Prognostic and Predictive Value of Circulating Tumor DNA in Patients with Advanced Colorectal Cancer and Chemotherapy

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Research

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Abstract

Background: Advancedcolorectal cancer (CRC) remains a challenging disease requiring a multidisciplinary approach, combining surgery with chemotherapy, yet most cases still has worse outcomes. Molecular variations were considered to be related to the clinical response. Moreover, emerging data suggest that circulating tumor DNA (ctDNA) may detect minimal residual disease (MRD) and reflect treatment efficacyacross diverse cancer types. However, most reported ctDNA measurements are based on monitoring a few hotspot mutations and their predictive value in advanced CRCs are still undetermined. Here, we carried out WES sequencing to explore the genomic landscape in CRC patients and developedctDNA fingerprints panelswhich are based on the top somatic mutated genes of each individual to evaluate the early changes of ctDNA fingerprints in chemo-treated advanced CRC patients as a marker of clinical benefits.

Methods: We enrolled 122 patients with CRC and analyzed their genetic profilinginclude somatic alterations and copy number variations. Meanwhile, we monitored their ctDNA fingerprints changes along the course of treatment by serial sampling of peripheral blood. Seventy-one of the patients were treated with standard chemotherapy, and theirctDNAfingerprints variations were used to assess the prognosis. We analyzed the correlation between ctDNA fingerprints levels and overall survival (OS) of the patients.

Results: Among the 122 enrolled patients,the*TP53* (70%), *APC* (59%) and *KRAS* (38%) are most frequently mutationsin CRC patients. The *AHNAK* mutation and HOXB-AS1 amplification are associated with survival times.We monitored the baseline ctDNAvalue and found thatthe ctDNA-high group has a shorter OS than that of the ctDNA-low group (HR, 2.89; CI 95% 1.45-5.79; p = 0.0027). The conclusion stands when only 48of the 122 patients, who had advanced CRC (stage IV) received chemotherapy (HR, 1.60; CI 95% 0.61-4.17; p = 0.037). Moreover, change of ctDNA fingerprints were associated with survival timesduring the course of chemotherapy, a decreased group has a favorable clinical benefit. We compared the performance of ctDNA fingerprints in predicting clinical outcomes with that of imaging-based diagnosis in 34 advanced CRC patients, andfound that 52.9% (18/34) of the patients had consistent outcomes between ctDNA fingerprints and imaging diagnosis.

Conclusions: This study analysis the genetic characteristics of CRC patients and explore molecular markers related to the prognosis. It is also confirming the association between baseline levels of ctDNA fingerprints and survival times in CRCs. More importantly, it suggests early change of ctDNA fingerprints level in plasma is a promising biomarker of chemotherapy efficacy and patient prognosis in advanced CRCs.

Background

Colorectal cancer (CRC) is a common malignant disease and causes around 700,000 deaths a year worldwide [1]. Accurate diagnosis and precise treatment are crucial for improving patient survival, especially for those with advanced stage disease. Imaging-based Response Criteria in Solid Tumors

(RECIST) is the gold standard for assessing initial disease and defining treatment, but its application is limited to some extent, such as low sensitivity and poor inter- and intra-observer reproducibility [2]. It's also difficult to distinguish disease progression from early treatment effects. Effective biomarkers therefore are needed in these situations for improving the accuracy of diagnosis and benefits of treatment. For example, *RAS* mutation and *HER2* amplification are known predictors of poor prognosis/treatment response in mCRC [3–5]. *BRAF V600E* mutation suggests a poor response to standard chemotherapy and some target agents (cetuximab) and with poor prognosis in CRC [6–7]. In contrast, *HER2 mutation* predicts a good response to HER2 targeting drugs [8–9]. The above known biomarkers have greatly improving the diagnosis and treatment of CRC. Unfortunately, to date, there is none effective prognosis marker in advanced CRC.

