



Enhanced tumor control with a combination of APG-157 and immune checkpoint inhibitors for head and neck cancer

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Abstract 2652

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OBJECTIVES

- To investigate the anti-tumor activity of APG-157 in combination with immune checkpoint inhibitors.
- Characterize tumor microenvironment with the combination treatment with APG-157 and immune checkpoint inhibitors
- Assess gut microbiome changes by APG-157 combined with immunotherapy.

BACKGROUND

- Natural botanical drugs containing flavonoids are under clinical investigation for the treatment of malignancies.
- A phase I clinical study for patients with locally advanced head and neck cancer with a new class of botanical drug APG-157 showed excellent tolerability, systemic absorption via sublingual administration, and enhanced immune cell infiltration into the tumor microenvironment and suppression of T cell inhibitory inflammatory cytokine production.
- We sought to evaluate the efficacy of tumor control for the combination of APG-157 and checkpoint blockade immunotherapy using a head and neck cancer mouse model

METHODS

- CCL23 and UM-SCC1 head and neck cancer cell lines were utilized to assess interferon and NF-KB pathway with APG-157/other derivatives of curcumin treatments in presence or absence of interferons.
- SCCVII (HPV-) and MEER (HPV+) head and neck cancer murine cell line models were utilized in *in vivo* tumor growth study. After optimization for route and cell number, we measured tumor growth after treating mice with following groups: i) control, ii) APG-157, iii) anti-PD-1, iv) anti-CTLA-4, v) APG-157 + anti-PD-1, vi) APG-157 + anti-CTLA-4, vii) anti-PD-1 and anti-CTLA-4 , viii) APG-157 + anti-PD-1 + anti-CTLA-4. Tumors were collected after 2 injections of immune checkpoint inhibitors and characterized for immunophenotype by multi-color flow cytometry, immunohistochemistry, and RNA-sequence analysis. Fecal microbiome was analyzed by 16S rRNA sequence for SCCVII model to assess the impact of APG-157 on the microbiome as we observed the shift of oral microbiome species in the human trial. (Figure 1 for *in vivo* study diagram.)

