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A predictive multi-organ-chip platform for cancer precision medicine using automated high-content substance testing

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Abstract

Since cancer is a condition that greatly varies between individuals, there is no universal approach of treatment. Hence there is a necessity for a rapid and tailored treatment for each individual. To allow for the standardization and throughput essential for patient testing, systems are needed that enable highly standardized automated handling of MPS. Here we use the HUMIMIC AutoLab to test drug treatment on human cancers. The individual results of treatments create a path for combination and novel therapies, resulting in prognoses that are more precise and subsequently increases individual patients' treatment success.

Experimental Design Principles

To demonstrate the methodology, two different cancer types were investigated: Patient derived gastric tumor organoids (GC) and diffuse large B-cell lymphoma (DLBCL) cell lines/ patient derived xenograft. The HUMIMIC Chip2 contains two independent circuits, each interconnects the cancer- and liver models by microfluidic channels. The automated setting that handles the multi-organ-chips is capable of substance treatment, sampling of supernatant containing metabolites and imaging 24/7. To test the individual responses to chemotherapeutic prodrugs, a co-culture of each tumor model with a 3D liver spheroid model composed of HepaRG and human hepatic stellate cells for drug metabolism was established in a multi-organ-chip.

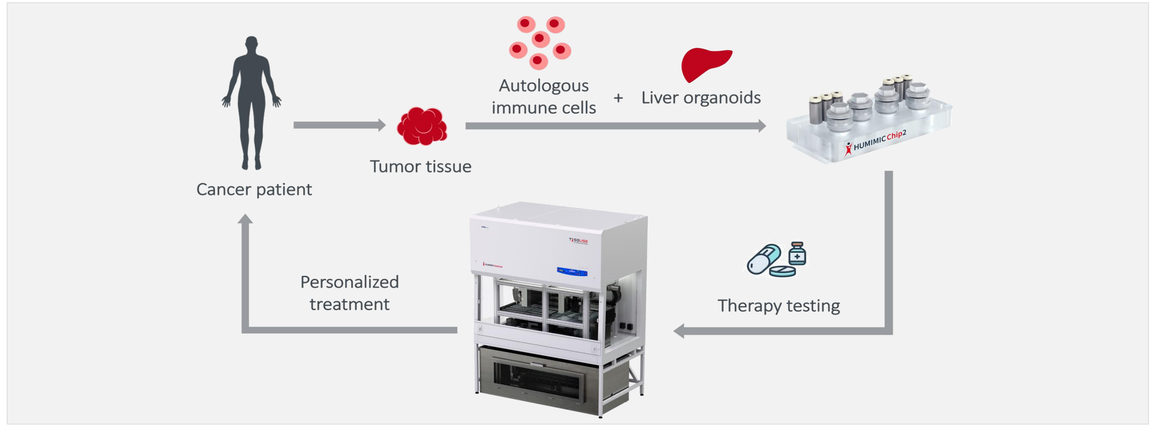

Fig. 1. Schematic overview of automated precision medicine platform.

Results

Morphologic, metabolic and immunohistochemical analysis showed that each co-cultures of GC and DLBCL together with the liver model were stable in the multi-organ-chip for at least 7 days (Fig 2/5). For the two patient derived gastric tumors, the data showed effectiveness of one 5-Flourouarcil (5-FU) prodrug, Capecitabine, commonly used in the therapy of gastric cancer, indicating biotransformation of the prodrug to their toxic metabolites via the liver model (Fig. 5). This resulted in a significantly decrease in the GC biomass of two individual patients. Another 5-FU prodrug, Tegafur, showed toxic effects independent of biotransformation. For the DLBCL lines and one PDX, data showed varying sensitivities of the cells when treated with biotransformed Cyclophosphamide, dependent on the original cancer subtype and genetic background of the patient (Fig. 4)

