FP-1039 (FGFR1:Fc), A Soluble FGFR1 Receptor Antagonist, Inhibits Tumor Growth and Angiogenesis

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INTRODUCTION

- FP-1039 is a soluble fusion protein consisting of the extracellular domain of human fibroblast growth factor receptor 1c (FGFR1) linked to the Fc portion of human IgG1.
- FGFR1 ligands, such as FGF1, FGF2, FGF4, possess not only mitogenic activity, but some also have potent angiogenic activities that promote tumor progression.
- FP-1039 prevents FGFR1 ligands from binding to any of their cognate receptors within the family of seven FGF receptors, and may mediate both direct anti-tumor and anti-angiogenic effects.
- In this study, we demonstrate:
  - *In vitro* activity of FP-1039 against A549, a lung carcinoma cell line and Caki-1, a renal carcinoma cell line
  - *In vivo* activity of FP-1039 against a Caki-1 xenograft model
  - Anti-angiogenic effect of FP-1039
  - Effect of FP-1039 on ERK activation, a downstream signal in the FGF-FGFR pathway
  - Differential *in vivo* activities of FP-1039 against primary patient-derived tumor xenograft models

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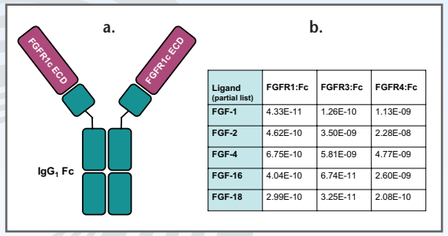
FP-1039 is a fusion protein engineered to neutralize multiple FGF family members and prevent their binding to multiple receptors. Most ligands that bind to FGFR1 also bind to FGFR3 and FGFR4.



b.

Ligand (partial list)	FGFR1:Fc	FGFR3:Fc	FGFR4:Fc
FGF-1	4.33E-11	1.26E-10	1.13E-09
FGF-2	4.62E-10	3.50E-09	2.28E-08
FGF-4	6.75E-10	5.81E-09	4.77E-09
FGF-16	4.04E-10	6.74E-11	2.60E-09
FGF-18	2.99E-10	3.25E-11	2.08E-10

Figure 1. a). Domain structure of FP-1039. FP-1039 is a recombinant fusion protein consisting of the extracellular domain of Fibroblast Growth Factor Receptor 1c (FGFR1c) fused to the Fc domain of IgG1.

b). Affinity of Fibroblast Growth Factor Receptors 1, 3, and 4 for selected FGF family members. k_a and k_d for fusion proteins consisting of the extracellular domains of FGFR1, FGFR3, and FGFR4 binding to recombinant, purified FGF ligands were determined using surface plasmon resonance (Biacore) technology. The receptor fusions were immobilized on a protein A surface, ligands were incubated, and rate constants measured using a Biacore T100. Kd is reported in the table.

FP-1039 significantly reduces the number of viable tumor cells *in vitro* in a concentration-dependent manner.

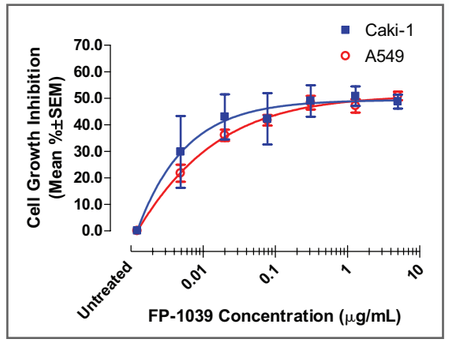


Figure 2. A549 cells (human lung cancer cell line) and Caki-1 cells (renal cancer cell line) were seeded at 20,000 cells and 1000 cells per well respectively. The cells were subsequently treated with either medium alone or with serially titrated FP-1039 up to 5 μg/mL for 5 days in a 37°C humidified incubator with 5% CO_2. The cells were then lysed with the CellTiter Glo reagent (Promega) and the luminescence of each well measured. The percent inhibition of luminescent density relative to untreated cells was calculated. Each point represents the average of triplicate wells.