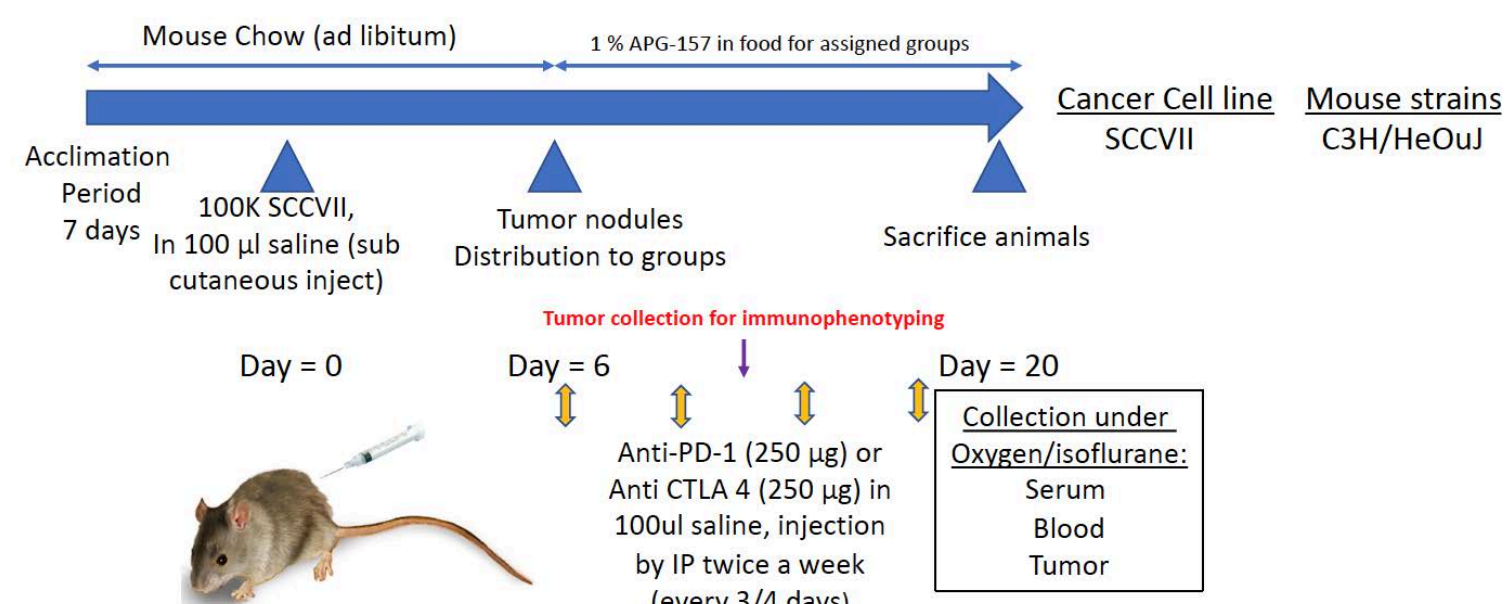


Figure 1: Diagram of *in vivo* study using SCCVII murine head and neck cancer cell line model.

RESULTS

Figure 2: APG-157 and curcumin derivatives suppress the proliferation of human head and neck cancer cells

