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Review Article

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Comparative Analysis of TP53 Mutation Burden in Tissue Biopsies and Circulating Tumor DNA from Patients with Colorectal Carcinoma: A Review

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Abstract

TP53 is the most frequently altered gene in colorectal carcinoma, and it can be read from a resected tumour, from an endoscopic biopsy, or from plasma. The word burden means something different in each. In tissue it refers to which residues are hit and how often; in plasma it refers to how much mutant DNA is circulating, which is a property of the disease rather than of the gene.

Objectives: To compare what TP53 sequencing of tissue and of circulating tumour DNA each report in colorectal carcinoma: mutation frequency and hotspot distribution in tissue, detection rates in plasma, agreement between the two compartments, and the prognostic weight attached to each.

Material and Methods: Narrative review of primary cohort studies, prospective observational series and meta-analyses indexed in PubMed and the major oncology journals. Studies were eligible if they reported TP53 mutation frequency or residue distribution in colorectal tissue, detection of TP53 or ctDNA in plasma, matched tissue-plasma concordance, or outcomes stratified by mutation or ctDNA status. No new patient data were generated. Every frequency, hazard ratio and survival estimate quoted is one published by the original investigators, and the figures and tables reproduce reported values only.

Results: TP53 is mutated in approximately 60 per cent of colorectal carcinomas. The disease is unusual in how tightly its mutations cluster: the four hotspot residues R175, R248, R273 and R282 account for about 37 per cent of all TP53 mutations in colorectal cancer against 17 per cent across all other cancers, and tumours carrying R273 progress to metastasis more often and survive less well than those carrying R175. In 433 patients tested in both compartments, TP53 was found in 59.6 per cent overall, in tissue in 49.7 per cent, in ctDNA in 36.7 per cent and in both in 27.8 per cent, giving an overall concordance of 67.2 per cent and a positive concordance of 45.0 per cent that rose with circulating tumour fraction. Detectable ctDNA after surgery carried hazard ratios for recurrence of 10.98 immediately post-operatively and 32.02 during surveillance, and in 2,240 patients followed prospectively, positivity in the residual-disease window gave hazard ratios of 11.99 for disease-free and 9.68 for overall survival.

Conclusion: Tissue sequencing characterises the mutation; plasma sequencing measures the disease. Discordance between them is dominated by tissue-positive, plasma-negative cases and reflects low circulating tumour fraction rather than genuine genomic disagreement. The prognostic signal from plasma is the larger of the two, but it is a signal about residual tumour rather than about TP53 itself, and the two readouts should be reported separately.

Abbreviations: CRC – Colorectal Carcinoma, ctDNA – Circulating Tumour DNA, cfDNA – Cell-Free DNA, MRD – Molecular Residual Disease, NGS – Next-Generation Sequencing, ddPCR – Droplet Digital Polymerase Chain Reaction, DFS – Disease-Free Survival, OS – Overall Survival, HR – Hazard Ratio, GOF – Gain of Function, ACT – Adjuvant Chemotherapy.

Keywords: TP53; Colorectal Carcinoma; Liquid Biopsy; Circulating Tumour DNA; Molecular Residual Disease

Introduction

Colorectal carcinoma is the second leading cause of cancer death worldwide, and roughly a third to a half of patients treated with curative intent will eventually relapse. Staging by TNM criteria and histopathological risk features remains the basis on which adjuvant chemotherapy is offered, and it is an imperfect basis: node-negative

patients recur, and many node-positive patients are cured by surgery alone [4,9].

TP53 sits at the centre of the molecular account of this disease. It is mutated in approximately 60 per cent of colorectal carcinomas, and the malignant progression of the disease is driven largely by the sequential accumulation of alterations in oncogenes and tumour suppressor genes, of which TP53 loss is a late and consequential step

[1,2]. Unlike a driver oncogene, however, mutant p53 is not a straightforward therapeutic target, and decades of effort to restore its function have yet to produce an approved agent [3].

What has changed is where the gene can be read. The same mutation recovered from a resected tumour can be tracked in cell-free DNA, and the two measurements have quietly come to mean different things. Sequencing the tumour tells you which residue is affected, and there is now evidence that the identity of the residue matters for outcome [1]. Sequencing plasma tells you whether mutant DNA is circulating, which depends far more on how much tumour is present than on which mutation it carries [5,6].

