Abstract #2597

Preclinical Efficacy of FP-1039 (FGFR1:Fc) in Endometrial Carcinoma Models with Activating Mutations in *FGFR2*

Authors: Thomas C. Harding, Servando Palencia, Li Long, Jeffrey T. Finer, Harold N. Keer, Kevin P. Baker, W. Michael Kavanaugh • Five Prime Therapeutics Inc., San Francisco, CA 94158*

INTRODUCTION FP-1039

- The Fibroblast Growth Factor (FGF) / Fibroblast Growth Factor Receptor (FGFR) signaling pathway has been widely implicated in the development and maintenance of many different cancers.
- FP-1039 **(Figure 1)** is a soluble fusion protein consisting of the extracellular domains of human FGFR1 linked to the Fc region of human Immunoglobulin G_1 (IgG_1).
- FP-1039 is designed to bind multiple FGF ligands and prevent them from activating multiple FGF receptors **(Figure 1)**.
- FP-1039 has multiple potential mechanisms of action in cancer:
  - — Direct anti-tumor activity in cancers dependent on the FGF pathway
  - — Inhibition of tumor angiogenesis
  - — Inhibition of cancer stem cell maintenance
- FP-1039 is currently in a Phase I clinical study to characterize safety and pharmacokinetics in cancer patients[1]

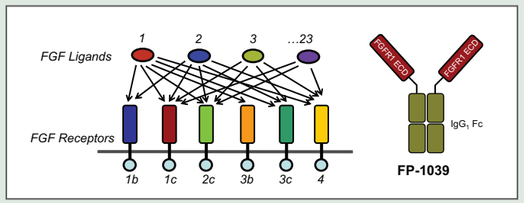


Figure 1. Targeting the FGF/FGFR Pathway with FP-1039. The FGF axis contains 4 FGF receptors with multiple splice forms and 22 FGF family ligands. There is selective binding of overlapping sets of ligands to the different receptors. FP-1039 is a soluble fusion protein consisting of the extracellular domains of human FGFR1 linked to the Fc region of human Immunoglobulin G_1 (IgG_1). It is designed to bind and sequester multiple FGF ligands and block activation of multiple FGF receptors. Previous data has demonstrated FP-1039 is capable of blocking tumor growth in murine and human preclinical tumor models[2].

Rationale of FP-1039 in Endometrial Cancer

- Endometrial cancer is the most common gynecological cancer in industrialized countries, with an estimated incidence of approximately 42,000 cases in the United States in 2009[3].
- Recent publications have reported somatic S252W/P253R *FGFR2* mutations in 7% of endometrial carcinomas[4, 5].
- Germline S252W/P253R *FGFR2* mutations are responsible for Apert syndrome, a congenital human skeletal disorder.
- Structure-function studies demonstrate that S252W FGFR2 has inappropriate, but ligand-dependent, activation due to:
  - — Binding to FGFs that do not bind wild-type FGFR2
  - — Enhanced affinity for multiple FGFs, compared to wild-type FGFR2
- **Hypothesis: Tumors containing the Apert syndrome S252W mutation in *FGFR2* will be sensitive to FGF ligand blockade by FP-1039**

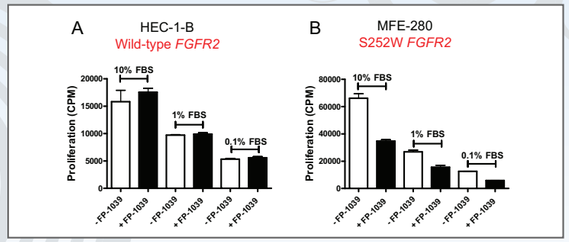


Figure 2. FP-1039 inhibits cell proliferation in endometrial carcinoma cells expressing S252W FGFR2 *in vitro*. Impact of FP-1039 on cell proliferation in **(A)** HEC-1-B (wild-type *FGFR2*) and **(B)** MFE-280 (*FGFR2* S252W) cell lines. Human endometrial carcinoma cell lines HEC-1-B and MFE-280 were obtained from DSMZ (Berlin, Germany) and ATCC (Manassas, VA), respectively, and sequenced to confirm FGFR2 mutation status. Cells ($5x10^4$) were plated in decreasing concentrations of FBS in the presence (filled bars) or absence (open bars) of FP-1039 at 10μg/ml. Cell proliferation was assessed 4 days post-plating using ^3H thymidine incorporation.

