Title: Reduction in Surgical Interventions for the Treatment of Recurrent Respiratory Papillomatosis by INO-3107 is Associated with Enriched Macrophage, Dendritic cell and T cell Signatures in Patient Airways

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Introduction: Recurrent respiratory papillomatosis (RRP) is a debilitating chronic disease of the airway caused by Human Papillomavirus (HPV) characterized by recurrent benign tumor growth with the potential for malignant transformation. The current standard of care requires frequent surgeries, the sequalae of which have long-standing negative impacts on airway and vocal function. Treatments that avoid surgery targeted against HPV6 and/or HPV11 (known etiological agents of RRP) are thus an area of need. Here we describe the results of a Phase 1/2 trial of INO-3107, a DNA medicine designed to elicit T-cell responses against HPV-6 and HPV-11, in adults with RRP (NCT04398433).

Methods: Peripheral blood mononuclear cells (PBMCs) were assessed by IFNγ ELISpot and TCRβ sequencing of complementarity determining region 3 (CDR3). Formalin fixed paraffin embedded papilloma tissue obtained prior to INO-3107 treatment as well as at the end of the 52-week study was assessed by RNA sequencing. Data were analyzed using Ingenuity Pathway Analysis (IPA) and R. Overall clinical response (OCR) was defined as a reduction in surgical interventions recorded on study as compared to the year prior to study.

Results: The OCR of the trial was 81.3%. Analysis of PBMCs longitudinally across the study via ELISpot confirmed induction of T cell activity specific to HPV-6 as well as HPV-11. TCRβ sequencing of CDR3 regions indicated significant T cell clonal expansion in all patients. IPA based assessments indicated a number of significantly enriched immune pathways in airway tissue at the end of study relative to pre-treatment, spanning both innate and adaptive immune responses. For the former, enriched pathways were inclusive of recruitment ($z=2.3$, $p=1.00e^{-10}$), migration ($z=2.4$, $p=6.50e^{-15}$), quantity ($z=2.3$, $p=1.37e^{-10}$), and response ($z=3.0$, $p=5.85e^{-07}$) of antigen presenting cells, indicative of an innate inflammatory response. Upregulated genes included macrophage and dendritic cell associated transcripts such as *IFNγ, CIITA, XCR1, CD14* and others. For the latter, homing ($z=2.4$, $p=7.77e^{-06}$), quantity ($z=2.7$, $p=6.27e^{-21}$), signaling ($z=3.9$, $p=3.16e^{-12}$), and cytotoxicity ($z=2.6$, $p=1.18e^{-06}$), of T cells were all significantly enriched, indicating a robust influx of activated T cells into airway tissue. T cell associated transcripts included *CD3D/E/G, PRF1, CD8A, GZMA*, and others.

Conclusions: INO-3107 provides clinical benefit to adults with RRP, is well tolerated, and generates an antigen-specific immune response against HPV types 6 and 11. In total, 100% of patients on trial demonstrated T cell activity in blood after administration of IN0-3107, inclusive of antigen specific cytokine production and significantly expanded T cell populations. Moreover, treatment induced an inflammatory response in patient airway tissues, inclusive of signatures of macrophages, dendritic cells and T cells. Importantly, this activity was associated with overall clinical response (81.3%) which is consistent with the proposed mechanism of action of INO-3107.