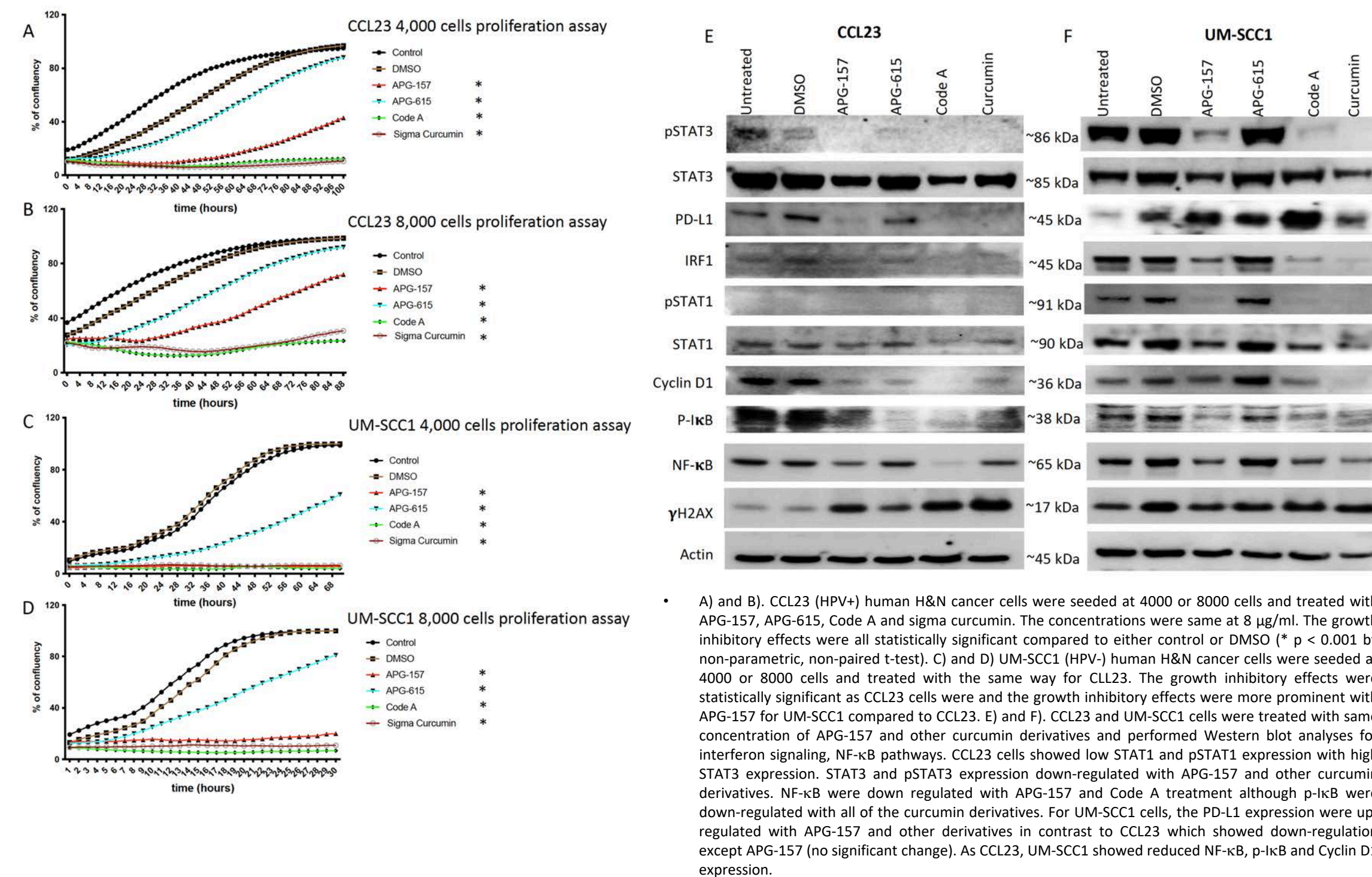
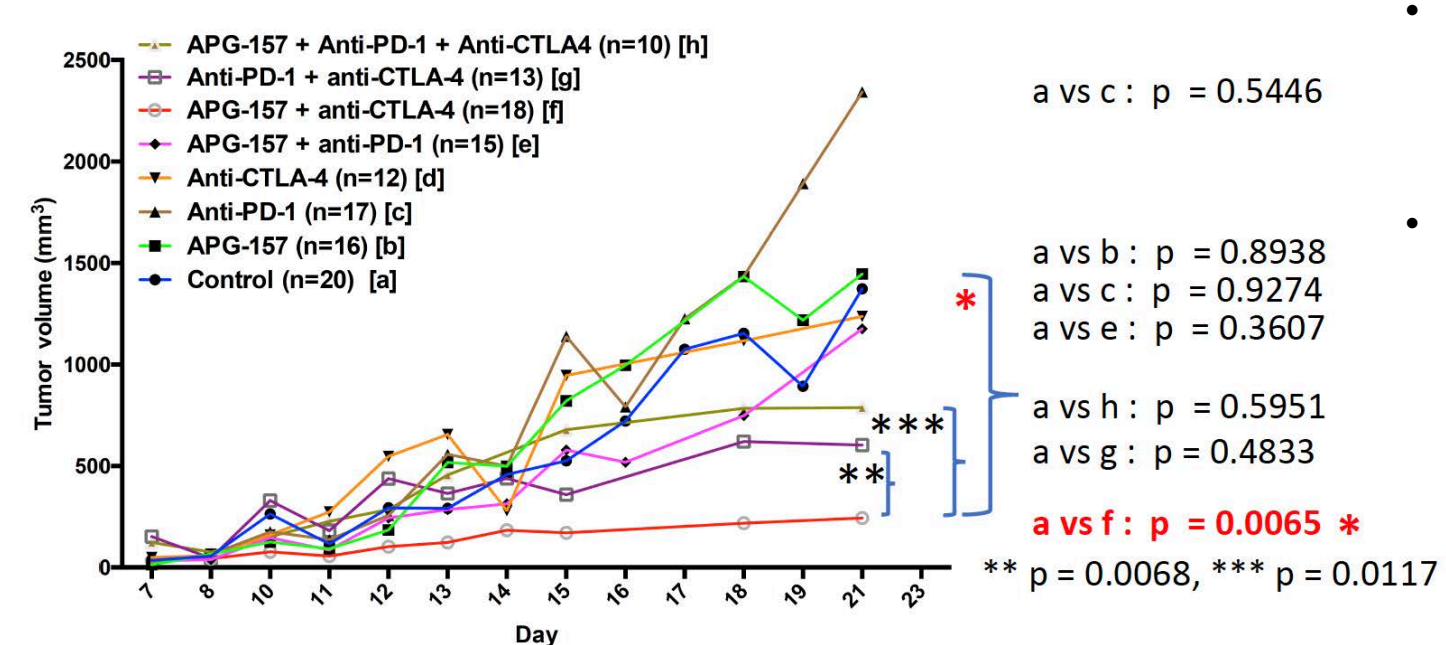


