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Evaluation of NXI-101 in non-small cell lung cancer (NSCLC) using patient-derived organotypic tumor spheroids (PDOTS): Targeting a novel tumorigenic pathway

Julia Vail[1], Hyung Gun Maeng[2], Ji Woong Jang[2], Kyungjin Boo[2], Ji-In Youn[2], Danielle Hagee[1], Emma Cui[1], Alyssa Martin[1], Aaron Goldman[3], Kyoung Wan Yoon[2], Michael A. Perricone[1]
[1]Xsphera Biosciences, Inc., Boston, MA, [2]NEX-I, Seoul, Korea, Republic of, [3]Harvard Medical School, Boston, MA

Background

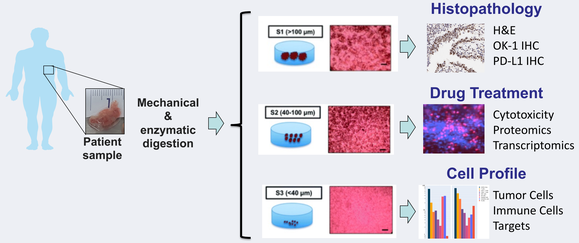
About Xsphera PDOTS Platform

Patient-derived organotypic tumor spheroids (PDOTS), developed at the Dana-Farber Cancer Institute (Boston, MA) and commercialized by Xsphera Biosciences, Inc. (Boston, MA), offer a powerful preclinical model for evaluating efficacy, mechanism of action, and patient response variability to test articles (1,2). PDOTS encompass key components of the tumor microenvironment (TME), in particular immune cells, making them an ideal model for assessing the performance of immunotherapeutics and investigating related drug resistance and immune evasion mechanisms.

About NXI-101

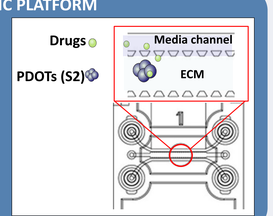
ONCOKINE-1 (OK-1), a soluble protein released by tumor cells, is thought to contribute significantly to immunotherapy resistance by suppressing tumor apoptosis and dendritic cell (DC) activation while elevating TGF-β levels. To counteract this, NEX-I (Seoul, South Korea) has developed NXI-101, a monoclonal antibody aimed at neutralizing OK-1. Expected outcomes of NXI-101 treatment include increased tumor cell death, enhanced DC activation and M1 polarization, and suppression of resistance mechanisms, offering a promising avenue for improving immunotherapy outcomes in cancer patients.

The Xsphera Platform



THE MICROFLUIDIC PLATFORM

- Hundreds of spheroids in each treatment channel
- Each spheroid contains tumor, stromal and immune cells and ECM components
- The platform preserves each patient's tumor microenvironment (TME) containing tumor cells and the immune contexture



Live tumor samples collected from patients diagnosed with Non-Small Cell Lung Cancer (NSCLC) are dissociated into spheroids using a mechanical and enzymatic digestion process. Patient derived organotypic tumor spheroids (PDOTS) 40 µm - 100 µm diameter are loaded into microfluidic devices containing an extracellular matrix to support the tumor microenvironment of the PDOTS. Following treatment with therapeutics, PDOTS cytotoxicity, growth, cytokine expression and RNA transcription are determined to study the post-treatment effects on tumor growth and immune responses. All graphs were produced in the Xsphera Cloud.

Results

Cytotoxicity and Target Expression

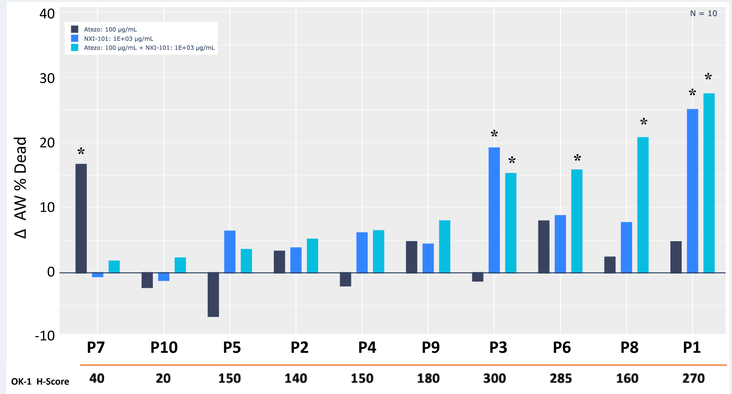


FIGURE 1. NXI-101 and NXI-101 + atezo elicited cytotoxic effects on select patient tumors. Change in area-weighted % dead (Δ AW % Dead) of NSCLC PDOTS expressed as difference from the media control. 3 days post-treatment with atezolizumab (atezo; anti-PD-L1), NXI-101 or NXI-101/atezo combination. The combination treatment elicited significant ($p<0.05$) cytotoxicity in 4 of 10 patient tumors. These tumors (P1, P3, P6, P8) were designated as "Responsive" tumors. While OK-1 H-Scores were correlated with NXI-101 cytotoxic response (Figure 5), PD-L1 H-Scores did not correlate with atezolizumab cytotoxic response. While atezo had 1 of 10 response (P7) by AW % Dead, atezo significantly reduced the live spheroid areas in 5 of 10 (data not shown).

