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Dissecting personalized PD-1 inhibitor efficacy using patient-derived 3D spheroids

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Background

Agents that invoke an immune response against tumors, such as immune checkpoint inhibitors (ICIs), have revolutionized cancer treatment. Understanding the efficacy of these agents involves more than just the characterization of patient-specific tumor cells, but also the supporting cells, such as immune cells, that encompass the tumor microenvironment (TME). Typically, selection of cancer therapy involves evaluation of the genomic landscape and expression profiling of the tumor cells alone. However, the composition of the TME has been shown to be a critical determinant in patient response to immune modulators. Thus, *ex vivo* profiling of ICI response for functional precision medicine should include a platform that provides a working replica of the patient's TME. We have developed a model utilizing all cells resident in a patient's tumor to engineer 3D spheroids for ICI response profiling. To highlight the importance of the TME, we sought to detect PD-1 inhibitor efficacy using tumor tissue from a cancer patient whose immune cells express PD-L1, but tumor cells are negative for PD-L1 and positive for MHC class I and II. To determine the role of immune and stromal cells in response to PD-1 inhibitor, we treated spheroids that were depleted of EpCAM+ cells. While PD-1 inhibitor increased cytosolic interferon gamma (IFNγ) and granzyme B in both CD4+ and CD8+ T-cells, the degree of response was decreased in the CD8+ T-cells suggesting CD4+ T-cell response is tumor cell independent but still regulated via the PD-1/PD-L1 signaling axis. To test the effect of MHC class I and class II in response to PD-1 inhibitor, MHC class I and class II blockade experiments were conducted. Only MHC class II blockade had a negative effect on CD4+ T-cell granzyme B expression, but that effect was abrogated by treatment with PD-1 inhibitor. MHC class I and II blockade individually blocked any impact of PD-1 inhibitor upon granzyme B expression in CD8+ T-cells. We then measured T-cell activation and cytokine secretion in relation to tumor cell killing following PD-1 inhibitor treatment. We found substantially increased CD25 on T-cells over the course of 96 hours but only modestly increased CD69 at 24 hours and 48 hours. Furthermore, we observed a concentration dependent increase in granzyme B and IFNγ secretion that peaked at 72 hours. We found treatment decreased EpCAM+ tumor cells at timepoints after 24 hours. These data demonstrate response from PD-1/PD-L1 blockade can be elicited in a tumor cell independent manner indicating a role for the TME in ICI efficacy. This work provides evidence that *ex vivo* recreation of the TME is critical to detect and accurately predict patient response to ICI therapies.

Pembrolizumab activates T-cells and decreases viable tumor cells

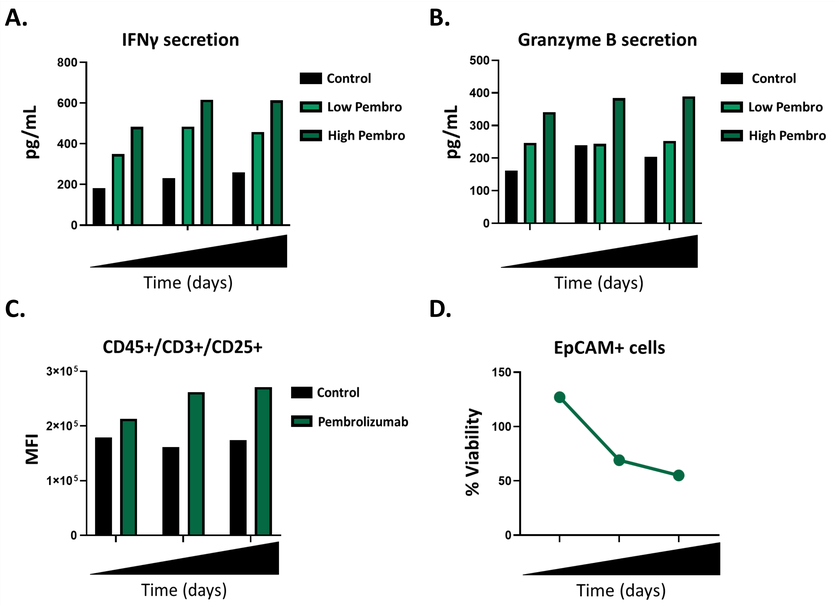


Figure 2. Detection of Pembrolizumab efficacy in ex vivo 3D cultures. 3D spheroids composed of primary human tumor cells and autologous immune cells were treated with pembrolizumab and monitored for the secretion of **(A)** IFNγ and **(B)** granzyme B over the course of multiple days. Treatment with pembrolizumab induced the dose-dependent secretion of both IFNγ and granzyme B. Additionally, at each time point, the 3D spheroids were monitored via flow cytometry for activated T-cells using the activation marker CD25 **(C)** and the presence of viable EpCAM+ tumor cells **(D)**. Treatment with pembrolizumab at high concentration resulted in activated T-cells and loss of viable tumor cells.

