



Growth inhibitory effect of PHRAD129, a Higher Order Therapeutics (HOT)TM drug derived from *Curcuma longa*, in SCCVII head and neck cancer syngeneic mouse xenograft tumor model system

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Abstract

Our recent phase I clinical study of APG-157, a higher order therapeutic (HOT) drug containing curcumin, in normal and head and neck cancer subjects showed an excellent safety profile, inhibitory effect on cancer-associated cytokines, alteration of the oral microbiota and a marked increase in CD4 and CD8 positive T cell infiltration into the tumor microenvironment. Based on these observations, we performed *in-vitro* growth studies on the head and neck cancer cell line UM-SCC1 and preclinical evaluation of SCCVII tumor cell line growth in a syngeneic mouse xenograft tumor model. We used PHRAD129, the drug substance used in formulating APG-157. In the UM-SCC1 cells, the treatment with PHRAD129 showed inhibition of expression of p-IkB and cyclin D1, indicating an inhibitory effect on NFkB. In the mouse model system, delivering PHRAD129 in food (1% w/w in normal mouse chow), in water (2.5mg/ml for 7 days and 10mg/ml for 3 more weeks, also containing 0.5% sucrose to compensate for the taste), or by IP (intra-peritoneal injection 5 days a week of 0.5 mg in 200ul pharmaceutical grade saline/mouse for 1 week and 1.0 mg in 200ul saline/mouse for 3 more weeks) showed tissue absorption of curcumin and its derivatives in the serum of mice fed with 1% PHRAD129 in the normal mouse diet. Further, mice fed with 1, 2 and 4% PHRAD129 diet did not show blood toxicity and there was a higher level of curcumin and derivatives in the serum of mice fed with 2 and 4% PHRAD diet. Microbial content through 16S RNA seq analysis of mouse fecal material showed the greatest alpha diversity from 1% to 2% PHRAD, with 2% and 4% PHRAD129 being similar in both alpha diversity and microbial genetic makeup. There was a significant decrease in *Lactobacillus* species and an increase in *Lachnospiraceae* NK4A136 species in 2 and 4% PHRAD129 fed mice in comparison to the 1% PHRAD diet. A preliminary study of one month feeding followed by SCCVII xenograft tumor cell injection showed inhibition of tumor growth in the 4% diet. Additional studies are required to confirm tumor cell growth inhibition by 4% PHRAD129 diet and draw conclusions on the relationship between gut microbial changes and *in vivo* tumor cell growth inhibition.

Materials & Methods

- Human and mouse cell lines: Human HNSCC cell lines CCL23 and UM-SCC1 and the mouse syngeneic head and neck cancer cell line SCC VII were grown in RPMI medium containing 1% streptomycin-penicillin and 10% FCS. The mouse studies were performed after approval from the IACUC committee of the VAGLAHS West Los Angeles.
- PHRAD129 treatment: Mice were provided normal chow as control and PHRAD129, mixed with food (1%), in water (2.5 mg/ml) or as intraperitoneal injection (0.5 mg in 200 µl saline). PHRAD129 concentration study included 1, 2 and 4% drug mixed in normal mouse chow.
- Blood curcumin measurement: Serum collected from the animals using specific collection tubes were used for the extraction of curcumin and its derivatives using the organic solvents. Mass spectrometric analysis was carried out and a concentration standard was established. Circulating curcumin and derivative concentrations were determined using the standard curve.
- Toxicity assay: Blood collected from the animals were subjected to the standard blood count and electrolyte measurement through IDEXX Inc., Davis, CA.
- Microbiome analysis: fecal material collection at the beginning and on weekly basis were used for DNA isolation and 16S microbial sequence analysis. Alpha diversity methods were used to identify differential microbiome content in the fecal samples representing gut microbiome.
- Subcutaneous SCCVII cell injections: Cells (100 X 10³) were injected on the right flanks of the animals and tumor growth was followed daily. Once tumor bumps were noticed (in 5 days), animals were distributed to different group treatments. Tumor volume ($V = L \times W^2/2$) was measured daily, and animals were sacrificed when the control tumor reached 1.5cm in one of the dimensions.

