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 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 (>120mm\(^3\)) C3.43 tumors. 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.

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 cytomolagus 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 combing 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

Abstract 1084 Figure 1 NTX-0250 induces complete regression of large tumors in mice
A) 10 C57BL/6 mice per group were inoculated with 1x10^6 C3.43 cells in the subcutaneous compartment. NTX-0250 or Mock-treatment, was initiated when tumors were >120mm\(^3\) (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. Cynomolagus 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\(\gamma\) ELISpot after one and three doses.