77 ANALYZING EFFECTS OF IMMUNOTHERAPIES, SUCH AS BISPECIFIC ANTIBODIES AND CELLULAR THERAPY, TARGETING EGFR EXPRESSION WITH A HIGH CONTENT SCREENING PLATFORM IN PATIENT-DERIVED ORGANOIDS

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Background While several immunotherapies have shown promising results for specific cancer indications in the clinic, the full potential and mechanisms that regulate the anti-tumor immune response remain to be uncovered. Patient-derived organoids (PDOs) used in advanced 3D co-cultures with immune cells offer good preservation of the original patient tumor and allow reconstitution of complex cellular interactions that occur in the tumor microenvironment. In this study, a high content imaging (HCI)-based analysis that allows testing of cancer immunotherapies in a physiological relevant 3D screening platform is presented.

Methods PDOs from different cancer indications, including colorectal, lung, breast, melanoma, and ovarian, were cultured in protein hydrogels. PBMCs isolated from healthy donors were labeled and added (naïve or activated with superantigens or CD3/CD28 beads) to the 3D cultures in the presence of immunotherapies, such as monoclonal and bispecific antibodies, or treated with CAR T cells alone targeting EGFR expression on the PDOs. Co-cultures were analyzed for immune cell migration, infiltration, and PDO killing via our proprietary image analysis platform, which segments tumor objects and immune cells in a 3D reconstituted stack.

Results Differences in the sensitivity of organoid models from different indications towards immune cell-mediated killing were measured by reduced organoid volumes and counts. Pre-activation or reactivation of immune cells within the 3D co-cultures increased the killing of the organoids. Moreover, targeting EGFR expression with either monoclonal or bispecific antibodies enhanced the immune cell-mediated killing effects on EGFR expressing PDOs. Additionally, quantitative readouts that are strictly dependent on a 3D environment, such as immune cell migration and infiltration, could be measured and analyzed.

Conclusions The platform of complex *in vitro* 3D co-cultured organoids presented here enables rapid, reproducible, physiologically relevant, and spatial readouts for testing various cancer immunotherapies. With our broad biobank of >600 PDOs, these assays are available for at least 17 different cancer indications. In combination with our HCI platform, immune cell-mediated tumor killing could be quantified and correlated with immune cell migration and infiltration, providing a deeper understanding of the mechanism of action of immunotherapies. Moreover, it allows the identification of specific subsets of tumors that share genetic or morphological characteristics, that could benefit from the therapy. Overall, the preclinical testing of cancer immunotherapies will benefit from the platform presented, bringing the promise of immunotherapies to more indications and patients.

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