cmFPA008, an Anti-Mouse CSF-1R Antibody, Combines with Multiple Immunotherapies to Reduce Tumor Growth in Nonclinical Models

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Abstract

The colony stimulating factor 1 receptor (CSF1R) signaling pathway drives the recruitment, differentiation, and survival of tumor-associated macrophages (TAMs) in the neoplastic microenvironment, promoting tumor progression and suppression of the immune response. As such, CSF1R represents an intriguing therapeutic target for immuno-oncology. FivePrime has developed FPA008, an IgG4 antibody with high affinity for CSF1R and the ability to block binding of both CSF1 and IL-34 to this receptor. In order to interrogate the impact of CSF1R signaling inhibition on tumor growth and the immune environment, we generated a surrogate antibody, cmFPA008, which targets mouse CSF1R and demonstrates equivalent affinity and ligand-blocking ability as FPA008.

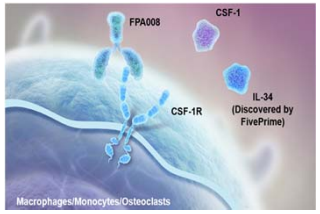
Consistent with other reports on molecules targeting this pathway, cmFPA008 as monotherapy results in statistically significant but modest growth inhibition in multiple preclinical tumor models, including MC38 colon adenocarcinoma and B16 melanoma. Utilizing a combination of flow cytometric, immunohistochemical, and gene expression analyses, we show that CSF1R inhibition induces a marked reduction of TAMs in syngeneic tumor models.

Given that the regulation of an anti-tumor immune response is complex, effective cancer therapy may require combining multiple immunotherapy agents. Therefore, we sought to determine whether inhibition of CSF1R when combined with other immuno-oncology therapeutics enhanced the anti-tumor impact. We observed increased PD-L1 (CD274) expression in the tumor after treatment with cmFPA008 monotherapy, thereby providing a rationale for a combination of FPA008 with a compound targeting the PD-1 pathway. Our results show that cmFPA008 significantly enhances anti-tumor efficacy when combined with an anti-PD1 antibody in multiple syngeneic tumor models. In addition, we show that co-administration of cmFPA008 with an agonistic anti-CD40 antibody significantly enhances tumor suppression compared to either therapy alone. Changes in tumor-infiltrating lymphocyte (TIL) populations upon treatment provide important insight into the mechanism of action of cmFPA008 monotherapy and in combination with other immunotherapies.

These results provide support for ongoing clinical efforts to evaluate FPA008 as an anti-cancer immunotherapy, particularly in combination with other immuno-oncology therapeutics. Five Prime has initiated a clinical trial in collaboration with Bristol-Myers Squibb (BMS) to investigate the efficacy of FPA008 in combination with the anti-PD1 therapeutic OPDIVO® (nivolumab) in six tumor types.

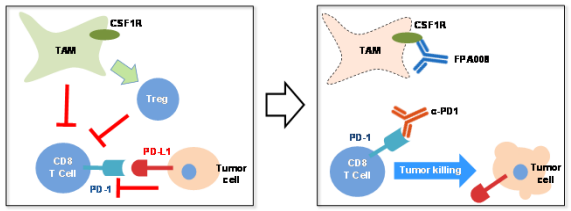
Background

FPA008 blocks the binding of CSF-1 and IL-34 to CSF1R



- FPA008 is a humanized IgG4 anti-CSF1R that blocks binding of both CSF1 and IL-34.
- FPA008 inhibits survival of CSF1R-dependent monocytes, macrophages, and osteoclasts.

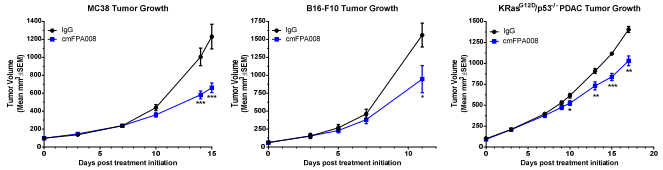
Potential mechanism of action of FPA008 in cancer immunotherapy



- Tumor-associated macrophages (TAMs) facilitate tumor growth by immuno-suppression, including direct and indirect inhibition of cytotoxic CD8 T cells.
- FPA008-mediated inhibition of CSF1R is anticipated to impact TAM function and/or viability, thereby reducing immuno-suppression and allowing anti-tumor T cell activation .
- Co-inhibition of CSF1R and PD-1/PD-L1 signaling is hypothesized to potentiate an anti-tumor immune response.