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Data & Results

The *in vitro* treatment of CCL23 and UM-SCC1 cell lines with PHRAD129 showed downregulation of NFkB, plkB and cyclin D1 confirming the inhibitory effect on NFkB signaling pathway by curcumin present in the drug (Fig. 1). There was upregulation of γH2AX indicating induction of DNA damage response (DDR). Systemic absorption studies in mice showed higher concentration of curcumin and its derivatives in animals treated with PHRAD159 in food (Figs. 2 and 3). While there was absorption a day or two after IP injections, there was no absorption when the drug was included in water. There was weight gain in water treatment samples possibly due to increased water consumption with 0.5% sucrose in the water. (Figure 4). Blood analysis did not reveal any toxicity pointing to the safety and applicability of PHRAD129 in tumor cell growth inhibition studies (data not shown). Fecal microbial analysis indicated statistically significant decrease in *Lactobacillus* species and an increase in *Lachnospiraceae* NK4A136 species (Figs. 5). Finally, preliminary tumor growth inhibition studies on SCCVII cells showed growth suppression in 4% PHRAD157 fed animals (Fig. 6).

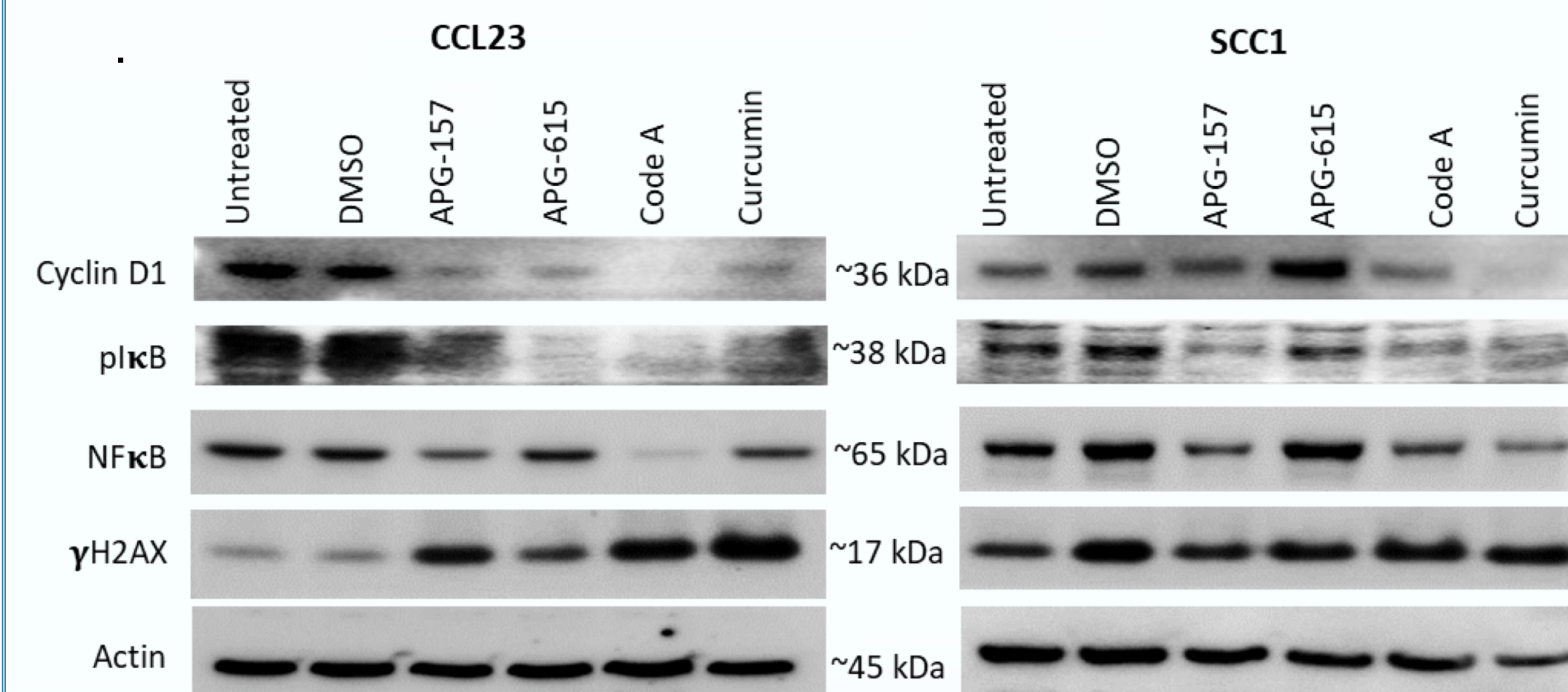


Figure 1. PHRAD129 treatment shows inhibition of NFkB in CCL23 cells. Although code A component of PHRAD129 and curcumin show inhibition of cyclin D1 expression, PHRAD129 shows an increased expression.