Circulating tumor DNA (ctDNA) are derived from cancer DNA fragments with tumor specific mutations [10]. They have been considered as biomarkers for tailored therapy efficacy in various solid tumors [11–12]. National Comprehensive Cancer Network (NCCN) guidelines currently recommended ctDNA can be used to evaluated the presence of minimal residual disease (MRD) and predicted recurrence risk in early-stage CRC patients after surgery [13–14]. Moreover, some studies also have illustrated that ctDNA could provide an indication of efficacy of other newer therapies, such as molecular targeted therapies [15] and immunotherapies [16–17] in advanced CRC patients. However, in the above studies, the utility of ctDNA was focused on a limited number of tumor hotspots in population, which lack specificity to individual patient and may be ineffective to patients carrying no or low mutations at the hotspots probed. To improve the detection specificity and sensitivity, we have developed ctDNA fingerprints that are designed based on the top somatic mutations in each individual patient [18]. We have showed the ctDNA fingerprints had higher sensitivity and specificity than conventional hotspots based ctDNA panels in a variety of cancers [18]. Nevertheless, their value as predicative biomarkers for advanced CRC (stage IV) patients received chemotherapy are yet to be determined.

In this retrospective study, we enrolled 122 CRC patients, 48 of them in stage IV. We designed ctDNA fingerprints panels for the patients individually and monitored their levels along treatment course to test whether ctDNA fingerprints are effective predictor of treatment response and prognosis of patients with advanced CRC received standard chemotherapy.

Materials And Methods

Patients and samples

This retrospective study enrolled 122 patients with histologically proven colorectal cancer and treated at our hospital between Nov. 2012 and Apr. 2019. All the patients were provided with formalin-fixed or paraffin embedded (FFPE) tumor tissue of primary or metastatic lesions for WES detection, and their baseline ctDNA were determined before the treatment and change of the ctDNA fingerprints monitored up to Aug. 2020 were retrospectively identified from hospital records and recruited. The study was conducted

in accordance with Good Clinical Practices and the Declaration of Helsinki and were approved by each Institution's Ethical Review Board prior to study initiation.

FFPE tumor primary tissues of the patients from diagnostic biopsy or surgical resection were collected. 10 ml peripheral whole blood were collected in EDTA tubes from patients. The blood was then centrifuged twice at 1500g for 10 min and the isolated plasma was stored at -80°C until use. The first time collection before the first cycle of chemo-therapy was defined as the baseline, and 2–8 times subsequent collections were made during the course of treatment according to the follow-up visit schedule as decided by the clinicians, typically 2–3 months in interval.

Whole-exome sequencing (WES) of tumor tissue and paired blood sample

Tumor tissue DNA were extracted from FFPE slides using MagMAX FFPE DNA/RNA Ultra kit (cat# A31881, Thermo Fisher Sci., USA). Germline (peripheral white blood cells) DNA were extracted from the plasma using Maxwell RSC blood DNA kit (cat# AS1400, Promega). A minimum of 10 ug DNA samples were sonicated by a Covaris L220 sonicator and hybridized to whole-exome probes in Agilent SureSelect XT Human All Exon V5 kit (cat# 5990-9857EN, Agilent Tech., USA). The enriched DNA were PCR amplified and converted to sequencing libraries, sequenced on an Illumina Novoseq platform, and analyzed as previously described [18].

Somatic tumor mutations, including single-nucleotide variants (SNV), indel, and copy number variation (CNV) were determined by comparing the WES results between paired tumor tissue and germline DNA. Clinical Interpretation of Variants in Cancer (ClViC) knowledge base, which includes 3331 predictive and prognostic marker mutations distributed in 414 genes, was used as the reference in mutation calling.

Tumor mutation burden (TMB) and microsatellite instability (MSI)

TMB was defined as the number of non-synonymous somatic mutations and substitutions detected in WES using a published algorithm and reported as the number of mutations per mega base [19]. MSI was defined by counting the tracts of DNA containing 1–5 bp repeating subunits and five or more repeats in GRCh37/hg19 and were identified using MISA. Customized MSI analysis was performed according to the algorithm as previously described [20].