Figure 3: Anti-tumor activity of APG-157 and combination treatment with ICI



- The best tumor control group was APG-157 and anti-CTLA-4 antibody combination treatment compared to control (red-color statistics).
- The statistical analysis was performed by Prism graph pad program, non-paired, non-parametric two-sided, Mann-Whitney t-test. The difference between treatment groups, APG-157 + anti-CTLA-4 vs Anti-PD-1+ anti-CTLA-4 or APG-157 + anti-CTLA-4 vs APG-157 + anti-PD-1 + anti-CTLA-4 were significantly different as the statistics show (double and triple asterix).

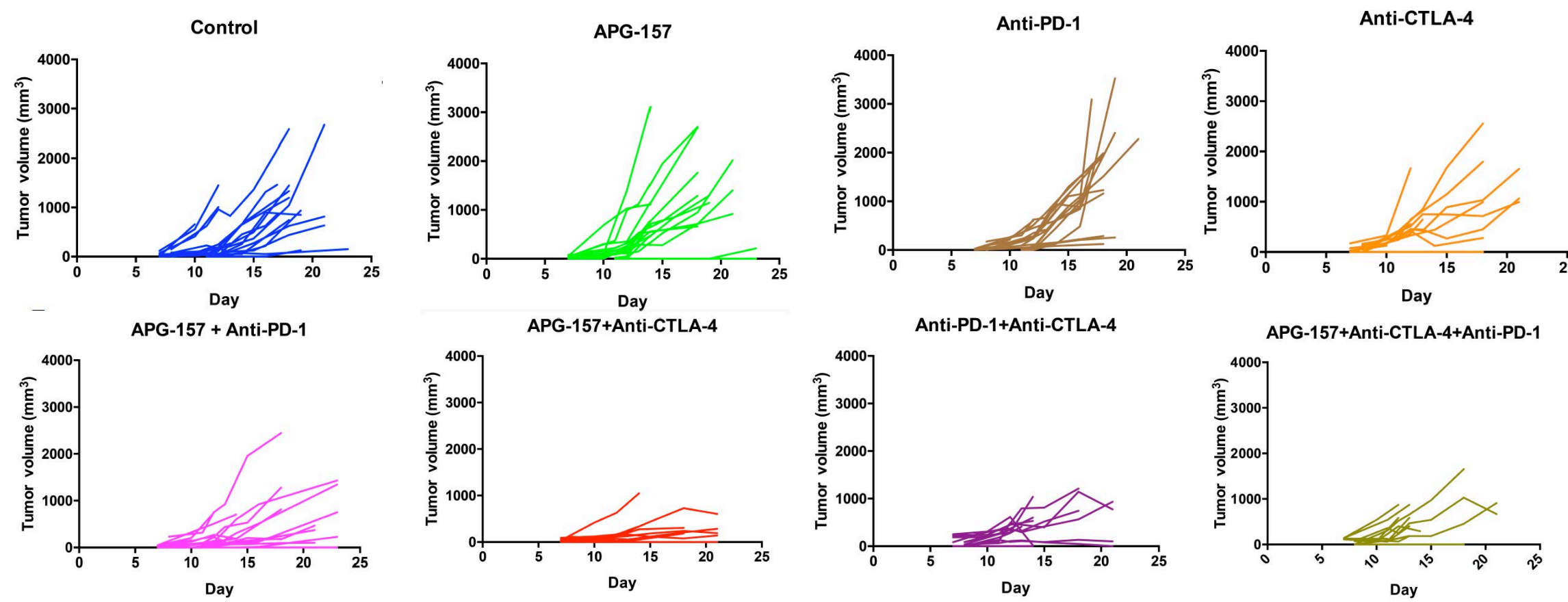


Figure 4: Increased T cell infiltration into Tumor Microenvironment assessed by flow.