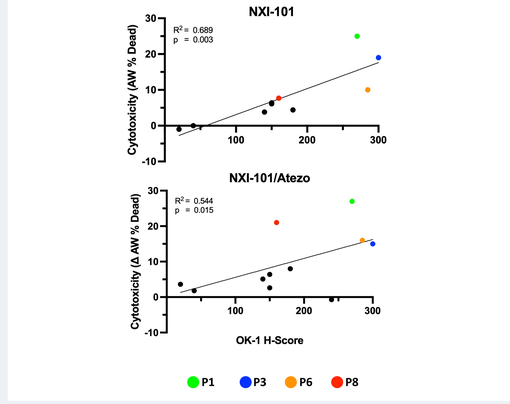


FIGURE 2. The cytotoxicity observed with NXI-101 was directly correlated with baseline target expression level. Simple linear regression of the cytotoxic response induced by NXI-101 with the baseline OK-1 H-Score ($p = 0.003$). The cytotoxicity values for NXI-101 were expressed as the difference in area-weighted % dead values from the control.

Molecular Pathways Associated with Response

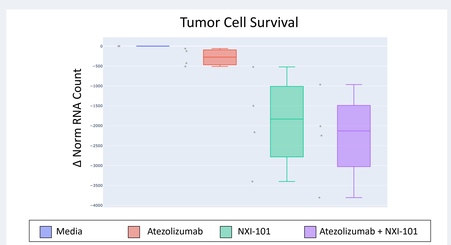


FIGURE 3. **Cytotoxicity is associated with decreased tumor cell survival in the cytotoxic responsive patient tumors.** Tumor cell associated RNA counts were decreased post-treatment relative to media in patients P1, P3, P6, P8. This indicates a decrease in tumor cell abundance. Both NXI-101 and NXI-101/atezo treatment groups had marked decreases. There were no differences in tumor associated RNA counts in the non-responsive patient tumors (data not shown).

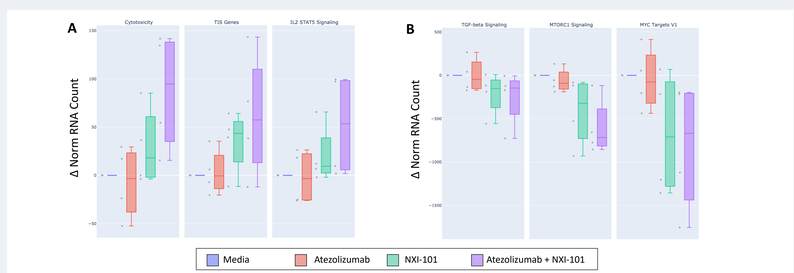


FIGURE 4. NXI-101 and NXI-101 + atezolizumab altered anti-tumor immune pathways in responsive tumors, with variable effects across patients. (A) Combination treatment notably boosted immune effector activity gene sets. (B) TGF-β suppression and c-Myc reduction with NXI-101 and NXI-101/atezo are consistent with anti-tumor responses. Pathway analysis utilized Nanostring, MSigDB curated gene sets, and Gene Set Enrichment Analysis.

Study Design

Treatment	Dose	Time	Endpoints
Media	-	3 Days	• Cytotoxicity (HPI Imaging) • Gene Expression (Nanostring)
Atezolizumab	100 µg/ml		
NXI-101	1 mg/ml		
Atezo NXI-101	100 µg/ml 1 mg/ml		

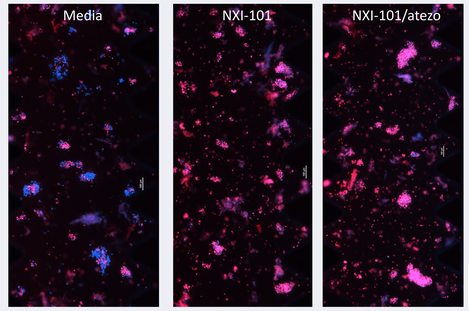


FIGURE 5. Fluorescence images of Hoechst/PI stained NSCLC PDOTS within the microfluidic channels 3 days post-treatment. Each channel contains hundreds of spheroids. NXI-101 and combination NXI-101/atezo treatment groups have a higher level of PI stain than the media control. Data from responsive sample P1 are shown.

Conclusions

- **Xsphera Ex Vivo Platform**: Proven as a reliable tool for assessing the efficacy and mechanism of action of immunotherapeutics, while also serving as a model for investigating factors contributing to patient variability in treatment responses.
- **Potent Cytotoxic Effects**: NXI-101/atezo combination enhanced the efficacy of each monotherapy and elicited potent cytotoxic effects in 40% of the patient tumors.
- **Correlation with Target Expression**: The magnitude of cytotoxic response induced by NXI-101 correlated significantly with expression levels of the target, OK-1, suggesting a targeted therapeutic effect.
- **Activation of Immune Pathways**: NXI-101 and NXI-101/atezo consistently modulated immune pathways in cytotoxic responsive patient tumors consistent with anti-tumor activities.
- **De-Risking Drug Development:** Xsphera's functional platform provides valuable data for exploring mechanisms of drug resistance and the potential for patient stratification as drugs progress towards clinical application.

References:
(1) Jenkins *et al.* (2018) Cancer Discovery 8 (2):196.
(2) Aref *et al.* (2018) Lab Chip, 2018, 18, 3129.