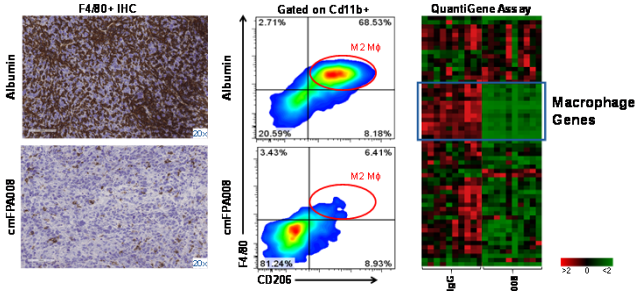
Results

CSF1R blockade reduces growth of multiple non-clinical tumor models



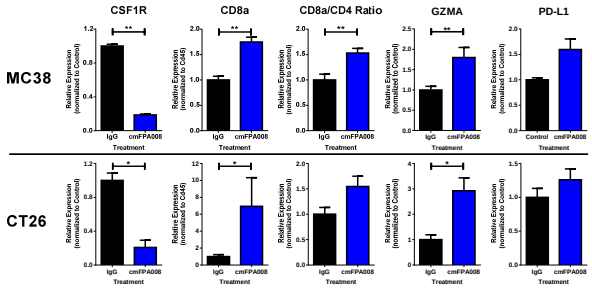
- Mice were administered cmFPA008, a rat anti-mouse CSF1R antibody engineered to express a mouse Ig tail (IgG1) that binds CSF1R and blocks CSF1 and IL-34 ligand-induced signaling with similar affinity to FPA008.
- Multiple subcutaneous tumor models demonstrated reduction in tumor growth when cmFPA008 was administered as a monotherapy in immunocompetent mice.
  - Statistical significance determined by unpaired, two-tailed t test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

cmFPA008 reduces tumor-associated macrophages (TAMs)



- Reduction in tumor-associated macrophages (TAMs) is observed in MC38 tumors following cmFPA008 administration.
- TAM depletion can be observed via immunohistochemistry (left), flow cytometry (center), and QuantiGene Plex expression analysis (right).

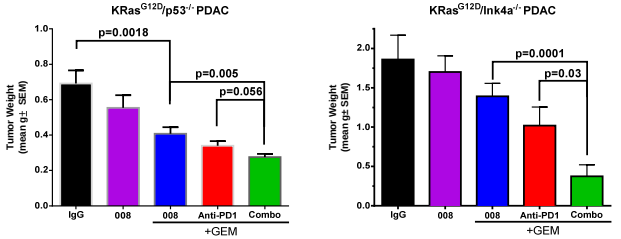
cmFPA008 treatment increases the frequency of cytotoxic T cells and the expression of PD-L1 in multiple mouse tumor models



- Immunocompetent, MC38 or CT26 tumor-bearing mice were administered cmFPA008 and tumor immune infiltration was assessed by QuantiGene Plex expression analysis.
- cmFPA008 treatment increased the abundance of CD8+ T cells relative to CD4+ T cells.
  - Increase in GZMA supports increased CTL activation upon cmFPA008 treatment.
- Tumor PD-L1 expression is increased following administration of cmFPA008.
  - Statistical significance determined by unpaired, two-tailed t test, * $p < 0.05$, ** $p < 0.01$.

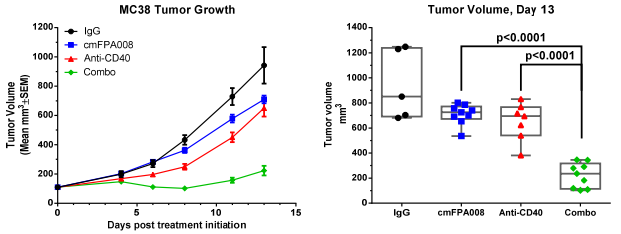
Results

Maximum efficacy in orthotopic pancreatic cancer models is achieved by the combination of cmFPA008 and anti-PD-1



- Pancreatic ductal adenocarcinoma (PDAC) cells were surgically implanted into the pancreas of immunocompetent mice.
- Tumor-bearing mice were treated with cmFPA008, anti-PD-1, or the combination together with gemcitabine (GEM).
- Combination therapy enhances anti-tumor efficacy compared to either cmFPA008 or anti-PD-1 alone.
  - Statistical significance determined by unpaired, two-tailed t test.

cmFPA008 combines with anti-CD40 immunotherapy to inhibit MC38 growth



- MC38 tumor-bearing mice were administered cmFPA008, agonist anti-CD40 (FGK45), or the combination.
- Combination therapy significantly inhibited tumor growth compared to either cmFPA008 or anti-CD40 alone.
  - Statistical significance determined by one-way ANOVA.

Conclusions

- Targeting the CSF1R pathway using cmFPA008 inhibits the growth of tumors in multiple models.
- Treatment with cmFPA008 results in marked reduction in tumor-associated macrophages (TAMs), shown by immunohistochemistry, flow cytometry, and gene expression analysis.
- cmFPA008 treatment induces an increase in the relative abundance and activation of cytotoxic CD8+ T cells, as well as an increase in PD-L1 in multiple tumor models.
- cmFPA008 and anti-PD-1 combine for greater anti-tumor efficacy than either immunotherapy alone.
  - Demonstrated in two independent orthotopic pancreatic ductal adenocarcinoma (PDAC) models, as well as in the subcutaneous MC38 tumor model
  - Five Prime and BMS have initiated a collaboration to evaluate the combination of FPA008 and OPDIVO in a Phase Ia/Ib clinical trial in multiple cancer indications.
- cmFPA008 can also enhance the anti-tumor efficacy of anti-CD40 agonist immunotherapies in nonclinical tumor models.

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