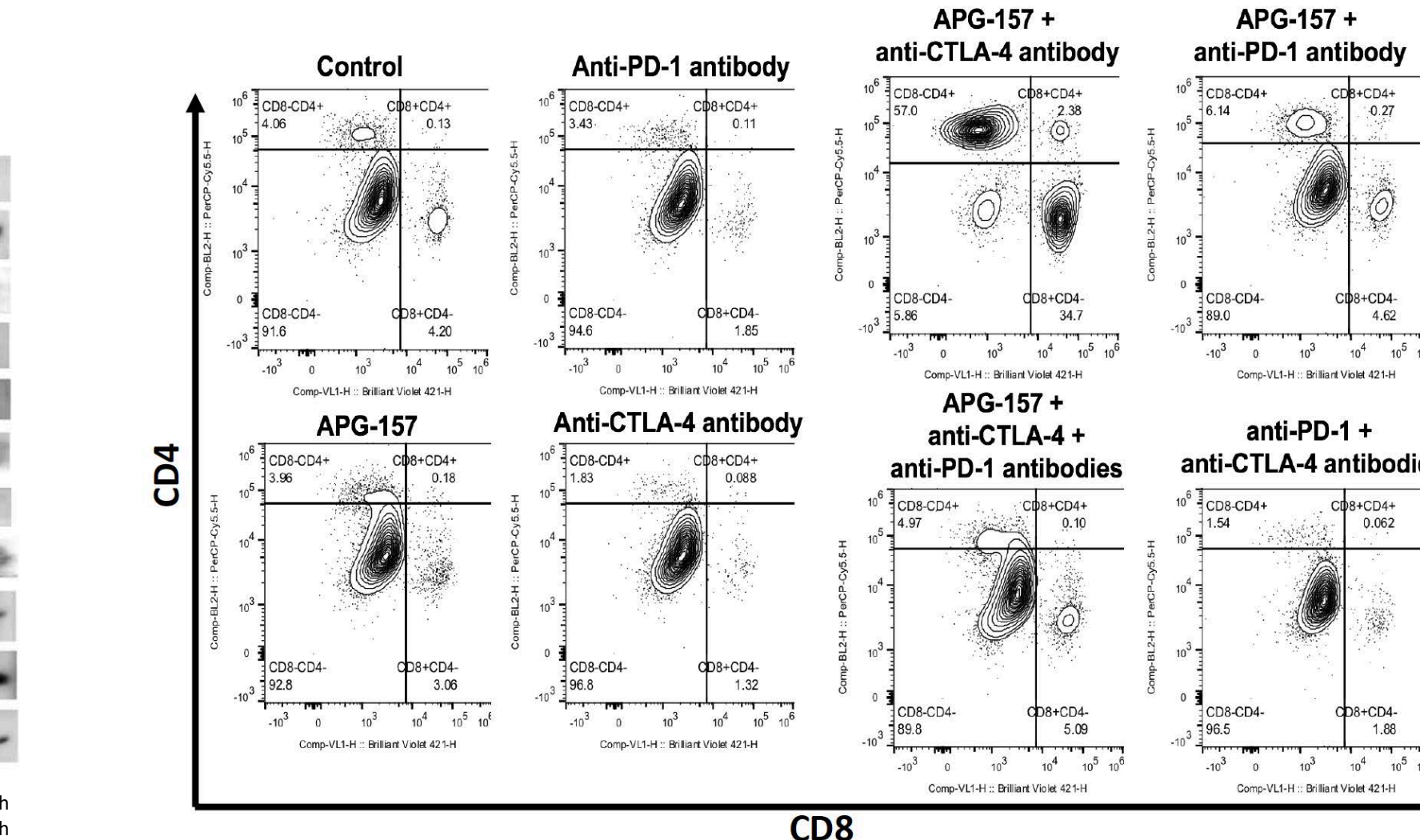


Figure 5: RNA-seq of tumor revealed significant down-regulation of EMT pathway genes with APG-157 and anti-CTLA-4 antibody treatment.

