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**

- All patients were included in this biomarker analysis. Patients received oral dovitinib (Ki-51797; Novartis, Basel, Switzerland) for patients with TKI-refractory GISTs (Kang et al., J. Clin. Cancer. 2013;2:186-195).
- 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 salivary serum protein markers involved in the VEGF and FGF pathways.
- Close evaluation 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 mutations, its utility is limited by the small size and the risk 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 (Biospeed Biotechnology, Germany).

**RESULTS**

### Serum Protein Biomarkers

- **BEAMing of ctDNA**
  - Free circulating DNA was isolated from baseline serum samples using a Qiagen DNA purification kit (Qiagen).
  - BEAMing assays were performed on serum ctDNA samples for detection of 20 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), 8 ROS1 mutations, and 1 BAVM mutation. BEAMing assays were conducted by Medical Link (Kang et al., Clin. Cancer Res. 2013;19:186-195).

- **Geneotyping of ctDNA by BEAMing**
  - **Primary kinase mutation**
    - Identified in five (17%) patients (two for KIT exon 11, and three for exon 15).
    - 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 (30%) of these patients had more than one secondary KIT mutation.
    - In two patients, additional mutations in other TKI-sensitive kinases (JAK, ABL, or PDGFR) were identified as well.

### Correlation between clinical outcomes and secondary mutation

- No significant correlation of secondary mutations with response rate, 24 weeks OS, and PFS was found. However, all 11 patients who achieved disease control by 24 weeks had no secondary ctDNA mutations, whereas tumor progressed within 24 weeks in all patients with secondary mutations.
- Secondary mutations had marginal relationship with poor OS (B) and PFS (A) significantly, whereas secondary mutations had significant relationship with poor OS (B) and PFS (A) significantly. Median OS (95% CI) was 5.6 months (4.0–8.4); p=0.02.
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**CONCLUSIONS**

- Dovitinib has pharmacological effects on both the VEGF and FGF pathways.
- Although baseline levels of candidate serum biomarkers were not predictive, changes in ctDNA 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.

*This research was funded by Novartis Pharmaceuticals and is part of the Novartis Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea.