Development of a 3D perfused melanoma-on-a-chip model to evaluate efficacy and toxicity of a CAR T-cell therapy

Daniel Rieger[1,2], Yassen Abbas[1] and Tomasz Kostrzewski[1]

[1]CN Bio Innovations, 332 Cambridge Science Park, Cambridge, CB4 0WN, UK

[2]Université Paris Cité, 85 boulevard Saint-Germain, 75006 Paris, France

Funded by the European Union

HORIZON EUROPE

Project funded by Schweizerische Eidgenossenschaft, Confédération suisse, Confederazione Svizzera, Confederaziun svizra; Swiss Confederation; Federal Department of Economic Affairs, Education and Research EAER, State Secretariat for Education, Research and Innovation SERI

UK Research and Innovation

Abstract

Preclinical models such as cancer-on-a-chip systems are at the forefront of cancer research; however, models that capture the full immunological cascade of immunotherapies such as CAR T cells remain scarce. These processes include circulation in the blood stream, transendothelial migration, tumor infiltration, and cytolysis of cancer cells. Recreating the physical and biochemical barriers imposed by the tumor microenvironment using advanced *in vitro* systems is critical for investigating CAR T cell performance against primary tumors, while simultaneously providing insights into potential adverse effects.

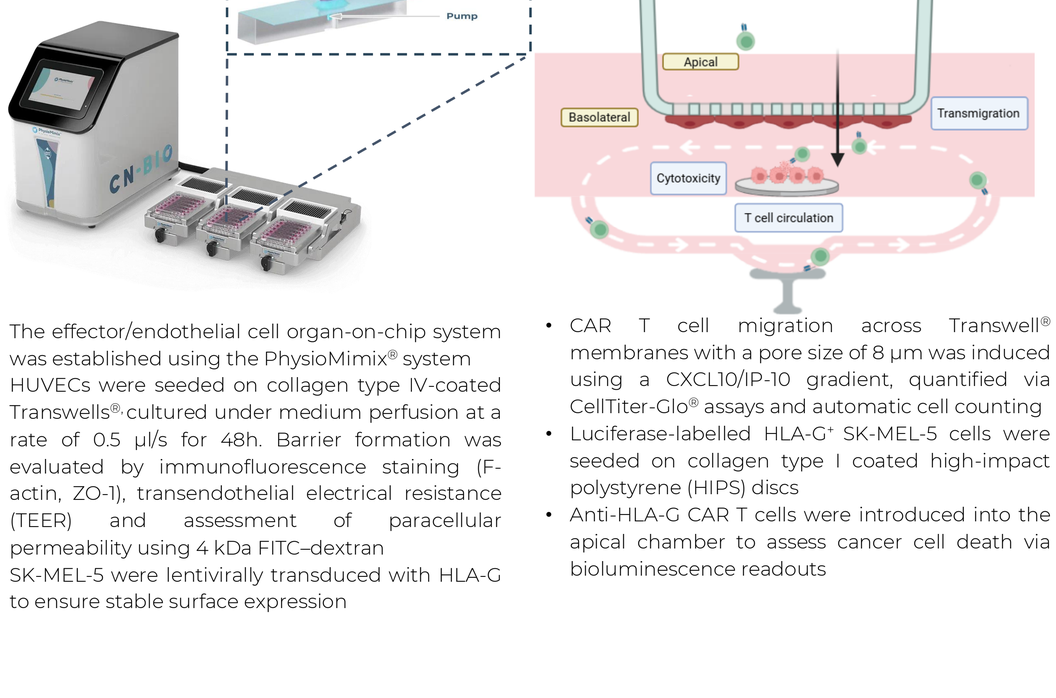
As part of Melomanes, an EU-wide research consortium dedicated to the development of an immunotherapy combining anti-HLA-G CAR T cells and magnetic nanoparticles capable of inducing localised hyperthermia, this project aims to develop an advanced melanoma-on-a-chip system to evaluate the therapeutic efficacy and safety of CAR T cells. This model is developed using the PhysioMimix® Core platform (CN Bio Innovations), enabling medium circulation to model blood flow. A Transwell®-based dual-compartment strategy enables chemokine-controlled immune cell migration across a monolayer of human umbilical vein endothelial cells (HUVECs). Such human-based preclinical models offer a pathway to improved predictive accuracy while reducing the reliance on *in vivo* studies.