Pembrolizumab induces tumor cell independent CD4+ T-cell activation

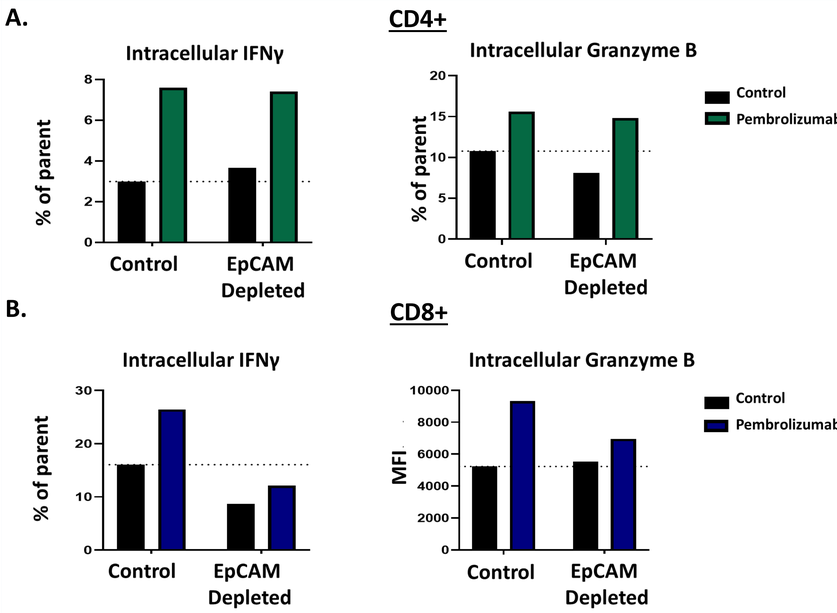
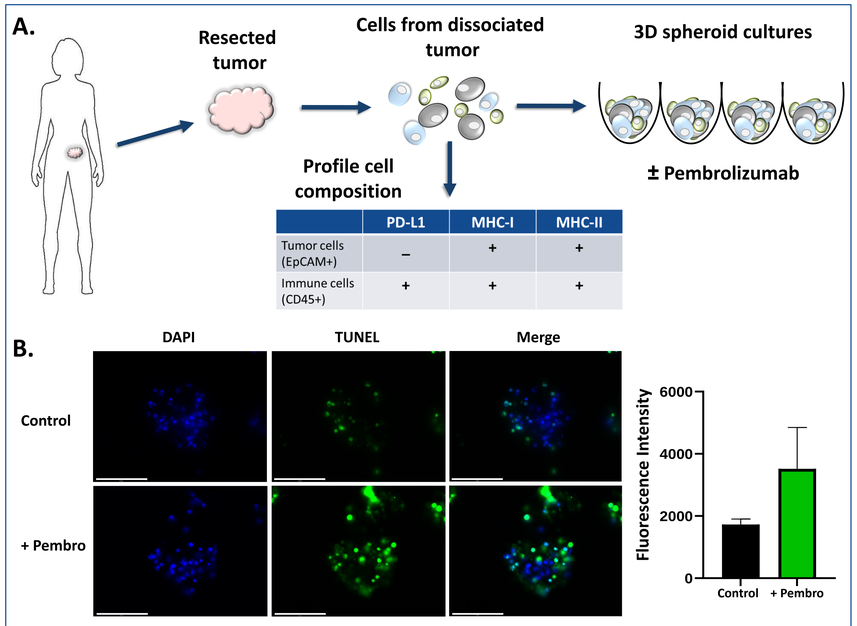


Figure 4. T-cell subsets are activated differently in the presence of pembrolizumab. The role of tumor cells in pembrolizumab dependent activation of **(A)** CD4+ or **(B)** CD8+ T-cells was evaluated by depleting EpCAM+ cells from the primary tissue. 3D spheroids were treated with pembrolizumab then cytokine production was monitored using flow cytometry. CD8+ T-cell activation is negatively impacted by tumor cell depletion. This implies that the tumor cells are likely involved in the activation of CD8+ T-cells. CD4+ T-cell activation is not impacted when tumor cells are depleted but CD4+ T-cells are regulated via the PD-1/PD-L1 signaling axis.

3D Model System



	PD-L1	MHC-I	MHC-II
Tumor cells (EpCAM+)	–	+	+
Immune cells (CD45+)	+	+	+

Figure 1. Patient–derived 3D spheroids for the profiling of immune checkpoint inhibitor efficacy. (A) Resected tumors are dissociated into single cells then re-engineered into 3D spheroids. A portion of the cells are evaluated for the presence of tumor cells, immune cells, and proteins involved in immune checkpoint blockade response. **(B)** Spheroids were treated with pembrolizumab for then stained for cell death using TUNEL. Scale bar = 75 µm. Fluorescence intensity was calculated,n=2.

