

## Abstracts

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### NTX-0250, A MULTIMODAL MRNA-BASED IMMUNOTHERAPY, ERADICATES LARGE ESTABLISHED TUMORS IN A STRINGENT MOUSE MODEL OF HPV16-DRIVEN CANCER

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**Background** Human papillomavirus (HPV) is a contagious cause of anogenital and oropharyngeal cancers developing from persistently infected and subsequently transformed basal keratinocytes of mucosal epithelium. More than 90% of cervical cancers and pre-cancerous cervical intraepithelial neoplasia (CIN) are linked to infections with high-risk HPV, with more than 50% of cancers linked to HPV16.<sup>1,2</sup> At least 25% of women with high-grade CIN lesions progress to in situ or invasive cancer, if untreated.<sup>3</sup> Current treatments for high-grade CIN can remove abnormal tissue but do not address underlying HPV infection, and 15% of women treated develop residual or recurrent high-grade CIN or cervical cancer.<sup>4</sup> Long-term efficacy may require induction of tumor-specific T cell responses combined with alleviated local immune suppression and increased tumor immune cell infiltration. Multimodal mRNA-based immunotherapies that deliver both antigens and immunomodulators in a single drug product represent a promising new approach for treatment of CIN and cervical cancer that can address current disease as well as the underlying cause (HPV infection). Here we report on pre-clinical efficacy of NTX-0250, a nanoparticle-formulated, multi-component mRNA drug that co-delivers a novel HPV16 antigen design with two potent immunomodulators.

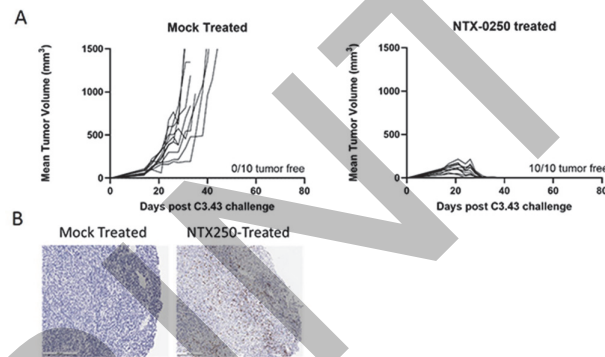
**Methods** To test efficacy, we utilized the well-established, clinically relevant, C3.43 tumor model (5). C3.43 is a progressive subclone of C3, HPV16-transformed B6 mouse embryo cell line that expresses HPV16 E6 and E7 antigens under the natural promoter.<sup>5</sup> Therapeutic efficacy of NTX-0250 was assessed in mice with large (>120mm<sup>3</sup>) C3.43 tumors. HPV16-specific T cells were assessed by flow cytometry on peripheral blood mononuclear cells (PBMCs). Mechanistic studies were performed by post-treatment tumor microenvironment characterization. To assess translational potency of NTX-0250, induction of HPV-specific T cell responses in cynomolgus monkeys was measured by flow cytometry and IFN $\gamma$  ELISpot on PBMCs

**Results** In tumor challenged mice, administration of NTX-0250 induces complete regression of large tumors resulting in long-term, tumor-free survival of 100% of treated animals (figure 1A). Complete responses are accompanied by strong tumor immune infiltration of CD8+, CD4+ APCs and NK cells and upregulation of IFN $\gamma$  in the tumor microenvironment (figure 1B). In cynomolgus monkeys, administration of NTX-0250 induces strong HPV16-specific responses (figure 2). **Conclusions** Here we report for the first time robust pre-clinical efficacy of a multimodal, mRNA-based therapeutic combining antigen- and immunomodulator-encoding mRNAs in a novel nanoparticle formulation. NTX-0250 treatment resulted in complete regression of large established murine tumors and robust induction of HPV-specific T cell responses in non-human primates.

## REFERENCES

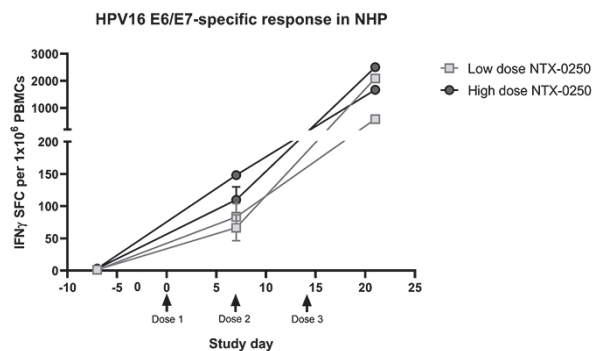
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**Abstract 1084 Figure 1** NTX-0250 induces complete regression of large tumors in mice

A) 10 C57/BL6 mice per group were inoculated with 1x10<sup>6</sup> C3.43 cells in the subcutaneous compartment. NTX-0250 or Mock-treatment, was initiated when tumors were >120mm<sup>3</sup> (18 after tumor inoculation). Mice received 3 doses of 1.5 $\mu$ g NTX-0250 with 7 days interval. Tumor growth was monitored for 90 days. B) Representative immunohistochemistry staining of C3.43 tumors 3 days post treatment with vehicle control (Mock treated) or NTX-0250. Slides were stained for infiltrating CD8+ T cells (Brown).



**Abstract 1084 Figure 2** NTX-0250 induces robust HPV16-specific responses in NHPs.

Cynomolgus monkeys (n=2) were immunized three times with low or high dose of NTX-0250. Induction of HPV16 E6 and E7-specific T cell responses were measured by IFN $\gamma$  ELISpot after one and three doses.

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