Potent and selective C-C chemokine receptor 4 (CCR4) antagonists inhibit regulatory T cell recruitment, increase effector T cell numbers, and potentiate anti-tumor responses in mice

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Background

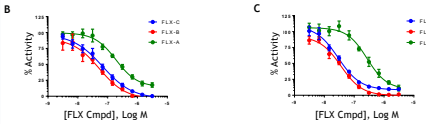
Regulatory T cells (T_{reg}) are essential for immune tolerance and T_{reg}-mediated suppression of effector T cells (T_{eff}) is important to control inflammation and prevent autoimmune diseases. However, the presence of T_{reg} in the tumor microenvironment (TME) has been shown to dampen anti-tumor immune responses. Human T_{reg} express CCR4, the receptor for the chemokines CCL17 and CCL22. 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. Furthermore, patients receiving immunomodulatory agents demonstrate an influx of T_{reg} in responding patients which may dampen optimal anti-tumor responses. CCR4 is an ideal target to selectively block T_{reg} recruitment into the TME.

We have developed a structurally unique series of small molecule antagonists of CCR4 with cellular potencies in multiple assays (e.g. chemotaxis of primary human T_{reg} in 100% serum) in the low double-digit nM range. Moreover, these compounds have excellent *in vitro* and *in vivo* ADME properties, consistent with convenient oral dosing. These CCR4 antagonists were tested in murine syngeneic tumor models alone and in combination with immunomodulatory agents. During and following treatment, CCR4 ligand levels, tumor infiltrating lymphocytes, and tumor volumes were evaluated.

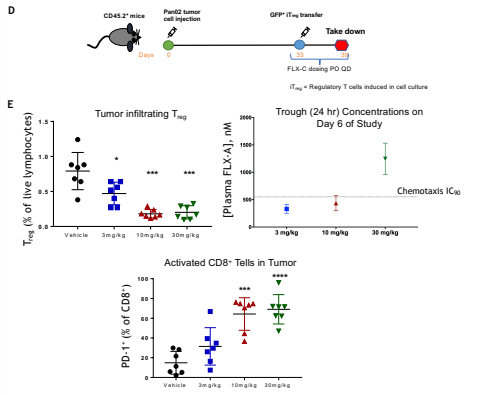
Potent CCR4 antagonists inhibit T_{reg} recruitment to tumors

A

	Human Treg Chemotaxis (100% Serum), IC_{50} (nM)	Mouse Treg Chemotaxis (100% Serum), IC_{50} (nM)	Mouse IV t1/2 (hr)	Mouse Cl (L/hr/Kg)	Mouse V_{ss} (L/Kg)	Mouse F (%)
FLX-A	154	192	2.1	12.6	21.1	7
FLX-B	33	42	7.6	0.72	5.7	26
FLX-C	31	63	19.6	0.23	6.2	22

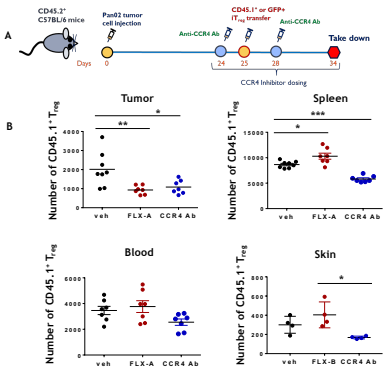


A) Potency and PK parameters for select FLX Bio CCR4 antagonists. Three different FLX Bio CCR4 antagonists demonstrated dose-dependent inhibition of CCL22-induced chemotaxis of **B)** mouse iT_{reg} and **C)** human iT_{reg} ,respectively.



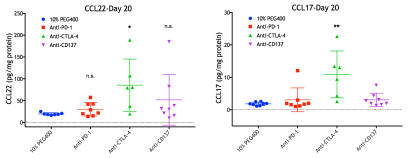
In vivo T_{reg} trafficking into tumor. D) Study design: Murine pancreatic tumor cells (Pan02) were subcutaneously inoculated into C57BL/6 mice. Tumor-bearing mice were dosed with FLX-C or vehicle prior to transfer of *in vitro*-induced T_{reg} (iT_{reg}) (I.V.). **E)** Analysis of TILs: Dose-dependent inhibition of T_{reg} trafficking into tumor but not in periphery (data not shown). Activated $CD8^+$ T cell numbers (measured by $PD\text{-}1^+$ staining) increased with higher dose of FLX-C.

CCR4 antagonism does not delete peripheral T_{reg} cells



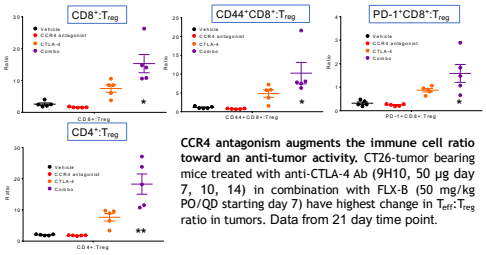
A) Experimental outline. Animals were dosed with either FLX-A (100 mg/kg, PO/BID), FLX-B (50 mg/kg, PO/QD) or depleting mouse anti-CCR4 Ab (2G12, 300 µg). **B)** CCR4 inhibitor selectively inhibits T_{reg} migration into the tumor but not in the periphery, spleen or skin. Anti-CCR4 antibody systemically reduced T_{reg} numbers. CCR4 antagonism provides a potential safety advantage compared to a depleting antibody. Both treatments show similar increase in activated CD8 T cell numbers in the tumor (Data not shown).

