

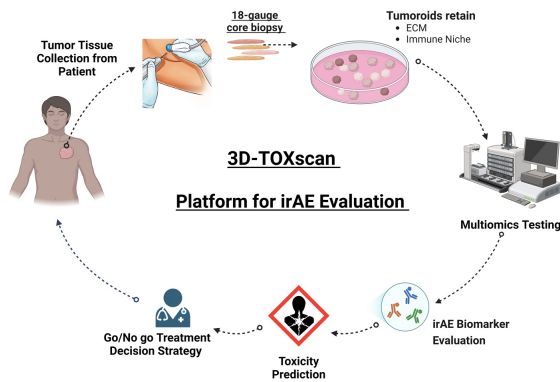
Background

Immunotherapy has revolutionized cancer treatment, yet the emergence of immune-related adverse events (irAEs) poses significant challenges to its widespread adoption. Predicting and managing irAEs remains a critical aspect of patient care. Tumoroid models, mimicking the tumor microenvironment, offer a promising platform to study these events in a controlled setting. In this study, we utilized tumoroid models composed of patient-derived cancer cells and immune cells to simulate the dynamic interactions between the tumor and the immune system. By subjecting these models to immune-stimulating agents, including checkpoint inhibitors, we monitored the development of irAEs while characterizing immune cell infiltration, cytokine release patterns, and tumor growth dynamics. Our results demonstrate a correlation between specific features of the tumoroid model and the occurrence and severity of irAEs observed in clinical settings. Utilizing machine learning algorithms, we developed predictive models capable of anticipating the likelihood and severity of irAEs associated with different immunotherapies and patient profiles. These models offer insights into the underlying mechanisms driving irAEs and facilitate the optimization of treatment strategies to minimize adverse events while maximizing therapeutic efficacy. Thus, tumoroid models represent a valuable tool for predicting irAEs associated with immunotherapy. By integrating patient-specific data and computational approaches, we can advance towards personalized immunotherapy regimens that balance efficacy with safety. This approach holds promise for improving patient outcomes and accelerating the translation of immunotherapeutic interventions into clinical practice.

Why use 3D-TOXscan for preclinical safety and efficacy evaluation?

Nilogen Oncosystems' 3D TOXscan plays a crucial role in advancing therapeutic toxicity screening through the use of ex vivo 3D tumoroid models. These models replicate the complexities of the tumor microenvironment, providing a unique opportunity to evaluate treatment-related side effects and improve personalized treatment approaches. With their close resemblance to physiological conditions, these models accurately depict how drugs interact within tumors and surrounding tissues, offering valuable insights into potential immune responses during the early stages of drug development. Consequently, 3D-TOXscan not only improves the screening of therapeutic agents but also holds promise for safer and more tailored treatment methods, ushering in a new era in precision oncology.

Nilogen Oncosystems' 3D-TOXscan platform



Questions can be explored using this model:

- What are the underlying mechanisms that link irAEs to antitumor responses?
- What are the implications of stopping immune checkpoint blockade (ICB) or using immunosuppression when irAEs occur?
- Is it viable to refine preclinical models of irAEs post-ICB by leveraging clinical data and modeling techniques to enhance our comprehension of irAE mechanisms?

High PD-L1 TPS qualifies patient for PD-L1 targeted therapy

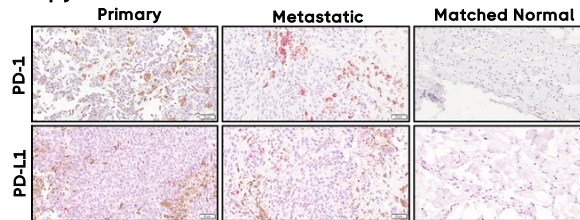


Figure 1: Immunohistochemistry (IHC) staining for PD-L1 in primary and metastatic melanoma. Images show moderate to intense PD-L1 staining (4+2+3), with a tissue positivity score (TPS) of 70-90% in both primary and metastatic melanoma tissues. Minimal to no PD-L1 staining was observed in the tumor-adjacent normal tissue derived from same patient.

3D-TOXscan recapitulates clinical observations

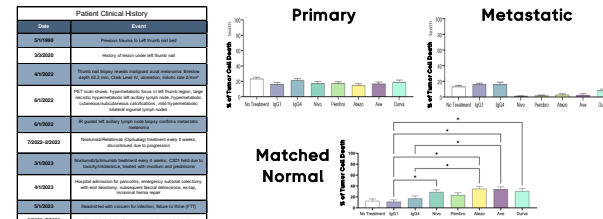


Figure 2: Clinical history of the patient showing resistance to immune checkpoint inhibitor. **Figure 3:** Treatment-Mediated Tumor Cell Killing (TCK) Activity 48 Hours Post-Treatment in Primary, Metastatic, and Matched Normal Melanoma Samples. Minimal response is observed in both primary and metastatic melanoma tissues across all treatments, with no significant changes in tumor cell killing. In contrast, matched normal samples show notable cell death, indicating a toxic response. Bars represent mean \pm SEM. Asterisks indicate *p < 0.05.

