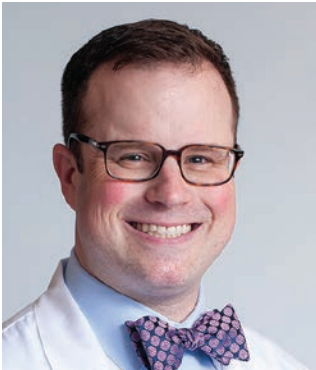


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Immunotherapy has transformed the treatment of metastatic melanoma and other cancers, allowing a new avenue of therapeutic options and prolonging lives of many patients. Unfortunately, while immunotherapy is highly effective in some patients, it does not work for every patient and there are no available tests to determine whether or not a patient will respond to immunotherapy before treatment begins. To understand why immunotherapy works for some patients and not others, **the Jenkins laboratory** uses sophisticated tools and techniques to study and investigate the complex and dynamic interactions between cancer cells and the immune system. Our solution to this problem involves a specialized 3-dimensional culture of a patient's own tumor enabling researchers to examine interactions between tumor cells and immune cells. The integration of this novel approach with other emerging technologies is helping us navigate the complex landscape of the tumor immune microenvironment and learn which patients will respond to immunotherapy as well as how to effectively treat cancer patients that do not respond immunotherapy alone.

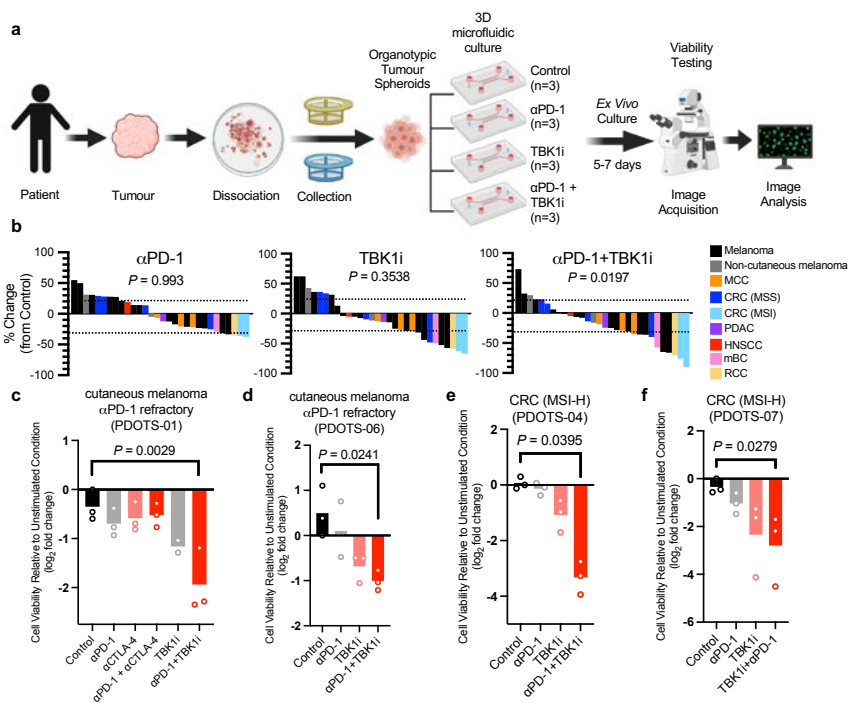
Precision cancer medicine currently focuses on knowledge of the cancer mutation repertoire and the tailored application of drugs that target altered genes or pathways in individual patients, such as use of BRAF inhibitors in patients with BRAF mutant melanoma. Immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway have shown dramatic and durable clinical responses in melanoma and others cancers, but robust predictive biomarkers are lacking and innate resistance is common. Thus, a critical need exists for more sophisticated ex vivo functional testing modalities that recapitulate human tumor biology to predict response to targeted and immune-based therapies and to develop personalized treatment plans in real-time.

Major focus areas of the Jenkins lab include (1) identifying and characterizing mechanisms of response and resistance to PD-1 blockade, (2) discovering novel therapeutic strategies to overcome resistance to PD-1 blockade, and (3) using the MDOTS/PDOTS as a functional precision

medicine platform for the development of novel combinations, and ultimately, personalized immunotherapy to tailor immunotherapy treatment to individual patients. Improved understanding of the response to immune checkpoint inhibitors within the tumor microenvironment will facilitate efforts to identify predictive biomarkers/models for immune checkpoint blockade in real-time, as well as future efforts to screen for therapeutic combinations that enhance the response to immune checkpoint blockade, and may ultimately provide a platform for the 'personalization' of immunotherapy.

Our novel approach for evaluating ex vivo response to PD-1 blockade utilizes murine- and patient-derived organotypic tumor spheroids (MDOTS/PDOTS) cultured in a 3-dimensional microfluidic system.

