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Preclinical efficacy of fibroblast growth factor ligand trap HGS1036 in lung carcinoma models with genomic amplification of *FGFR1*

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INTRODUCTION – HGS1036

- 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.
- HGS1036 (formerly 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).
- HGS1036 is designed to bind multiple FGF ligands and prevent them from activating multiple FGF receptors (**Figure 1**)
- HGS1036 does not inhibit the "hormonal" FGFs (FGF-19, -21 and -23) that play key roles in bile acid secretion, phosphate homeostasis and metabolism
- HGS1036 has multiple potential mechanisms of action in cancer including direct anti-tumor activity, inhibition of angiogenesis and cancer stem cell maintenance
- HGS1036 has completed a Phase I dose-escalation study to characterize safety and pharmacokinetics in cancer patients[1].

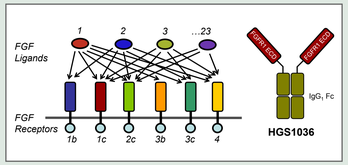


Figure 1. Targeting the FGF/FGFR Pathway with HGS1036.

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. HGS1036 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 HGS1036 is capable of blocking tumor growth in murine and human preclinical tumor models[2].

Rationale of HGS1036 in Lung Cancer

- Lung cancer is the most common cause of cancer-related death in industrialized countries. In 2012, it is estimated that cancer of the lung and bronchus will be responsible for 164,770 deaths in the US[3].
- Recent publications have reported amplification of the *FGFR1* gene in 22% of squamous non-small cell lung (NSCLC) carcinoma and 27-33% of small cell lung cancer[4, 5, 6].
- Amplification of the *FGFR1* gene in lung cancer cell lines is associated with over-expression and increased signaling of the FGFR1 receptor protein resulting in increased cell proliferation and tumorgenesis[5].
- Hypothesis: Lung tumors containing the amplification of the *FGFR1* gene will be sensitive to FGF ligand blockade by HGS1036

A

Lung cancer cell line	Sub-type	*FGFR1* copy number/cell
DMS53	SCLC	5
DMS114	SCLC	10
NCI-H1581	NSCLC	6
NCI-H1703	NSCLC	8
NCI-H520	NSCLC	6

B

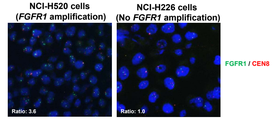


Figure 2. *FGFR1*-amplified lung cancer cell lines examined.

(A) A panel of lung cancer cell lines was identified by public database analysis (CONAN, Tumorscape) and *FGFR1* amplification status confirmed by using a *FGFR1* QuantiGene® Plex DNA Assay (Panomics/Affymetrix, Santa Clara, CA). Abbreviations: SCLC – small cell lung cancer; NSCLC – non-small cell lung cnacer. (B) Representative photomicrographs of *FGFR1* FISH assay results on an *FGFR1* amplified (NCI-H520) and non-ampilifed (NCI-H226) lung cancer cell lines.

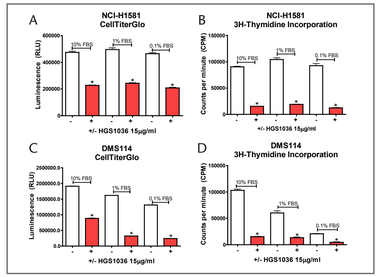


Figure 3. HGS1036 inhibits *in vitro* cell proliferation in lung cancer cells with *FGFR1*-amplification.

Impact of HGS1036 on cell number and proliferation in A) & C) NCI-H1581 and B) & D) DMS114 cell lines. Human lung cancer cell lines ($5x10^4$) were plated in decreasing concentrations of FBS in the presence (filled bars) or absence (open bars) of HGS1036 at 15 μg/mL. Cell number (A, B) and proliferation (C,D) was assessed 5 days post-plating using CellTiterGlo and ^{3}H thymidine incorporation, respectively. * indicates P <0.01 as determined by t-test.

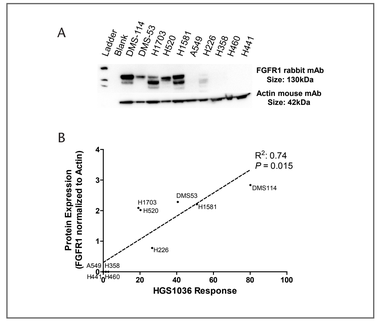


Figure 4. FGFR1 protein expression in a panel of lung cancer lines is positively correlated with *in vitro* HGS1036 sensitivity.

Protein expression of FGFR1 and ß-Actin was quantified from Western blots using densitometry analysis (ImageJ Software). The sensitivity of the cell lines *in vitro* in response to 15 μg/mL HGS1036 in 1% FBS was determined by calculating percent inhibition using CellTiterGlo. Pearson correlation was used to evaluate the association between FGFR1 protein expression and sensitivity to HGS1036.

