Liquid Biopsy as a Reliable Alternative for Comprehensive Genomic Profiling in Advanced-Stage Colorectal Cancer with Insufficient Tissue DNA

Zoran Gatalica[1]*, Zhihong Zhang[2], Bo Yang[2], Ying-Hsuan Shih[2], Inga Rose[1], Sarah Rapisardo[3], Semir Vranic[4]

[1] Reference Medicine, Phoenix, AZ, [2] Burning Rock Dx, Irvine, California, [3] Duke University, Durham, NC, [4] College of Medicine, QU Health, Qatar University, Doha, Qatar

Introduction

Reported failure/unsuccessful rates for tumor tissue NGS vary widely by cancer type, specimen site, and pre-analytic quality, ranging from 5–25% in real-world series, with outliers being higher in more challenging specimens.

Comprehensive genomic profiling (CGP) using circulating tumor DNA (ctDNA) offers potentially critical insights for treatment-naïve advanced colorectal cancer (CRC) patients whose tissue biopsies lack sufficient DNA quality.

Methods and Samples

Plasma samples from 56 advanced-stage (III–IV) CRC patients (collected 2022–2024) were analyzed using a 520-gene ctDNA panel (Burning Rock OncoCompass™ Plus). This next-generation sequencing assay detects single-nucleotide variants, insertions/deletions, gene fusions, and copy number variations, and it assesses microsatellite instability (MSI) status, tumor mutational burden (TMB) and Human Leukocyte Antigen (HLA). All blood samples were drawn before initiation of therapy, and genomic findings were reported with a focus on clinically actionable alterations. The assay can be performed as plasma-only with effective differentiation of germline, somatic, and Clonal Hematopoiesis of Indeterminate Potential (CHIP) variants.

Results

CGP was successful in all 56 cases (100% success rate). Frequently mutated genes included *TP53* (39%), *APC* (39%), *LRP1B* (12%), and *KRAS* (36%). MSI-high (MSI-H) status was observed in 8.9% of cases, while 12.5% demonstrated high TMB (≥10 mutations/Mb). Two cases (3.6%) harbored *POLE* mutations, displaying hypermutation phenotypes (TMB ranging from 76.8 to 238.3 mutations/Mb). Notably, a *TPM3-NTRK1* fusion was identified in one patient with a high-grade CRC, which was also classified as TMB-high and MSI-high, highlighting actionable genomic alterations amenable to TRK and immune checkpoint inhibitors. There were notable associations between high TMB and MSI-H status with higher tumor grades and advanced stages, suggesting these molecular markers could provide prognostic and predictive insights.

Cohort Characteristics

Parameter	Value
Diagnosis	Colorectal adenocarcinoma (100%)
Population	White/Caucasian (56, 100%)
Age (mean, range)	56 years (52-83 years)
Sex	Male (48%) Female (52%)
Tumor grade	Grade 2 (74%) Grade 3 (26%) Unknown (nine cases)
AJCC TNM stage	IIIA (7%) IIIB (64%) IIIC (27%) IV (2%)

Table 1. Clinicopathologic and demographic characteristics of the cohort.

Genomic Findings

Molecular marker	Value (%)
Tumor mutational burden (TMB)*	High* (12.5%) Low (87.5%)
Microsatellite stability (MS) status	MSI-H (8.9%) MSI-S (91.1%)
Mutational profile	*TP53* (39%) *APC* (39%) *KRAS* (36%) *LRP1B* (12%) *NRAS* (11%) *BRAF* (9%) *TPM3-NTRK1* fusion (one case)

* Defined as ≥10 mutations/Mb

Table 2. Molecular features of the cohort obtained by the liquid biopsy.

Mutational Landscape

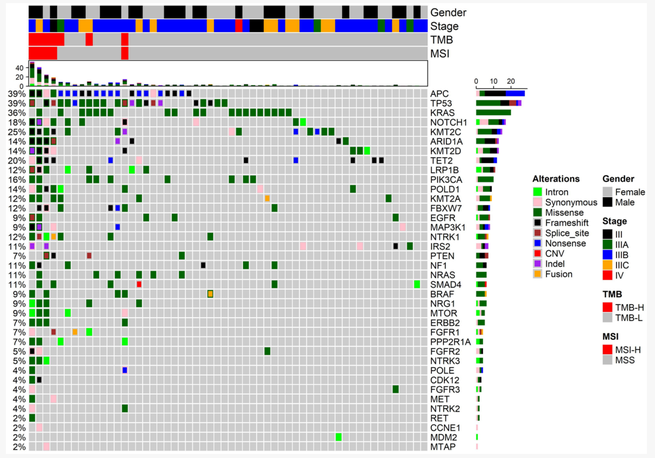

Figure 1. Heatmap displays the prevalence and types of identified genetic alterations for a set of key genes in all samples..

POLE-mutated Case Characteristics

Case	Molecular characteristics
#1: 61-year-old female, grade 3, stage IIIB colorectal cancer	MSI-H, TMB-H* (76.8 mutations/Mb), *BRAF* mutation
#2 75-year-old male, grade 3, stage IIIB colorectal cancer	MSI-H, TMB-H* (238.3 mutations/Mb)

* Defined as ≥10 mutations/Mb

Table 3. Two high-grade colorectal carcinomas harbored POLE mutations followed by high TMB and MSI-H.

CNV Concordance

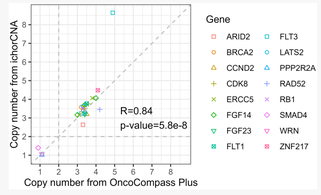

Figure 2. Comparison of CNV calling shows high concordance between the OncoCompass Plus ctDNA assay and ichorCNA (CN estimated using off-target reads) results.

Conclusions and Key Takeaways

- **Our study demonstrates that plasma-based CGP is a reliable alternative to tissue-based sequencing in advanced CRC.**
- **Liquid biopsy identified clinically important genomic alterations in all patients when tissue DNA was unavailable or inadequate.**
- **These results support the integration of ctDNA liquid biopsy into routine diagnostic workflows and treatment decision-making for advanced CRC, particularly when traditional tissue biopsies are insufficient.**