FPA144: A Therapeutic Antibody for Treating Patients with Gastric Cancers Bearing *FGFR2* Gene Amplification

#5446

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ABSTRACT

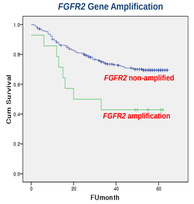
A subset of patients with gastric cancer have an amplification of the receptor tyrosine kinase fibroblast growth factor receptor 2 (FGFR2) gene. The amplification is most common in the diffuse type of gastric cancer and its presence correlates with poor patient prognosis. Although it has been reported that there is high expression of FGFR2 protein in patients with the amplification, it is unknown which of the two major FGFR2 isoforms, FGFR2b or 2c, is expressed. In this study we demonstrate by both quantitative PCR (qPCR) and immunohistochemistry (IHC) using FGFR2b selective antibodies, that it is the FGFR2b isoform, and not FGFR2c, that is overexpressed in gastric cancer tumors that contain the FGFR2 amplification.

FivePrime Therapeutics has developed an FGFR2b-specific antibody, FPA144, to treat patients with gastric cancers bearing amplification of the *FGFR2* gene. FPA144 is glycoengineered for enhanced antibody-dependent cell-mediated cytotoxicity (ADCC). FPA144 causes tumor growth inhibition by 72 to 100% in *FGFR2* amplified gastric cancer xenograft models with *FGFR2* gene amplification that overexpress FGFR2b. In the SNU-16 model, FPA144 reduces the levels of FGFR2b protein expressed in the tumors by approximately 50%, and decreases both FGFR2b phosphorylation and phosphorylation of the downstream effector, FRS2. The anti-tumor effect of FPA144 is additive with the standard of care chemotherapies 5-fluorouracil/cisplatin and paclitaxel in the OCUM-2 gastric cancer xenograft model. Since this molecule, unlike the FGFR tyrosine kinase inhibitors, blocks signaling by only the FGFR2b and not the other FGFRs, we expect a favorable toxicology profile, either alone or in combination with chemotherapy. We anticipate starting clinical trials of FPA144 by the end of 2014.

BACKGROUND

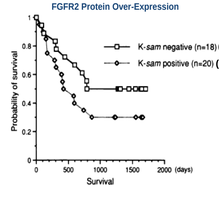
- Gastric cancer is the second leading cause of cancer death worldwide
  - The fourth most common malignancy in the world
  - More than 70% of gastric cancer cases occur in Eastern Asia
- Cumulative data indicate *FGFR2* gene amplification occurs in approximately 5% of gastric cancer
- *FGFR2* gene amplification or FGFR2 protein over-expression is associated with lower survival in gastric cancer patients

FGFR2 Gene Amplification



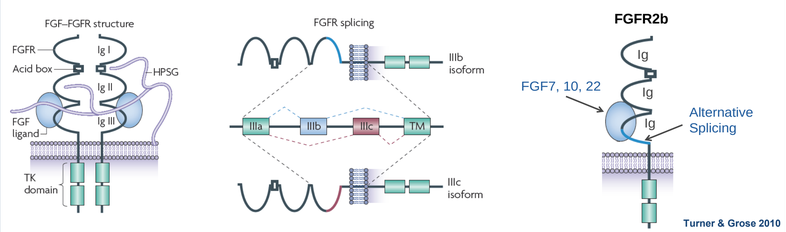
Jung *et al.* 2012; FGFR2 FISH; P=0.012

FGFR2 Protein Over-Expression



Hattori *et al.* 1996; FGFR2 IHC; P=0.15

FGFR2 RNA SPLICING GENERATES THE FGFR2b ISOFORM



- FGFR1, FGFR2 and FGFR3 undergo alternative splicing that results in b and c isoforms of the receptor
  - The IIIb ("b") isoform is restricted to epithelial lineages and the IIIc ("c") isoform is preferentially expressed in mesenchymal lineages
- *FGFR2* RNA splicing in the third-Ig like domain generates the FGFR2b isoform

FPA144 - A HUMANIZED MONOCLONAL ANTIBODY TO FGFR2b FOR GASTRIC CANCER

- Engineered to directly kill tumor cells via enhanced ADCC
- Blocks FGFR2b receptor activation
- Down-regulates FGFR2b receptor levels
- Causes tumor regression in animal models
- Lead indication is FGFR2b over-expressed gastric cancer
- Companion diagnostic will identify patients most likely to respond (AACR Abstract #2845)
  - Potential to target 15% of gastric cancer patients
- Phase I testing to begin by the end of 2014

A. FPA144 Mediates Targeted Cell Killing in OCUM2

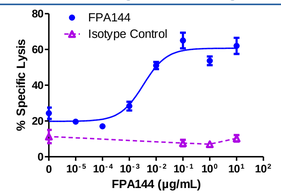


Figure A: OCUM2 is an *FGFR2* amplified, FGFR2b overexpressed, gastric cancer cell line that is sensitive to FPA144 killing by ADCC. ADCC was detected by lactate dehydrogenase (LDH) release.