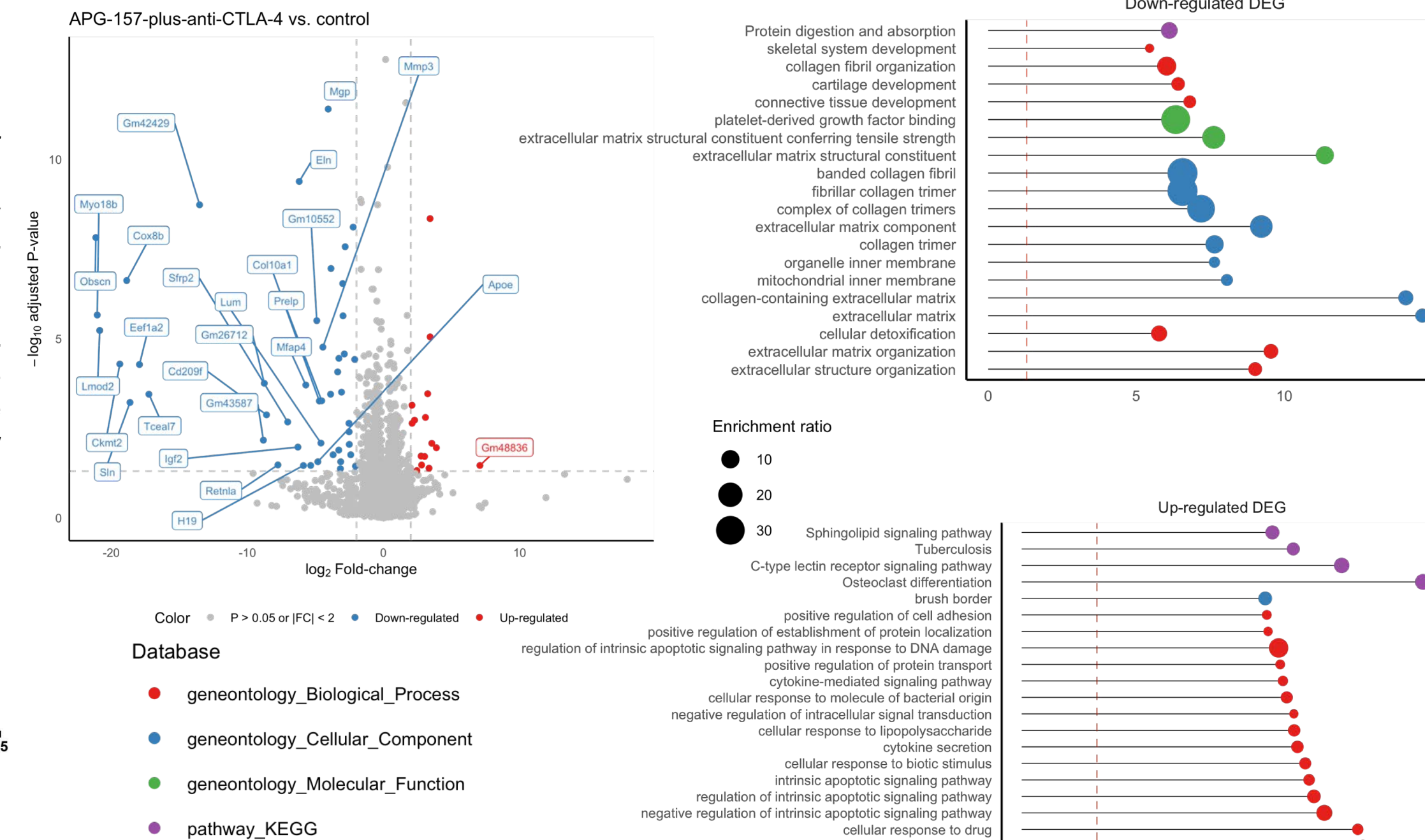


Figure 6: CD8+ T cell depletion abolishes anti-tumor activity of APG-157 and anti-CTLA-4 treatment.