We demonstrated that organotypic tumor spheroids isolated from fresh mouse and human tumor samples retain autologous lymphoid and myeloid cell populations, including antigen- experienced tumor



TBK1 inhibition enhances sensitivity to PD-1 blockade using PDOTS. a, Schematic of PDOTS preparation. b, Waterfall plots for PDOTS (n = 30, indicated tumor types) treated with anti-PD-1 (250 µg/ml pembrolizumab), TBK1i (1 µM) or combined anti-PD-1 + TBK1i. Mean values (bars) for each sample are shown. Statistical analysis was performed using one-way ANOVA (matched) with Dunnett's multiple-comparison test compared with the control. MCC, Merkel cell carcinoma; CRC, colorectal cancer; MSS, microsatellite stable; PDAC, pancreatic ductal adenocarcinoma; HNSCC, head and neck squamous cell carcinoma; mBC, metastatic breast cancer; RCC, renal cell carcinoma. (ref: Sun et al., Nature 2023)

infiltrating CD4 and CD8 T lymphocytes, and respond to PD-1 blockade in short-term ex vivo culture (Jenkins et al., *Cancer Discovery* 2018; PMID: 29101162).

Our findings demonstrated the feasibility of ex vivo profiling of PD-1 blockade and offer a novel functional approach for the selection of immunotherapeutic combinations. The ultimate goals of these efforts are to identify and characterize novel features of response/resistance to PD-1 blockade and to identify novel therapeutic strategies to overcome resistance to anti-PD-1 therapy, ultimately to bring forward into human clinical trials.

Recently, we identified the innate immune kinase TANK-binding kinase 1 (*TBK1*) as a candidate immune-evasion gene in a pooled genetic screen. Using a suite of genetic and pharmacological tools across multiple experimental model systems, we confirm a

role for *TBK1* as an immune-evasion gene. Targeting *TBK1* enhances responses to PD-1 blockade by decreasing the cytotoxicity threshold to effector cytokines (TNF and IFN γ). *TBK1* inhibition in combination with PD-1 blockade also demonstrated efficacy using patient-derived tumor models, with concordant findings in matched patient-derived organotypic tumor spheroids and matched patient-derived organoids. Tumor cells lacking *TBK1* are primed to undergo RIPK- and caspase- dependent cell death in response to TNF and IFN γ in a JAK-STAT-dependent manner. Taken together, our results demonstrate that targeting *TBK1* is an effective strategy to overcome resistance to cancer immunotherapy.

Selected Publications:

Sun Y, Revach O-Y, Anderson S, Kessler EA, Wolfe CH, Jenney A, et al. Manguso RT, **Jenkins RW**. Targeting *TBK1* to overcome resistance to cancer immunotherapy. *Nature*. 2023;615:158–67.

Revach OY, Liu S, **Jenkins RW**. Targeting TANK-binding kinase 1 (*TBK1*) in cancer. *Expert Opin Ther Targets*. 2020 Nov;24(11):1065-1078.

Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, **Jenkins RW**, Lieb DJ, Chen JH, Frederick DT, Barzily-Rokni M, Freeman SS, Reuben A, Hoover PJ, Villani AC, Ivanova E, Portell A, Lizotte PH, Aref AR, Eliane JP, Hammond MR, Vitzthum H, Blackmon SM, Li B, Gopalakrishnan V, Reddy SM, Cooper ZA, Paweletz CP, Barbie DA, Stemmer-Rachamimov A, Flaherty KT, Wargo JA, Boland GM, Sullivan RJ, Getz G, Hacohen N. Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma. *Cell*. 2018 Nov 1;175(4):998-1013.

Aref AR, Campisi M, Ivanova E, Portell A, Larios D, Piel BP, Mathur N, Zhou C, Coakley RV, Bartels A, Bowden M, Herbert Z, Hill S, Gilhooley S, Carter J, Cañadas I, Thai TC, Kitajima S, Chiono V, Paweletz CP, Barbie DA, Kamm RD, **Jenkins RW**. 3D microfluidic ex vivo culture of organotypic tumor spheroids to model immune checkpoint blockade. *Lab Chip*. 2018 Oct 9;18(20):3129-3143.

Jenkins RW, Aref AR, Lizotte PH, et al. Ex Vivo Profiling of PD-1 Blockade Using Organotypic Tumor Spheroids. *Cancer Discov*. 2018;8(2):196-215.

Deng J, Wang ES, **Jenkins RW**, et al. CDK4/6 Inhibition Augments Antitumor Immunity by Enhancing T-cell Activation. *Cancer Discov*. 2018;8(2):216-33.