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Down regulation of HPV 16 and NF-kB and upregulation of gigaxonin and immune markers in APG-157 treated head and neck cancer: A phase 2A clinical investigation

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Abstract

Persistent HPV 16 expression and NF-κB activation drive inflammation and immune suppression in head and neck squamous cell carcinoma (HNSCC). APG-157 is an oral botanical immunomodulator possessing anti-inflammatory and tumor-microenvironment (TME) reprogramming activity. Our Phase 2A clinical study evaluated its molecular and immunologic effects in HNSCC patients. Fourteen patients received APG-157 (200 mg TID for 4 weeks) under IRB approval at VAGLAHS. Paired pre- and post-treatment saliva and tumor biopsies (n=24) were analyzed for HPV 16 E7 integration and expression by qPCR/RT-qPCR, and for the expression of p16, NF-κB, gigaxonin, E-cadherin, and Snail. Paired PBMCs from 6 patients underwent single-cell RNA sequencing (scRNA-seq) to characterize systemic immune modulation. PCR identified presence of HPV 16 E7 sequences in 2/7 OC and 4/7 OPC pre-APG-157 samples. Expression of p16 by IHC showed p16 positivity in 4/7 OC and 7/7 OPC samples. APG-157 treatment reduced HPV 16 E7 integration and expression levels in 4 of 6 HPV 16 E7 pre-APG-157 positive samples in the saliva and tumor, confirming a direct antiviral effect. NF-κB expression decreased concordantly, whereas gigaxonin increased, suggesting restoration of proteasomal regulation and suppression of inflammatory signaling. Expression of epithelial integrity markers (E-cadherin↑, Snail↓) improved, indicating a shift towards a less invasive phenotype. scRNA-seq of PBMCs revealed post-treatment expansion of activated B cells and effector T-cell clusters consistent with systemic immune activation. APG-157 demonstrating dual antiviral and immunoregulatory activity could serve as monotherapy and as a combinatorial agent with chemoradiation or immune checkpoint inhibitors.

Methods and Materials

- Samples were collected from a Phase 2A clinical trial evaluating APG-157 in head and neck cancer patients under IRB approval (VAGLAHS, Los Angeles) and FDA IND authorization (NCT05312710), with informed consent obtained from all participants.
- Gene expression was quantified using RT-qPCR with Thermo Fisher one-step RT-qPCR kits on ABI 7600 instrumentation, using GAPDH as the internal control and triplicate measurements for each sample.
- Statistical analyses were performed using Prism software, with paired one-sided Student's t-tests used to assess significance; results are presented as mean ± SE, with $p < 0.05$ considered statistically significant.

References

1. Basak SK, Bera A, Yoon AJ, Morselli M, Jeong C, Tosevska A, et al. A randomized, phase 1, placebo-controlled trial of APG-157 in oral cancer demonstrates systemic absorption and an inhibitory effect on cytokines and tumor-associated microbes. Cancer. 2020;126:1668–82.
2. Veena MS, Gahng JJ, Alani M, et al. Gigaxonin Suppresses Epithelial-to-Mesenchymal Transition of Human Cancer Through Downregulation of Snail. Cancer Res Commun. 2024;4:706–22.
3. Shin DS, Basak S, Veena MS, et al. Enhanced CTLA-4 blockade anti-tumor immunity with APG-157 combination in a murine head and neck cancer. Cancer Med. 2024;13:e7212.

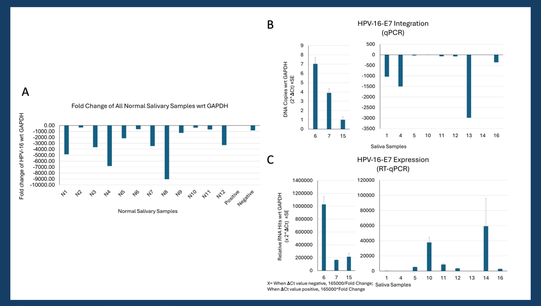

Figure 1. HPV 16 E7 gene integration correlates with expression in salivary cells.

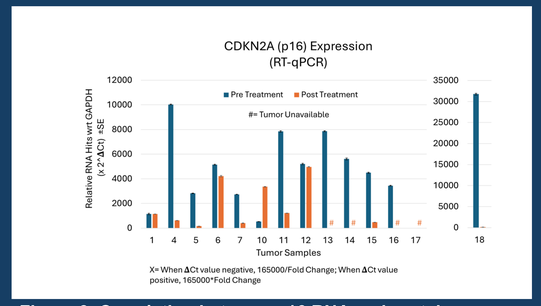

Figure 3. Correlation between p16 RNA and protein expression in tumor cells.

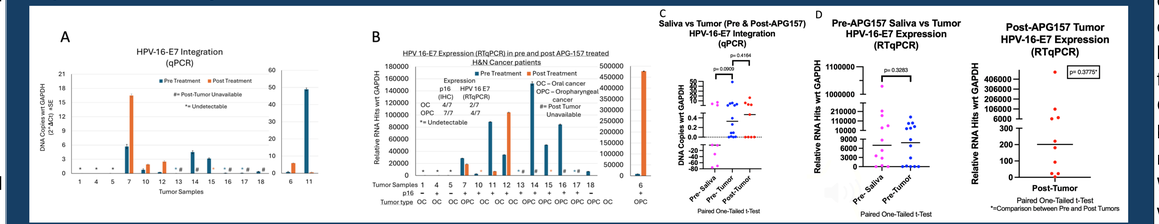

Figure 2. Tumor cell HPV 16 E7 expression correlates with salivary cells.

