Potent and selective C-C chemokine receptor (CCR4) antagonists potentiate anti-tumor immune responses by inhibiting regulatory T cells (T_{reg})

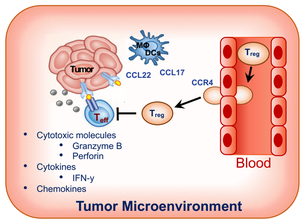
Oezcan Talay, Lisa Marshall, Cesar Meleza, Maureen K. Reilly, Omar Robles, Mikhail Zibinsky, Abood Okal, Lisa Seitz, Jenny McKinnell, Scott Jacobson, Erin Riegler, Emily Karbaz, David Chian, Angela Wadsworth, Paul Kassner, David Wustrow, Jordan S. Fridman

FLX Bio, Inc. South San Francisco, CA

Abstract

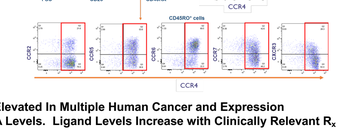
Naturally suppressive CD4$^+$ Foxp3$^+$ T_{reg} are essential for immune tolerance. Although T_{reg}-mediated suppression of effector cells is important to control inflammation and prevent autoimmune diseases, the presence of T_{reg} in the tumor microenvironment (TME) has been shown to dampen anti-tumor immune responses. Human T_{reg} express CCR4, the receptor for the chemokines CCL17 and CCL22. These chemokines are produced by tumor cells, tumor-associated macrophages and dendritic cells, as well as by effector T cells (T_{eff}). Preclinical and clinical data supports a role for CCR4-mediated recruitment and accumulation of T_{reg} in the TME which can be associated with poor prognosis. Further, recent longitudinal studies in patients receiving IO agents demonstrate an influx of T_{reg} in responding patients which may dampen optimal anti-tumor responses. Therefore, CCR4 is an ideal target to selectively block T_{reg} recruitment into the TME.

We have developed structurally unique series of small molecule antagonists of CCR4. These antagonists have cellular potencies in multiple assays (e.g. chemotaxis of primary human T_{reg} in 100% serum) in the low double-digit nM range. Representative compounds are selective against other chemokine receptors, GPCRs and ion channels, including the hERG channel, and lack inhibition of common human CYP450 enzymes. Moreover, compounds have excellent *in vitro* and *in vivo* ADME properties, consistent with convenient oral dosing. In preclinical syngeneic tumor models, these CCR4 antagonists block T_{reg} migration and support expansion of activated T_{eff}. In contrast to the non-selective approach of depleting anti-CCR4 antibodies, our compounds reduce T_{reg} in the tumor, but not in peripheral tissues such as blood, spleen or skin. In preclinical efficacy studies, CCR4 antagonists potentiate the anti-tumor effects of various checkpoint inhibitors and immune stimulators such as anti-PD-L1 and anti-CTLA-4 antibodies. We observe enhanced tumor growth inhibition and increased tumor regressions when these agents are combined with CCR4 antagonists, without any gross toxicity. Further characterization of these CCR4 antagonists and their anti-tumor effects will be described.

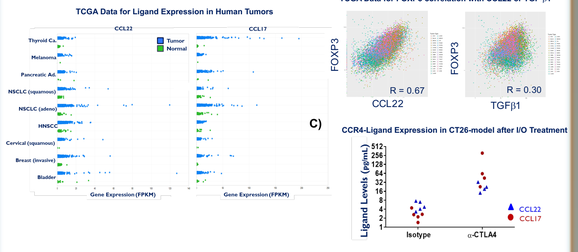


CCR4 is a Dominant Regulator of T_{reg} in Human Tumors

A) CCR4 Is The Most Prevalent Chemokine Receptor On Human Effector T_{reg}



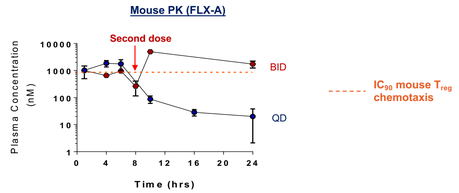
B) CCR4 Ligand Expression is Elevated In Multiple Human Cancer and Expression Correlates with FOXP3 mRNA Levels. Ligand Levels Increase with Clinically Relevant R_x