Isolation of cell-free DNA (cfDNA) and design and characterization of ctDNA fingerprints

Isolation of cell-free DNA (cfDNA) and the bioinformatic design of ctDNA fingerprints specific to individual patient were done according to the published procedures [18, 21]. Briefly, cfDNA were extracted from 10 ml plasma aliquots saved during clinical visits that were not previously frozen. 8 ng cfDNA were used as the template in the below multiplex PCR to detect ctDNA fingerprints. Somatic single nucleotide variations (SNVs) in each patient detected by WES was used to estimate clonal clusters using SciClone

tools [22] with the following parameters: tumor purity as determined by pathologists, and tumor copy number alterations (CNA), loss of heterozygosity (LOH) ratio in somatic mutation regions, and somatic variant allele frequency (VAF). The CNA and LOH were inferred from WES data as well using VarScan v2.4.2 tools [23]. Then, multiplex PCR primers specific to the top 9–37 somatic mutations within the high frequency clonal clusters were designed by Ion AmpliSeq Designer (ThermoFisher Scientific) and used as multiplex PCR primers. ctDNA fingerprints specific to individual patient were amplified from the above cfDNA template using the multiplex PCR primers described and KAPA2G Fast Multiplex Mix kit (cat# KK5802, KapaBiosystems, Wilmington, MA, USA). The PCR products were further amplified and converted to sequencing libraries using Ion AmpliSeq Library kit (cat# 4475345, ThermoFisher Sci., USA). The libraries were sequenced on an Ion Torrent S5 sequencer (Thermo Fisher Sci., USA).

Sequencing data of ctDNA fingerprints were processed and reported as cancer cell fraction (CCF) as previously described [18]. Same as ref. 18, The detection limit of CCF is 0.25%. When ctDNA fingerprints CCF evaluation was compared to diagnostic imaging examination, we defined the CCF measured within +/-10 days of diagnostic imaging examination as the definitive CCF so the two kinds of evaluation were done at close enough clinical time-frame and directly comparable. We also defined ΔCCF as the value of the median of CCF after therapy minus the baseline CCF in ctDNA fingerprints measurements of individual patients.

Statistical analysis

Disease assessment was performed every 8 weeks until documented progression. Response was assessed according to RECIST criteria, version 1.1. The OS was defined as the time elapsed from the first cycle treatment of chemotherapy until death. Surviving patients were censored at the last follow-up date (Aug. 2020). Survival curves were drawn with the Kaplan–Meier method and compared with the log-rank test. The Mann–Whitney U-test was used to assess the variables correlated with baseline of ctDNA levels, such as gender, ages and metastatic sites. All statistical analysis was performed using R. Events with p value < 0.05 are considered significant.

Results

Characteristics of patients

The study design and patient enrollment are presented in Fig. 1. A total of 122 patients (78 men and 44 women; median age, 52 years) were enrolled who had sought treatment for colorectal cancer (CRC) between Nov. 2012 and Apr. 2019, and had custom ctDNA fingerprints test done and monitored up to Aug. 2020. All the patients had whole-exome sequencing done on their primary tumor samples. Of them, 102 (83.6%) patients received at least one dose of chemotherapy (FOLFOX). 31 patients were excluded from subsequent analysis because they were lost of follow-up or had a follow-up time shorter than 6 months. In the remaining 71 patients, 48 patients were in advanced disease (stage IV) at the time of diagnosis and the other 23 patients were in lower or uncertain stages. 38 of the advanced stage patients had their ctDNA fingerprints monitored for 3 times or more, up to 8 times.

Genetic landscape of CRC and their predictive role for prognosis

In order to design and monitor the ctDNA fingerprints for each individual, we applied WES sequencing to eligible patients. After quality filtering, the genetic profiling including somatic mutations, DNA copy number variations (CNV) and mutational significance were assessed subsequently. Among the 122 CRC patients, 96.72% had at least one genomic alteration (Fig. 2). We identified *TP53* (70%), *APC* (59%) and *KRAS* (38%) as the most frequently cancer-associated mutations (Fig. 2A), consistent with previous studies [24]. Compared with TCGA and MSK CRC database, *APC* mutation frequency was significantly lower (59.0% vs 76.9% for TCGA and 59.0% vs 79.0% for MSK), similar to the result observed by Li et al (59.0% vs 65.1% for CCRC) (Fig. 2B). Furthermore, we found that *KCNK15* and *ABHD16B* were the most common genes with CNV amplified, and the number of CNV amplified genes was greater than the number of CNV deleted genes (Figure S1).