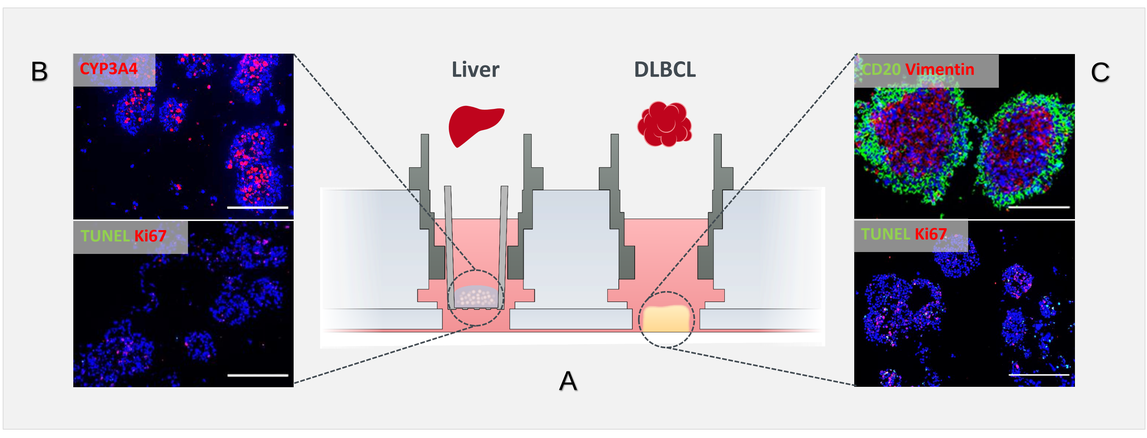

Fig. 2. Set-up of DLBCL biochip with immunohistological stainings. (A) Status quo system set-up scheme of one circuit. **(B)** IHC staining of liver model 7 days after dynamic co-culture: upper figure- CYP3A4 staining; lower figure- TUNEL/Ki67 staining. **(C)** Upper figure- IHC staining of DLBCL cell line model (U2932+YK6) before dynamic co-culture: CD20 (DLBCL marker)/Vimentin (YK6 marker) staining; lower figure- TUNEL/Ki67 staining after 7 days of dynamic culture. Scale: 100µm.

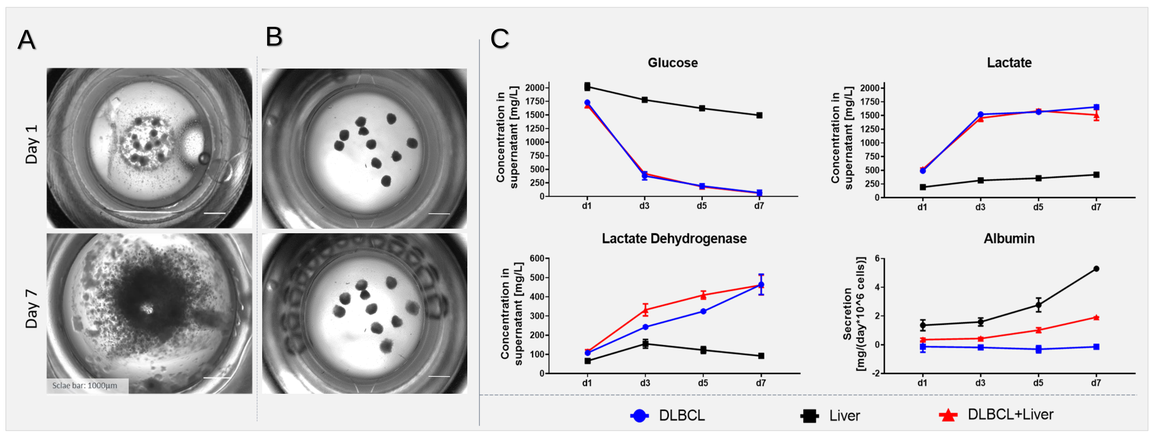

Fig. 3. Co-culture of DLBCL (U2932) and liver model over 7 days. Representative brightfield images taken by the AutoLab of **(A)** DLBCL model (U2932+YK6) and **(B)** liver model from beginning of experiment to end. Scale bar: 1000µm **(C)** Metabolic data at every medium exchange time point. Values are a mean of 3 biological replicates ± SD.