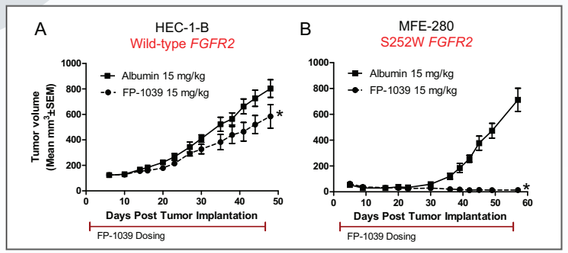


Figure 3. FP-1039 inhibits tumor growth in an endometrial carcinoma model expressing S252W FGFR2. Efficacy of FP-1039 in a HEC-1-B **(A)** and MFE-280 **(B)** human endometrial cancer xenograft model. Five million HEC-1-B or MFE-280 cells were implanted subcutaneously over the right flank of SCID mice (N=10 per group). FP-1039 or albumin was administered intra-peritoneally twice a week at a dose of 15 mg/kg. * indicates P <0.01 as determined by t-test of area-under-the-curve.

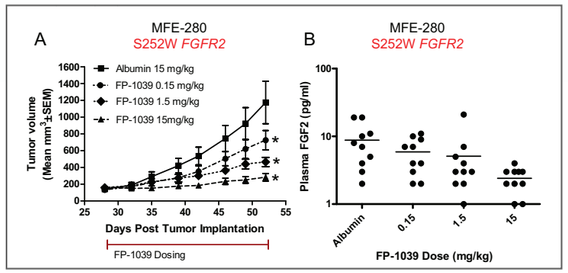


Figure 4. (A) FP-1039 displays therapeutic dose-dependent efficacy in a model of endometrial carcinoma with an FGFR2 S252W mutation. Five million MFE-280 cells were implanted subcutaneously over the right flank of SCID mice (N=10 per group). Once tumors reached 150-200mm³ following implantation, FP-1039 or albumin was administered intraperitoneally twice a week at the doses indicated. * indicates P <0.01 as determined by t-test of area-under-the-curve. **(B) Response of S252W FGFR2 endometrial carcinoma xenografts is correlated with circulating plasma FGF-2.** Circulating plasma levels of FGF-2 were determined at end-of-study, 72 hours post-FP-1039 dosing. Reduction in circulating FGF-2 levels by FP-1039 was significant (P<0.01) at all dose levels compared to albumin control group.

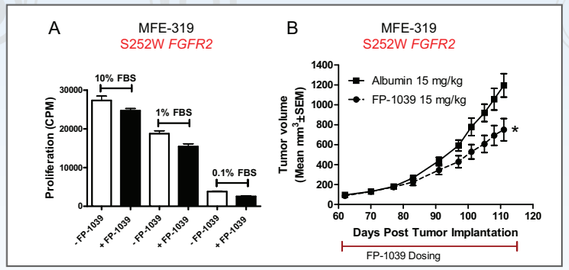


Figure 5. The MFE-319 cell line is partially dependent on the FGF signaling pathway for cell growth and survival. FP-1039 activity in the S252W FGFR2-bearing endometrial carcinoma cell line MFE-319, *in vitro* **(A)** and *in vivo* **(B)**. The human endometrial carcinoma cell line MFE-319 was obtained from DSMZ (Berlin, Germany) and sequenced to confirm FGFR2 mutation status. Experiments were performed as outlined in Fig.2 and Fig.3. * indicates P <0.01 as determined by t-test of area-under-the-curve. Efficacy of FP-1039 was comparable to PD173074, a small-molecule tyrosine kinase inhibitor of FGFRs that inhibits FGF receptor signaling whether or not FGF ligands are bound (data not shown). This suggests that, MFE-319 cells unlike MFE-280 cells, are not highly dependent on FGF pathways for survival.