We then evaluated the genomic alteration as predictive biomarker for CRC patients, and found that *AHNAK*, *FRG1*, *MUC2* and *XIRP2* mutations are associated with survival times (Fig. 2D, Fig. S2). Various of sites of *AHNAK* mutations had been identified and the median OS of patients with *AHNAK*-wt and *AHNAK*-mut were 30 months and 35 months respectively (HR 0.66; 95% CI 0.35–1.23; p = 0.013) (Fig. 2C). The CNV of HOXB-AS1 also has been suggested as a candidate biomarker of clinical benefits (HR 0.45; 95% CI 0.21–0.94; p = 0.025). Finally, we verified the cytoband 1q31.3, 2q24.3, 2q32.1 and 3q24.3 etc. were associated with OS (Fig. 2E), suggesting these alterations were candidate prognostic markers for CRC.

Prognostic impact of ctDNA fingerprints at baseline

The ctDNA fingerprints of each patients were designed following the method we described previously and their abundance were reported as cancer cell fraction (CCF) [18]. In 122 enrolled CRC patients at the baseline, 81 (66.4%) had positive CCF (CCF ≥ 0.25%), and 41 (33.6%) had undetectable CCF (CCF < 0.25%) (Table S1).

We first classified the 122 patients to two groups according to their baseline CCF values, a ctDNA-high group (CCF > median) and a ctDNA-low group (CCF < median). The start time of OS is set at the time of the initiation of chemotherapy, and the cut-off date is Aug. 2020. There is a strong correlation between CCF and overall survival (OS) based on univariate analysis. The OS of the ctDNA-low group is significant longer than the ctDNA-high group (HR, 2.89; 95% CI 1.45–5.79; p = 0.0027). The median OS is 33 months in the ctDNA-low group and 28 months in the ctDNA-high group, respectively (Fig. 3A). Conversely, if we divided the patients according to their OS, the group with OS longer than the median (30 months) has a significantly higher ctDNA levels than the group with OS shorter one (Fig. 3B). Moreover, we analyzed the correlation between disease stage at diagnosis of the patients with the baseline ctDNA level. Thirty-one patients had no available stage data and were excluded from this analysis. In the remaining 91 patients, 75 were at advanced stage (stage IV, n = 75), and 16 were at lower stages (stage II, n = 5; stage III, n = 11).

The advanced stage patients had a significantly higher ctDNA levels than those at the lower stages (Fig. 2C). In addition, no significant difference in the ctDNA concentration was detected to be associated with the patients' other characteristics, such as gender and ages (Table 1) in multivariant analysis.

Table 1 Clinicopathological characteristics of patients and association with baseline ctDNA levels

Parameter	No.	ctDNA level, median (range %)	P value
Sex (median age, years)			
Male (53)	78	0.47 (0.07-95.41)	—
Female (49)	44	0.99 (0.07-90.24)	0.617
Age, years			
> 55	50	1.44 (0.07-95.41)	—
< 55	72	0.5 (0.09-91.21)	0.229
Stage			
II	5	1.2 (0.23-12.23)	—
III	11	0.27 (0.12-14.78)	0.59 —
IV	75	0.15 (0.07-91.21)	0.45 0.194 —
Other	31	0.24 (0.07-95.41)	0.789 0.524 0.244
Primary tumor sites			
Colon	60	1.1 (0.07-91.21)	—
Rectum	50	0.41 (0.07-75.82)	0.056 —
Other	12	0.11 (0.07-95.41)	0.842 0.133 —
Metastatic			
Yes	98	1.44 (0.07-91.21)	—
No	24	0.27 (0.07-95.41)	0.385

ctDNA fingerprints monitoring in patients after chemotherapy

In order to evaluate the utility of ctDNA fingerprints as a therapeutic biomarker, we analyzed 102 CRC patients who had received chemotherapy (Fig. 1). As mentioned above, the start point OS of patients was corrected using the initiation of chemo-therapy instead of diagnostic time so can assess treatment