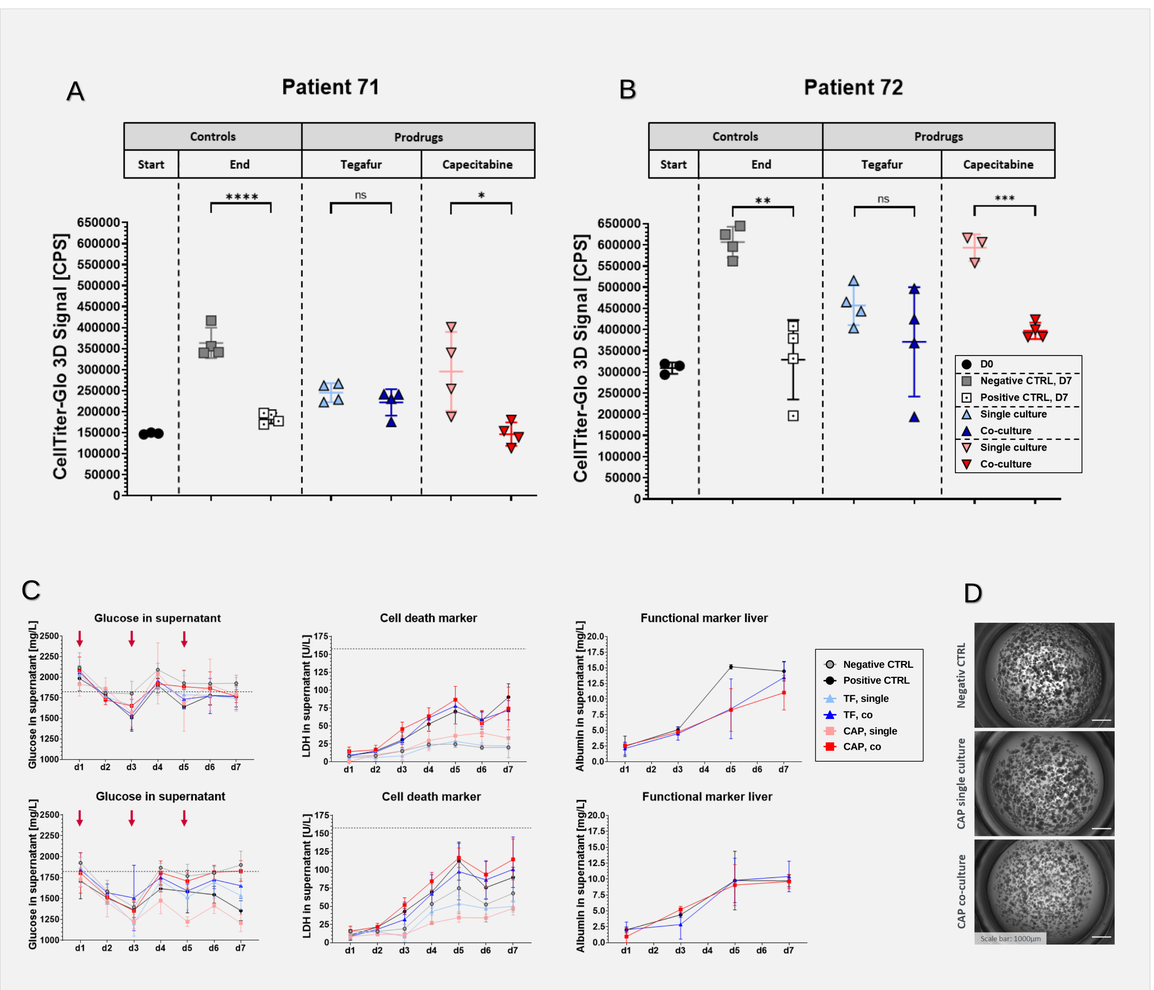

Fig. 5: Effect of two 5-FU prodrugs on two patient derived gastric cancer models in co-culture with liver model for 7 days. (A) Cell viability assay (CellTiter–Glo 3D) data of treated patient 71 with Tegafur (TF) and Capecitabine (CAP). **(B)** Cell viability assay data of patient 72. Values for (A, B) are a mean of 4 biological replicates ± SD; each value is a mean of 4 technical replicates. **(C)** Metabolic data at every medium exchange time point. Upper panel- patient 71 GC model; lower panel- patient 72 GC model. Values are a mean of 4 biological replicates ± SD. Arrows indicate 50% medium exchange. **(D)** Representative image of negative CTRL, single culture CAP and co-culture CAP for patient 72 on day 7.

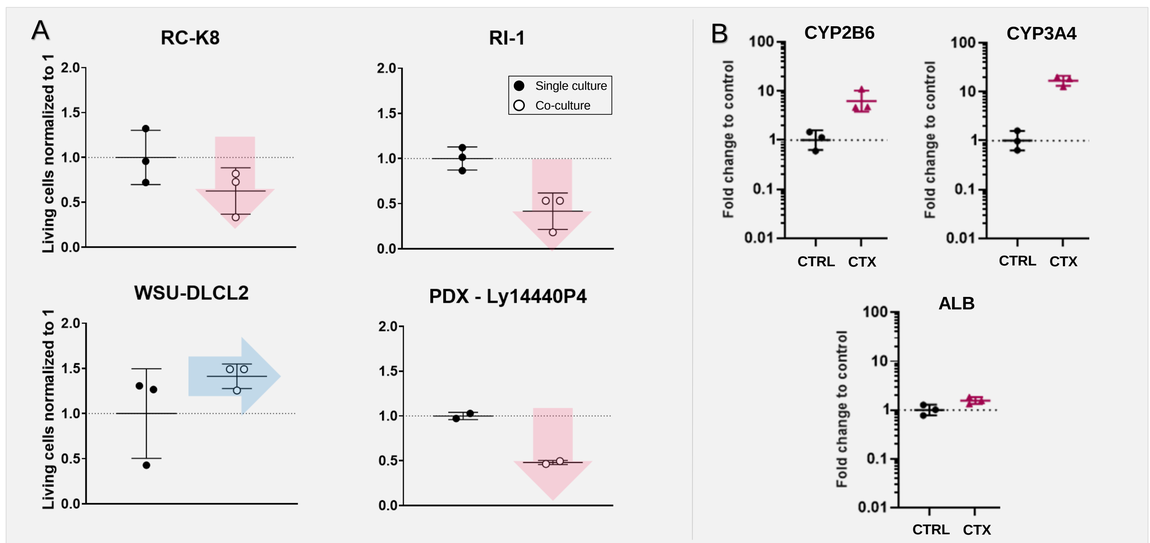

Fig. 4. Cyclophosphamide (CTX) biotransformation. (A) Effect of co-culture and different sensitivities towards Cyclophosphamide treatment of three different DLBCL cell lines and one PDX in dynamic culture over 7 days. Each replicate is shown and normalized to mean of living cell number. **(B)** Upregulation of major Cytochrome P450 enzymes responsible for biotransformation of CTX and functionality marker Albumin after 24h of substance exposure.

Summary and Outlook

Metabolism and distribution of compounds was demonstrated in this precision medicine platform on a variety of cancer models over a period of one week. In the future, prodrug absorption and metabolite excretion will be studied as well. Combining intestine, liver and kidney alongside with the cancer, the ADME-capable HUMIMIC Chip4 can be the MPS of choice. Adding relevant parts of the immune system will be an important step towards improved modeling of the patient's physiology. The standardization of assays and reduction of manual workload, which this automation system entails has enormous potential for future therapeutic approaches based on predictive medicine. A wide variety of combinations and concentrations of pharmaceuticals and immunotherapies should be tested and compared with clinical data on the corresponding patient, to further proof the reliability of this system.

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