Perfused patient-derived tumor organoid models with autologous immune cells for preclinical drug development

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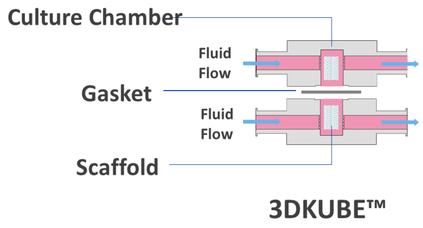
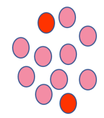
Background

The paradigm shift towards inclusion of primary patient samples in preclinical drug development bottlenecks at the tissue procurement step due to an on-going lack of 'research-ready' tumor samples. Organoid technology was developed as a solution to this hurdle. Their ability to expand in culture makes them a renewable resource similar to cell lines while recapitulating key properties of the primary tissue, including tumor heterogeneity, structural organization and drug responses. As preclinical drug development continues to adopt organoid-based screening technology and immune-oncology agents continue to be advanced, there is high demand for more complex organoid-based models capable of more accurately predicting individual patient drug responses. Due to their development process, the vast majority of organoids do not contain immune cells, resulting in the need to utilize allogeneic immune cells for any immune cell involved interrogation of the models. Maintaining the immune cells for incorporation with the organoid model can overcome this hindrance. Additionally, perfused organoid models offer a complex, ex vivo model with the ability to replicate the mechanical forces imparted by blood flow and interstitial pressure. Compared to 2D and static 3D cultures, perfused 3D culture models show enhanced proliferative capacity and can be sustained as long-term cultures. In our previous studies, DNA methylation signatures in patient-derived breast microtumors grown in Kiyatec's 3DKUBE™ perfusion bioreactor matched the primary tissues of derivation. Given the need for more complex biology when assessing immune-oncology agents, the physiological relevance in maintaining gene expression *ex vivo,* and the capability to sustain long-term viability, the impact of autologous immune cells and perfusion on the success of organotypic culture was investigated across a panel of primary patient tumors. Immune cells were profiled for status and activity and viability assays were used to evaluate static and perfused 3D organoid models for differences in viability and morphology. Drug responses were measured using our KIYA-Predict™ platform, comparing organoid drug responses to primary tissue drug responses. Results of this study provide insight on alternative methods of organotypic culture and evidence for the utilization of more complex tissue modeling in preclinical drug development.

Methods

Perfused Cell Culture



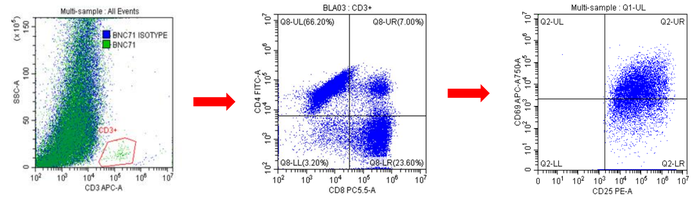


1° Patient Tumor Organoids

Patient-derived organoids (PDOs) were cultured in the 3DKUBE™ perfusion bioreactor as static (sMT) or perfused (pMT) microtumors.

Viability was measured at Days 0,7 and 14

T-cell Characterization

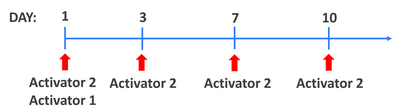


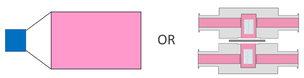
CD3+ | CD4 CD8 | CD25 CD69 CD279

Isolated and expanded T cells were characterized based on CD marker expression

T-cell Expansion

Autologous CD3+ TILs





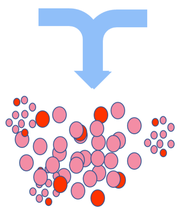
STATIC PERFUSION

Autologous CD3+ T cells were cultured under static and perfused conditions under activating conditions

Cells were counted after 3-, 7- and 10-days culture.

T-cell Activation

1° Patient Tumor Organoids



Autologous CD3+ TILs

Flow Cytometry Assay for T-cell Activation

- CD107a Degranulation
- TNFα production
- Activation Markers: CD137, CD154

Co-culture

T cell reactivity toward tumor was assayed to compare the effects of static and perfusion-based expansion protocols on T cell functionality