This distinction is often blurred by the term mutation burden, which is used in the tissue literature to describe the concentration of mutations at particular residues and in the liquid biopsy literature to describe the quantity of mutant DNA in the circulation. The aim of this review is to set the two compartments side by side and report, for each, what has actually been measured in colorectal carcinoma and what it predicts.

Material and Methods

Search strategy: PubMed and the archives of the major oncology and gastroenterology journals were searched for primary cohort studies, prospective observational series, clinical trials and meta-analyses addressing TP53 alterations or circulating tumour DNA in colorectal carcinoma. Search terms combined TP53 and p53 with colorectal carcinoma, liquid biopsy, circulating tumour DNA, molecular residual disease, concordance, hotspot mutation and overall survival. Reference lists of retrieved reviews were screened by hand for additional primary sources.

Eligibility: Studies were included if they reported at least one of the following in patients with histologically confirmed colorectal carcinoma: TP53 mutation frequency or residue-level distribution in tumour tissue; detection of TP53 alterations or ctDNA in plasma; matched comparison of tissue and plasma genotyping in the same patients; or clinical outcomes stratified by TP53 status or by ctDNA detection. Preclinical studies were cited only where they bear directly on the interpretation of a clinical finding. Studies without extractable numerical outcomes were excluded.

Data extraction and handling: Design, sample size, compartment sampled, assay platform, reported frequencies and reported effect estimates with their measures of uncertainty were extracted as published. No pooling, re-analysis or recalculation was performed and no patient-level data were obtained. Detection rates from different assay platforms are reported separately rather than combined, since analytical sensitivity differs substantially between them. Figures 1 to 4 and Tables 1 and 2 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

TP53 mutation frequency and hotspot burden in tissue: TP53 is mutated in approximately 60 per cent of colorectal carcinomas [1]. More striking than the frequency is the concentration. Missense mutations at the four most commonly affected residues, R175, R248, R273 and R282, account for approximately 37 per cent of all TP53 mutations in colorectal cancer, against approximately 17 per cent of TP53 mutations across all other cancer types combined (Figure 1) [1]. Mutations at R175 or R273 alone are carried by over 20 per cent of TP53-mutated colorectal tumours, compared with roughly 10 per cent in other cancers [1].

This clustering is what gives residue-level analysis any statistical purchase in this disease. In most tumour types the hotspots are too diluted across nearly two hundred mutated codons for subgroup comparison to be meaningful [2,3].

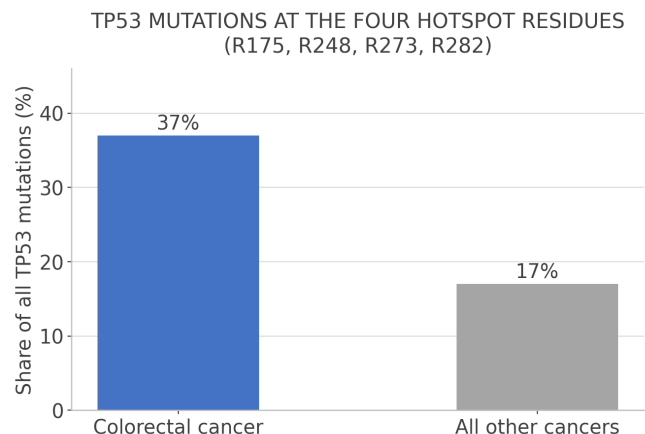


Figure 1: Proportion of all TP53 mutations occurring at the four hotspot residues, in colorectal cancer against all other cancer types [1]

Which residue is mutated changes the outcome: Comparing structural mutants at R175 with DNA-contact mutants at R273 in colorectal tumours, R273 cases were more likely to progress to metastatic disease and had shorter survival. In the TCGA colorectal series analysed for that comparison, 132 cases carried R175 mutations and 121 carried R273 mutations; in an independent institutional cohort, 66 R175 and 68 R273 cases were compared for metastatic pattern at presentation [1]. R273 mutants were associated with a distinct transcriptional programme implicating oncogenic signalling, a pattern shared with the R248Q and R248W hotspot mutants [1].

Older tissue series support the broader point that TP53 status carries prognostic weight independent of stage. In 79 patients undergoing radical resection of colorectal hepatic metastases, mutation was associated with worse outcome on multivariate analysis (HR 1.80, 95% CI 1.07-3.06, $p = 0.028$), and the effect was larger among chemotherapy-treated patients (HR 2.54, 95% CI 1.12-5.75, $p = 0.026$) [7]. The separation is real but modest, and it is not of a size that would change management on its own.