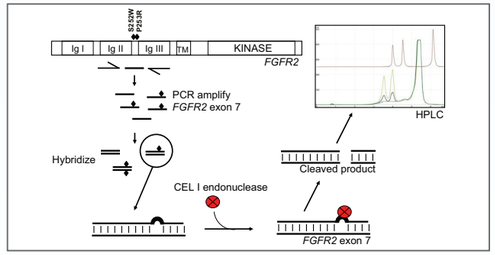


Figure 6. Schematic outline of an *FGFR2* exon 7 mutation assay compatible with FFPE endometrial cancer specimens. Exon 7 of the *FGFR2* gene is PCR amplified using sequence specific primers. PCR products are then denatured, re-hybridized and incubated with the heteroduplex specific endonuclease CEL I. Points of heteroduplex formation (i.e. *FGFR2* S252W) are cleaved by CEL I into 2 fragments that can be visualized using HPLC. Following mutation detection by HPLC, FFPE DNA samples are sequenced for confirmation.

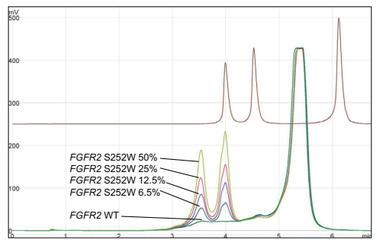
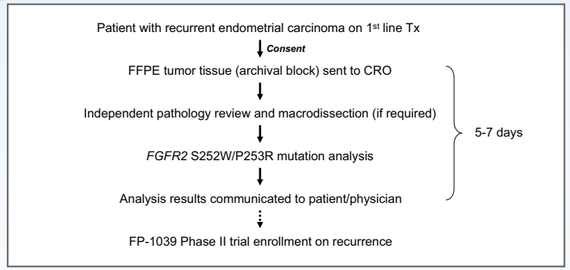


Figure 7. The *FGFR2* S252W mutation can be detected in FFPE endometrial carcinoma cell blocks. FFPE cell blocks were prepared containing varying ratios of HEC-1-B (wild-type *FGFR2*) and MFE-319 (**FGFR2** S252W) cells. Samples were then cut into 10μm sections, DNA extracted and the FGFR2 exon 7 assay performed as outlined in Fig. 6. The FGFR2 S252W mutation was clearly detectable as 2 peaks by HPLC down to a level of 6.5%. Comparable results were observed for FGFR2 S252W mutation detection sensitivity using the MFE-280 cell line (data not shown).

Hypothetical Outline of *FGFR2* Exon 7 Mutation Screening in Endometrial Carcinoma Patients



SUMMARY

- FP-1039 is an FGFR1:Fc fusion protein that binds multiple FGF ligands and prevents them from activating multiple FGF receptors.
- The ligand-dependent *FGFR2* mutation S252W / P253R is observed in endometrial cancer specimens at a frequency of ~7%[4, 5].
- FP-1039 has single-agent therapeutic activity in mice with established human MFE-280 endometrial cancer xenografts bearing the S252W *FGFR2* mutation.
- Additional determinants of an FP-1039 anti-tumor response exist in tumors with *FGFR2* S252W and are currently under investigation.
- A *FGFR2* S252W / P253R genomic mutation assay compatible with FFPE patient samples has been developed.
- FP-1039 is currently in a Phase I dose-escalation clinical study to characterize safety and pharmacokinetics in cancer patients.
- Future Plans:
  - – Single agent efficacy of FP-1039 in a genomically selected population
  - – Identify additional indications with ligand-dependent FGFR mutations

REFERENCES

1. Tolcher AW, et al. *AACR-NCI-EORTC: Molecular Targets and Cancer Therapeutics 2009*; A103.
2. Long L, et al. AACR 2009; A2789.
3. Jermal A, et al. CA *Cancer J Clin* 2009;59:225-49.
4. Pollock PM, et al. *Oncogene* 2007;26:7158-62.
5. Dutt A, et al. Proc Natl Acad Sci U S A 2008;105:8713-7.

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