Pembrolizumab stimulates CD4+ T-cells which increases their helper function

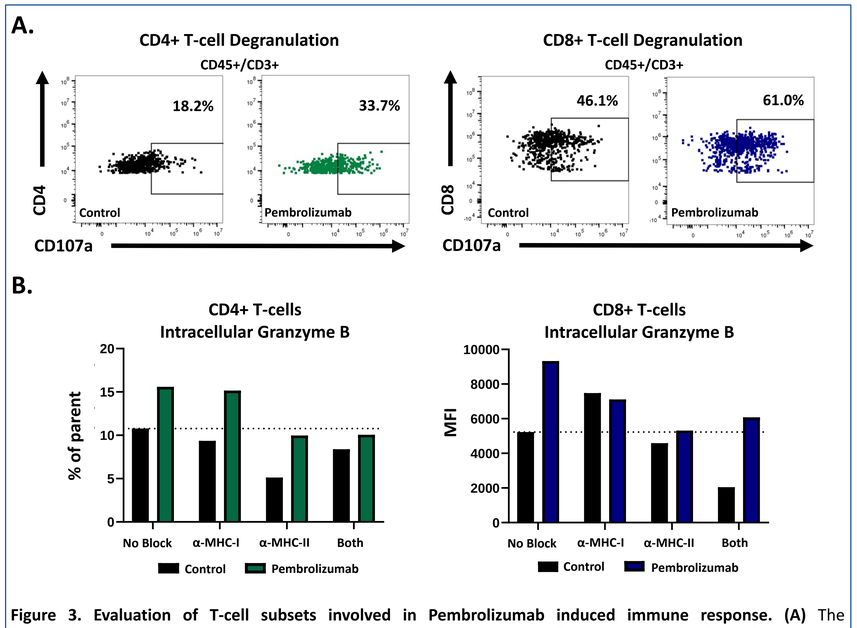
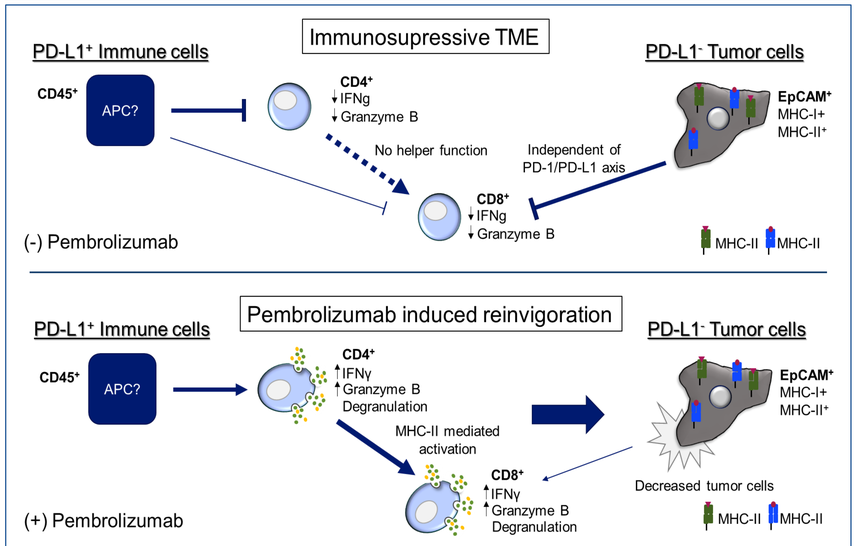


Figure 3. Evaluation of T-cell subsets involved in Pembrolizumab induced immune response. (A) The degranulation marker CD107a was detected on CD4 and CD8 positive T-cells following pembrolizumab treatment. The contribution of MHC class I and MHC class II was determined using blocking antibodies following pembrolizumab treatment. MHC class I blockade did not impact CD4+ T-cells but MHC class-II blockade did reduce basal and pembrolizumab induced granzyme B production. MHC class-II blockade completely blocks CD8+ T-cell granzyme B production suggesting CD4+ T-cells contribute to their activation.

Conclusions



- **KIYATEC's 3D models can be used to interrogate the mechanism of action of immune checkpoint inhibitors**
- **Pembrolizumab induced reinvigoration is CD4+ T-cell dependent.**
- **Pembrolizumab liberates CD4+ T-cell inhibition from PD-L1 expressing immune cells.**
- **CD4+ T-cell activation results in increased helper function leading to enhanced CD8+ T-cell activation and reduced tumor cell viability.**