Objectives

- Build a melanoma-on-a-chip model using the human cancer cell line SK-MEL-5
- Develop a human endothelial barrier using HUVECs and investigate endothelial activation in response to TNF-α
- Assess the impact of basolateral perfusion on the formation of an endothelial barrier
- Evaluate chemokine-mediated CAR T cell motility and transendothelial migration across the endothelial layer as a function of endothelial activation state
- Investigate CAR T cell cytotoxic efficacy under varying conditions of perfusion and endothelial activation

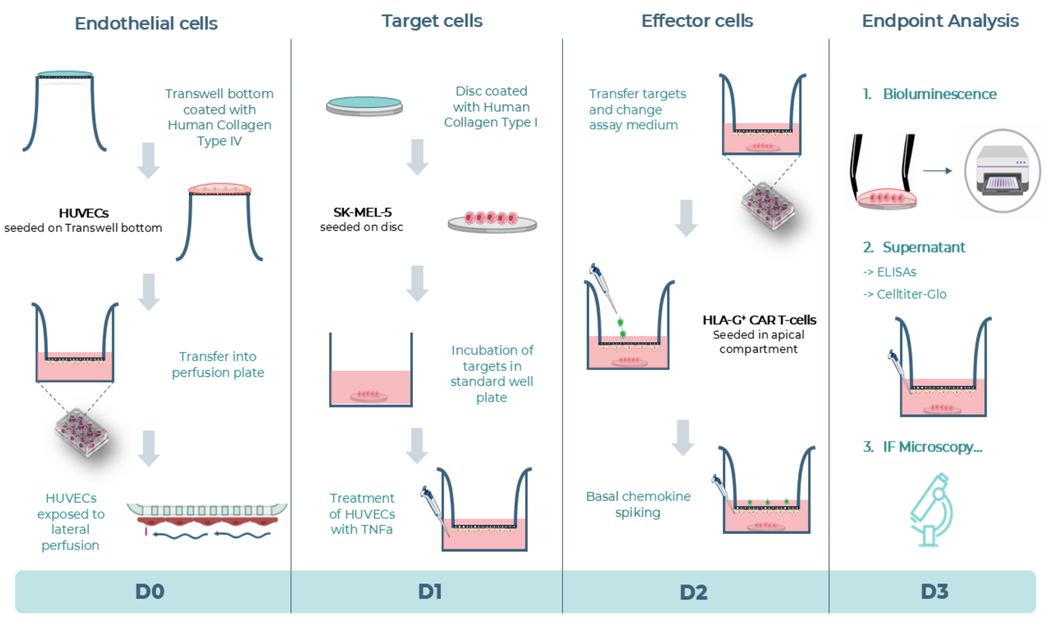
Methods

Fig 1. Establishment of melanoma-on-a-chip model



- The effector/endothelial cell organ-on-chip system was established using the PhysioMimix® system
- HUVECs were seeded on collagen type IV-coated Transwells®, cultured under medium perfusion at a rate of 0.5 µl/s for 48h. Barrier formation was evaluated by immunofluorescence staining (F-actin, ZO-1), transendothelial electrical resistance (TEER) and assessment of paracellular permeability using 4 kDa FITC–dextran
- SK-MEL-5 were lentivirally transduced with HLA-G to ensure stable surface expression
- CAR T cell migration across Transwell® membranes with a pore size of 8 µm was induced using a CXCL10/IP-10 gradient, quantified via CellTiter-Glo® assays and automatic cell counting
- Luciferase-labelled HLA-G⁺ SK-MEL-5 cells were seeded on collagen type I coated high-impact polystyrene (HIPS) discs
- Anti-HLA-G CAR T cells were introduced into the apical chamber to assess cancer cell death via bioluminescence readouts

Fig 2. Time course for the generation of the model



Results

Endothelial cells seeded on the basal side of the collagen-coated Transwells formed tight barriers. Treatment with TNF-α prompted VCAM-1 upregulation, loss of cell adhesion, altered cell morphology and increased permeability of the endothelial layer (Fig. 3). Permeability and TEER assays showed a peak barrier integrity after 48h of exposure to perfusion (Fig. 4). CAR T cells exhibited transmembrane migration across Transwells depending on the endothelial activation state, chemokine concentration, and medium perfusion (Fig. 5). CAR T cell-induced cancer cell death was governed by effector-to-target ratio and medium perfusion (Fig. 6a-b). CAR T cells retained cytotoxicity after transmigration but showed reduced killing efficiency compared to direct exposure (Fig. 6c).

