Treatment response monitoring using cell-free DNA fragmentation profiling

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Background & objectives

Blood cell-free DNA (cfDNA) tests have become broadly adopted for minimal invasive cancer characterization and treatment monitoring. However, available assays require deep-targeted sequencing to detect cancer-specific mutations at low mutant allele frequency (MAF). We recently developed a tumor-agnostic, mutation-independent approach that utilizes low-coverage whole genome sequencing to provide real-time estimates of tumor fraction during treatment. Here, we report analyses of DELFI (DNA evaluation of fragments for early interception) Tumor Fraction (DELFI-TF) applied to patients with metastatic colorectal cancer (mCRC) enrolled in the phase III study CAIRO5 (NCT02162563).

Methods

In total, 692 longitudinal plasma samples from 153 mCRC patients were sequenced at low coverage. In patients with tumor-tissue-proven RAS/BRAF mutations, the tumor fractions were quantified as the cfDNA MAF of the RAS/BRAF variant measured by droplet digital PCR (ddPCR). Using genome-wide fragment-sequencing statistics, a probabilistic model was trained against RAS/BRAF MAFs. Longitudinal changes in DELFI-TF scores during first-line therapy (DELFI-TF slopes) were assessed to predict treatment response and survival outcomes.

Results

The DELFI-TF scores strongly correlated with RAS/BRAF cfDNA MAF measured by ddPCR (*Pearson*, r=0.85, p<0.001). Baseline DELFI-TF correlated with liver metastases dimensions reported in CT scans (*Pearson*, r=0.49, p<0.001) and clinical response (*Wilcoxon*, p<0.05). Patients with low or negative DELFI-TF slopes presented with longer progression-free survival (13.4 months vs 10.4 months, HR = 2.03, 95% CI 1.25 to 3.32, Log-rank p<0.01) and overall survival (59.4 months vs 29.1 months, HR = 3.05, 95% CI 1.58-5.90, Log-rank p<0.001).

Tissue-informed focal and arm-level copy number changes were detected 4-12 weeks after liver metastases resection. Most patients with a molecular relapse were diagnosed earlier than clinical recurrences identified by conventional imaging.

Conclusion

Genome-wide cfDNA fragmentation profiling reliably estimates plasma tumor fractions with performance comparable to standard methods used for treatment response assessment and clinical outcome prediction.