FP-1039 displays potent *in vivo* anti-angiogenic activity.

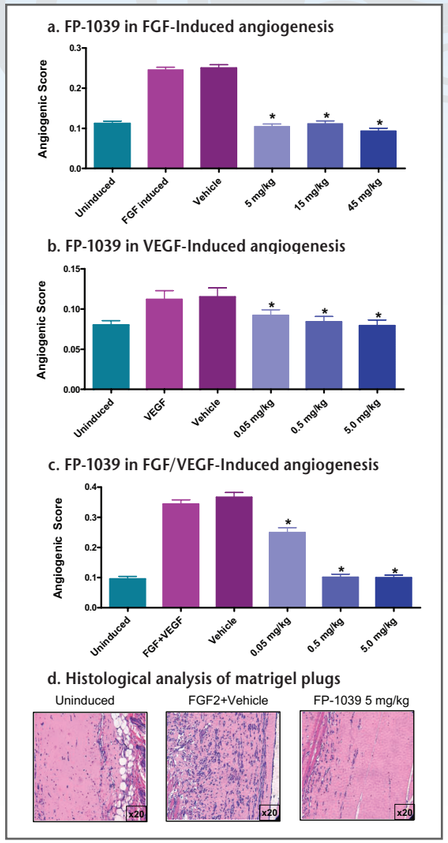


Figure 3. 0.5 ml matrigel plugs were implanted subcutaneously into C57BL/6 mice in the abdominal region. Test article was delivered by tail vein injection, 1, 4, and 7 days post matrigel implantation. On day 9, the plugs were excised and processed for histology, and the angiogenic response was quantified as an angiogenic score based on neovascularization in five random fields along the peripheral edge of each plug.

a). Uninduced control group (group 1: no FGF-2) and induced groups (groups 2-6: 500ng/ml FGF-2 in matrigel). Group 3 was treated with vehicle, and groups 4-6 were treated with FP-1039 at 5 mg/kg, 15 mg/kg and 45 mg/kg respectively, once every 3 days. **b).** Uninduced control group (group 1: no VEGF) and induced groups (groups 2-6: 100ng/ml VEGF in matrigel). Group 3 was treated with vehicle, and groups 4-6 were treated with FP-1039 at 0.05 mg/kg, 0.5 mg/kg and 5 mg/kg respectively, once every 3 days. **c).** Uninduced control (group 1: no FGF-2 or VEGF) and induced groups (group 2-6: 250 ng/ml FGF-2 + 100ng/ml VEGF in matrigel). Group 3 was treated with vehicle, and groups 4-6 were treated with FP-1039 at 0.05 mg/kg, 0.5 mg/kg and 5 mg/kg respectively, once every 3 days. **d).** Representative 20X photographs of H&E stained sections of matrigel plugs demonstrating neovascularization and cell migration response.

* Statistically significant difference between an FP-1039-treated group compared to the vehicle-treated group. Data analyzed by one way ANOVA followed by a Tukey test.

** The study was performed by Paragon Bioservices, Inc.

FGFR1:Fc (FPT039 and FP-1039) significantly inhibits tumor growth of a human renal carcinoma cell line (Caki-1) xenograft model in a dose dependent manner.

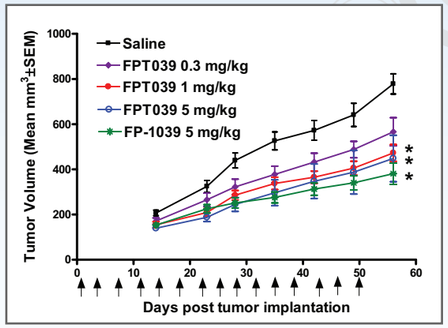


Figure 4. Human renal carcinoma Caki-1 cells were implanted subcutaneously into the right flank of CB17-SCID mice at 1.5 x 10^7 cells/200 μl/mouse. One day after tumor implantation, the mice were randomized and treated intravenously with either saline, FPT039 or FP-1039 twice a week at the doses indicated in the graph. Tumor volume at each time point was analyzed by a Kruskal-Wallis test followed by a Dunn's multi-group comparison.