Immunomodulatory agents induce CCR4 ligand upregulation



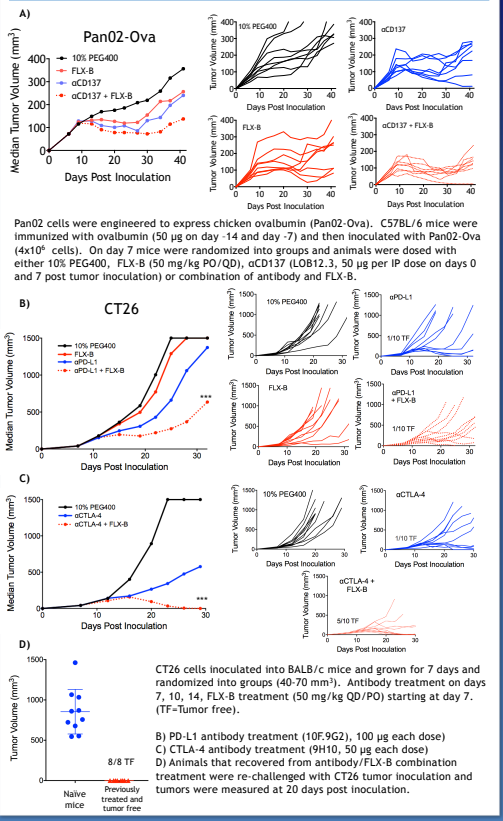
Both CCL17 and CCL22 ligand levels are increased after dosing with IO agents in colon tumor (CT26)-bearing mice. Anti-PD-1(J43; 200 µg/mouse first dose, then 100ug), anti-CD137 (LOB12.3; 150 µg/mouse first dose, then 100ug) or anti-CTLA-4 (9H10; 150 µg/mouse/first dose, then 100ug) were given on days 4, 7 and 11 following tumor inoculation. Tumors were harvested at day 20 and ligands measured by ELISA.

CCR4 antagonism increases T_{eff}:T_{reg} ratio in tumors



CCR4 antagonism augments the immune cell ratio toward an anti-tumor activity. CT26-tumor bearing mice treated with anti-CTLA-4 Ab (9H10, 50 µg day 7, 10, 14) in combination with FLX-B (50 mg/kg PO/QD starting day 7) have highest change in T_{eff}:T_{reg} ratio in tumors. Data from 21 day time point.

CCR4 antagonists potentiate anti-tumor effects of immune modulators



Pan02 cells were engineered to express chicken ovalbumin (Pan02-Ova). C57BL/6 mice were immunized with ovalbumin (50 µg on day -14 and day -7) and then inoculated with Pan02-Ova ($4x10^6$ cells). On day 7 mice were randomized into groups and animals were dosed with either 10% PEG400, FLX-B (50 mg/kg PO/QD), αCD137 (LOB12.3, 50 µg per IP dose on days 0 and 7 post tumor inoculation) or combination of antibody and FLX-B.

CT26 cells inoculated into BALB/c mice and grown for 7 days and randomized into groups (40-70 mm^3). Antibody treatment on days 7, 10, 14, FLX-B treatment (50 mg/kg QD/PO) starting at day 7. (TF=Tumor free).

B) PD-L1 antibody treatment (10F.9G2), 100 µg each dose)
C) CTLA-4 antibody treatment (9H10, 50 µg each dose)
D) Animals that recovered from antibody/FLX-B combination treatment were re-challenged with CT26 tumor inoculation and tumors were measured at 20 days post inoculation.

Results and Conclusions

We have developed specific and potent CCR4 antagonists that block Treg migration and preclinically, these CCR4 antagonists block T_{reg} migration and support expansion of activated T_{eff} in the tumor. Our antagonists reduce T_{reg} in the tumor, but not in peripheral tissues such as blood, spleen or skin; which presents a potential safety advantage to the non-selective approach of depleting anti-CCR4 antibodies. In preclinical efficacy studies, treatment with various checkpoint inhibitors and immune stimulators (e.g., anti-CTLA-4 or anti-CD137) induce the upregulation of CCR4 ligand expression. Combination therapy with CCR4 antagonist and immunomodulatory agents reduced intratumoral T_{reg} number and increased number of activated and total T_{eff}, resulting in an increase in the intratumoral ratios of both $CD4^+$ and $CD8^+$ T_{eff} to T_{reg}. The change in these T_{eff} to T_{reg} ratios is greater for our CCR4 antagonist in combination than with the immunomodulatory agent alone and correlates with enhanced tumor growth inhibition and increased tumor regression.

Combination therapy with CCR4 antagonist and immunomodulatory agents overcome T_{reg}-mediated suppression in tumors and tips the balance toward tumor rejection.

Unless otherwise indicated, significance tests and p-values refer to test compared to vehicle control group. P values represented as follows: 0.05 > * > 0.01 > ** > 0.001 > *** > 0.0001 > ****