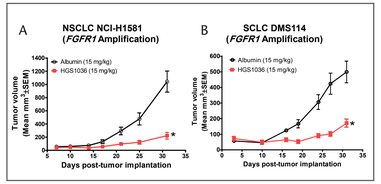
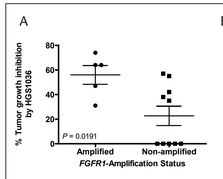


Figure 5. HGS1036 inhibits tumor growth in *FGFR1*-amplified lung xenograft models.

Efficacy of HGS1036 in a NCI-H1581 (A) and DMS114 (B) human lung cancer xenograft models. Five million cells were implanted subcutaneously over the right flank of SCID mice (N=10 per group). HGS1036 or albumin was administered intra-peritoneally twice a week at a dose of 15 mg/kg starting at day 1 post-tumor implantation. * indicates P <0.01 as determined by t-test of area-under-the-curve.

A



B

Gene	Ratio	*P*-value
FGFR1	2.968	0.01276
FGFR1c	4.842	0.01603
FGFR3c	19.25	0.02488
FGF2	20.83	0.04462

Figure 6. Relationship of *FGFR1* gene amplification (A) and RNA expression (B) with HGS1036 anti-tumor efficacy *in vivo*.

(A) HGS1036 *in vivo* anti-tumor efficacy in a panel of lung cancer xenograft models was compared between *FGFR1* gene amplified (N=5) and *FGFR1* non-amplified (N=10) models. HGS1036 efficacy was determined by percent tumor growth inhibition of area-under-the-curve comparing HGS1036-treated and control groups. (B) The RNA expression of HGS1036-related genes (FGFs / FGFRs) was determined for 35 xenograft models using qRT-PCR and correlated with HGS1036 anti-tumor efficacy. The significance of gene expression and *in vivo* HGS1036 efficacy (*P*-value) was determined by Mann-Whitney. Ratio represents the value of RNA expression in HGS1036 responder / non-responder.

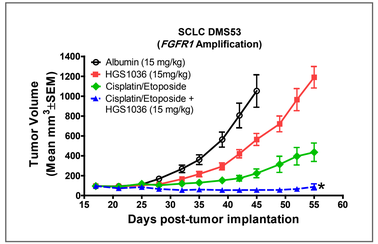


Figure 7. Combination of HGS1036 with cisplatin and etoposide in the DMS53 tumor model.

Five million DMS53 cells were implanted subcutaneously over the right flank of SCID mice (N=10 per group). HGS1036 or albumin was administered intraperitoneally (i.p.) at 15 mg/kg twice per week starting on day 17 post-tumor implantation. Cisplatin was administered i.p. at 3 mg/kg once per week starting on day 17 post-tumor implantation. Etoposide was administered i.p. at 4mg/kg daily for 3 consecutive days per week, starting at day 17 post-tumor implantation. Chemo was dosed at maximum tolerated dose (MTD). * indicates P < 0.01 comparing cisplatin + etoposide to cisplatin + etoposide + HGS1036 as determined by t-test of the area-under-the-curve.

SUMMARY

- HGS1036 is an FGFR1:Fc fusion protein that binds multiple FGF ligands and prevents them from activating multiple FGF receptors..
- Amplification of the *FGFR1* gene has been reported in 22% of squamous NSCLC and 27-33% of SCLC.
- HGS1036 has single-agent activity in mice with human lung cancer xenografts bearing *FGFR1*-amplification.
- HGS1036 addition to standard-of-care chemotherapy (SOC) regimens used in lung cancer shows increased efficacy.
- HGS1036 has completed a phase I dose-escalation clinical study to characterize safety and pharmacokinetics in cancer patients.
- Future Plans:
  - Examine safety of HGS1036 in combination with SOC regimes in a phase Ib clinical study
  - Determine additional biomarkers of HGS1036 response

References

1. Tolcher AW, *et al.* (2012) Final results of a dose escalation study of HGS1036 (previously FP-1039), an FGF "trap" (FGFR1:Fc) in patients with advanced malignancies. 10th International Congress on Targeted Anticancer Therapies; #L12.3
2. Long L, *et al.* (2009) Preclinical Antitumor Efficacy of FP-1039, A Soluble FGF Receptor 1:Fc Conjugate, as a Single Agent or in Combination with Anticancer Drugs. *AACR 100th Annual Meeting;* #2789
3. Siegal R, *et al.* (2012) Cancer statistics, 2012. *CA Cancer J Clin* 1:10-29.
4. Weiss J, et al. (2010) Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci Transl Med* 2: 62ra93.
5. Voortman J, *et al.* (2010) Array comparative genomic hybridization-based characterization of genetic alterations in pulmonary neuroendocrine tumors. *Proc Natl Acad Sci U S A* 107: 13040-13045
6. Dutt A, *et al.* (2011) Inhibitor-Sensitive FGFR1 Amplification in Human Non-Small Cell Lung Cancer. *PLoS One* 6: e20351.

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