LB218/13

Advanced Ex Vivo Platform for Target Expression and Drug Response Analysis:
Evaluating the Potent Effects of NMC-521 mAb on Fresh Mouse- and Patient-Derived Organotypic Tumor Spheroids

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Background

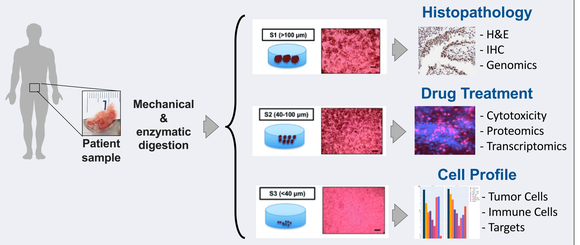
About Xsphera PDOTS Platform

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 NMC-521

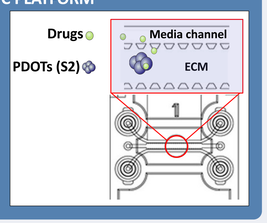
In tumor cells, NMC-1, typically a nuclear protein, undergoes alternative splicing, leading to a membrane-bound variant on the cell surface. NMC-521, a monoclonal antibody (mAb), was developed by NomoCan Pharmaceuticals (New York, NY) to target NMC-1 as a first-in-class therapeutic selectively targeting cancers that express NMC-1. NMC-521 is an IgG1 mAb with anti-tumor activities that include antibody dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) as well as robust activation of an NK-mediated tumor killing. On this poster, NMC-521 refers to the α-hu mAb as well as the murine surrogate mAb against the NMC-1 target.

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 the tumor microenvironment (TME) containing tumor cells and the immune contexture



Live tumor specimens collected from murine CT-26 tumors or human endometrial cancer patients were dissociated into spheroids using a mechanical and enzymatic digestion process. Murine- or patient-derived organotypic tumor spheroids (MDOTS or PDOTS) of 40 µm - 100 µm diameter were size fractionated and loaded into microfluidic devices with an extracellular matrix to support the tumor microenvironment of the PDOTS. Following treatment with therapeutics, cytotoxicity is measured by imaging and RNA transcription profiles are measured to study the post-treatment effects on tumor growth and immune responses.

Results

Murine CT26 Tumors

EX VIVO CT26 TUMOR SPHEROID PLATFORM (MDOTS)

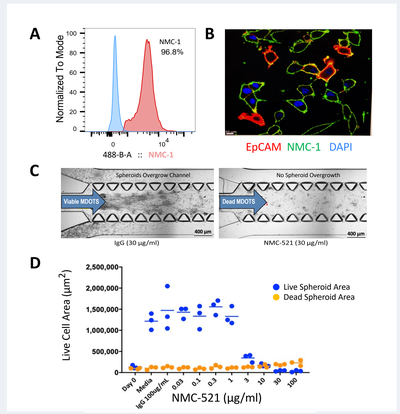


FIGURE 1. NMC-521 elicited cytotoxicity on CT26 MDOTS *ex vivo* in a dose-dependent manner. A-B. NMC-1 target expression on CT-26 cell line assessed by flow cytometry and IF. **C.** NMC-521 suppresses tumor spheroid outgrowth and enhances tumor spheroid killing ex-vivo in the CT-26 MDOTS model. **D.** NMC-521 elicited cytotoxicity (Live Cell Area) in dose-dependent manner.

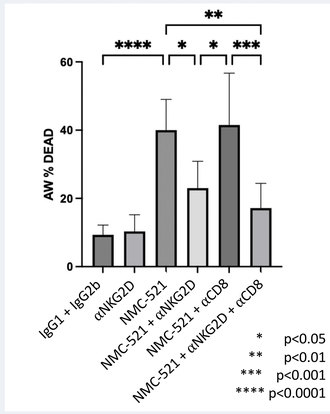


FIGURE 2. Mechanism of NMC-521 involved NK cell-mediated killing. CT26 MDOTS treated with NMC-521 elicited significant cytotoxicity relative to IgG (p<0.0001). Efficacy was partially reduced by neutralizing NK cell activity by co-administration with αNKG2D mAb (p<0.05), whereas αCD8 neutralizing mAb did not reduce activity of NMC-521. Mean ± sd shown.