Secretome profiling reflects clinical observations

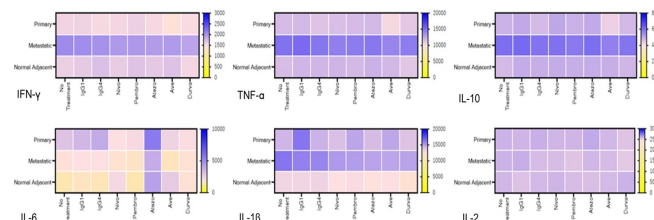


Figure 4: This figure displays heatmaps representing the expression levels of various cytokines (IFN- γ , TNF- α , IL-10, IL-6, IL-1 β , and IL-2) in primary, metastatic, and normal adjacent tissues across different treatment conditions. The color gradient in each heatmap indicates cytokine expression levels, with yellow representing lower expression and blue indicating higher expression levels. These heatmaps allow for a comparative analysis of how different immunotherapies modulate cytokine production in various tissue types, providing insights into immune responses under each treatment condition.

Comparison of Different Therapeutic Response Using High Content Imaging

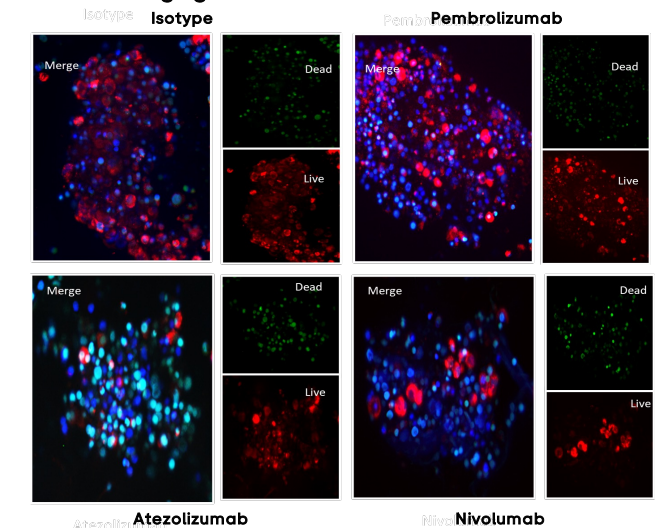


Figure 5: Fluorescence microscopy images comparing the effects of isotype control, Pembrolizumab, Atezolizumab, and Nivolumab on cell viability. Each treatment is represented with merged images, highlighting nuclei (blue), and separate panels showing dead cells (green) and live cells (red). Pembrolizumab and Nivolumab demonstrate a higher proportion of dead cells (green), suggesting greater efficacy in reducing cell viability, while Atezolizumab shows a more balanced distribution of live and dead cells, indicating a different therapeutic effect.

	Set 1		Set 2		Set 3		Set 4		Set 5	
	Melanoma	Matched normal	NSCLC	Matched normal	Melanoma	Matched normal	RCC	Matched normal	RCC	Matched normal
Nivo	1	N	0	N	0.5	N	1	N	0.5	Y
Pembro	0.5	N	0	N	0.5	N	0	Y	0	N
Atezo	0	N	0	N	0.5	N	1	N	0	N
Nivo +Atezo	0	N	n/a	n/a	n/a	n/a	0.5	N	0	N
Pembro +Atezo	0.5	N	n/a	n/a	n/a	n/a	0	N	0	N

Tumor Response score	Complete Response	1
	Partial Response	0.5
	No Response	0
Matched normal Toxicity Score	Toxicity	Y
	No Toxicity	N

Table 1: Tumor Response and Toxicity Evaluation in Various Cancer Types with Different Therapeutic Regimens. The table summarizes tumor response scores (Complete Response: 1, Partial Response: 0.5, No Response: 0) and toxicity outcomes (Y: Toxicity, N: No Toxicity) across different cancer models (Melanoma, NSCLC, RCC) treated with various immunotherapies, including Nivolumab (Nivo), Pembrolizumab (Pembro), Atezolizumab (Atezo), and their combinations. Response and toxicity are assessed in both cancerous and matched normal tissues.