Engineered 3D tissues facilitate preclinical immunotherapy studies in fully human platforms

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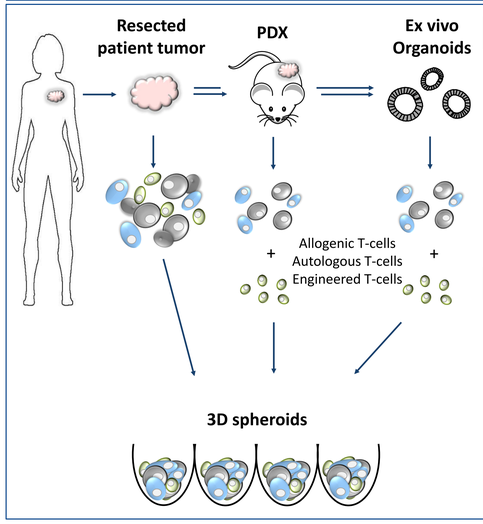
Background

Cancer treatment has evolved with the advent of immunotherapies. This drug class now plays a key role in supplementing or even replacing chemotherapies as first line treatments. Immunotherapies such as immune checkpoint inhibitors, bispecific antibodies, and chimeric antigen receptor T-cells (CAR-T) are just a few of the therapeutic strategies currently utilized to promote an anti-cancer response. Preclinical testing of current and novel immune modulators is necessary to expand our knowledge of these drugs and better predict their efficacy. Animal models have numerous deficiencies and complications that make them insufficient for accurate representation of human response, including an insufficient human immune system and a lack of genomic heterogeneity. Thus, there is an urgent need to develop preclinical models that incorporate human tumors and autologous immune cells together to create an accurate microenvironment for effective monitoring of tumor and immune cell interactions. We have developed a tumor-type agnostic platform in which 3D cell cultures are engineered with cells from resected human tumors. This *ex vivo* model not only recapitulates the tumor immune microenvironment (TIME), but it includes multiple downstream read-outs to address a single question such as whether an agent or combination of agents stimulates an immune response resulting in an anti-tumor effect. Our model can incorporate tumor and immune cells directly from the TIME or tumor cells from patient-derived xenografts or organoids which are then supplemented with allogenic or autologous immune cells or even CAR-T cells. Immune response can be detected via T-cell activation, degranulation assays, and immune cell-mediated tumor cell killing which can be multiplexed with anti-tumor activity assays. We can measure a reduction in viable tumor cells via flow cytometry following treatment or at increasing effector to target cell ratios, detect fluctuations in production and secretion of granzyme B indicating T-cell cytotoxic activity, and evaluate reduced cell viability for synergy assessments of drug combinations. We have detected increased secretion of IFNγ with combination drug treatment compared to single agent treatment. Macrophage polarization can be monitored with co-culture of CD14+ cells with tumor cells in our system inducing an M2 phenotype from an M1 phenotype. Finally, we can detect dendritic cell maturation following stimulation by measuring increased expression of MHC class II and CD103. Taken together this model system supports complex cell-cell interactions necessary to detect immunotherapy response and represents an ideal preclinical testing platform.

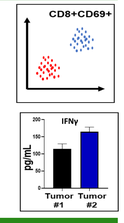
3D spheroid models and assays



T-cell activation

Read-outs:

1. Activation markers
2. Cytokine production/secretion
3. Degranulation assay
4. T-cell subset evaluation
5. Immune cell proliferation & clustering

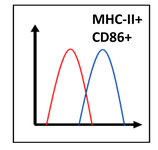


Dendritic cell activation

Read-outs:

Increased expression of dendritic cell markers:

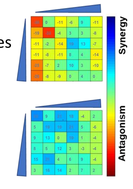
CD45+/CD11C+/HLA-DR+/CD86+



Immune cell-mediated cytotoxicity

Read-outs:

1. Expression of cytolytic molecules
2. Specific tumor cell killing
3. Determine T-cell dependence
4. Monitor 3D spheroid morphology over time
5. Synergy assessment



Macrophage polarization

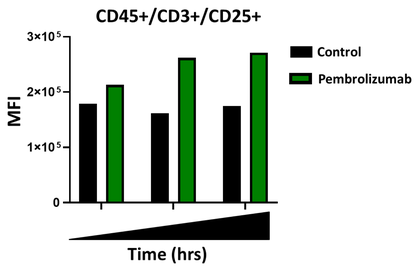
Read-outs:

Increased expression of macrophage markers:

<u>M1 markers:</u>
CD45+/CD14+/HLA-DR+/CD86+
<u>M2 markers:</u>
CD45+/CD14+/CD168+/CD206+

T-cell activation

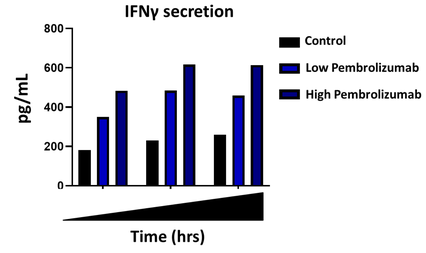
1. Detection of activation markers



Method: A primary ovarian cancer tissue sample was evaluated for change in the T-cell activation marker CD25 following pembrolizumab treatment using flow cytometry.