* Statistically significant difference compared to saline control group. N=13-17/group.

↑ Dosing date

** FPT039 is an earlier research version of FP-1039. FP-1039 contains a deletion, rendering it more resistant to proteolysis.

Treatment with FP-1039 decreases tumor vascularization and inhibits the phosphorylation of Erk1/2 in the human renal carcinoma Caki-1 xenograft model.

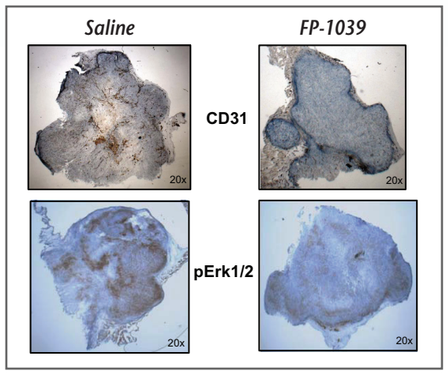


Figure 5. At the end of the study described in Figure 4, three tumors from animals treated twice weekly with saline and three tumors from animals treated twice weekly with FP-1039 at 5 mg/kg were collected for histological analysis. Frozen sections were probed with anti-mouse CD31 monoclonal antibody or anti-phospho-Erk1/2 polyclonal antibodies. CD31 and pErk1/2 were visualized by DAB staining (brown color) and the cell nuclei were counter-stained with hematoxylin (blue color). Shown are representative sections from each staining.

Treatment with FP-1039 significantly inhibits tumor growth in primary human breast cancer tumor xenografts with certain cell surface expression marker patterns but not in other xenograft models with different cell surface marker expression patterns.

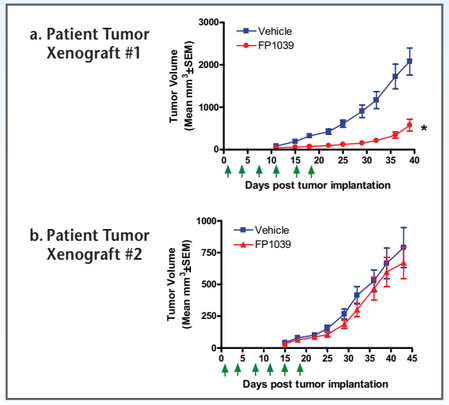


Figure 6. This study was performed by Oncotest GmbH. Breast cancer patient-derived xenograft tumor chunks were implanted subcutaneously into both flank areas of nu/nu mice. One day after tumor implantation, mice were randomized and treated intravenously with either saline or FP-1039 at 15 mg/kg, twice a week for six doses. Tumor volume at each time point was analyzed by a Student's t-test.

* Statistically significant difference compared to saline control group. N=11-15/group.

↑ Dosing date

SUMMARY

- FP-1039 is a potent inhibitor of both FGF and VEGF induced angiogenesis.
- Treatment with FP-1039 demonstrates a dose dependent anti-tumor effect in a Caki-1 xenograft model.
- Treatment with FP-1039 demonstrates differential efficacy in primary patient-derived tumor xenografts that highly express certain FGF ligands, certain FGF receptors or both. These response differences may help identify patient tumor types that are most likely to respond to treatment with FP-1039.
- Conclusions: These data indicate the potential for FP-1039 to be a selective and potent anti-cancer drug for the treatment of certain cancers in which the FGF-FGFR pathway plays an important role in supporting the tumor/host microenvironment, while possibly avoiding some of the dose limiting toxicities identified previously with an anti-FGFR1c antibody or oral tyrosine kinase inhibitors that non-selectively block multiple tyrosine kinases.

FP-1039 is currently in preclinical toxicology testing to enable a first-in-human study scheduled to commence in 2008.