

69 A HIGH CONTENT SCREENING PLATFORM FOR TESTING IMMUNOTHERAPIES IN PATIENT-DERIVED ORGANIDS

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Background Patient-derived organoids (PDOs) are advanced 3D cell culture models, which have high predictive value of patient drug responses in the clinic. In this study a high throughput screening platform is presented, which allows testing of cancer immunotherapies in co-cultures of human organoids with immune cells. Incorporating diverse cellular players from the immune system and stromal compartment, such as cancer associated fibroblasts (CAFs), allows the reconstitution of complex cellular interactions that occur in the tumor micro-environment (TME). Our proprietary, automated high content imaging (HCI)-based analysis provides visualization and quantification of immune cell-mediated effects in this complex physiological relevant 3D screening platform. Functional readouts such as migration of immune cells towards organoids, infiltration into the organoids and their killing are obtained, allowing for a better understanding of the immunomodulatory profile of immuno-oncology drugs.

Methods PDOs, obtained from HUB Organoid Technology, generated from different cancer indications among colon and ovarian were cultured in protein hydrogel. Partially HLA-matched immune cells isolated from different healthy PBMC donors were labelled and added (naïve or activated) to the 3D culture after incorporation of different suppressive populations, including pre-labelled CAFs and myeloid cells. High content microscopy combined with morphometric image analysis software was used to quantify the capacity of immune cells to infiltrate and subsequently kill the organoids.

Results Differences in the sensitivity and kinetics of organoid models from the same indication and from different indications towards the killing by immune cells was profiled. Pre-activation or reactivation of immune cells within the 3D co-cultures increased the sensitivity towards killing of the organoids, while incorporation of suppressive cellular players within the TME could reduce immune cell mediated killing effects. Moreover, testing of different immune cell donors, although showing slight donor-to-donor variability, indicated an overall consistency of organoid sensitivity towards immune cell-mediated killing effects. Additionally, quantitative readouts strictly dependent on a 3D environment such as immune cell migration and infiltration could be measured and analyzed upon treatment with different immune modulators such as T cell engagers.

Conclusions HCI of complex *in vitro* 3D co-cultured organoids enables rapid, reproducible, physiologically relevant and spatial readouts for testing various cancer immunotherapies within different cancer indications. Visualization and quantification of these complex cellular interactions within the TME via our high content screening platform is a powerful tool for immunotherapeutic drug development in patient-relevant models to select the most promising candidates, better understand their mechanism of action, and ultimately stratify patient cohorts for clinical success.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0069>