response more precisely. 31 patients were therefore excluded from subsequent analysis due to loss of follow-up. Of the remaining 71 patients, 29 patients (40.8%) were deceased and 42 patients (59.2%) were alive at the cut-off date. To explore the predictive roles of baseline ctDNA in the 71 patients included, we did Kaplan-Meier analysis. The ctDNA-low group had statistically significant better prognosis than the ctDNA-high group (HR, 2.59; CI 95% 1.23–5.46; p = 0.0088) (Fig. 4A).

Based on the study by Zhang *et al*., the dynamics of blood ctDNA is a predictor of immunotherapy benefits in a variety of advanced cancers [17]. We then sought to explore the predictive value of ctDNA fingerprints in CRC patients who received chemotherapy. We defined the baseline as CCF measured before the start of treatment in each patient. For patients who had multiple measurements post chemotherapy, we took the median values to represent their post chemotherapy levels. The ΔCCF of patients were defined and determined to reflect change of ctDNA fingerprints during the course of treatment (Figure S3). Of the 71 patients with OS and CCF data, 23 (32.4%) had a positive baseline (CCF ≥ 0.25%) and increased CCF (ΔCCF > 0) and had the worst outcomes; 27 (38.0%) had a positive baseline (CCF ≥ 0.25%) but decreased CCF (ΔCCF < 0) and had an intermediate outcomes; and 21 (29.6%) had undetectable CCF at both the baseline and post-treatment (CCF < 0.25%) and had the best OS (Fig. 4B; increased vs undetectable, p < 0.0001).

ctDNA fingerprints monitoring in advanced CRC patients after chemotherapy

Since baseline CCF as predictive biomarker has been confirmed in 122 CRC patients and the subset of 71 patients received standard chemotherapy CRC patients, we postulate that baseline ctDNA fingerprints can also be a benefit predictor for advanced CRC patients received chemotherapy. To explore this postulate, we focused on the 48 patients diagnosed with advanced CRC and split them into two groups based on the median of their CCF values. A significant association was found between the baseline CCF and OS. The patients in the ctDNA-high group (baseline CCF > median) had a shorter OS after chemotherapy, while the patients in the ctDNA-low group (ctDNA levels < median) had a longer OS (HR, 0.63 95% CI 0.23–1.64; p = 0.037). The median OS was 54 months for the ctDNA-low group and 51 months for the ctDNA-high group (Fig. 4C).

We further evaluated whether monitoring ctDNA change along the course of treatment have clinical value to patients. The 48 advanced CRC patients had paired ctDNA fingerprints measurements at the baseline and post chemotherapy. We classified them into three groups based on the dynamic of their ctDNA fingerprints.16 (33.3%) patients had positive baseline (CCF ≥ 0.25%) and increased CCF (ΔCCF > 0). They had a significantly shorter OS. 18 (37.5%) patients had positive baseline (CCF ≥ 0.25%) and decreased CCF (ΔCCF < 0) and 14 (29.2%) patients had undetectable CCF (CCF < 0.25%). Both the later two groups had better clinical outcomes (Fig. 4D-E; increased vs undetectable p = 0.0035). The dynamics of ctDNA fingerprints can also be characterized by the difference of their levels between the definitive test (the first done concurrent or within 10 days standard clinical evaluation) and the baseline, which we had previously defined as ΔCCF and used to predict treatment response [18].

