Analysis of serum protein biomarkers and circulating tumor DNA for activity of dovitinib in patients with tyrosine kinase inhibitor (TKI)-refractory gastrointestinal stromal tumors (GIST)

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BACKGROUND

➢We previously performed a phase II trial of dovitinib, a novel TKI targeting c-KIT, fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor β (PDGFRβ) for patients with TKI-refractory GISTs (Kang et al., Br J Cancer, 2013;109:2309).

➢An exploratory translational analysis was performed for patients enrolled in this study. As the main mechanism of action of dovitinib is thought to involve anti-angiogenesis, we examined soluble serum protein markers involved in the VEGF and FGF pathways.

➢Clonal evolution of GISTs harboring secondary kinase mutations results in acquired resistance to TKIs. Although genotyping of tumor tissues is the gold standard method for detection of mutation, its utility is relatively limited in a salvage setting, considering the risks of complications associated with the invasive procedure and potential failure to fully assess the molecular heterogeneity of the tumor. We therefore performed mutational analysis on circulating tumor DNA (ctDNA) in serum using a novel sequencing technique, BEAMing (Beads, Emulsions, Amplification, and Magnetics).

METHODS

➢Among 30 patients with TKI-refractory GISTs enrolled in the prior phase II study, all patients were included in this biomarker analysis. Patients received oral dovitinib 500 mg once daily with a 5 days-on/2 days-off schedule in a 28-day cycle.

➢*Serum Protein Biomarkers*

- Pre-dose serum samples were collected on day 1 of cycle 1 (n=30) and cycle 2 (n=28) and ELISA was used to assess serum levels of each candidate protein biomarkers of anti-angiogenesis.
- VEGF165 (Invitrogen, USA), VEGF-A (ebioscience, USA), soluble VEGFR (sVEGFR)-1, -2 (ebioscience), placental growth factor (PlGF; R&D, USA), interleukin-8 (IL-8; R&D), basic fibroblast growth factor (bFGF; Invitrogen) and FGF23 (Millipore, USA).

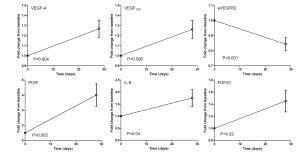
➢*BEAMing of ctDNA*

- Free circulating DNA was isolated from baseline serum samples using a QIAamp DNA purification kit (Qiagen).
- BEAMing assays were performed on serum ctDNA samples for detection of 29 KIT mutations (5 in exon 11, 1 in exon 9, 2 in exon 13, 2 in exon 14, 18 in exon 17, and 1 in exon 18), 5 PDGFRα mutations, and 1 BRAF mutation. BEAMing assays were conducted by Inostics GmbH (Hamburg, Germany).

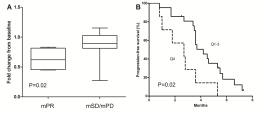
RESULTS

➢*Serum Protein Biomarkers*

- At the end of first cycle, dovitinib significantly increased the levels of VEGF165 (1.26-fold), VEGF-A (1.27-fold), PlGF (6.0-fold), FGF23 (1.45-fold), and IL-8 (1.75-fold), whereas sVEGFR-2 levels decreased 0.8-fold from baseline.

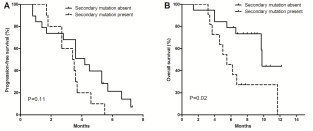


- Correlation between changes in sVEGFR-2, and metabolic response by PET-CT (A) and PFS (B), Q; quartile

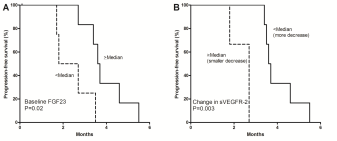


➢*Genotyping of ctDNA by BEAMing*

- **Primary kinase mutation**
  - Identified in five (17%) patients (two for *KIT* exon 11, and three for exon 9).
  - 100% concordant with the results of mutation analyses from tumor tissue taken from the corresponding patients.
- **Secondary kinase mutation**
  - Identified in 11 (37%) patients. All identified mutations involved *KIT* exon 17.
  - Three (27%) of these patients had more than one secondary *KIT* mutation. In two patients, additional mutations in other *KIT* exons were detected (each for exon 18, and exon 13). Different mutations of *KIT* exon 17 were simultaneously identified in two patients.
  - None of baseline characteristics was significantly associated with the presence of secondary mutations, except age (mutation detected vs not detected, 63 vs 54 years; p=0.01).
- **Correlation between clinical outcomes and secondary mutation**
  - No significant correlation of secondary mutations with response rate, 24-week DCR, and metabolic response rate. However, all 4 patients who achieved disease control by 24 weeks had no secondary serum ctDNA mutations, whereas tumor progressed within 24 weeks in all patients with secondary mutations.
  - *Secondary mutations had marginal relationship with poor PFS (A)*
    - Median 3.4 months (95% CI, 2.2–4.6 months) vs. 4.2 months (95% CI, 2.8–5.6 months), HR=1.9 (95% CI, 0.8–4.5); p=0.11
  - *Secondary mutations had significant relationship with poor OS (B)*
    - Median 5.5 months (95% CI, 3.8–7.2 months) vs. 9.8 months (95% CI, 9.6–10.0 months), HR=3.1 (95% CI, 1.1–8.4); p=0.02



- **Serum protein biomarkers in patients with secondary mutations**
  - Baseline FGF23 (≤median vs. >median; A) was significantly associated with PFS (median 3.6 months [95% CI, 3.2–4.0 months] vs median 1.8 months [95% CI, 0.8–2.8 months]–4.0]; p=0.02).
  - Change in sVEGFR-2 from baseline (≤median vs. >median; B) significantly correlated with PFS (median 2.7 months [95% CI, 0.8–4.6 months] vs. 3.5 months [95% CI, 2.6–4.4 months]; p=0.003)
  - In patients with no secondary ctDNA mutations, no serum protein biomarkers correlated with PFS.



CONCLUSIONS

- Dovitinib has pharmacological effects on both the VEGFR and FGFR pathways.
- Although baseline levels of candidate serum biomarkers were not predictive, changes in sVEGFR-2 levels were associated with dovitinib-mediated antitumor activity including metabolic response and PFS.
- Secondary resistant mutations were readily detectable in ctDNA by BEAMing and the majority of these mutations are located in the activation loop.
- The presence of secondary *KIT* mutations in serum-derived ctDNA was significantly associated with reduced OS.

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