Background
Antibiotics (ATB) induce intestinal dysbiosis and decrease the efficacy of immune checkpoint inhibitors (ICI).1,2 DAV132 is an orally administered colon-targeted ATB adsorbent designed to prevent ATB-induced dysbiosis.3 We investigated whether DAV132 co-administered with ATB could protect gut microbiota diversity and composition. Moreover, in murine avatar tumor model, we assessed anti-PD-1 efficacy through fecal microbiota transplantation (FMT) in germ-free (GF) or antibiotic-treated specific pathogen-free (SPF) mice.

Methods
Twenty-four human healthy volunteers (HV) were randomized to receive either ceftazidime-avibactam (CZA, 2g/0.5g q8h IV for 5 days) or CZA+DAV132 (12g PO tid for 7 days). CZA plasmatic and fecal pharmacodynamic levels were measured using HPLC-MS/MS. Microbiome was profiled with 16S and shotgun metagenomics at different timepoints. FMT in GF or ATB-treated SPF mice was performed using fecal samples from 3 HV and 2 HV respectively, in each group before (D1) or after 6 days (D6) of CZA+/-DAV132; subsequently mice were inoculated with MCA-205 tumor and frequently mice were inoculated with MCA-205 tumor and treated intraperitoneally with anti-PD-1, 4 times every 3 days. Immunological population of tumor infiltrating lymphocytes were analyzed by flow cytometry.

Results
DAV132 did not impact plasmatic CZA concentrations, but significantly reduced ceftazidime-avibactam concentration in feces compared to HV treated with CZA alone (p<0.001). DAV132 significantly prevented the reduction in microbiota alpha-diversity at D6 (p=0.0019) and was associated with a more rapid return to baseline microbiota composition (figure 1). Significantly more bacteria associated with better response to ICI were preserved in the DAV group compared to CZA, among which Faecalibacterium prausnitzii and several Allstipes spp. FMT in GF mice transplanted with feces collected at D1 exhibited a significant anti-PD-1 activity. This anti-tumor response was inhibited in mice transplanted with D6 feces from any of the 3 CZA-treated HV. Conversely, the anti-tumor response was maintained in mice transplanted with D6 feces from any of the 3 HV treated with CZA + DAV132 (figure 2). Similar results were observed upon FMT using samples from HVs into ATB-treated SPF mice. Flow cytometry on tumor T cell infiltrates demonstrated that CZA decreased CD8+ T cell infiltration and CD8+/Tregulatory ratio, compared to CZA + DAV132 treated HVs (figure 3).

Conclusions
DAV132 strongly prevented CZA-induced dysbiosis in HV without influencing plasmatic concentrations. In avatar mice FMT from HV treated with CZA+DAV132 was able to preserve anti-PD-1 cancer efficacy. These results provide rationale to launch clinical trials combining DAV132 in patients on ATB amenable to ICI.

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REFERENCES

Ethics Approval
All animal studies were approved by the Institutional Animal Care Committee (CIPA) and carried out in compliance with the Canadian Council on Animal Care guidelines (Ethics numbers: C18029BRs).
volunteers selected from each group of treatment (CZA ± DAV132) were transplanted in 10 germ-free mice, 5 being treated with ISO-PD-1 and 5 with aPD-1. Statistics at sacrifice were performed on n=15 mice except for the groups CZA+DAV132/ISO-PD-1 (n=14) and CZA+DAV132/aPD-1 (n=11) before treatment and the group CZA+DAV132/ISO-PD-1 (n=14) at D6.

Abstract 1306 Figure 3  DAV132 preserves local immune response. Antibiotic-depressed anti-tumor CD8+ response and CD8+/Treg ratio are preserved by DAV132. * p < 0.05, Mann-Whitney U Tests. Statistics were performed on n = 10 mice (from 2 healthy volunteers) per group.