From the logistic perspective in clinics, using CCF of the definite test, instead of the median value of multiple follow-up tests, is much more feasible for oncologists and will help them to make clinical decision in a timely fashion. We therefore tested ΔCCF, defined as the CCF measured at the definitive test minus the CCF measured at the baseline of individual patient, for the use of a predictive marker of early chemotherapeutic response and resistance. Of the 48 patients with advanced CRC and received chemotherapy, 39 were included in the subsequent analysis because the other 9 had undetectable ctDNA at both the baseline and post-chemotherapy measurements and were excluded. The 39 patients were divided into three groups according to their ΔCCF (Fig. 4F). 12 patients had ΔCCF > 3.0 and also had the shortest OS. 13 patients had ΔCCF < -3.0 and the best clinical outcomes. Additionally, the mortality rate between the groups are different. 11 out of the 12 patients with ΔCCF > 3.0 deceased while only 4 out of the 13 patients ΔCCF < -3.0 did so (Fig. S4). Moreover, we evaluated the degree of tumor mutation burden (TMB) and microsatellite instability (MSI) at the baseline of treatment. Only 10 TMB-high patients (TMB > 350) and 6 MSI-high patients (MSI > 3.5%) were identified and none of the group show significant association with the OS (Fig. 4F). Therefore, ΔCCF (R^2 = 0.773) is a more accurate prognostic biomarker than TMB (R^2 = 0.029) and MSI (R^2 = 0.033).

ctDNA fingerprints as a diagnostic and prognostic marker post-chemotherapy

We tested the correlation of the changes of ctDNA fingerprints level with the standard imaging-based conventional clinical evaluation for tumor response to chemotherapy in the advanced CRC patients. In total, 34 patients with were included in the analysis because among the 38 advanced CRC patients with ctDNA fingerprints monitored longitudinally (≥ 3 times), 4 had no evaluable imaging data. Among the 34 patients, 24 had detectable ctDNA (70.6%), while the other 10 (29.4%) had undetectable ctDNA both in baseline and post treatment (Fig. S1). Of the 24 patients with positive CCF, 9 showed increased ctDNA levels (ΔCCF > 0) and the other 15 showed decreased ctDNA levels (ΔCCF < 0). The tumor response imaging clinical data of those 34 patients were provided in the Supplementary Table S2. Based on the trends of ΔCCF and clinical imaging performance of the 34 patients, they were divided into three groups (Fig. 5). A "consistent" group (18 patient, 53.0%), which includes patients with disease progression by imaging standard also had increased ΔCCF or patients with disease remission also had decreased/undetectable ΔCCF. An “inconsistent” group (5 patients, 14.7%) which includes patients with opposite trends of imaging diagnosis as that predicted from ΔCCF; and a third “uncertain” group (11 patients, 32.4%) which includes patients with temporal progression or remission disease by imaging diagnosis although their ΔCCF stayed in one direction. These results demonstrated that at least over half of patients can benefit from ctDNA fingerprints test to portend disease progression and support its use as a diagnostic biomarker in combination with imaging-based diagnosis.

Discussion

Genetic changes drive phenotypic change and eventually clinical manifestation and outcomes of disease. Although histological diagnosis remains the cornerstone of disease progression, it is now well recognized that molecular-related diagnosis can be identified on the bias of genomics and transcriptomics. In this study, we revealed genetic landscape of patients with CRC based on WES, and explored the mutation status of *AHNAK* and HOXB-AS1 were associate with OS. Previous studies showed that *AHNAK* is a tumor suppressor can regulate cell cycle via TGFβ/Smad signaling pathway in breast cancer [25], and HOXB-AS1 is a long noncoding RNA can promote proliferation, migration, and invasion of glioblastoma [26], while their predictive role in CRC had not been reported.

Drug resistance, recurrence and metastasis are the major causes of death in most cancers but they are often inevitable consequences of late stage cancers. Therefore, it is critical to have sensitive and convenient markers to monitor disease and report drug resistance, recurrence or metastasis as early as possible. Clinical imaging is a gold standard to detect large tumor mass but it lacks sensitivity for tumors below a size threshold and may miss distal metastasis unless a full body scan is done, which is rare in clinical practice without good prior evidence of metastasis [27–28]. Tumor mutational burden (TMB), defined as the number of somatic mutations of tumor is also considered as a prognostic and predictive biomarker in cancer therapies [29]. However, the reduction of TMB is typically a slow process. The association between clinical parameters and concentration of ctDNA were therefore suggested as an alternative prediction marker [30–31].