Co-occurring mutations change what a TP53 result means: A TP53 result read on its own can mislead, because its prognostic weight depends on what else the tumour carries. In 433 patients with metastatic colorectal cancer, SMAD4 mutation was present in 70 (16.2 per cent) and was not by itself associated with worse survival on multivariate analysis (HR 1.25, 95% CI 0.90-1.73, $p = 0.18$). Where SMAD4 and TP53 were mutated together, however, overall survival was markedly worse (HR 2.5, 95% CI 1.44-4.36, $p = 0.001$), with a median of 24.2 months against 42.2 months for the rest of the population ($p = 0.002$) (Figure 2) [17].

The implication cuts against reporting TP53 as a standalone variable. In that cohort neither gene carried its apparent prognostic weight independently; the signal lived in the combination. Whether the same holds for TP53 read from plasma, where co-mutations are recovered less completely, has not been tested [5,17].

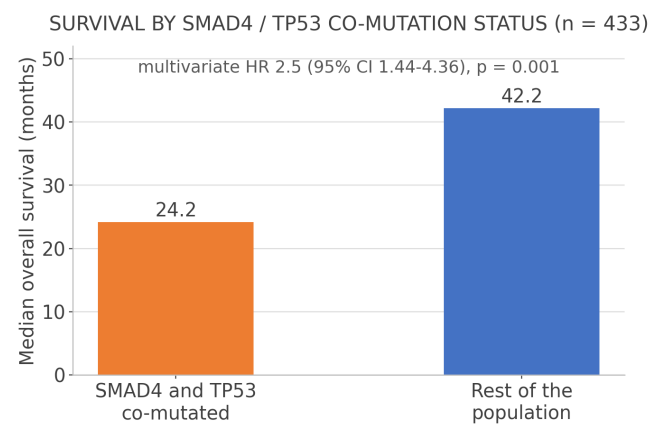


Figure 2: Median overall survival by SMAD4 and TP53 co-mutation status in metastatic colorectal cancer (n = 433) [17]

Study	n	Sample	Principal reported finding
Hassin et al. 2022 [1]	323	Tissue (TCGA)	Hotspot residues carry 37% of TP53 mutations; R273 worse than R175
Bieg-Bourne et al. 2020 [5]	433	Tissue + plasma	Overall TP53 concordance 67.2%; rises with ctDNA fraction
Molleví et al. 2007 [7]	79	Tissue	TP53 mutation independently prognostic after hepatic resection
Chen et al. 2021 [8]	240	Tissue + plasma	Serial ctDNA predicts relapse a mean 5.01 months before imaging
Nakamura et al. 2024 [9]	2,240	Plasma	MRD-window ctDNA positivity: DFS HR 11.99, OS HR 9.68

Table 1: Principal studies reviewed, with the compartment sampled and the finding as reported by the original investigators

TP53 in the two compartments compared directly: The clearest matched comparison comes from 433 patients who underwent next-generation sequencing of both tissue and plasma. TP53 alterations were found in 258 patients overall (59.6 per cent): in tissue in 215 (49.7 per cent), in ctDNA in 159 (36.7 per cent), and in both compartments in 116 (27.8 per cent) (Figure 3) [5]. The asymmetry is the point. Plasma recovers substantially fewer alterations than tissue, and the union of the two exceeds either alone, which means neither compartment is a complete substitute for the other.

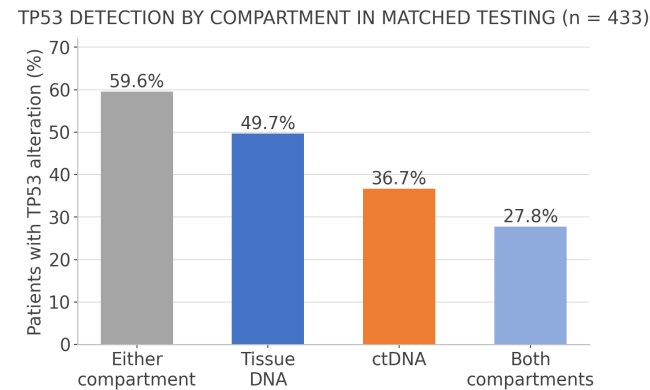


Figure 3: TP53 alterations detected in tissue, in ctDNA, in both and in either compartment among 433 patients sequenced in both [5]