FGFR2b IS THE DOMINANT ISOFORM EXPRESSED IN *FGFR2*-AMPLIFIED XENOGRAFT MODELS AND GASTRIC CANCER PATIENTS

B. *FGFR2b* is the Primary Isoform Expressed in Gastric Samples tested by qPCR

C. FGFR2b is the Primary Isoform Expressed in Gastric Cancer Patient Samples tested by qPCR and IHC

D. *FGFR2b* mRNA Levels Correlate to *FGFR2* SISH and FGFR2b IHC Results

Patient Sample:	SISH Results:	qPCR Results*:	FPR2-D IHC:
1	no amp	Low FGFR2b mRNA	-
2	no amp	Low FGFR2b mRNA	-
3	amplified	Low FGFR2b mRNA	+
4	amplified with polysomy	Low FGFR2b mRNA	+
5	"borderline amplification"	Low FGFR2b mRNA	+
6	amplified	Moderate FGFR2b mRNA	+
7	amplified	High FGFR2b mRNA	+
8	amplified, heterogeneous	High FGFR2b mRNA	+
9	amplified	High FGFR2b mRNA	+
10	amplified	High FGFR2b mRNA	+
11	amplified	High FGFR2b mRNA	+

* "Low": relative expression < 1, "Moderate": 1 < relative expression < 3, "High": 3 < relative expression

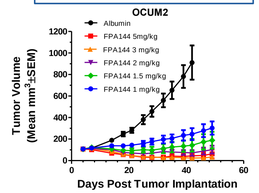
Figure B: qPCR analysis on both a *FGFR2*-amplified xenograft and a gastric cancer patient sample confirms that *FGFR2b* is the dominant *FGFR2* isoform expressed.

Figure C: qPCR analysis on additional gastric cancer patient samples further validates that *FGFR2b* is the dominant isoform expressed in these samples. IHC performed on these respective samples using FPR2-D, a mouse monoclonal antibody specific for FGFR2b, confirms that FGFR2b is also highly expressed at the protein level.

Figure D: The correlation between *FGFR2* SISH and FPR2-D IHC analysis is supported by FGFR expression profiling. Gastric cancer patient samples that exhibit *FGFR2* amplification and strong FGFR2b IHC staining also show over-expression of *FGFR2b* mRNA.

FPA144 IS ACTIVE IN *FGFR2*-AMPLIFIED GASTRIC CANCER XENOGRAFTS

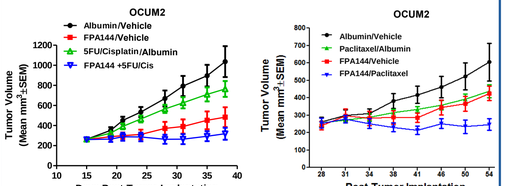
E. Tumor Regression is Observed with FPA144



Dose	Complete Regression
5 mg/kg	7/15
3 mg/kg	1/15
2 mg/kg	0/15
1.5 mg/kg	0/15
1 mg/kg	0/15

(Dosing twice weekly, IP)

F. FPA144 Has Additive Activity in Combination with Chemotherapy

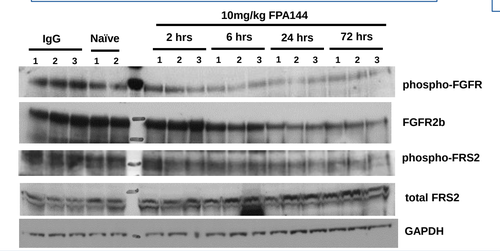


- FPA144 dose = 5 mg/kg twice weekly
- 5-FU (5-fluorouracil) dose = 33 mg/kg
- Cis (cisplatin) dose = 2.3 mg/kg
- Paclitaxel dose = 18 mg/ kg
- Therapeutic dosing started when tumors reached ~250 mm^3

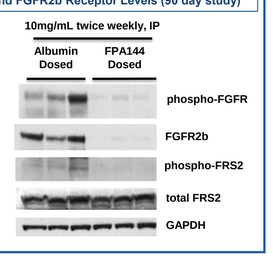
FPA144 DOWN-REGULATES FGFR2b RECEPTOR LEVELS AND DECREASES FGFR2b AND FRS2 PHOSPHORYLATION

- SNU-16 is an *FGFR2*-amplified, FGFR2b-overexpressed gastric cancer cell line
  - FPA144 dosed therapeutically results in a 72% tumor growth inhibition *in vivo* in this model
  - Western blot analysis was performed on SNU-16 xenografts at the time points indicated below

G. FGFR2b and FRS2 Phosphorylation Decreases Within 2 Hours After FPA144 Treatment; FGFR2b Receptor Levels Decrease at 6 Hours



H. Continued Dosing with FPA144 Results in Decreased FGFR2b and FRS2 Phosphorylation and FGFR2b Receptor Levels (90 day study)



SUMMARY

- FPA144 is a glycoengineered antibody that selectively binds the FGFR2b isoform of FGFR2
- Expression profiling by qPCR shows that *FGFR2b* is the dominant *FGFR2* isoform expressed in gastric cancer xenografts and patient samples
- IHC, using FPR2-D, a mouse monoclonal antibody to FGFR2b, confirms that FGFR2b protein is in fact over-expressed in gastric cancer patient samples
- FPA144 treatment causes tumor regression with monotherapy and has additive anti-tumor activity when combined with standard-of-care chemotherapeutics, 5-fluorouracil + cisplatin, and Paclitaxel in non-clincal models
- FPA144 disrupts FGFR2b signaling *in vivo*
  - Decreases FGFR2b and FRS2 phosphorylation as early as 2 hours after dosing
  - FGFR2b receptor is down-regulated as early as 6 hours after dosing

REFERENCES

- Jung, EJ, *et al.* Fibroblast growth factor receptor 2 gene amplification status and its clinicopathologic significance in gastric carcinoma. *Hum Pathol.* 2012;43(10):1559-66
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