Regulatory T cells (T<sub>reg</sub>) are a major immune-suppressive cell found in the tumor microenvironment (TME). FLX Bio has developed potent and selective small molecule antagonists of CCR4, a key T<sub>reg</sub> Chemotactic receptor that helps them migrate into tumors. Epstein-Barr Virus (EBV) positivity is seen in approximately 95% of Nasopharyngeal Carcinoma, 40% of classical Hodgkin's Lymphoma, and 10% of Gastric Carcinomas. The EBV gene LMP1 has been shown to drive high expression of the CCR4 ligands, CCL17 and CCL22. EBV-positive tumors may be particularly sensitive to treatment with the FLX Bio CCR4 antagonist in the clinic.

EBV-Associated Tumors Increase Regulatory T cell Recruitment via CCR4 Ligand Expression and are a Promising Indication for Treatment with Small Molecule CCR4 Inhibitors

Expression of LMP1 in Human EBV+ B Cells Induces CCL22

LMP<sup>+</sup> Tumors Produce High Levels of CCL22 in Mouse Models

Summary and Conclusion

- EBV<sup>+</sup> tumors in humans, including Nasopharyngeal, Classical Hodgkin's Lymphoma, and Gastric Carcinoma show expression of the CCR4 ligands, CCL22 and CCL17, and increased T<sub>reg</sub> infiltration, as measured by FOXP3 expression.
- Supporting observations that the Epstein-Barr Virus (EBV) LMP1 gene can drive expression of CCR4 ligands, we find high production of CCL22 by EBV<sup>+</sup> RAJI cells in vitro and in vivo, but not by EBV<sup>+</sup> LMP1<sup>-</sup> DAUDI cells or EBV<sup>+</sup> LMP1-siRNA RAJI cells. RAJI supernatant drove migration by CCR4<sup>+</sup> CEM cells that could be blocked by a FLX Bio CCR4 small-molecule antagonist.
- An engineered LMP1<sup>+</sup> CT26 mouse cell line produced significant levels of CCL22 in vivo but not in vitro, suggesting that, directly or indirectly, LMP1 also has effects on non-B cell tumors. Further studies will be performed to demonstrate antitumor effects of the FLX Bio CCR4 small-molecule antagonist in this system.
- Thus, EBV<sup>+</sup> tumors may modulate the tumor microenvironment by recruiting suppressive T<sub>reg</sub> via CCL17 and CCL22. EBV<sup>+</sup> tumor patients may represent a population preferentially sensitive to CCR4-antagonist treatment.