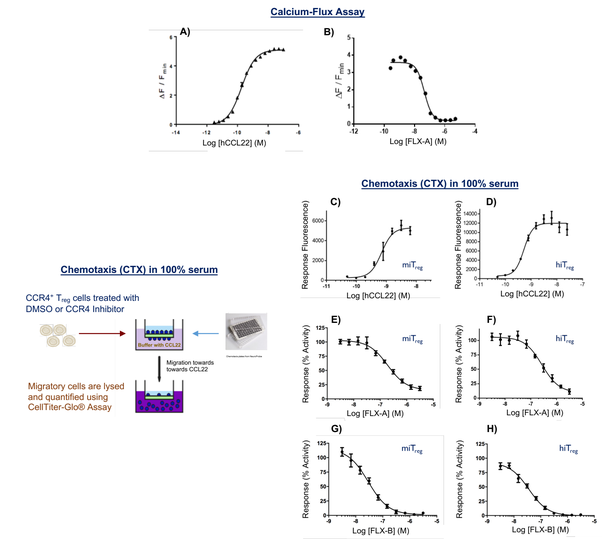
Effector T_{reg} are the most suppressive subpopulation of T_{reg} and they express the highest amount of CCR4 (Tanaka and Sakaguchi, Cell Research, 2017). **A)** CCR4 is the most prevalent chemokine receptor on human T_{reg}. **B)** The expression of CCR4 ligands (CCL22 and CCL17) is elevated in a spectrum of human tumor tissues and ligand expression correlates with FOXP3 mRNA - a marker for T_{reg}. **C)** CCR4-ligand is upregulated in "ligand-low" syngeneic tumors (representative data shown in CT26) after treatment with checkpoint inhibitor.

FLX Antagonist can be Dosed to Cover the Mouse T_{reg} Chemotaxis IC_{90}

	Serum Free Ca^{++} Flux, IC_{50} (μM)	10% Serum Ca^{++} Flux, IC_{50} (μM)	CTX 100% Serum, IC_{50} (μM)	Mouse IV t1/2 (hr)	Mouse Cl (L/hr/Kg)	Mouse V_{ss} (L/Kg)	Mouse F (%)
FLX-A	0.055	0.103	0.164	2.1	12.6	21.1	7
FLX-B	0.021	0.093	0.065	7.6	0.72	5.7	26

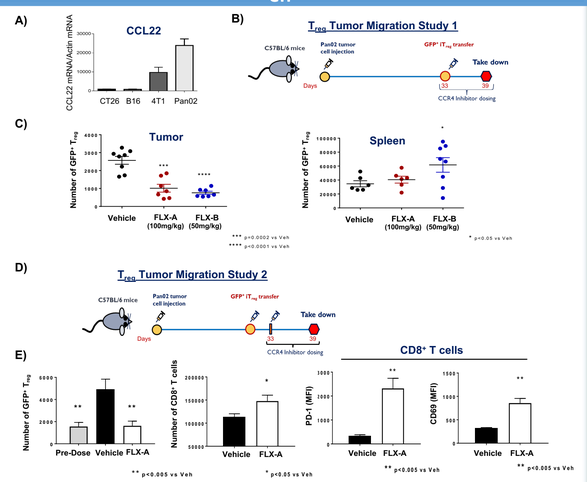


FLX Antagonist are Highly Potent Against Mouse and Human T_{reg} Chemotaxis



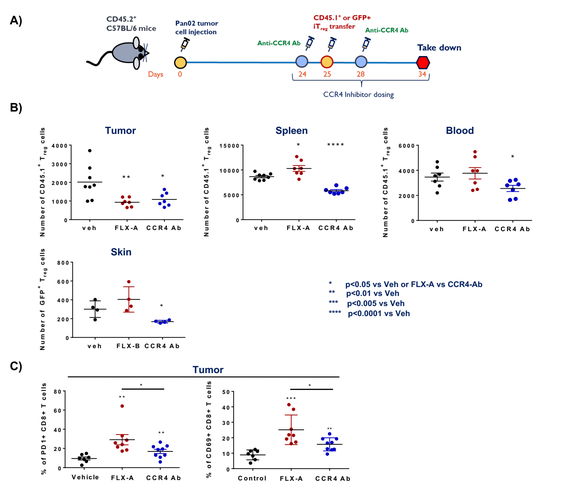
A) Ca^{2+} flux in CCR4-expressing Chem5-hCCR4 cells is induced by CCL22. **B)** Compound FLX-A demonstrated dose-dependent inhibition of CCL22-induced Ca^{2+}-flux. **C, D)** Chemotaxis of murine induced T_{reg} (miT_{reg}) and human induced T_{reg} (hiT_{reg}) is stimulated by CCL22. **E, F)** FLX-A demonstrated dose-dependent inhibition of CCL22-induced chemotaxis of miT_{reg} and hiT_{reg} ,respectively. **G,H)** FLX-B demonstrated dose-dependent inhibition of CCL22-induced chemotaxis of miT_{reg} and hiT_{reg} ,respectively.

