Institut für Biotechnologie
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TissUse
Emulating Human Biology

OncoChip

Development of a microfluidic human test platform for immune-oncology therapy testing

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INTRODUCTION

So far, there is no human test system for preclinical immune-oncology therapy. Predictive testing of new immune-oncological therapies is currently limited to clinical trials. Therefore, this work focus on first steps towards an establishment of a short-term co-culture of a human intestinal equivalent, a tumor-infiltrated model (TIM) and circulating immune cells interconnected through a microfluidic vasculature on a HUMIMIC Chip2 – the so called *OncoChip*.
The *OncoChip* supports "oral" and "intravenous" administration of substances since modern immune-oncology therapies are combination therapies of oral-given drugs and intravenously-given monoclonal antibodies.
Specific in this study, the immune background is controlled by antibody-dependent cell mediated cytotoxicity (ADCC). ADCC is mostly mediated by Natural killer cells (NK cells) which are part of the innate immune system and are also working in allogenic systems. The tumor-model is based on a non-small cell lung cancer (NSCLC) cell line calling H292 cells. NSCLC-H292 cells are supposed to have a strong EGFR overexpression which is a target of monoclonal antibodies such as Cetuximab. Previously, there was contradictory research of the treatment of NSCLC-H292 cells with Cetuximab. Here, this work focused on the theory that Cetuximab not only works with the overexpression of EGFR but also by ADCC.

SET-UP & RESULTS

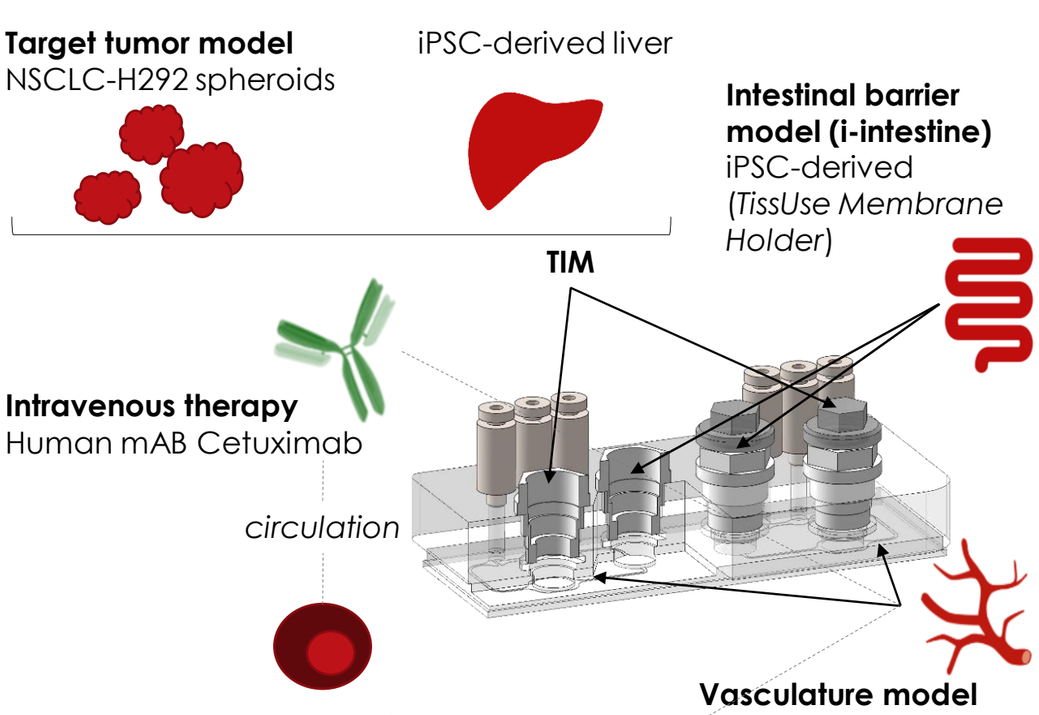

Fig. 1: OncoChip set-up. First, endothelialization was performed with i-ECs emulating the vasculature. After three days (day -2), TIM was added. It consist of 25 x NSCLC-H292 spheroids and 40 x i-Liver spheroids. TIM was lying on i-ECs in the outer insert of Chip2. The i-Intestine was inserted in the inner insert. On day 0 the NK cells were injected directly on the TIM. The ratio NK cells to tumor NSCLC-H292 cells in Chip2 was 3:1. This was adapted to the beforehand calculated ratio in static conditions. Static ADCC works with a ratio of 1:1. Since Chip2 has a larger culture area for circulating NK cells, especially because of the second insert and the channel area, the ratio for Chip2 was adapted comparing its cell culture area to the area of a 96-well. Chip2 were either injected with NK cells or treated as control and/or treated with 10 µg/mL Cetuximab or without any treatment.

