3D Tissue Models to Predict Patient-Specific Responses to immuno-Oncology Agents

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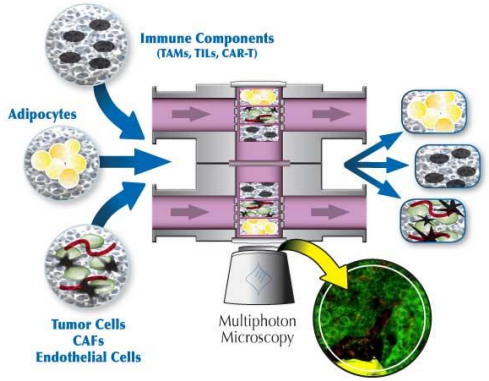
Abstract

While immuno-oncology based therapies are rapidly becoming the treatment of choice for many tumor types, the assay systems to accurately test them in preclinical development are lacking. This is primarily due to the complexity and function of the immune system and its interaction with tumors in terms of cell types, cellular recruitment and organization, and microenvironment. The ultimate *in vitro* systems would 1. Combine patient specific tumor cells with autologous immune cells to counteract cytotoxicity due to interactions between allogeneic lymphocytes and tumor cells, 2. Be designed to force recruitment of lymphocytes and macrophages by the tumor cells and their microenvironment, 3. Promote macrophage polarization and lymphocyte activation that recapitulates the patient's tumor by recapitulating the microenvironment, and 4. Result in tumor cell death that correlates to clinical response, biomarker expression (PD-1, PD-L1, CTLA-4, etc.), and therapy mechanism of action. To develop these systems, we have focused our work on numerous areas with 2 presented here, 1. Checkpoint inhibitors, pembrolizumab and Ipilimumab, in solid tumors such as ovarian cancer and melanoma, and 2. Lymphocyte and Macrophage migration, activation, and polarization in breast cancer. For checkpoint inhibitor studies, we have screened primary ovarian cancer tissues, melanoma, and matching lymphocytes for the expression of PD-1, PD-L1, and CTLA-4 by histology and flow cytometry. Both negative and positive tissues have then been utilized in 3D tissue models to examine the effects of drug upon the tumor cells and the other cell types within the model. We have seen correlation between Pembrolizumab binding to lymphocytes and PD-1 expression and shown further correlation to the expression of PD-L1 on the matching tumor cells and drug efficacy. In breast cancer we have used our complex, multi-cell type models to examine macrophage and lymphocyte migration and recruitment into the tumor. We have also examined the polarization of macrophages in these systems and their impact upon tumor cell viability with the tumor cells promoting M2 macrophage polarization and the M2 macrophages promoting tumor cell viability compared to M1 macrophages. Secreted cytokines as measured by multiplex technology have also supported an immune protective environment with M2 macrophages as seen by changes in IFNγ, IL-10, and TNFα compared to the presence of M1 macrophages. Our data to date reveals that these complex 3D in vitro models have the ability to recapitulate *in vivo* biology and biomarkers correlated drug response. These models can be used to both predict individual patient response to immune-oncology agents and test new agents for efficacy in a preclinical setting.

Models



Solid Tumor Response to Checkpoint Inhibitors

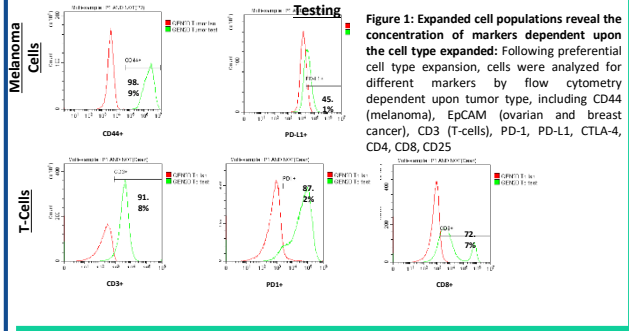


Figure 1: Expanded cell populations reveal the concentration of markers dependent upon the cell type expanded: Following preferential cell type expansion, cells were analyzed for different markers by flow cytometry dependent upon tumor type, including CD44 (melanoma), EpCAM (ovarian and breast cancer), CD3 (T-cells), PD-1, PD-L1, CTLA-4, CD4, CD8, CD25

