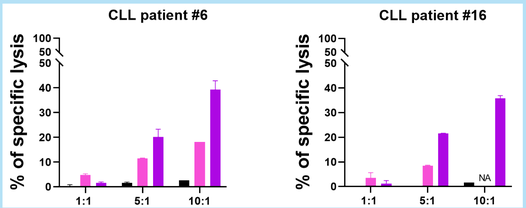
B leukemic tumor cells are defined as $CD19^+$ $CD5^+$ cells (60% - 80%) of total PBMCs in patients

Enhanced CLL patient tumor cell killing with CAR19 iNK

Functional QC on CD19⁻ K562 tumor cells



Killing of $CD19^+$ CLL patient tumor cells



Methods: *Evaluation of tumor cell death by FACS (viability dye) after 4 hours of co-culture at various E:T ratios*

- **CAR19 iNK cells show higher killing capacity of resistant primary B leukemic patient tumor cells as compared to WT iNK**

Conclusion

- iPSC-derived NK cells (iNK cells) offer a highly attractive alternative for patient-derived NK cell therapy, both from a therapeutic efficacy and safety perspective
- Robust feeder-free 3D differentiation protocol to produce iNK cells together with a freezing/thawing protocol in place
- Validation of the possibility to boost iNK cells function via genetic editing
- iNK cells are showing phenotypic properties and single cell RNA sequencing profiles equivalent to blood-derived NK cells
- iNK cells are fully functional with the ability to form lytic immunological synapses leading to an efficient killing of cancer cell lines and CLL patient tumor cells