Previous studies demonstrated that ctDNA could not only be detected in a high proportion in patients with CRC, but also were more easily detected in patients with advanced cancer compared patients with localized tumor [32]. In cancer patients, the plasma ctDNA is carrying specific tumor genetic mutation [33] and this tumor-originated DNA is considered as a surrogate marker of tumoric alterations [30]. Meanwhile, recent study in clinical trial operations confirmed that the utility of ctDNA genotyping had significantly shortened the time required to screen patients and improved the trail enrollment rate than other tumor tissue-based tests [34]. However, limited number of tumor hotspots were mainly applied to explore the roles of ctDNA in most of studies, which reduce sensitivity and specificity due to cancer heterogeneity and individual genetic mutations bias. In order to overcome these defects and add a new option of monitoring late stage cancers, we have developed patient specific ctDNA fingerprints as it can achieve higher sensitivity and specificity, and at the same time is minimally invasive to patients, allowing multiple samplings over time [18].

We applied ctDNA fingerprints to monitor late stage CRC patients and focused on those who had received chemotherapy because chemotherapy is the curial standard treatment for advanced CRC patients. We measured both the baseline and post chemotherapy levels of ctDNA fingerprints (CCF) and found notable association between the efficacy of chemotherapy with the baseline CCF as well as the trend of CCF changes. These results support that plasma ctDNA fingerprints is a potential predictive biomarker of chemotherapy in late stage CRC, in line with our previous report in pan-cancer [18]. Our findings were similar to other studies in the literature on ctDNA and systemic therapies in prostate cancer [35], advanced stage melanoma [36], non-small cell cancer [37] and gastric cancer [38], which all showed that

the patients with detectable ctDNA had an extremely rapid disease progression and recurrence risk. It also provides a concept that raising individual treatment strategies are necessary to strive more effective clinical outcomes for patients with high ctDNA concentration.

Moreover, ctDNA seems to be more sensitive to changes of malignant tumor burden than imaging, suggest it could assist in monitoring clinical response of patients after treatment. In this study, we evaluated the predictive roles of ctDNA fingerprints compared to the clinical imaging and showed that about 52.9% patient disease progression prognosis based on ctDNA were consistent with the imaging diagnosis (Fig. 5). This observation was also similar to previous study by Boysen AK *et al.*, who analyzed 35 mCRC patients and found that 4 patients (4/5) with positive ctDNA who had recurrent, 17 patients (17/30) with undetectable ctDNA who had none recurrent, suggested about 60% consistency (21/35, 60%) when using ctDNA as a biomarker for predict recurrent disease [39]. Taken together, our data demonstrated that dynamics of ctDNA level during the course of treatment reflect disease biology and is associated with prognostic outcomes and could serve as a prognostic marker complementary to imaging after treatment.

Limitations of our study include the lack of paired tumor biopsy and small size of sample for the assessment of ctDNA kinetics.

Conclusions

CtDNA measurement is taking on an increasingly important role in clinics. Besides serving as easily accessible and reliable source for genotyping, ctDNA can also be used to predict and monitor drug response and study resistance mechanisms. Our analysis contributes to the understanding of the roles of ctDNA fingerprint as a prognostic and predictive biomarker and its potential to complement radiologic endpoints and adjudicate radiologically equivocal benefits.

Abbreviations

ctDNA: Circulation tumor DNA; OS: Overall survival; MRD: Minimal Residual Disease; CAN: Copy Number Alterations;CCF: Cancer Cell Fraction; TMB:Tumor Mutation Burden; MSI: Microsatellite Instability.

Declarations

Ethical approval and consent to participate

The study was conducted in accordance with Good Clinical Practices and the Declaration of Helsinki and were approved by each Institution's Ethical Review Board prior to study initiation.

Consent for publication

All authors have agreed to publish this manuscript.

Availability of supporting data

All the data that support the findings of this study are available from the corresponding authors upon reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

Xiu Liu, YibinXie, Kai Ou and Xuan Zhang carried on the study design and contributed to sample preparation. Xuan Zhang, Xiu Liu and Che Long analyzed the clinical data and wrote manuscript. Jun Liu and Lin Yang revised the manuscript and approved final manuscript.