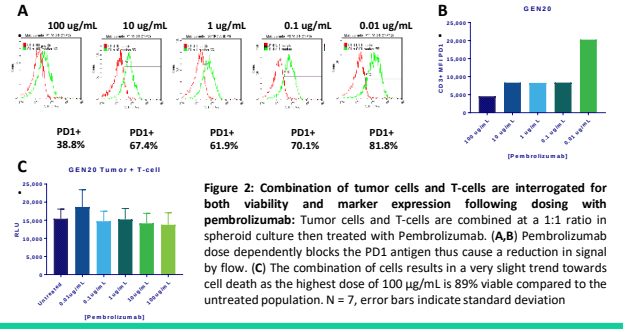
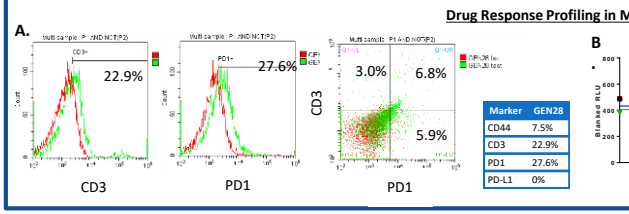


Figure 2: Combination of tumor cells and T-cells are interrogated for both viability and marker expression following dosing with pembrolizumab: Tumor cells and T-cells are combined at a 1:1 ratio in spheroid culture then treated with Pembrolizumab. (**A**,**B**) Pembrolizumab dose dependently blocks the PD1 antigen thus cause a reduction in signal by flow. (**C**) The combination of cells results in a very slight trend towards cell death as the highest dose of 100 µg/mL is 89% viable compared to the untreated population. N = 7, error bars indicate standard deviation



Marker	GEN28
CD44	7.5%
CD3	22.9%
PD1	27.6%
PD-L1	0%

Drug	GEN28
Pembrolizumab	>100
Ipilimumab	>100
Epacadostat	37.28
Vemurafenib	169.4

Figure 3: Direct drug response profiling: Directly Following tumor dissociation, cells were analyzed for cell markers as mentioned in figure 1 (**A**). This particular patient had a relatively large population of CD3+/PD1+ dual positive cells. (**B**) Immediately following dissociation, the cells were allowed to form spheroids and were then dosed for 72 hours. While there was no response to the PD1 and CTLA-4 inhibitors, there was a response to both Epacadostat and Vemurafenib. N=7.

Immune Cell Migration In Vitro

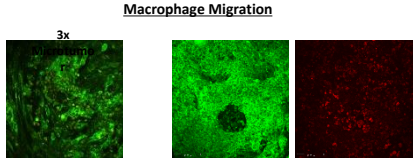


Figure 4: 3D Microtumors were examined by multiphoton microscopy for proliferation and macrophage migration: Tumor cells (green) and fibroblasts were grown in their own scaffold in perfusion culture for 1 week after which a second scaffold was combined with them in the culture chamber containing PKH26 stained CD14+ PBMCs. After 2 weeks in culture the macrophages had invaded into the tumor compartment.

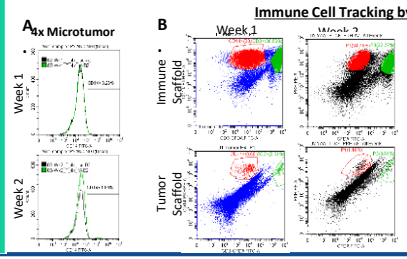


Figure 5: 3D Microtumors were dissociated and analyzed for cell markers by flow cytometry to examine both immune cell migration and CD14+ cell differentiation: (**A**) 4x microtumors consisted of a fat scaffold, a tumor/fibroblast scaffold and an immune cell scaffold containing only CD14+ monocytes. (**B**) 5x microtumors included T-cells in the immune scaffold. Additionally, for the 5X, the monocytes were labeled with PKH26 (red) and the T-Cells with CFDA (green). After 1 and 2 weeks in culture, the tumor/fibroblast scaffolds were dissociated and examined for monocytes by CD14+ (4x) or monocytes and T-cells by their fluorescent labels (5x). For both models, infiltration increased with time.

Conclusions

- We can isolate and expand both tumor cells and immune cells without marker-based selection bias successfully in ovarian cancer, melanoma, and rare tumors.
- Pembrolizumab effectively blocks PD1 antigen sites in a dose dependent manner in isolated and expanded melanoma T-cells in 3D spheroid cultures within 72 hours, We have also seen this result in ovarian cancer.
- We can track T-cell and macrophage migration within our 3D microtumors via multiphoton microscopy and flow cytometry

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With Special Thanks to the Biorepository

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