DEVELOPMENT OF A 3D ORGANOID AUTOLOGOUS TIL CO-CULTURE PLATFORM FOR HIGH THROUGHPUT IMMUNO-ONCOLOGY STUDIES

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Background The preclinical screening of immune-modulatory therapies suffers from the absence of models that recapitulate in vivo heterogeneous tumor microenvironment (TME). 3D tumor organoid cultures provide a model that closely mimics in situ tumor architecture and is being aggressively used to evaluate therapeutic efficacy ex vivo. A vastly heterogeneous TME impacts patient treatment response, and there is a dearth of human tumor models (2D or 3D), that mimic in vivo diversity of TME, including infiltrating immune populations. 3D organoid cultures typically contain neoplastic epithelium; however, they fall short in representing tumor to tumor-infiltrating lymphocytes (TILs) interactions, limiting their ability to generate a clinically relevant response to immunotherapeutics. Addition of immune cells from unrelated donors to organoids can simulate that microenvironment but is complicated by T cell alloreactivity. Here we describe 3D patient-derived xenograft organoid (PDXO) co-cultures with matching autologous human TILs to recapitulate the tumor-specific immune response, leveraging confocal high content analysis and luminex multiplex assays. This platform allows the evaluation and high throughput screening of novel immune targeting agents to determine impacts on patient-derived T cell function, T cell infiltration, and tumor cytotoxicity.

Methods Surgical resections from patients were used to generate patient-derived xenografts and tumor-infiltrating lymphocytes in parallel. PDX were dissected and digested to establish PDXO. TILs and organoids from the same patient were fluorescent labeled and cultured together for four days to evaluate tumor infiltration and drug cytotoxicity in 3D cultures. CellInsight CX7 high content imaging platform was used to trace TILs and cancer cells and evaluate T cell infiltration and tumor cell killing in the presence and absence of immunomodulatory therapies.

Results PDXO were established to mimic in vivo tumor biology. Tumor-specific TILs were successfully characterized by flow cytometry. Co-culture resulted in TIL infiltration in organoids from day one in culture and increased over four days. Cytotoxicity and TIL infiltration were quantified using fluorescent dyes via high throughput imaging platform. Significantly enhanced TIL infiltration was observed in autologous co-cultures compared to non-autologous co-cultures. The established unique autologous PDXO immune organoid co-cultures could be used as an improved simulation of the modulatory activity of therapeutic agents in patient-specific T cells against their own tumors.

Conclusions Patient autologous TILs – PDXO co-culture platform is an advanced model for evaluating 1H therapies with the tumor-specific immune microenvironment. The platform provides an opportunity for precision medicine and high throughput drug screening of immuno-modulatory therapies.

Ethics Approval The study was approved by Champions Oncology’s Institutional Animal Care and Use Committee (IACUC).

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Background T-cell based immunotherapies such as CAR-T, bispecific mAb, transgenic T cells and checkpoint blockade have profound efficacy in multiple tumor types but share a common limitation – target antigen (Ag) escape. One approach to address this limitation has been therapy directed at a ‘parallel’ target (e.g. CD22 after CD19 loss), however, these lineage markers are frequently lost together. Here, we describe an alternate, broadly applicable, approach: potentiating fasL/fas-signaling to increase localized bystander killing of Ag tumor cells and thereby prevent Ag escape.

Methods We used a CRISPR/Cas9 library to screen for tumor expressed molecules that inhibit or facilitate T-cell killing. We then evaluated one candidate -fas using murine transgenic T cells, murine and human CAR-T cells, bispecific mAb redirected PBMC, and tumoral RNAseq data from a large CAR-T clinical trial.

Results GFP-specific (JEDI) CD8 T cells were co-cultured with on-target (GFP*) and bystander (mCherry*) lymphoma cells that had been transfected with a CRISPR/Cas9 library; this screen revealed several tumor-expressed candidate molecules inhibiting or facilitating T-cell killing. Notably, we observed a marked dependence on fas for on-target tumor killing and then, surprisingly, an exquisite dependence on fas for localized bystander tumor killing. Because bystander tumor killing appeared critically fas-dependent, we hypothesized that potentiating fas-signaling might increase bystander killing. An in vitro screen of small molecules that modulate fas-pathway revealed several candidates, including inhibitors of histone deacetylases (HDAC), inhibitors of apoptosis proteins (IAP) and Bcl-2 family members in murine and human systems (figure 2). To validate these candidates, we demonstrated that HDACi increased GFP-specific T cell killing of both on-target and bystander lymphoma cells, in a completely fas-dependent manner (figure 3). Similar ly, using a bispecific antibody-based system, we demonstrated increased, fas-dependent, T cell killing of both on-target and bystander human lymphoma cells with inhibitors of IAP and bcl-2 family members (e.g. MCL1).