3D-PREDICT - An *Ex vivo* Precision Oncology and Clinical Trial Enrichment Platform

NILOGEN ONCOSYSTEMS

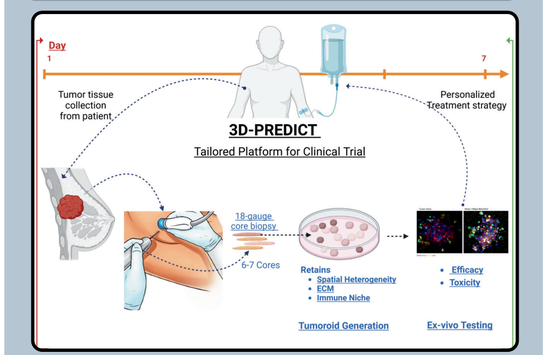
Background

Immuno-oncology (IO) therapy using checkpoint inhibitors has shown superior response rates, response duration, and overall survival compared to chemotherapy. However, accurately predicting the optimal FDA-approved IO therapies, pharmaceutical agents, combinations, and delivery sequences for individual patients remains a significant challenge in personalized medicine.

Nilogen Oncosystems' 3D-PREDICT is an innovative *ex vivo* therapeutic investigative platform. It offers a functional model of a patient's tumor to directly assess susceptibility to various therapeutic approaches. Derived from core biopsies, the 3D-PREDICT model maintains tumor heterogeneity and preserves the tumor microenvironment, including stromal components and cell-cell and cell-extracellular matrix interactions.

Using the 3D-PREDICT platform, we compared the efficacy of different immunotherapeutic approaches across various solid tumor indications *ex vivo*.

3D-PREDICT Platform



This method allows forward-looking assessment of treatments like cytotoxic agents and immunotherapy drugs for cancer. Healthcare professionals can use it to accurately match patients with the most effective and least harmful treatments, improving outcomes. Additionally, it can reduce healthcare costs by minimizing trial and error in treatment selection, leading to evidence-based decisions tailored to individual patient needs and better overall outcomes.

Ethics Approval: This study was approved by Vanderbilt University Ethics Board; approval number 031078 and Ohio State University Ethics Board: 2014J0130

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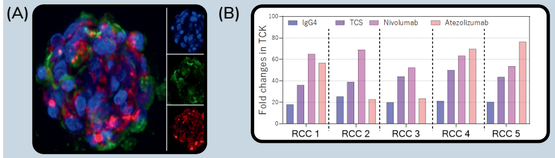
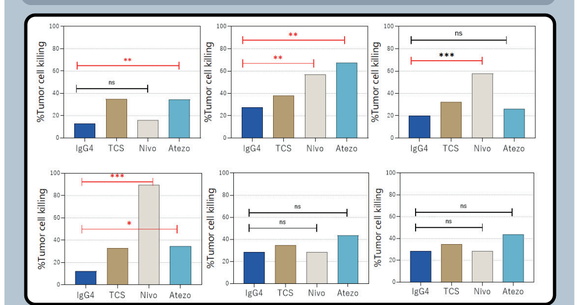
3D-PREDICT Platform Optimization



Optimization of the 3D-PREDICT Platform. (A) Tumoroids from 18-gauge core biopsies from renal cell carcinoma patients were generated. Scale bar, 100 µm. (B) Pooled tumoroids were treated *ex vivo* with various standard of care options as indicated. Treatment-mediated tumor cell killing (TCK) activity was evaluated using high content confocal imaging and Nilogen's proprietary algorithm for data analysis. Quantification of relative TCK under indicated treatment conditions.

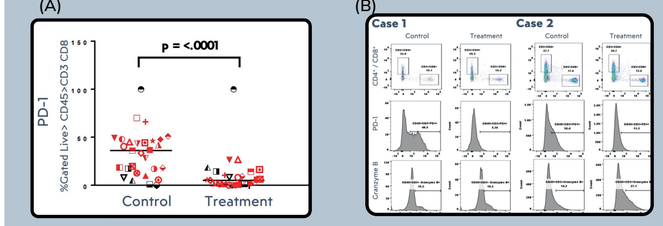
The 3D-PREDICT *ex vivo* tumoroid platform presents a crucial resource in the realm of clinical trials by offering a distinctive means to forecast patients' responses to treatments. This platform holds the potential to serve as a pivotal clinical diagnostic tool, aiding healthcare professionals in guiding clinical care effectively. Additionally, it can facilitate the stratification of patient recruitment into clinical trials based on their predicted response to treatments.

3D-PREDICT Platform Sensitivity



Sensitivity of the 3D-PREDICT Platform. Tumoroids from 18-gauge core biopsies from different solid tumor patients were generated. Pooled tumoroids from the different tumors were then treated *ex vivo* with various standard of care options as indicated (*TCS*, T-cell stimulator; *Nivo*, nivolumab; *Atezo*, atezolizumab). TCK activity was evaluated using high content confocal imaging and Nilogen's proprietary algorithm for data analysis. Quantification of relative TCK under indicated treatment conditions. *p<0.05, **p<0.01, ***p<0.001, ns, p>0.05.