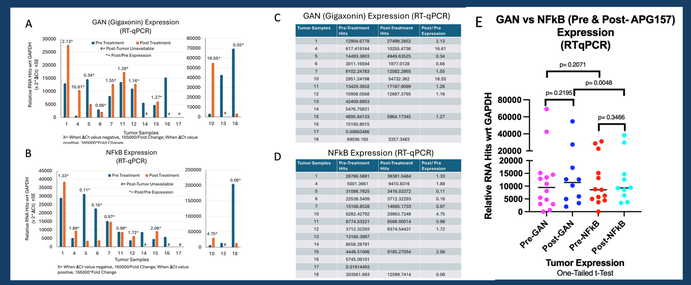

Figure 4. APG-157 treatment leads to activation of gigaxonin and downregulation of NFκB.

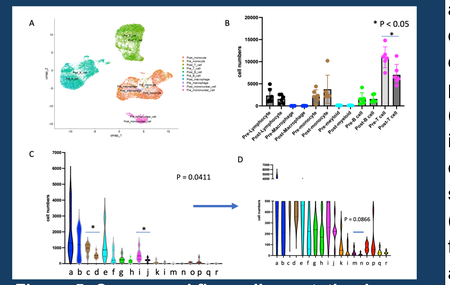

Figure 5. Coarse and fine cell annotation by single cell analysis.

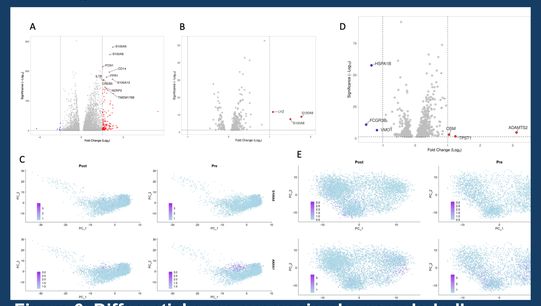

Figure 6. Differential gene expression by pseudo-bulk analysis.

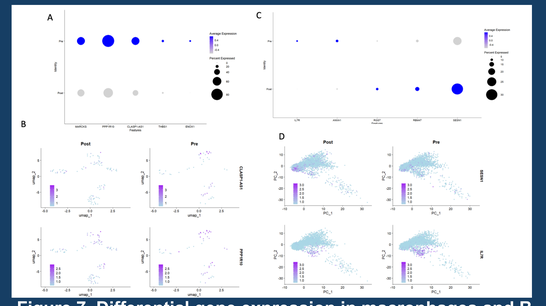

Figure 7. Differential gene expression in macrophages and B cells.

Results and Discussion

We have used qPCR to identify DNA copy numbers (integration), and RTqPCR to determine RNA copies in the saliva and tumor cells (Figs. 1-2). We show that there is a direct correlation between viral integration and expression, and a correlation between saliva and tumor cells (Fig.3). Our results indicate that saliva could be a useful non-invasive biospecimen for the detection of HPV 16 in head and neck cancer. We also show that APG-157 inhibits HPV 16, pointing to the utility of this botanical drug in cancer adjuvant therapies (Fig. 2). NF-κB is a transcription factor that is associated with the expression of growth promoting cytokines IL-6, IL-8, and IL-1β and the transcription factor Snail that downregulates expression of e-cadherin. Recurrent and metastatic cancers showed epithelial to mesenchymal transition (EMT) where there was upregulation of Snail and downregulation of e-cadherin. Suppression of metastatic cancer cells through inhibition of Snail expression leads to suppression of EMT pathway, resulting in the development of less aggressive cancer. Thus, suppression of NF-κB, the oncogenic transcription factor involved in Snail expression, could lead to tumor cell growth suppression. Our studies have also shown that a statistically significant inverse relationship between gigaxonin and NF-κB resulting in cancer cell growth suppression that might be reflected in the inhibition of HPV 16 in salivary and tumor cells (Fig. 4). We did not observe significant global changes in major immune cell populations when comparing the pre- and post-APG-157 treatment samples. However, we did see a statistically significant decrease in T cell population by coarse cell annotation, and fine cell annotation revealed that CD4 positive T cell was decreased in post APG-157 treatment samples, and there was a trend towards decrease in T regulatory cells (Fig. 5). The differential gene expression analysis by pseudo-bulk analyses for the whole population and subset of immune cells (monocyte, macrophage, dendritic and B cells) show that the function of these immune cells is up-regulated (evidenced by up-regulated genes related to immune cell activation as well as genes associated with negative feedback regulation), yet some of the genes implicated in auto-immune diseases were down-regulated (Figs. 6-7). Based on these results, we speculate that APG-157 may enhance the functions of immune cells, yet it can down-regulate the genes associated with the activities of immune cells that might cause immune related disease. One caveat is that we do not have single cell RNA data for tumor biopsy samples to assess the changes of the immune cell population and its associated functional modulation by APG-157 in the tumor microenvironment. However, based on our previous phase I clinical study[1] and recent study using animal models[2, 3], we believe that our findings provide a rational combinatorial cancer treatment approach for the use of APG-157 with current or novel immune checkpoint inhibitors.

Acknowledgements

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