Concordance and what drives it: Overall concordance between plasma and tissue for TP53 in that cohort was 67.2 per cent, but

positive concordance, which excludes the large number of negative-negative pairs, was 45.0 per cent [5]. Three findings explain most of the residual disagreement. Higher circulating tumour fraction was associated with higher concordance ($p < 0.001$). Positive concordance was significantly higher when the tissue biopsy came from a metastatic rather than a primary lesion ($p = 0.03$). And positive concordance was significantly lower in non-colorectal cancers than in colorectal cancer ($p = 0.02$), which is consistent with the comparatively high ctDNA shedding of this tumour type [5]. Time interval between the two samples did not change overall concordance, although shorter intervals trended toward better positive concordance [5].

Read together these say that discordance in colorectal cancer is largely a sampling and sensitivity problem rather than evidence that the tumour genome differs between compartments.

Measure	Value	Factor tested	Reported effect
Overall concordance	67.2%	ctDNA fraction	Higher fraction, higher concordance ($p < 0.001$)
Positive concordance	45.0%	Biopsy site	Higher if metastatic lesion ($p = 0.03$)
TP53 in either compartment	59.6%	Tumour type	Lower in non-colorectal cancers ($p = 0.02$)
TP53 in both compartments	27.8%	Sampling interval	No change in overall concordance

Table 2: Tissue-plasma concordance for TP53 and the factors reported to affect it, from the matched 433-patient cohort [5]

Circulating tumour DNA as residual disease: The strongest clinical signal from plasma in colorectal carcinoma is not about TP53 at all but about whether any tumour DNA remains detectable after treatment. In a prospective multicentre series of 240 patients with stage II or III disease, ctDNA was detectable before surgery in 154 (64.2 per cent). Positivity at day 3 to 7 after surgery carried a hazard ratio for recurrence of 10.98 (95% CI 5.31-22.72, $p < 0.001$); at the first sampling point after adjuvant chemotherapy the hazard ratio was 12.76 (95% CI 5.39-30.19); and during surveillance it reached 32.02 (95% CI 10.79-95.08) (Figure 4) [8]. Serial sampling identified recurrence with an overall accuracy of 92.0 per cent and a mean lead time of 5.01 months over radiological imaging [8].

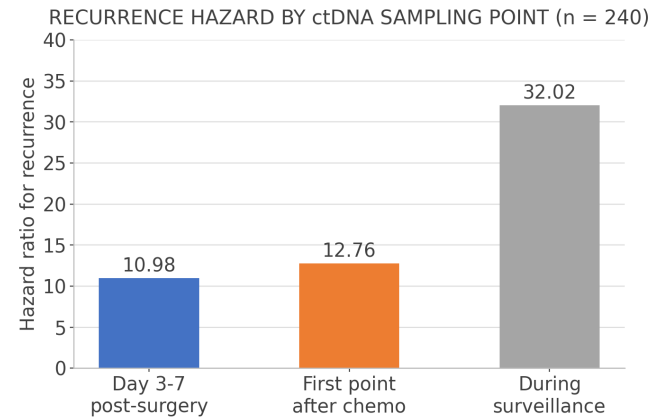


Figure 4: Hazard ratio for recurrence associated with ctDNA positivity at three sampling points in stage II-III colorectal cancer (n = 240) [8]

These findings have been reproduced at scale. In an updated analysis of a prospective observational cohort of 2,240 patients with stage II-III colon cancer or stage IV colorectal cancer followed for a median of 23 months, ctDNA positivity during the molecular residual disease window was associated with a hazard ratio of 11.99 for disease-free survival and 9.68 for overall survival, both $p <$

0.0001. Among patients who did recur, ctDNA positivity remained associated with shorter overall survival (HR 2.71, $p < 0.0001$) (Figure 5) [9]. A meta-analysis of prospective residual-disease studies across resectable cancers reached the same conclusion in pooled form [10].