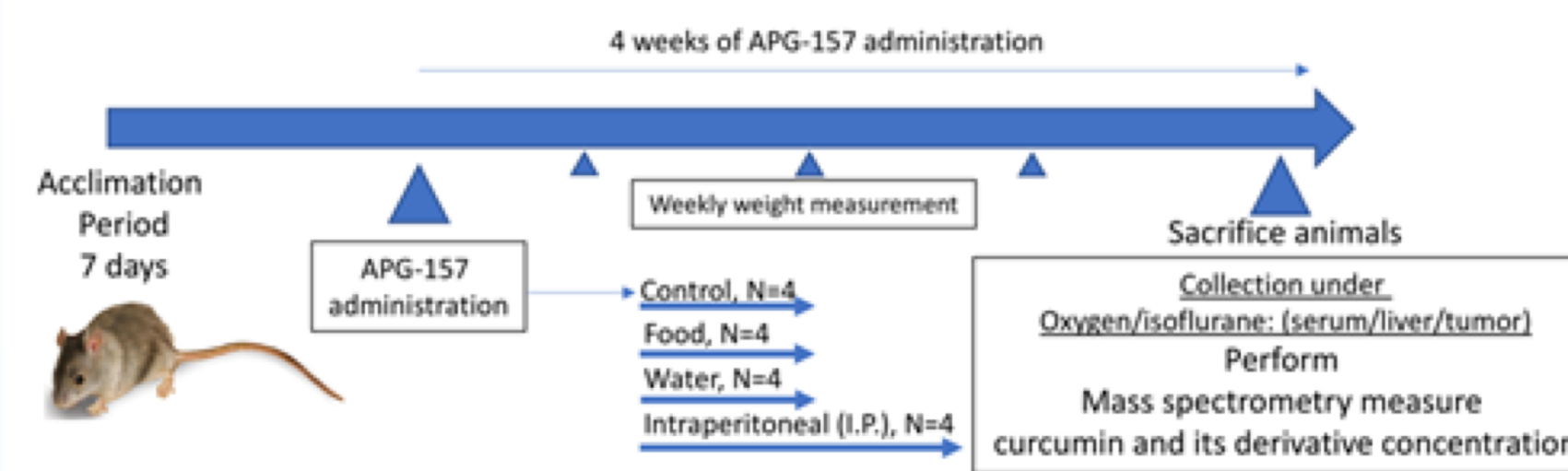


Figure 2. Flow chart of the mouse investigation of PHRAD129.