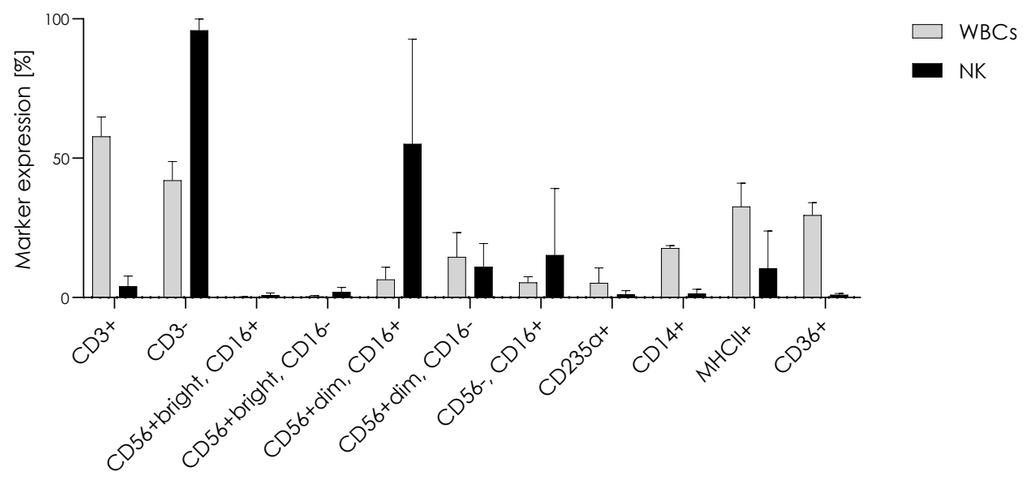

Fig. 2: Flow cytometry analysis of White Blood Cells (WBC) vs. NK cells negatively isolated of WBCs. Shown are gates of single, living cells and specific marker expression [%] on day -1 of assay. n = 4 biological replicates.

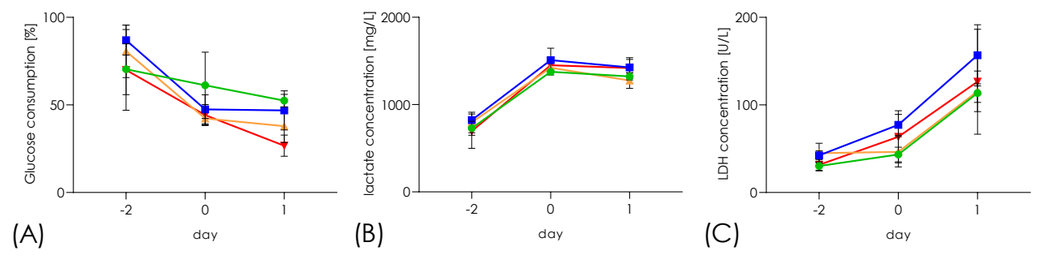

Fig. 4: Metabolic activity assays of basolateral supernatant of OnocChip and respectively control groups over the time. ■: NK + Cet, ■: NK w/o Cet, ■: w/o NK + Cet, ■: w/o NK w/o Cet. (A) Glucose consumption [%]. (B) Lactate concentration [mg/L]. (C) LDH concentration [U/L]. Biological replicates: n between 2 and 3.