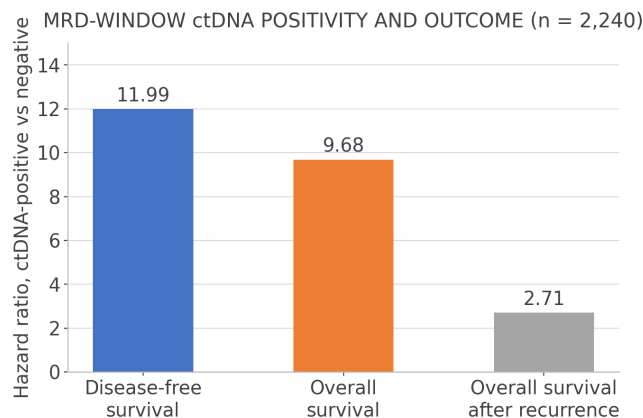


Figure 5: Hazard ratios for ctDNA-positive against ctDNA-negative patients in the molecular residual disease window (n = 2,240) [9]

Discussion

The two compartments are answering different questions, and the phrase mutation burden obscures that. In tissue, burden means the concentration of mutations at particular residues, and colorectal carcinoma is one of the few settings where that concentration is high enough for residue-level comparison to be informative [1]. In plasma, burden means how much mutant DNA is circulating, which is governed by tumour volume, vascularity and turnover [5,8].

This explains why the prognostic separations reported from the two compartments differ so much in size. Tissue TP53 status produces hazard ratios in the region of two [7]. Plasma ctDNA positivity after surgery produces hazard ratios of ten and above [8,9]. The larger number is not evidence that the plasma assay is a better test of TP53; it is evidence that residual tumour is a stronger predictor than any single genotype. Reporting the two as though they were competing measurements of the same thing invites a misreading of both.

There is a third reading problem, which is that TP53 is rarely alone. The co-mutation data show a gene whose prognostic weight is contingent on context: mutated alongside SMAD4 it marks a subgroup with survival cut almost in half, while either alteration reported in isolation looks unremarkable [17]. Since plasma sequencing recovers subclonal and co-occurring alterations less completely than tissue, the compartment that gives the stronger prognostic signal is also the one less able to supply the context that makes a TP53 result interpretable.

Clinical implications

For risk stratification after curative resection, the residual-disease signal is now well enough replicated to be acted on in trial settings, and the lead time of roughly five months over imaging is clinically meaningful [8]. Its use to escalate or de-escalate adjuvant chemotherapy is the subject of ongoing prospective work [9,10].

For TP53 specifically, the tissue finding with the clearest implication is that residue identity is not neutral. If R273 tumours behave more aggressively than R175 tumours at comparable stage, then reporting TP53 as a binary mutated or wild-type discards information that is already in the sequencing report [1]. Whether that information should change management is a separate question, and one that no prospective study has yet addressed.

Study limitations

The limitations are those of the underlying literature. The matched tissue-plasma concordance data come predominantly from pan-cancer cohorts in which colorectal cases are a subgroup, so colorectal-specific concordance estimates rest on smaller numbers than the headline figures suggest [5]. Detection rates are not comparable across studies, because assay depth, plasma volume and input DNA differ and are often under-reported. Residue-level survival comparisons draw on registry and institutional cohorts assembled for other purposes, with the attendant risk of stage imbalance between mutation groups [1]. Few of the subgroup analyses reported here were prespecified, so multiplicity is a genuine concern.

Two gaps deserve naming. First, no study reviewed here separates the prognostic contribution of TP53 genotype from that of circulating tumour fraction within the same patients, which is the comparison the title of this review implies and the literature does not yet support. Second, concordance is almost always reported at the gene level rather than the variant level, so an agreement counted as concordant may involve different residues in the two compartments.

Conclusion

TP53 is mutated in roughly 60 per cent of colorectal carcinomas, and this disease is unusual in how tightly those mutations cluster at four residues, which makes residue-level prognostic analysis possible here in a way it is not elsewhere. Matched testing shows that plasma recovers fewer TP53 alterations than tissue and that the gap narrows as circulating tumour fraction rises, indicating a sensitivity limit rather than genomic divergence. The strongest prognostic signal available from plasma concerns residual disease rather than TP53 genotype, and it is considerably stronger than anything TP53 status contributes on its own. Tissue and plasma results should therefore be reported separately and interpreted for what each actually measures.

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Conflict of Interest

No competing interests are declared by the authors.

Ethics Statement

Only previously published studies are drawn on here. No new patient data were collected and no people or animals took part, so ethical review was not applicable.

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