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

¹Ole Haabeth*, ²Diane DaSilva, ¹Colin McKinlay, ¹Weiqun Liu, ¹Edward Lemmens, ¹Christopher Rae, ¹Adrienne Sallets, ¹Daniel Frimansson, ¹Sangeeta Nath, ¹Nicole Peck, ¹Ou Li, ¹Nicole Fay, ²Ruben Prins, ¹Meredith Leong, ²W Martin Kast, ¹Samuel Deutsch. ¹Nutcracker Therapeutics, Emeryville, CA, USA; ²Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, USA

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.^{1,2} At least 25% of women with high-grade CIN lesions progress to in situ or invasive cancer, if untreated.³ 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.⁴ 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.

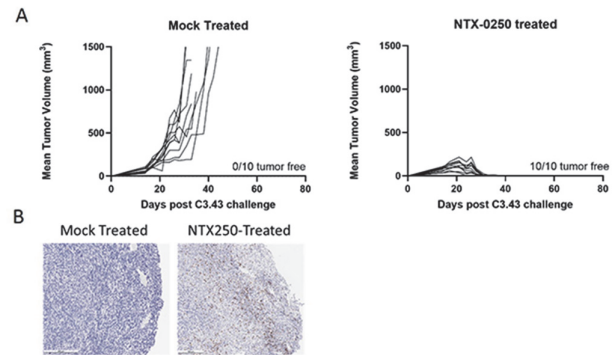
Methods To test efficacy, we utilized the well-established, clinically relevant, C3.43 tumor model (5). C3.43 is a progressive subclone of C3, HPV16-transformed B6 mouse embryo cell line that expresses HPV16 E6 and E7 antigens under the natural promoter.⁵ Therapeutic efficacy of NTX-0250 was assessed in mice with large (>120mm³) 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 γ 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 γ 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

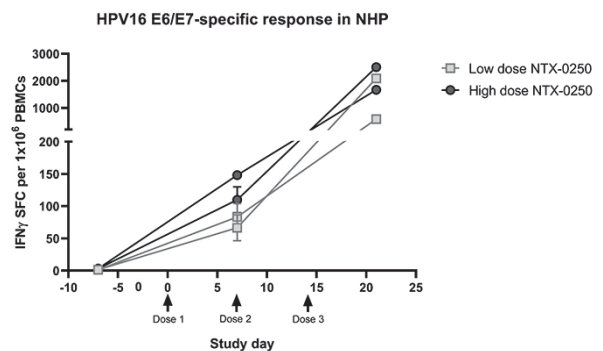
1. https://www.who.int/health-topics/cervical-cancer#tab=tab_1

- da Silva RL, da Silva Batista Z, Bastos GR, *et al.* Role of HPV 16 variants among cervical carcinoma samples from Northeastern Brazil. *BMC Women's Health* 2020;**20**:162.
- Tao L, Han L, Li X, *et al.* Prevalence and risk factors for cervical neoplasia: a cervical cancer screening program in Beijing. *BMC Public Health* 2014;**14**:1185.
- Risk of recurrent high-grade cervical intraepithelial neoplasia after successful treatment: a long-term multi-cohort study. Mariëlle Kocken 1, Theo J M Helmerhorst, Johannes Berkhof, Jacqueline A Louwers, Mariëlle A E Nobbenhuis, Aagje G Bais, Cornelis J A Hogewoning, Afra Zaal, René H M Verheijen, Peter J F Snijders, Chris J L M Meijer. *Lancet Oncol* 2011;**12**(5):441–50
- Feltkamp MC, Smits HL, Vierboom MP, *et al.* Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. *Eur J Immunol* 1993;**23**:2242–9.



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

A) 10 C57/BL6 mice per group were inoculated with 1×10^6 C3.43 cells in the subcutaneous compartment. NTX-0250 or Mock-treatment, was initiated when tumors were >120mm³ (18 after tumor inoculation). Mice received 3 doses of 1.5 μ 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 cells responses were measured by IFN γ ELISpot after one and three doses.

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