Results: An increase in CD25 on T-cells following pembrolizumab treatment at three time points was observed.

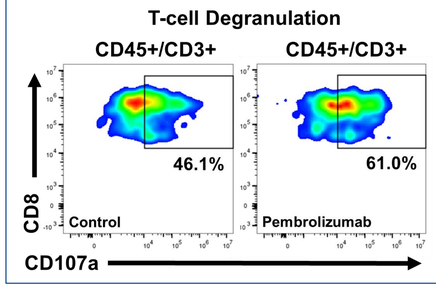
2. Detection of secreted cytokines



Method: A primary ovarian cancer tissue sample was evaluated for secretion of IFNγ following pembrolizumab treatment.

Results: An increase in secreted IFNg in a concentration dependent manner at three time points was observed

3. Degranulation Assay

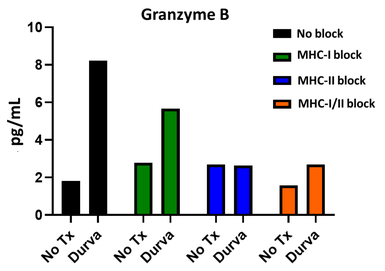


Method: A primary ovarian cancer tissue sample was evaluated for T-cell degranulation following pembrolizumab treatment.

Results: Degranulation was detected in CD8+ T-cells after treatment with pembrolizumab.

Immune cell-mediated cytotoxicity

1. Expression of cytolytic molecules

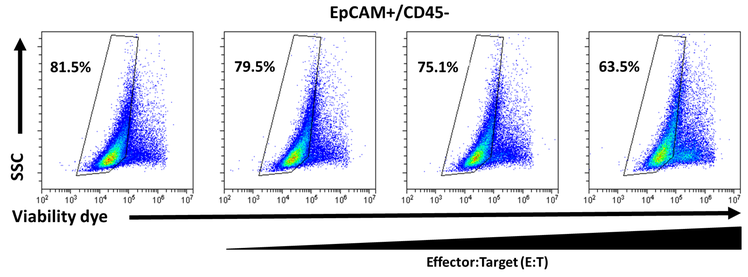


Method: A primary NSCLC tissue sample was treated with MHC blocking antibodies then stimulated with durvalumab. Supernatants were evaluated for secreted granzyme B.

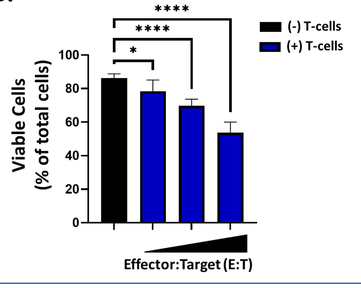
Results: MHC-II blocking abolished durvalumab mediated granzyme B secretion demonstrating this patient specific durvalumab response is MHC class II restricted.

2. Specific tumor cell killing

A.



B.

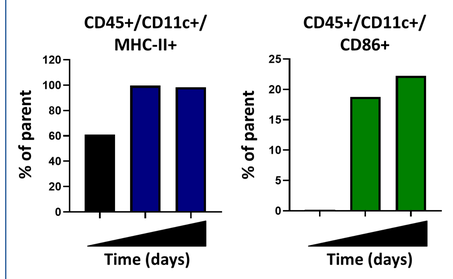


Methods: Reduced viable tumor cells were detected via flow cytometry **(A)** at increasing E:T ratios. This was directly correlated to a reduction in viable cells as measured in a 384-well plate-based assay **(B)**.

Results: Our platform supports a plate-based read-out as a good proxy to direct measurement of tumor cell viability while also allowing for flow-based measurements. *p=0.02; ****p<0.0001

Modulation of antigen presenting cell function

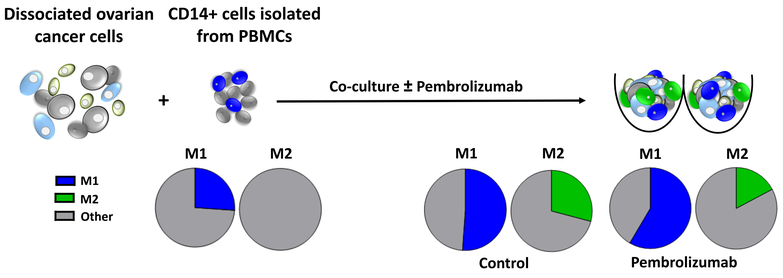
1. Dendritic cell activation



Method: Dendritic cells from a primary ovarian cancer tissue sample were monitored over the course of multiple days for changes in antigen presenting protein abundance.

Results: Dendritic cells from this patient sample are functional and increase expression of antigen presenting proteins in 3D culture.

2. Macrophage polarization



Method: Determine if primary tumor cells co-cultured with monocytes can induce macrophage polarization.

Results: Co-culture polarizes macrophages and pembrolizumab treatment shifts the phenotypes to M1>M2 compared to control.

Conclusions

- KIYATEC's 3D model systems support complex cell-cell interactions necessary to detect immunotherapy response
- KIYATEC's primary cell derived 3D model systems can be used to measure more than just tumor cell death