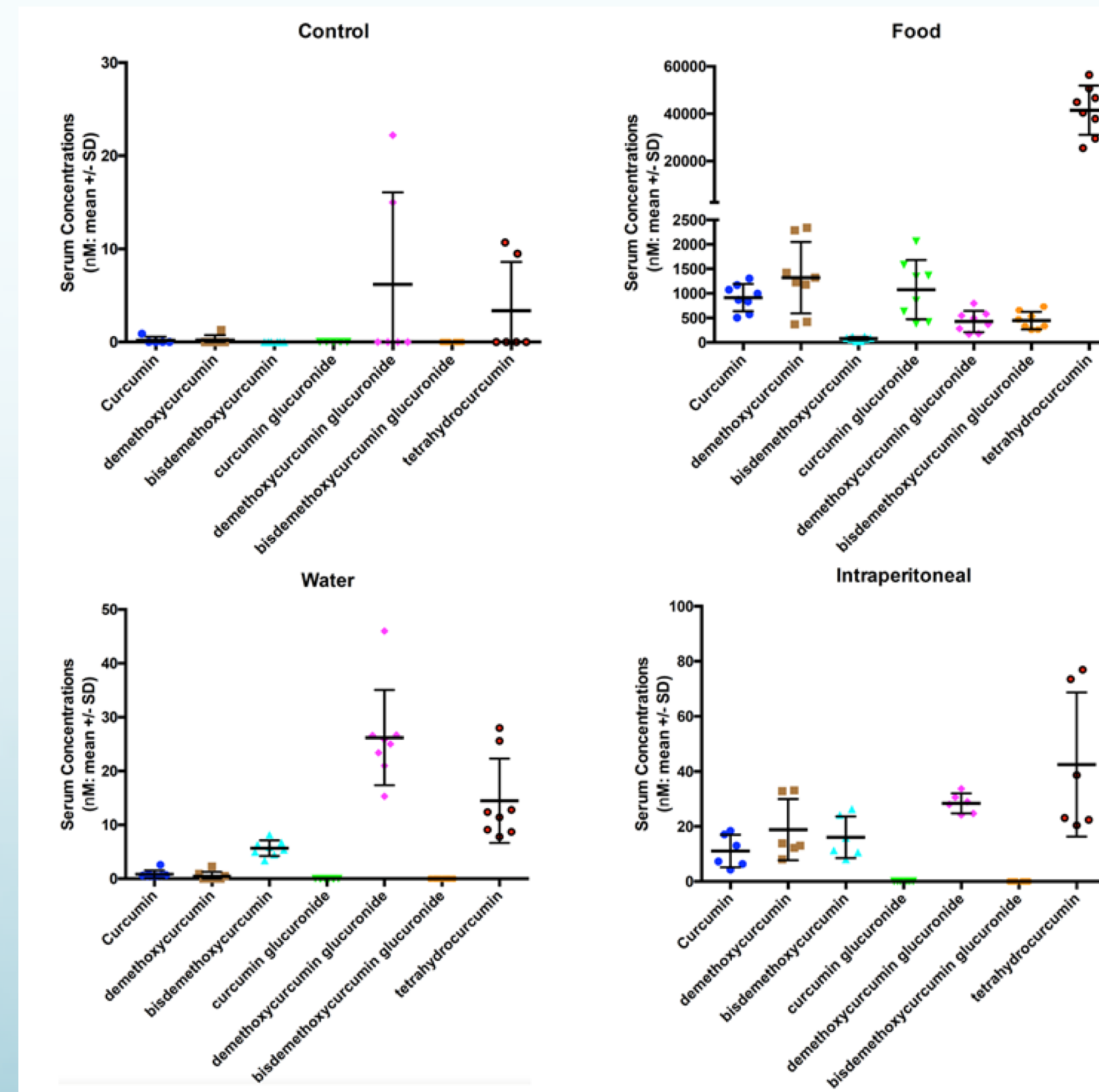


Figure 3. Systemic absorption of curcumin and its derivatives are seen with 1% PHRAD129 in mouse chow. There is increased presence of dimethoxy curcumin glucuronide in the control and PHRAD129 in water, a byproduct of curcumin metabolism.

Discussion & Conclusion

Our studies suggested that there was systemic absorption of curcumin and its derivatives when PHRAD129 was provided with food. This result confirmed our earlier phase 1 clinical studies where transoral treatment of APG157 resulted in systemic absorption. As was shown in our earlier studies PHRAD129 treatment led to the inhibition of NFkB signaling pathway pointing to its utility in preclinical and clinical tumor cell growth inhibition studies. This was further confirmed by the observation of tumor growth inhibition in mice treated with the drug in comparison to the untreated and 1 and 2% PHRAD129). Finally, we identified changes in microbiome content of the gut, increased presence of *Lactospiraceae* NK4A136, a species that has previously been shown to be playing a role in tumor cell growth inhibition in animal studies.