Fig 3. Optimization of an endothelial barrier on the basolateral side of the Transwell membrane

Fig 4. Endothelial barrier integrity was evaluated by measuring the apparent permeability P_{app} using FITC–dextran and monitoring TEER

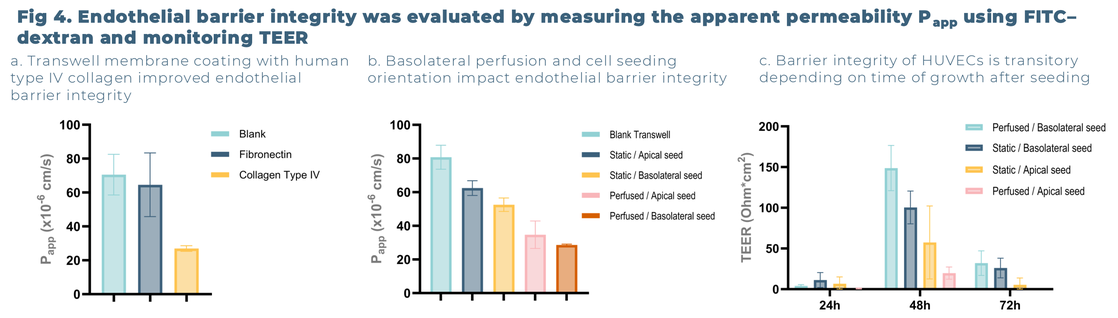


Fig 5. Immune cell trafficking across a Transwell membrane depends on endothelial barrier phenotype and chemokine signalling

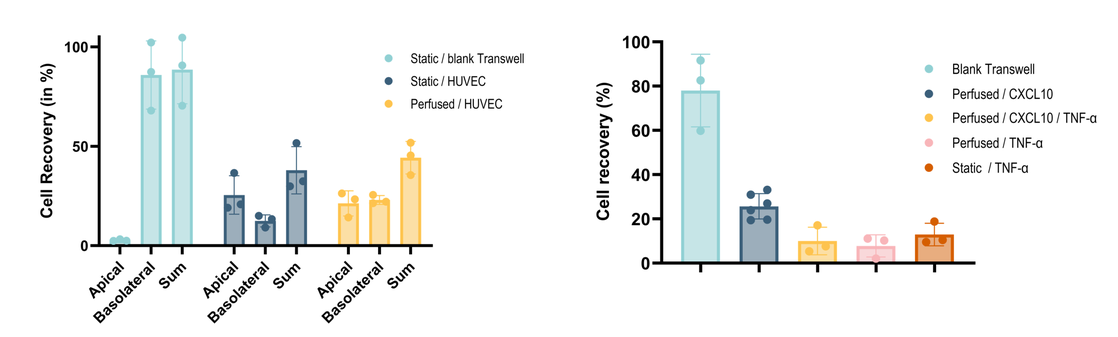
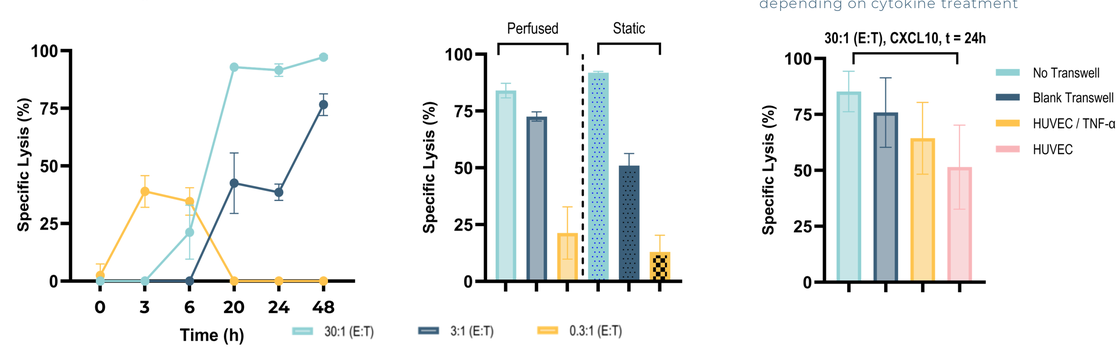


Fig 6. Killing efficiency of anti-HLA-G CAR T cells on SK-MEL-5 is controlled by basolateral perfusion and endothelial barrier–restricted effector interaction



Conclusion

- Here, we established a melanoma-on-a-chip model incorporating primary anti-HLA-G CAR T cells and endothelial and cancer cell lines, recapitulating key aspects of the immunological cascade of CAR T cells in a dynamic system
- Permeability assays demonstrated that optimized ECM coating and perfusion improve barrier formation of HUVECs
- The use of luciferase-based assays for cytotoxicity evaluation demonstrated significant immune-mediated tumor cell lysis depending on effector-to-target (E:T) ratio, basolateral perfusion and target antigen expression
- Access-limiting barriers formed by TNF-α-treated or untreated HUVECs seeded on Transwells yielded a reduced lysis rate of target SK-MEL-5 melanoma cells compared to barrier-free co-culture
- This platform provides a physiologically relevant preclinical tool for evaluating CAR T cell trafficking and efficacy in solid tumors, with potential applications in therapy optimization and safety assessment.

visit **cn-bio.com**

Download this poster

Scan the QR code with your mobile phone or tablet or visit

cn-bio.com/R1672