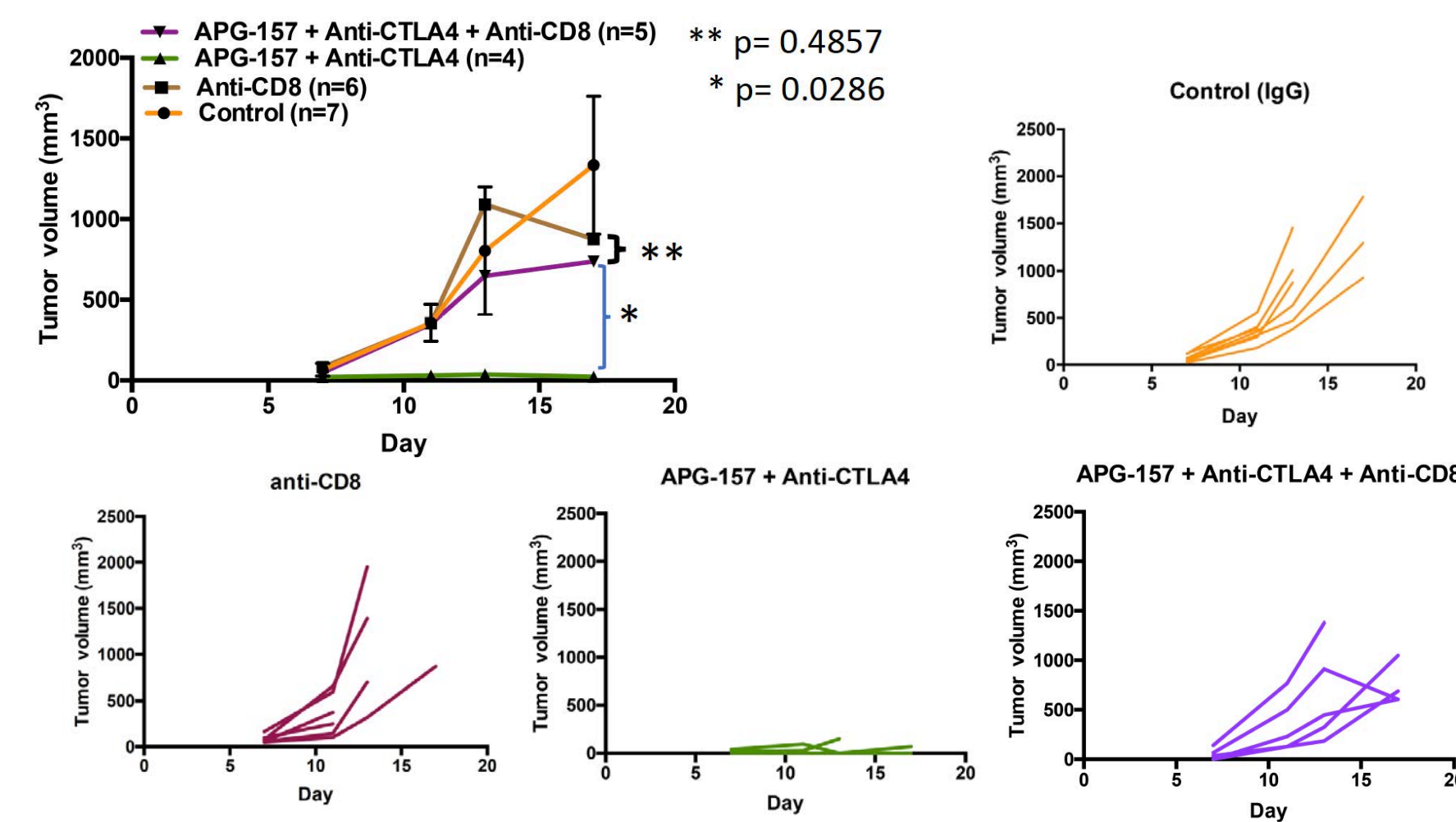
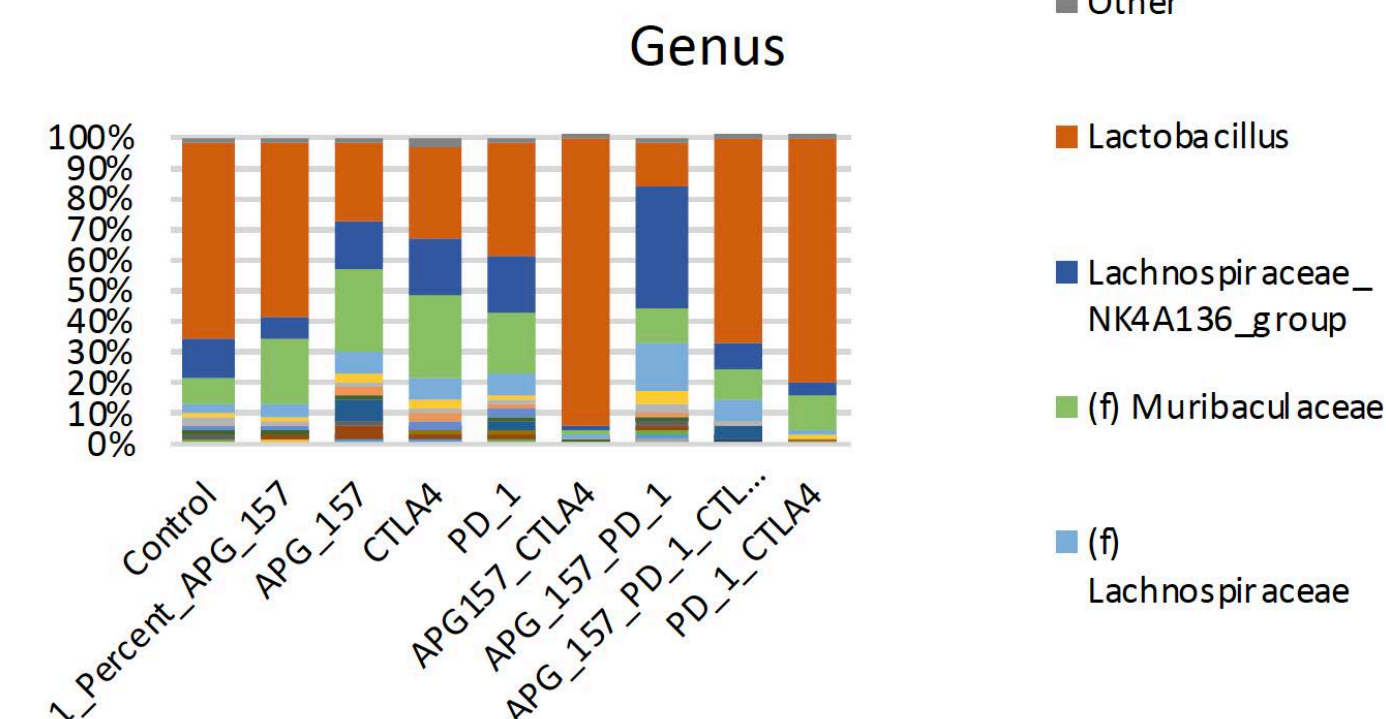


Figure 7: Gut microbial changes mediated by APG-157 and immune checkpoint inhibitors



- Gut microbiome was assessed by 16sRNA sequence from the feces we collected for each treatment group. There was substantial increased in Lactobacillus for APG-157 and anti-CTLA-4 antibody treatment group.

CONCLUSIONS

- The data supports immunomodulatory effect of APG-157 and anti-CTLA-4 antibody treatment that result in increased immune cell infiltration.
- Gut microbiome change mediated by APG-157 and anti-CTLA-4 warrants further investigation to understand mechanism and contribution to anti-tumor activity.
- This study supports further clinical investigation using APG-157 and immune checkpoint inhibitor combination treatment for head and neck cancer .

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