The development and validation of the UltraSEEK GIST Panel on the MassARRAY System for monitoring and detection of primary and secondary KIT mutations in plasma-derived DNA during targeted treatment of patients with gastrointestinal stromal tumors

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Abstract

Background
Gastrointestinal stromal tumors (GIST) with an incidence rate of 1-2 cases/100.000/year harbor tumor-specific cKIT or PDGFRA mutations in 85-90% of cases. Most patients with cKIT exon 11 mutations respond to imatinib with 10-years OS of ~80% with localized GIST and PFS of ~24 months in the metastatic setting. Approximately 2/3 of cases with acquired imatinib resistance harbor additional cKIT mutations in exons 13/14/17/18 that respond well to sunitinib, regorafenib or ripretinib. For response monitoring and follow-up in GIST, repetitive imaging is performed. However, there is compelling evidence demonstrating that detection of circulating tumor DNA is associated with tumor progression.

Objective
The aim of this study is to develop a plasma-based assay that detects most primary and secondary cKIT mutations with a short turn-around-time and lower costs compared to NGS assays. Here, we report the design and validation of a novel MassARRAY-based UltraSEEK GIST panel for monitoring treatment response and detection of secondary cKIT mutations.

Methods
We designed a MassARRAY-based UltraSEEK GIST panel that covers 116 different primary and secondary cKIT and PDGFRA mutations. For the analytical validation, we selected DNA extracted from FFPE tissues from 30 GIST with/without different known cKIT mutations as determined using NGS analysis. The analytical sensitivity was determined using diluted tissue-DNA and cfDNA of GIST plasma samples with different cKIT-exon 11 variants that were quantified with a sensitive cKIT-exon 11 drop-off ddPCR assay. For the detection of secondary cKIT mutations, plasma samples were used from 59 GIST patients during progression under treatment (GALLOP study).

Results
The accuracy of UltraSEEK GIST panel is 97%. The overall concordance in plasma was 85% between the UltraSEEK GIST panel and cKIT-exon 11 drop-off ddPCR assay (sensitivity 85%; specificity 84%). Discordant cases showed lower cKIT-mutant ctDNA levels. Secondary mutations were detected in 18 of 59 GIST-patients (30%). When numerous plasma samples were obtained for the same patient during long-term treatment, up to 6 different secondary cKIT mutations were identified.

Conclusion
Our preliminary validation results of the novel UltraSEEK GIST panel show promise as a rapid and inexpensive plasma-based screening assay for monitoring and detecting secondary KIT mutations for appropriate treatment-decision-making.

Conflicts of interest

E.S. reports lectures for Bio-Rad, Roche, Illumina, Lilly, Janssen Cilag (Johnson&Johnson) and Agena Bioscience (honoraria paid to UMCG institution); he is consultant in advisory boards for GSK, AstraZeneca, Astellas Pharma, Roche, Lilly, Illumina, Agena Bioscience, Janssen Cilag (Johnson&Johnson), Sinnovisionlab, Diaceutics, CC Diagnostics, Sysmex-Inostics and Protyon (all consulting fees paid to UMCG institution); and received unrestricted grants (all paid to UMCG institution) from MSD/MERCK, Biocartis, Agena Bio-science, BMS, Bio-Rad, Roche, CC Diagnostics, SNN/EFRO and Abbott; regarding his presentation as invited speaker by Agena bioscience, this company reimburse all cost for traveling/attending the ISMRC meeting.