Matched Organoid-Immune Cells

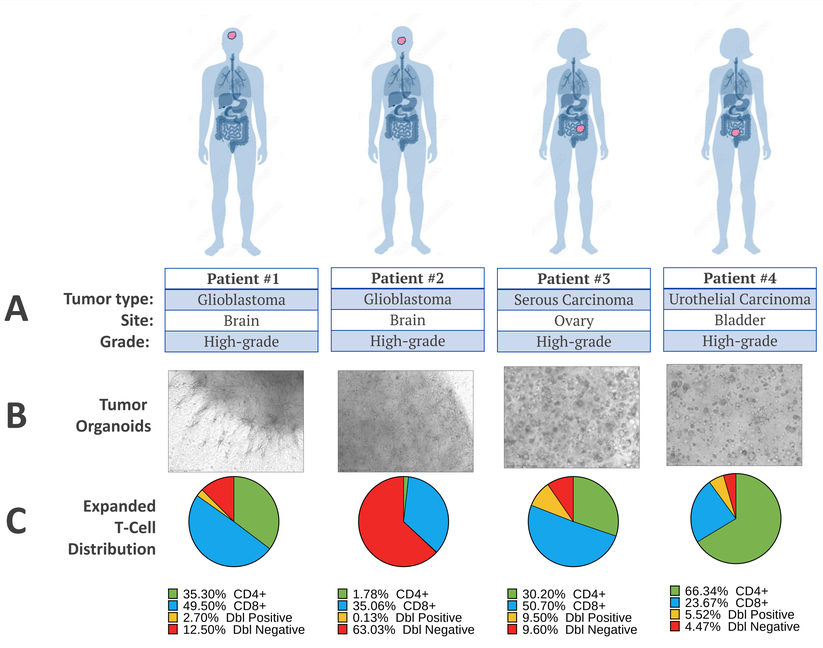


Figure 1. Patient and Tissue Characteristics (**A**) Characteristics of primary patient samples used for the derivation of organoids and autologous T-cells. (B) Brightfield (4x magnification) images of the resulting organoid samples and (**C**) phenotypic distribution of T-cell types in expanded CD3+ TIL's isolated from primary patient tissues.

Perfusion Cell Culture

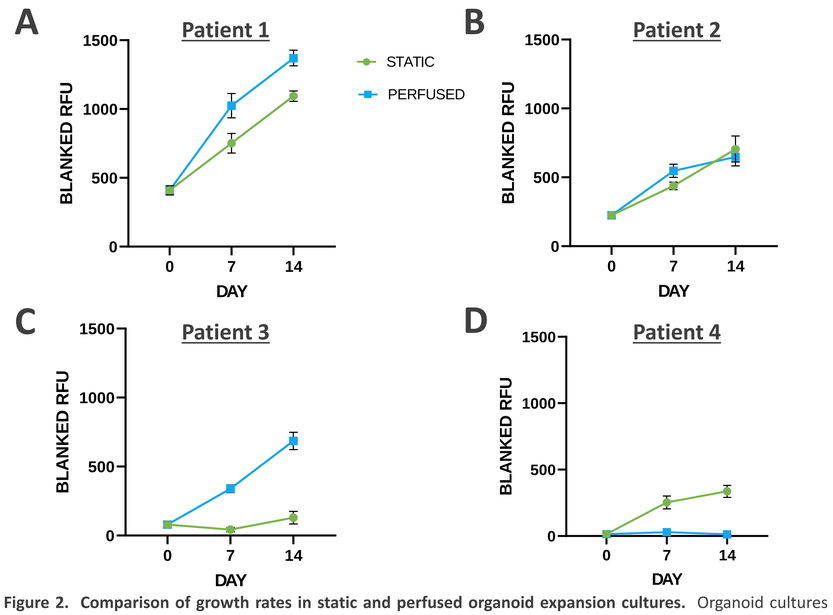


Figure 2. Comparison of growth rates in static and perfused organoid expansion cultures. Organoid cultures were seeded as microtumors and cultured under static (green line) and perfused (blue line) flow conditions. Baseline viability measurements were performed at Day 0 and compared to subsequent readings obtained at days 7 and 14 for each culture condition. Results are mean of 4 independent scaffolds/ condition. (**A**) Patient 1, glioblastoma organoids (**B**) Patient 2, glioblastoma organoids (**C**) Patient 3, ovarian serous carcinoma organoids (**D**) Patient 4, urothelial carcinoma organoids

T-cell Activation

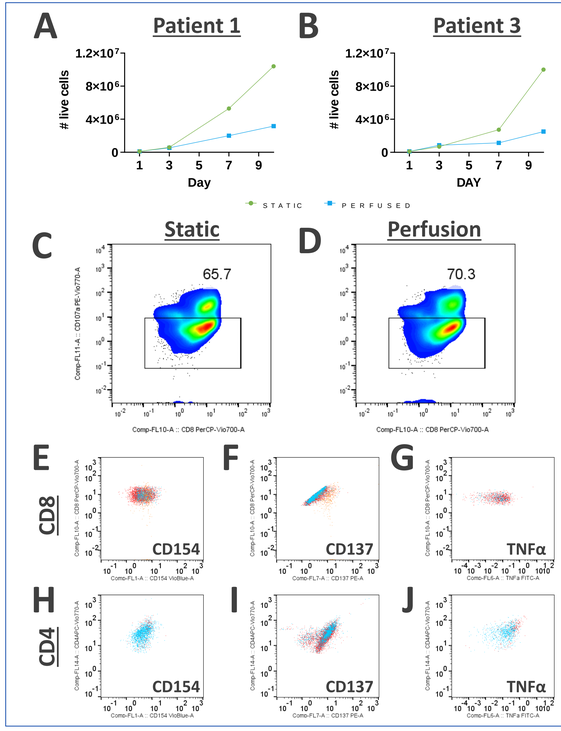


Figure 3. Expansion rate and tumor reactivity in perfusion cultured autologous T cells. Autologous T cells were expanded as static (green line) or perfusion (blue line) cultures for 10 days. Representative growth curves are shown for Patient 1 (**A**) and Patient 3 (**B**). Tumor cell reactivity was measured by flow cytometry. Representative plots are shown for Patient 1. T cell degranulation was estimated based on loss of CD107a staining in (**C**) static and (**D**) perfusion cultured T cells. Additional activation markers were assayed in CD8+ (**E-G**) and CD4+ (**H-J**) T cell populations. Static (red dots) and perfusion (blue dots) cultures showed similar levels of activation following 16h co-culture with autologous tumor organoid cells. (x,y) = E: CD154, CD8; F: CD137,CD8; G: TNF alpha, CD8; H: CD154, CD4; I: CD137, CD4; J: TNF alpha, CD4.