IN VIVO CT26 TUMOR MODEL

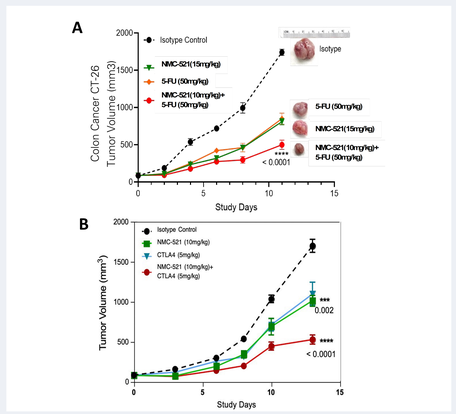


FIGURE 3. NMC-521 suppressed the growth of tumors in the CT26 BALB/c murine syngeneic tumor model. A. Tumor growth was significantly suppressed in the NMC-521 alone or in combination with 5-FU treated mice. The combination treatment had greater efficacy than either of the monotherapies as was significantly reduced from the isotype control, **B.** Synergistic effect of CTLA4 with NMC-521 in tumor growth inhibition. p<0.0001.**The results with the *ex vivo* CT26 MDOTS platform corroborate *the in vivo* CT26 tumor model and provide a potential MOA.**

Patient-Derived Organotypic Tumor Spheroids (PDOTS)

Treatment	Dose	Time	Endpoints
IgG1	100 µg/ml	3 Days	• Cytotoxicity (HPI Imaging) • Gene Expression (Nanostring)
Nivolumab	40 µg/ml		
NMC-521	50 µg/ml		
Nivolumab + NMC-521	50 µg/ml 40 µg/ml		

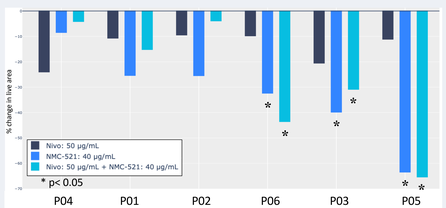


FIGURE 4. NMC-521 induced cytotoxicity in 3 of 6 patient tumors using the PDOTS *ex vivo* platform. The live spheroid areas were decreased relative to IgG1 control in patient tumors (P06, P03, P05). Combination with nivolumab did not improve the cytotoxic response of NMC-521.

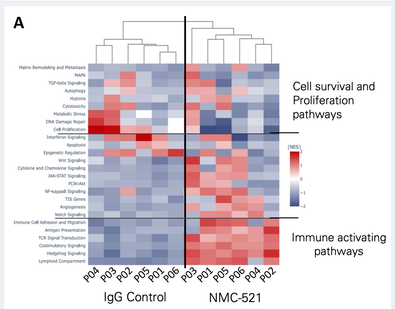


FIGURE 5. The drug treatments modulated the molecular immune profile in cytotoxicity responsive patients using Nanostring IO360 panel. A. Post-treatment pathway enrichment analysis (log 2 norm RNA count) of all patient tumors treated with NMC-521 (*right side*) compared to IgG control (*left side*). The heatmap clearly illustrates the robust activation of immune-activity related pathways (*high NES score*) and reduction of cell survival pathways in response to NMC-521 compared to IgG control. **B.** Nivolumab, NMC-521, and their combination consistently elevated the RNA counts of CD8+ T cell and NK cell associated RNAs in the cytotoxic responsive patients identified in Figure 4. Both NMC-521 and the combination treatment showed greater potency in enhancing these cell signatures compared to nivolumab alone.

PDOTS Imaging

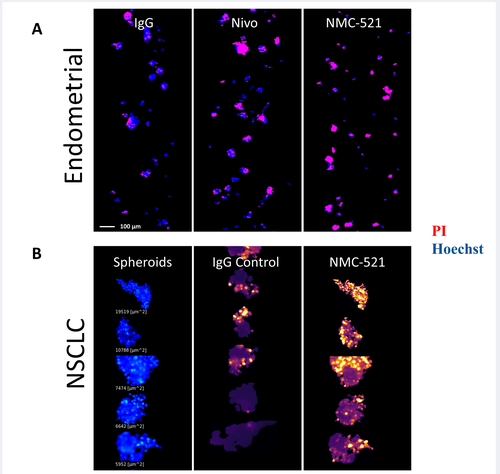


FIGURE 5. Fluorescence images of PDOTS demonstrating the cytotoxic effects of NMC-521. Hoechst-PI stained endometrial (A) and non-small cell lung cancer (B) PDOTS from shown from a portion of the microfluidic channel. The PDOTS had were viable (limited PI stain) in the IgG and nivolumab treatment groups, while the NMC-521 treatment arm had smaller spheroids and a high proportion of PI-stained spheroids. (Figure-5B is a high projected image of Hoechst-PI staining).

Conclusions

- **Xsphera Ex Vivo Platform**: The Xsphera *ex vivo* platform is a proven tool for assessing the efficacy and mechanism of action of immunotherapeutics and serving as a model to investigate factors contributing to variability in treatment responses.
- **Potent Cytotoxic Effects**: NMC-521 was highly effective in the *ex vivo* platform using murine CT26 MDOTS and mirrored the effects in the syngeneic in vivo CT26 mouse tumor model.
- **Mechanism of Action (MOA):** A potential MOA for NMC-521 was identified using neutralizing antibodies: neutralizing NK cell antibodies reduced cytotoxicity, while CD8+ T cell neutralization had no effect.
- **De-Risking Drug Development**: NMC-521 elicited cytotoxic effects in 3 of 6 patient tumors tested and immune pathways were activated in the responsive patient tumors. NMC-521 has clinical potential as a naked mAb or as an ADC-based therapeutic.

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