CCR4 Antagonism Blocks T_{reg} Recruitment and Increase T_{eff} in Tumors



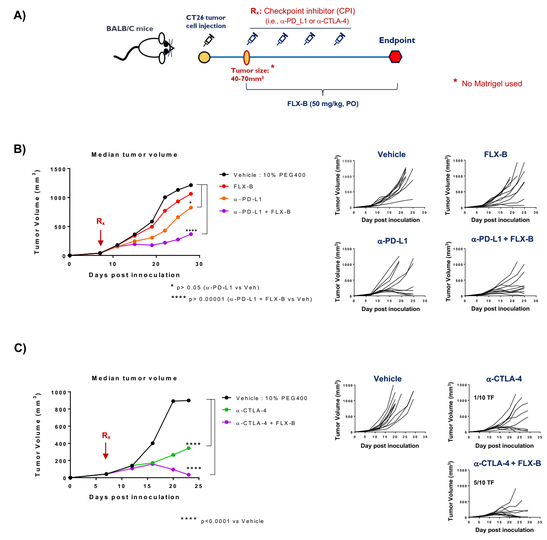
Exogenous GFP$^+$ T_{reg} were transferred into tumor-bearing mice and tumor-trafficking was analyzed. **A)** Pancreatic carcinoma Pan02 cells express high basal level of CCL22 and CCL17 (data not shown). **B)** Experimental outline T_{reg} Tumor migration study 1. **C)** Significant and selective inhibition of T_{reg} cell migration into the tumor. **D)** Experimental outline T_{reg} Tumor migration study 2. **E)** Decreased number of T_{reg} and increased number of activated CD8 T cells (shown as MFI for PD-1 and CD69) in the tumor of CCR4-inhibitor treated animals.

CCR4 Antagonism is a Selective Approach to Blocking T_{reg} Recruitment



A) Experimental outline. Animals were dosed with either FLX-A or depleting mouse anti-CCR4 Ab. **B)** CCR4 inhibitor selectively inhibits T_{reg} migration into the tumor but not in the periphery. Anti-CCR4 antibody systemically reduced T_{reg} numbers. **C)** Both treatments show similar increase in activated CD8 T cell numbers.

FLX CCR4 Antagonists Potentiate Various IO Agents



Tumor efficacy studies in Colon Carcinoma (CT26) tumor model using CCR4 inhibitor alone or in combination

A) Study outline. **B)** Mice treated with CCR4 inhibitor or anti-PD-L1 showed modest efficacy while combination significantly reduced tumor growth. **C)** Mice treated with anti-CTLA-4 alone showed significant tumor growth inhibition (TGI) with one tumor-free animals. TGI was significantly improved when combined with CCR4 inhibitor with 6 tumor-free animals.

Conclusions

- **CCR4 is the most prevalent chemokine receptor expressed on T_{reg} and high CCR4 expressing Treg are the most potent suppressive sub-population**
- **CCL22 and CCL17, the cognate CCR4 ligands, are elevated in human tumors and their expression levels correlate with FOXP3, a T_{reg} marker**
- **FLX CCR4 antagonists potently and selectively block agonist activation in both a calcium flux assay and chemotaxis assay run in 100% human serum**
- **FLX CCR4 antagonists selectively block T_{reg} migration into PAN02 tumors**
- **Unlike depleting antibodies (e.g. αCCR4) T_{reg} in skin, blood and spleen are not reduced with FLX CCR4 antagonists**
- **The addition of FLX CCR4 antagonists to clinically relevant antibodies, such as αPD-L1 or αCTLA4, results in dramatically improved tumor growth inhibition and regression rates**
- **These data demonstrate that a potent and selective CCR4 antagonist may be a tumor-selective and attractive approach to antagonize Treg function and support a productive anti-tumor immune response**
- **A FLX CCR4 antagonist is scheduled to begin clinical trials in 2017**