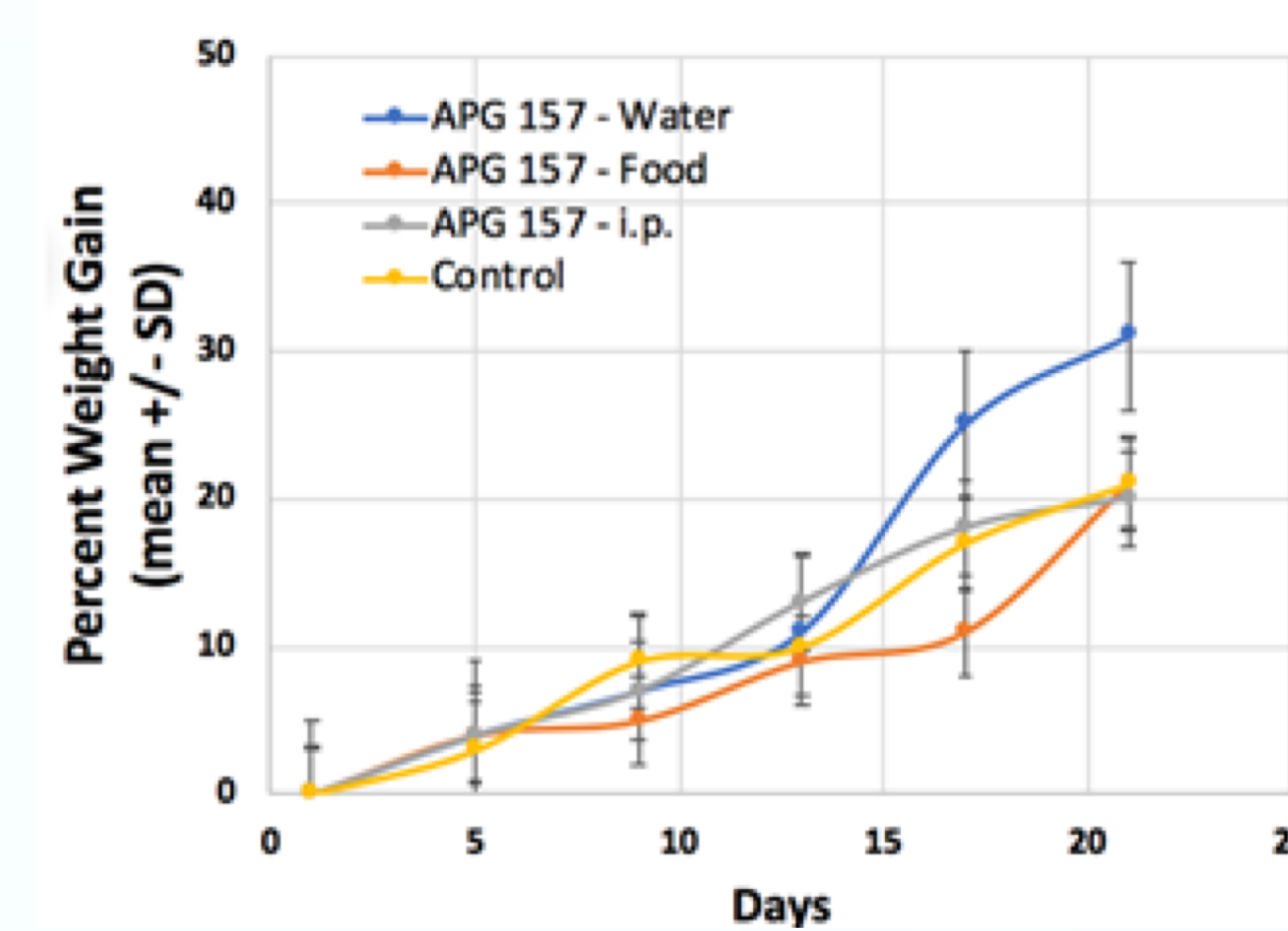


Figure 4. PHRAD129 treatment in water shows weight gain compared to other treatments. These animals were obese in appearance.