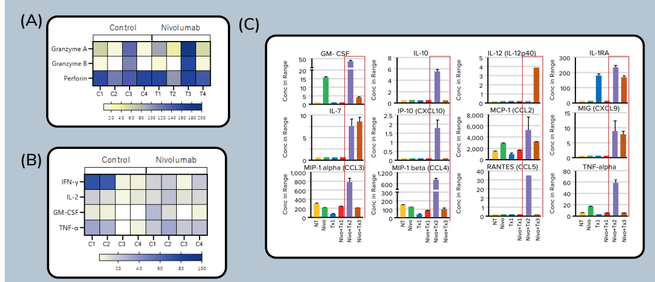
Immune Response Using the 3D-PREDICT Platform



Immunophenotyping of NSCLC tumoroids post-treatment. (A) In a 30-sample cohort of tumoroids generated from NSCLC patients, pembrolizumab treatment downregulated expression of PD-1 in CD3+CD8+ T cells.

(B) In tumoroids generated from two different NSCLC patients, a similar distribution of CD4+/CD8+ T cells was observed when we compared control and pembrolizumab-treated conditions. We assessed PD-1 occupancy using an antibody that binds to the same epitope as pembrolizumab. If pembrolizumab is bound to PD-1, our flow antibody cannot bind. In both cases, we observed a robust decrease in PD-1 occupancy following treatment with pembrolizumab. Concurrently, we observed an increase in T cell activation as assessed by Granzyme B expression, with a moderate increase in Case 1 and a robust increase in Case 2.

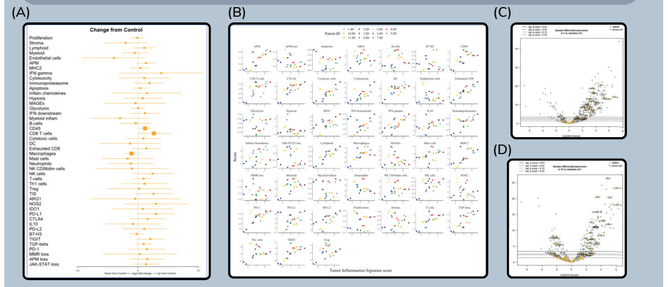
Inflammatory Response Signature using the 3D-PREDICT Platform



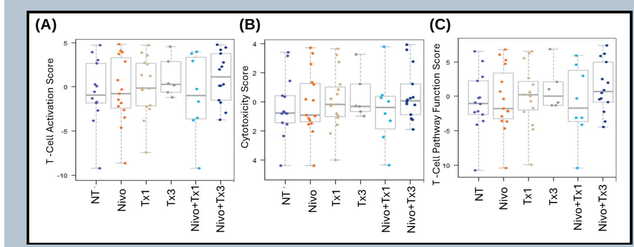
Treatment-mediated immune cell activation was observed with 3D-Predict platform. Multi-analyte inflammatory cytokine signature comparison was performed in harvested post-treatment supernatants using the Meso Scale Discovery U-PLEX multiplex assays. (A, B) Granzyme A/B and perforin (A), and IFN-g, TNF-α, IL-2 and GM-CSF (B) exhibited differential response in NSCLC patients (n=4). (C) Array of pro inflammatory analytes were evaluated in nivolumab mono and combo therapy treated tumoroids generated from NSCLC patients (n=10) derived tumoroids showing differences in tumor responsiveness.

Ex vivo 3D tumor models represent a powerful tool for identifying biomarkers of therapeutic toxicity and advancing personalized cancer treatment.

Biomarker Discovery Using the 3D-PREDICT Platform



Gene expression profiles in nivolumab-treated NSCLC samples (n=10) exhibited differences with tumor responsiveness. (A) Forest plot shows differential mRNA expression detected using NanoString's IO 360 panel. (B) Comparative signature plot depicts tumor inflammation signature score in the different samples. (C, D) Volcano plots showing treatment-induced differential expression of cytotoxicity related (C) and chemokine response (D) genes.



Pathway score profiles in nivolumab mono and combo therapy treated patients-derived tumoroids show differences with tumor responsiveness. We compared pathway enrichment scores under different treatment conditions using Box plots. We observed differences in scores for T-cell activation status (A), cytotoxic gene expression (B), and T-cell pathway function (C) when compared between treatment groups.

We can incorporate assays that can be utilized to capture drug-mediated changes in expression to lead clinically relevant biomarker discovery.

Summary

- The 3D-PREDICT platform provides a unique resource to predict patients' response to treatment, potentially serving as a clinical diagnostic to guide clinical care and stratifying patient recruitment into clinical trials.
- This would allow prospective assessment of therapeutic efficacy *ex vivo* to match each patient to the most effective and least toxic therapy, to improve outcomes, reduce costs, and assign therapeutics on a more rational basis.