Background FLX475 is a potent and selective CCR4 antagonist, designed to block immunosuppressive regulatory T cell (Treg) migration into the tumor microenvironment (TME), which has the potential to overcome immune resistance and broaden clinical efficacy to a variety of conventional and immunotherapy-based approaches. In a recent interim clinical update from the ongoing FLX475 immunotherapy-based approaches, evidence of monotherapy and combination activity were reported. Here, we present biomarker data from patients with multiple tumor types treated with FLX475 as monotherapy and in combination with pembrolizumab. These data support the beneficial effects of FLX475 in modification of the TME and promotion of anti-cancer immunity.

Methods and Results As determined by flow cytometry, a small but significant increase in proportion of circulating Treg (CD4+CD25+CD127−/low) was observed in patients by day 8 of treatment. Immunohistochemistry (IHC) revealed that FLX475 monotherapy increased CD8/Foxp3 density ratio, increased distance between CD8+ and Foxp3+ cells, and reduced migration of Foxp3+ cells from stroma to viable tumor regions. RNAseq data derived from tumor biopsies prior to (n = 33), and after approximately 6 weeks of treatment (n = 22, paired samples) with FLX475+/−pembrolizumab were compared to published biopsy data from anti-PD-(L)1 treated patients. Transcriptomic profiles from tumor biopsies of FLX475 monotherapy treated patients exhibited significant changes in immune pathways to resemble profiles from patients with favorable clinical outcome to anti-PD-(L)1 treatment. Analysis of paired biopsies from both FLX475/pembrolizumab and anti-PD-1 regimens showed significantly increased T cell signatures. However, FLX475/pembrolizumab prevented coordinated increase of Treg cell signatures observed in the TME of patients treated with anti-PD-1 alone. Consistent with this finding, increased expression of CCR4 and its ligands CCL17 and CCL22 were observed in biopsies of patients receiving anti-PD-1 treatment but not FLX475/pembrolizumab. To identify patients more likely to benefit from FLX475/pembrolizumab therapy, baseline transcriptomic profiles were analyzed. Patients with clinical benefit (CR, PR, and stable disease >6 months) were found to have elevated Treg populations compared to those without clinical benefit (PD and SD <6 months). This phenomenon was not observed in external anti-PD-1 datasets.

Conclusions FLX475 monotherapy and in combination with pembrolizumab result in beneficial changes in the TME. FLX475 monotherapy appears to modify the TME toward a phenotype associated with response to anti-PD-(L)1. Baseline markers associated with favorable outcome are different for the combination treatment compared to anti-PD-1 monotherapies suggesting that more patients might benefit from FLX475/pembrolizumab combination than pembrolizumab alone.

Ethics Approval All patients provided informed consent prior to inclusion in the study, and the protocol was approved by local institutional review boards for each clinical site. IRB