

**Title:** Treatment of Recurrent Respiratory Papillomatosis with DNA immunotherapy INO-3107 induces activation and enrichment of pro-inflammatory CD4+ T cell responses in blood and airways of patients with clinical response

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**Introduction:** Recurrent respiratory papillomatosis (RRP) is a chronic, debilitating disease of the airway primarily caused by infection with human papillomavirus (HPV) types 6 and/or 11 and characterized by recurrent, benign tumor growth with potential for malignant transformation. Current standard of care consists of repeated surgical removal of papillomas, which can lead to lasting airway damage and impaired vocal function. Thus, a non-surgical approach to treat RRP is paramount. Previously, we described CD8+ T cell responses associated with clinical response to INO-3107, a DNA immunotherapy designed to generate T cells capable of targeting HPV-infected cells, in adult RRP patients during a Phase 1/2 trial (NCT04398433). Here, we describe CD4+ T cell responses in these patients.

**Methods:** INO-3107 was administered during study weeks 0, 3, 6 and 9. Peripheral blood mononuclear cells (PBMCs) obtained at screening and post-treatment were assessed by multiparametric flow cytometry and RNA sequencing. Formalin-fixed, paraffin-embedded papilloma tissue obtained prior to INO-3107 treatment and at the end of the 52-week study (EOS) was subjected to RNA sequencing. RNA sequencing data additionally underwent single sample gene set enrichment analysis (ssGSEA). Clinical response was defined as any reduction in frequency of RRP surgical interventions in the 52 weeks following dose 1 of INO-3107 compared to the 52 weeks prior.

**Results:** Following INO-3107 treatment, activated HPV-specific CD4+ T cells were found to be elevated in PBMCs of responders compared to non-responders. ssGSEA assessment of airway tissue revealed enrichment in CD4+ T cell signatures, inclusive of Th1 and effector memory populations, when comparing screening to EOS. Enrichment of CD4+ T cell signatures was significantly higher in responders but not in non-responders. Enrichment of a Th2 signature was observed in post-treatment PBMCs from non-responders as compared to screening. Expression of HLA class II genes in airway tissue increased significantly from screening to EOS in responders but decreased in most non-responders. Conversely, expression of these genes in post-treatment PBMCs increased in non-responders but decreased in responders.

**Conclusions:** Mechanisms of immune escape may play a role in mediating clinical responses in recurrent respiratory papillomatosis (RRP) as evidenced by enrichment of Th2 cells in PBMCs and decreased expression of HLA class II genes in airway tissue of non-responders. However, DNA immunotherapy INO-3107 induced activation and enrichment of pro-inflammatory CD4+ T cell responses, inclusive of Th1 and effector memory signatures, in blood and airways of RRP patients. These immune responses were associated with clinical responses during the 52-week trial. Additionally, responder airways displayed increased expression of HLA class II genes responsible for proper binding, processing, and presentation of HPV antigens to CD4+ T cells, which may, in part, activate CD8+ T cell responses described previously (doi: 10.1038/s41467-025-56729-6).