Liquid Biopsy: the application of allele-specific PCR (AS-PCR) and mutation-directed SNaPshot assay to detect BRAF and NRAS mutant DNA in peripheral blood from metastatic melanoma patients

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Introduction

Liquid biopsy provides a non-invasive method to identify tumor signatures in peripheral blood. Clinical assays that can detect tumor-derived cells or circulating DNA in patients with metastatic melanoma may provide a valuable tool for diagnosis of disease, monitoring of response to therapy, and detection of relapse. The optimal assay will encompass high sensitivity and specificity with the greatest dynamic range of quantification.

Malignant melanoma signatures in circulating nucleic acids have been documented in the form of primary BRAF and NRAS activating mutations in peripheral blood.

Methods

Allele-specific PCR (AS-PCR) and SNaPshot assays were designed and evaluated to determine their sensitivities to detect BRAF V600E and NRAS Q61 mutations in tissue and plasma samples. Platform comparisons were conducted to validate assay performance using cell line DNA diluted into normal lymphocyte DNA. Stimulated patient samples were created by spiking dilutions of restricted digested gDNA into healthy donor plasma. Concordance between both mutation detection methods was evaluated using patient FFPE tumor tissue and plasma samples.

Results

The TaqMan panel for BRAF and NRAS demonstrates sensitivities to ≤0.5% mutant diluted into wild-type. SNaPshot provided similar results in the low input range down to ~6 mutant cells, however pre-amplification of the target limits the ability to quantify the result. When applied to a randomly selected set of metastatic melanoma patient plasma and matching FFPE tissue samples the TaqMan and multiplex SNaPshot assays from showed excellent concordance between the platforms for all 5 mutations tested. In a pilot study of 6 tumors, there was no difference in sensitivity to detect mutations using macrodissection of FFPE tissues prior to analysis.

Conclusions

The TaqMan and SNaPshot panels are highly sensitive platforms to detect BRAF V600E and NRAS Q61 mutations in blood and tissue samples. There was excellent concordance in the analysis of FFPE samples, and preliminary data suggests there is no substantial benefit to performing macrodissection prior to DNA extraction. TaqMan assays have a superior ability to quantify mutant and wild-type ratios over a large dynamic range compared to SNaPshot assays which rely on a non-quantitative PCR pre-amplification step. These preliminary results are being confirmed with an expanded set of blood and FFPE samples from metastatic melanoma patients.

References


Supported by 1U01FD004203 and 5R21CA154786