Antibody-Based Inhibition of CSF-1R as a Component of Combination Immunotherapy in Preclinical Models

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Abstract
The colony stimulating factor 1 receptor (CSF-1R) signaling pathway promotes tumor progression via the recruitment, differentiation, and survival of immunosuppressive, M2 polarized, tumor-associated macrophages (TAMs). FivePrime has developed cabiralizumab (FP008), an IgG4 antibody against CSF-1R that blocks the ability of both CSF-1 and IL-34 to bind and activate this receptor, thereby modulating the immune response to tumorigenesis. In order to investigate the impact of CSF-1R signaling inhibition in preclinical models, we generated a surrogate antibody, cmFP008, that targets mouse CSF-1R and demonstrates equivalent affinity and ligand-blocking ability as FP008. Utilizing a combination of flow cytometry and immunofluorescence analyses, we have identified alterations in the tumor microenvironment that occur upon CSF-1R inhibition, including significant reduction of immunosuppressive M2 TAMs and an increase in tumor PD-L1 expression. Interestingly, we observe an increase in CD8+ T cell number and activation upon TAM depletion, followed by a subsequent increase in putative monocytic (CD11b+Ly6G-/Ly6C+) and granulocytic (CD11b+Ly6G+Ly6C-) MDSC populations. Moreover, we have used murine syngeneic tumor models to examine the anti-tumor impact of CSF-1R inhibition in combination with other immunomodulatory agents. Our results show that, when added to PD-1/PD-L1 blockade, cmFP008 can significantly enhance anti-tumor efficacy. We are currently exploring the effects of combining cmFP008-induced TAM depletion with additional immunomodulatory agents, including T cell agonists. Our preclinical results demonstrate that inhibition of the CSF-1R pathway can combine with various immunomodulatory agents with distinct mechanisms of action. FivePrime has initiated a clinical trial in collaboration with Bristol-Myers Squibb (BMS) to investigate the use of cabiralizumab in combination with nivolumab (anti-PD-1, OPDIVO) in six different tumor types.

Anti-Cancer Mechanism of Action of cabiralizumab
Cabiralizumab (FP008) blocks the binding of CSF-1 and IL-34 to CSF-1R

- Cabiralizumab (FP008) is a humanized IgG4 anti-CSF-1R that blocks binding of both CSF-1 and IL-34.
- Cabiralizumab inhibits survival of CSF-1R-dependent monocytes and macrophages.

Combination of cabiralizumab with anti-PD-1 in cancer immunotherapy

- Tumor-associated macrophages (TAMs) facilitate tumor growth by immune-suppression, including direct and indirect inhibition of cytotoxic CD8+ T cells.
- Cabiralizumab-mediated inhibition of CSF-1R is anticipated to impact TAM function and/or viability, thereby reducing immune-suppression and allowing anti-tumor T cell activation.
- Co inhibition of CSF-1R and PD-1/PD-L1 signaling is hypothesized to potentiate an anti-tumor immune response.

cmFP008 Reduces Growth and M2 TAMs in the MC38 Model

- cmFP008 is a surrogate mouse IgG1 antibody developed at Five Prime that binds to murine CSF-1R with comparable affinity to cabiralizumab.
- MC38 bearing mice were administered a single dose of cmFP008 at 20 mg/kg, and tumors were analyzed via flow cytometry and immunofluorescence to examine changes in ILs.
- cmFP008 significantly reduced MC38 tumor volume beginning on Study Day 18.
- Immunosuppressive M2 macrophages (TAMs; CD11b+Ly6G-Ly6C-F4/80+CD206+) are depleted within 48 hours of cmFP008 dosing, with near complete depletion 4 days after treatment.
- TAMs begin to repopulate the tumor microenvironment after Day 17, corresponding with reduced cmFP008 drug exposure.
- Statistical analysis was performed by 2-tailed t-test, (* p < 0.05, ** p < 0.01, *** p < 0.001).

The Combination of cmFP008 and anti-PD-1 Induces Potent Anti-Tumor Activity

- cmFP008 treatment induces an increase in tumor PD-L1 expression near the tumor periphery.
- The expression of PD-L1 in the tumor periphery is correlated with an increase in CD8+ T cells at the tumor margin.
- cmFP008 and anti-PD-1 together induce a greater reduction in MC38 tumor volume than either therapeutic alone.
- We have shown previously that cmFP008 and anti-PD-1 exert superior anti-tumor activity in combination with gemcitabine in a preclinical model of pancreatic ductal adenocarcinoma (Bellovin, et. al., STIT Annual Meeting, 2015).
- Statistical analysis by one-way ANOVA (*** p < 0.001, *** p < 0.001).

TAMs are Depleted via cmFP008-Induced Apoptosis

- cmFP008 treatment results in depletion of immunosuppressive M2 TAMs from MC38 tumors, as shown by reduction in F4/80 immunofluorescence, with kinetics similar to those demonstrated via flow cytometry.
- TAMs undergo apoptosis following cmFP008 administration, as demonstrated by the presence of activated caspase-3 staining in F4/80+ cells within tumors.

Summary and Conclusions

- cmFP008 significantly reduces MC38 tumor growth as monotherapy and when administered in combination with anti-PD-1.
- Treatment with cmFP008 results in depletion of immunosuppressive M2 tumor-associated macrophages (TAMs) via apoptosis, as well as significant, but transient changes in CD68+ cells and putative MDSC populations.
- cmFP008 monotherapy induces an increase in CD8+ T cells in MC38 primarily at the tumor periphery where PD-L1 staining is the most intense.
- The anti-tumor activity of cmFP008 in combination with anti-PD-1 in MC38 correlates with a significant depletion of M2 TAMs, increase in CD8+ T cells, and a favorable CD8+/CD4+ T cell ratio compared to either therapeutic alone.

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