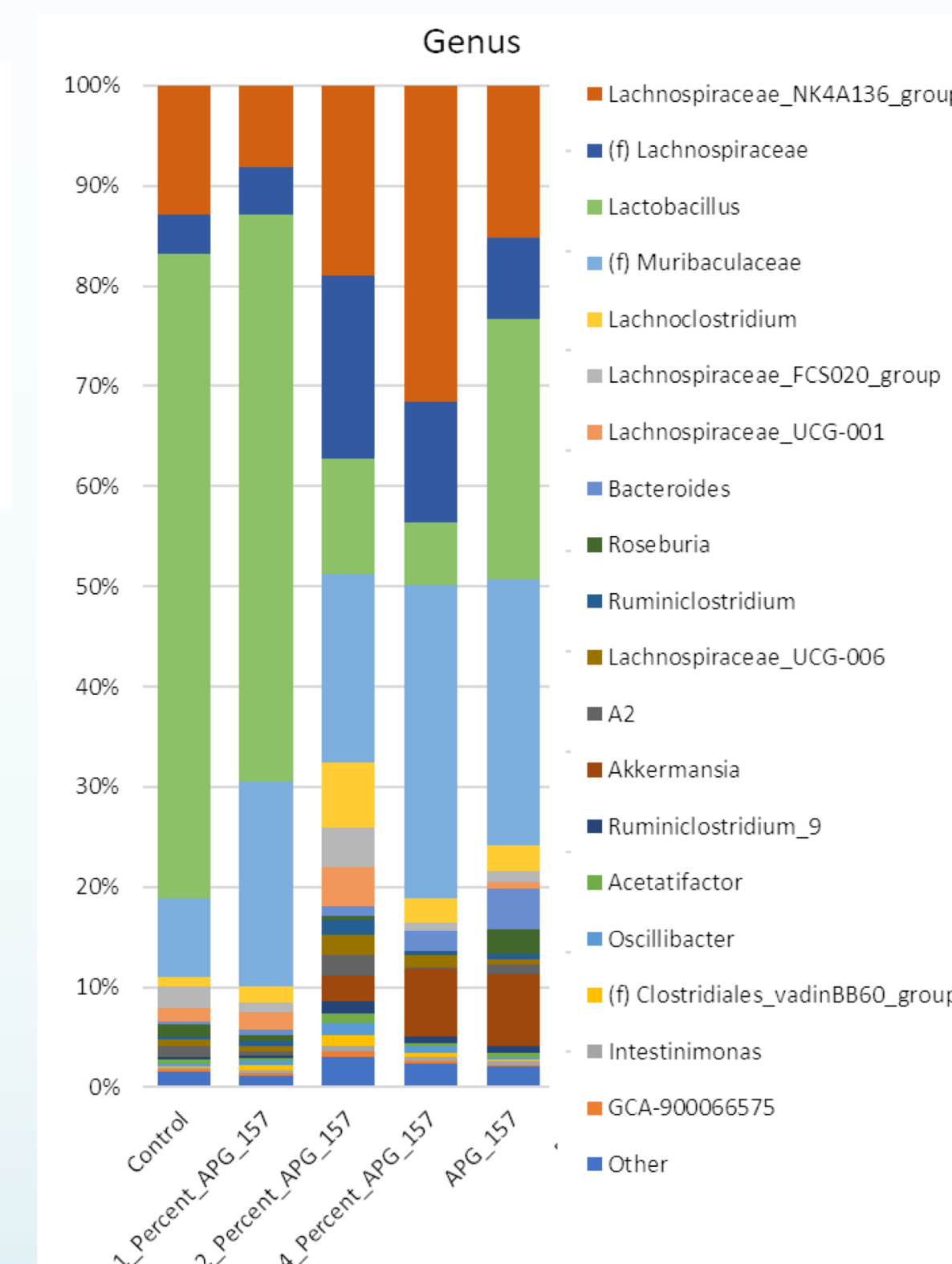


Figure 5. PHRAD129 treatment leads to gut microbial changes, decreased *Lactobacillus* and increased *Lachnospiraceae* NK4A136 species.

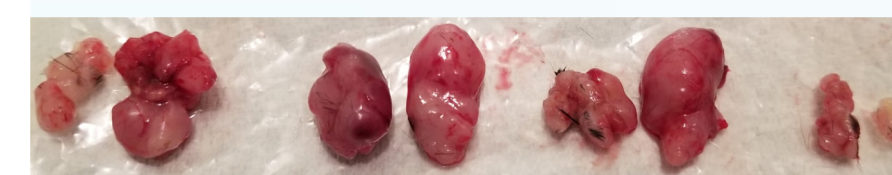


Figure 6. Reduction in tumor growth is seen with 4% PHRAD129 treatment in comparison to control and 1, & 2% PHRAD129.

References

- Kim, S.G., Veena, M.S., Basak, S. K., Han, E., Tajima, T., Gjertson, D.W., et al. Curcumin Treatment Suppresses IKKβ Kinase Activity of Salivary Cells of Patients with Head and Neck Cancer: A Pilot Study. *Clinical Cancer Res.* 17:5953-5961, 2011. PMID: PMC317
- Basak SK, Bera A, Yoon AJ, Morselli M, Jeong C, Tosevska A, Dong Ts 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 Apr 15;126(8):1668-1682. doi: 10.1002/cncr.32644. Epub 2020 Feb 5. PMID: 32022261
- Yoon AJ, Wu H, Pan RD, Teter B, Cipolla J, Chang E, Avila LZ, Basak S et al. A sensitive LC-MS assay using derivatization with boron trifluoride to quantify curcuminoids in biological samples. *Anal Biochem.* 2020 May 1;596:113636. doi: 10.1016/j.ab.2020.113636. Epub 2020 Feb 17. Anal Biochem. 2020. PMID: 32081619