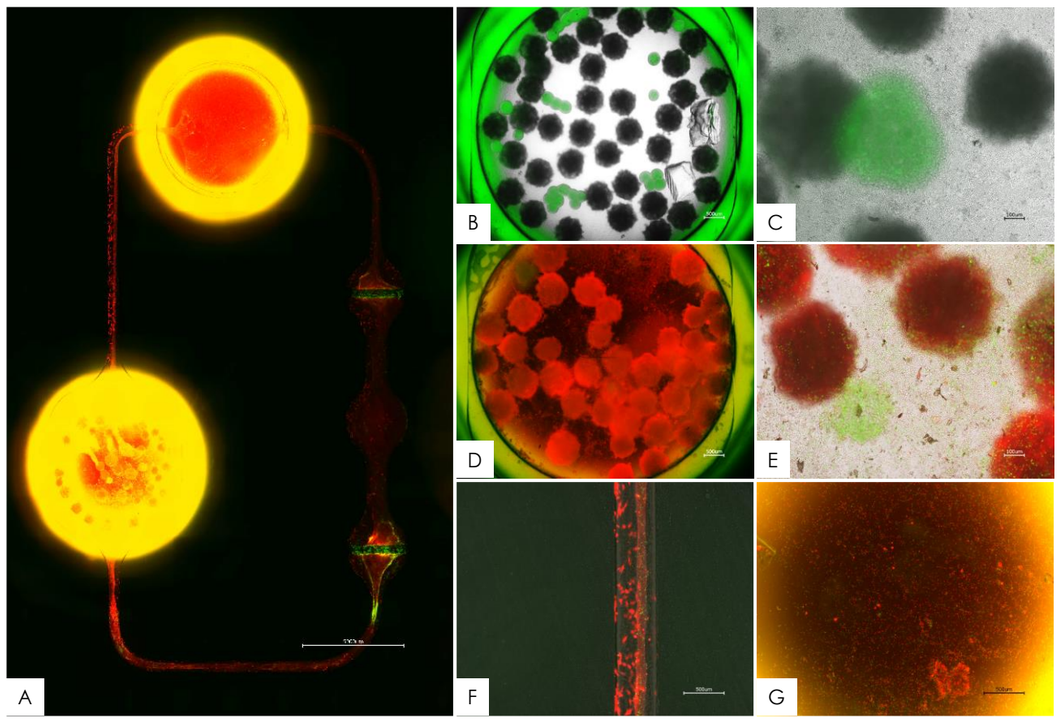

Fig. 3: OncoChip combined with different stainings of group 1 (NK cells, TIM, iECs, i-intestine, treated with 10 µg/mL Cetuximab). (A) Full OncoChip with CAM-live (red) and CellTox™Green-dead (green) co-staining on day 1 of assay. Scale bar: 5000 µm. **(B)** Immuno-phase contrast microscopy of CMFDA-NSCLC-H292 (green) and i-Liver spheroids in the inner insert of Chip2 on day -2 of assay. Scale bar: 500 µm. **(C)** Immuno-phase contrast microscopy of CMFDA-NSCLC-H292 (green) and i-Liver spheroids in the inner insert of Chip2 on day -2 of assay. Scale bar: 100 µm. **(D)** Inner insert with TIM on day 1 of assay stained with CAM-live (red) and CellTox™Green-dead (green) co-staining. Scale bar: 500 µm. **(E)** Inner insert of TIM on day 1 of assay stained with CAM-live (red) and CellTox™Green-dead (green) co-staining overlay with phase contrast microscopy. Visible are also NK cells (suspension cells). Scale bar: 100 µm. **(F)** Channel region with iECs stained with CAM-live (red) and CellTox™Green-dead (green) co-staining overlay with phase contrast microscopy. Scale bar: 500 µm. **(G)** Outer insert under i-intestine membrane holder (basolateral) with NK cells on day 1 of assay stained with CAM-live (red) and CellTox™Green-dead (green) co-staining overlay with phase contrast microscopy. Scale bar: 100 µm.

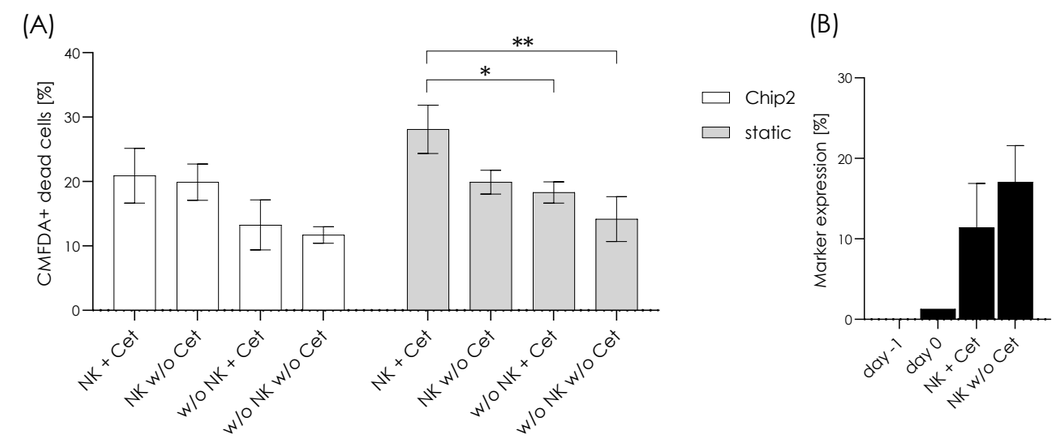

Fig. 5: Flow cytometry analysis of OncoChip and control groups. (A) Cytotoxicity assay. Cells were gated for single, CMFDA+, dead cells [%]. Chip2 culture (on day 1 of assay) vs. static co-culture (after 6 h of assay). In static culture NK + Cet shows the highest percentage of dead CMFDA+ cells with approx. 28 % which correlates with ADCC effect. Same tendency is visible for Chip2 culture. Biological replicates: n is varying between 2 and 3. Static analysis was performed with 2way ANOVA using Tukey's multiple comparisons test. P-values smaller than or equal to 0.05 were considered significant. * indicates $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$ and ****: $p \leq 0.0001$. **(B) CD107a+ cells [%].** Gated for living, CMFDA-, CD31-, CD3-, CD56dim+, CD16+. Biological replicates: day -1 n = 1, day 0 = n = 1, day 1 (NK + Cet) = 3, day 1 (NK w/o Cet) = 3.

Summary and Outlook

For the first time, ADCC on-chip was modelled. The OncoChip project highlighted first steps towards highly complex co-culture systems including NK cells. The optimization of a medium composition supporting all tissues was challenging. Here, standard NK cell medium was used, as this medium supported the activity of immune cells. Still, other models were suffering. Comparing Chip2 to static culture showed differences in cytotoxicity assay working with ADCC.
Nevertheless, CD107a+ expression is increasing in Chip2 culture.
With further improvements of co-culture medium, ratio of NK cells to tumor cells in Chip2 culture and optimal Cetuximab concentration completely working OncoChip for short-term assay looks promising.

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