Results Pg infection increased PD-L1 expression on Het-1A cells within 24 hours of infection and increased PD-L1 mRNA within 4 hours of infection. PD-L1 expression level correlated with cellular bacterial burden on the cells in a dose-dependent manner. PD-L1 expression was decreased by the Kgp inhibitor, atuzaginstat, or an Rgp inhibitor, COR613, and PD-L1 expression was completely blocked when both gingipain inhibitors were used together (figure 1). Pg also induced expression of PD-L1 on the surface of infected SCC-25, SH-SY5Y, and RAW cell lines. Western blot analysis and qPCR revealed that Kgp inhibition, but not Rgp inhibition, was able to inhibit the non-canonical activation of b-catenin and down regulation of classical wnt pathway effectors at both the mRNA and protein level.

Abstract 676 Figure 1 Gingipain inhibitors block PD-L1 induced by Pg

Pg grown with and labeled by red fluorescent membrane-incorporated dye was pre-treated with vehicle or the compounds listed for 30 min. Het-1A cells were infected (MOI = 20) for 24 hours, washed, fixed and stained for visualization of the nuclei (DAPI, blue), PD-L1 protein (anti-PDL1 primary and secondary antibodies, green), and Pg infection (red). Images were captured with immunofluorescent confocal microscopy.

Conclusions In host cells infected with Pg, gingipains mediate the induction of PD-L1 as a mechanism of immune evasion through the non-canonical activation of the wnt pathway. Further studies to elucidate induction mechanisms are in progress. In esophageal cancer and other cancers infected with Pg, combining gingipain inhibitors with anti-PD-1 therapy may improve treatment outcomes.

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EVALUATION OF ANTI-PD1 EFFICACY IN GERM-FREE AND ANTIBIOTIC-TREATED SPF MICE BEARING MC38 TUMORS

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Background Increasing evidence has indicated the important role of gut microbes in mediating normal and pathologic immune responses to cancer in both patients and animal models. There is growing effort in modulating microbiota composition to improve the outcome of cancer immunotherapy. To

investigate the immunomodulatory roles of microbiota-based therapeutics preclinically, germ-free (GF) mice are often used because they are free of microorganisms. However, logistic challenges and inherited physiological deficits in GF mice are also generally acknowledged. Alternative approach of depleting gut microbiota in using specific pathogen-free (SPF) mice with broad-spectrum antibiotics has also been adopted. Potential challenges with this approach are possible acquisition of antibiotic-resistant bacteria and potential expansion of fungi. Here we report on the efficacy assessment of anti-PD-1 mAb on MC38 syngeneic tumors in both GF mice and antibiotic-treated SPF mice.

Methods C57BL/6 mice were inoculated subcutaneously with MC38 tumor cells. In the GF study, GF mice (Taconic, provided by Cyagen) were housed in germ-free isolators at a Cyagen facility, and a cohort of SPF mice (Taconic) were used as controls. Both GF and SPF mice were randomized for isotype or anti-PD-1 mAb (mDX400) treatment when the tumors were established (80–120 mm³) and were continuously monitored for tumor growth over time. In the antibiotic treatment study, different antibiotic regimens were administered to SPF mice (Lingchang) in drinking water starting 2 weeks prior to MC38 tumor inoculation and continued throughout the study. Mice were treated with vehicle control or anti-PD-1 mAb (RMP1-14; CrownVivo™).

Results Tumor growth is significantly faster in GF than SPF mice, and mDX400 slowed the tumor growth rate in both GF and SPF mice. The tumors achieved complete regressions on 4 out of 10 GF mice as compared to 6 out of 10 SPF mice, yet the difference of mDX400 efficacy in GF vs SPF mice did not reach statistical significance. In antibiotic-treated SPF mice, none of the antibiotic regimens showed significant impact on MC38 tumor growth nor anti-PD-1 efficacy in SPF mice, which was contrary to most reported data. Immune profiling on tumor infiltrating lymphocytes in these mice and microbiota analysis by 16S rRNA gene amplicon sequencing are ongoing and the data will be presented at the meeting.

Conclusions We have observed faster tumor growth in GF mice, however, the efficacy of anti-PD-1 antibody is not impacted by GF condition or treatment with broad-spectrum antibiotics. These results are different from previously published work, indicating high variability in preclinical models. Ongoing analysis with antibiotic-treated mice will provide further insight.

Ethics Approval Animal experiments were conducted in accordance with animal welfare law, approved by local authorities, and in accordance with the ethical guidelines of CrownBio (Taicang).

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ADDITION OF A SINGLE BACTERIA FACILITATES ANTI-TUMOR IMMUNITY AND LONG-TERM SURVIVAL IN COLORECTAL CANCER

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Background Colorectal cancer remains one of the most common and deadliest cancers worldwide and effective therapies are lacking. While immunotherapy has revolutionized treatment for many cancers, the overwhelming majority of