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Figures

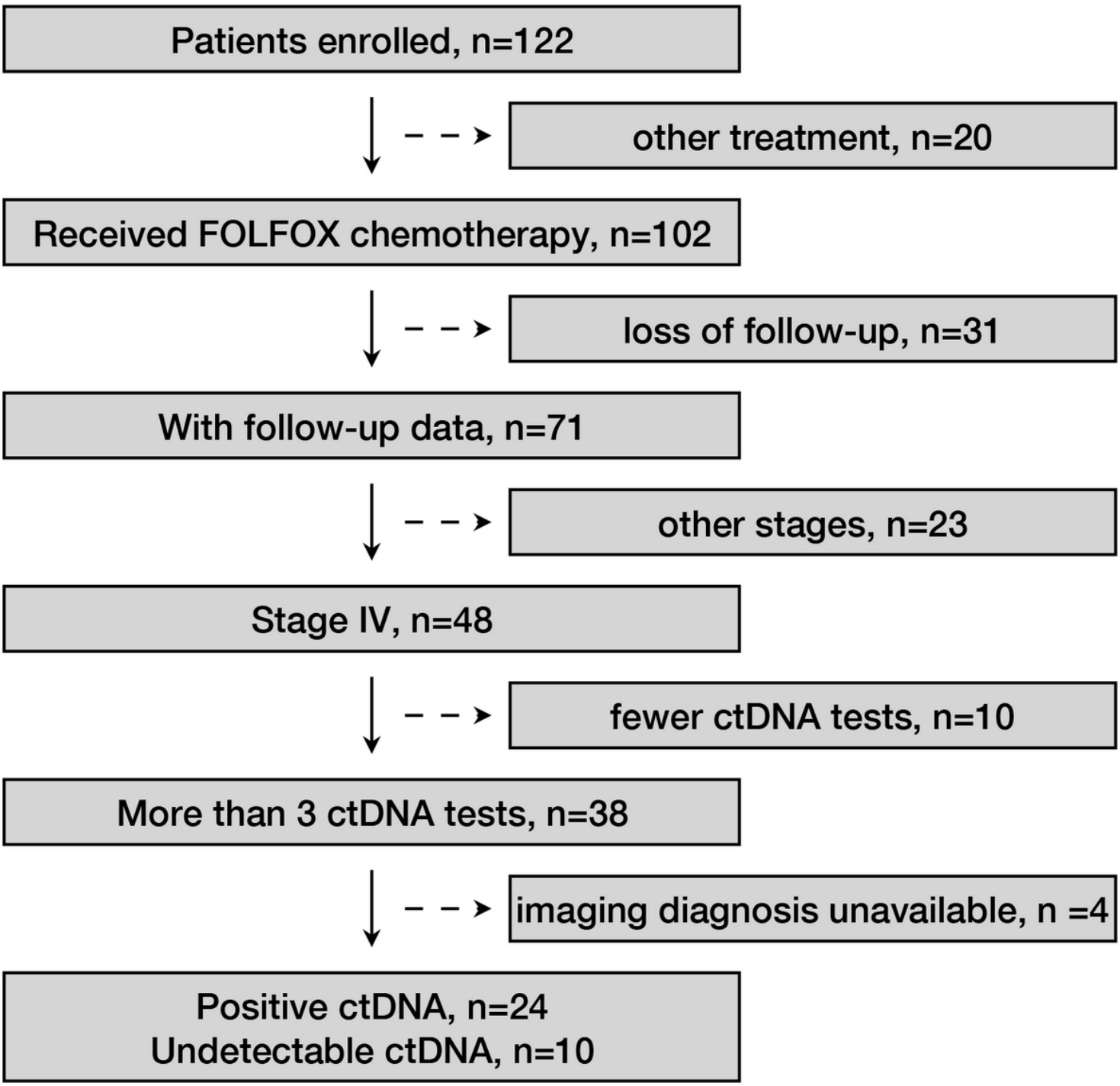


Figure 1

Diagram of patients and study work flow. The number of patients enrolled, and numbers of included and reason of exclusion of patients in different analysis endpoints are indicated.

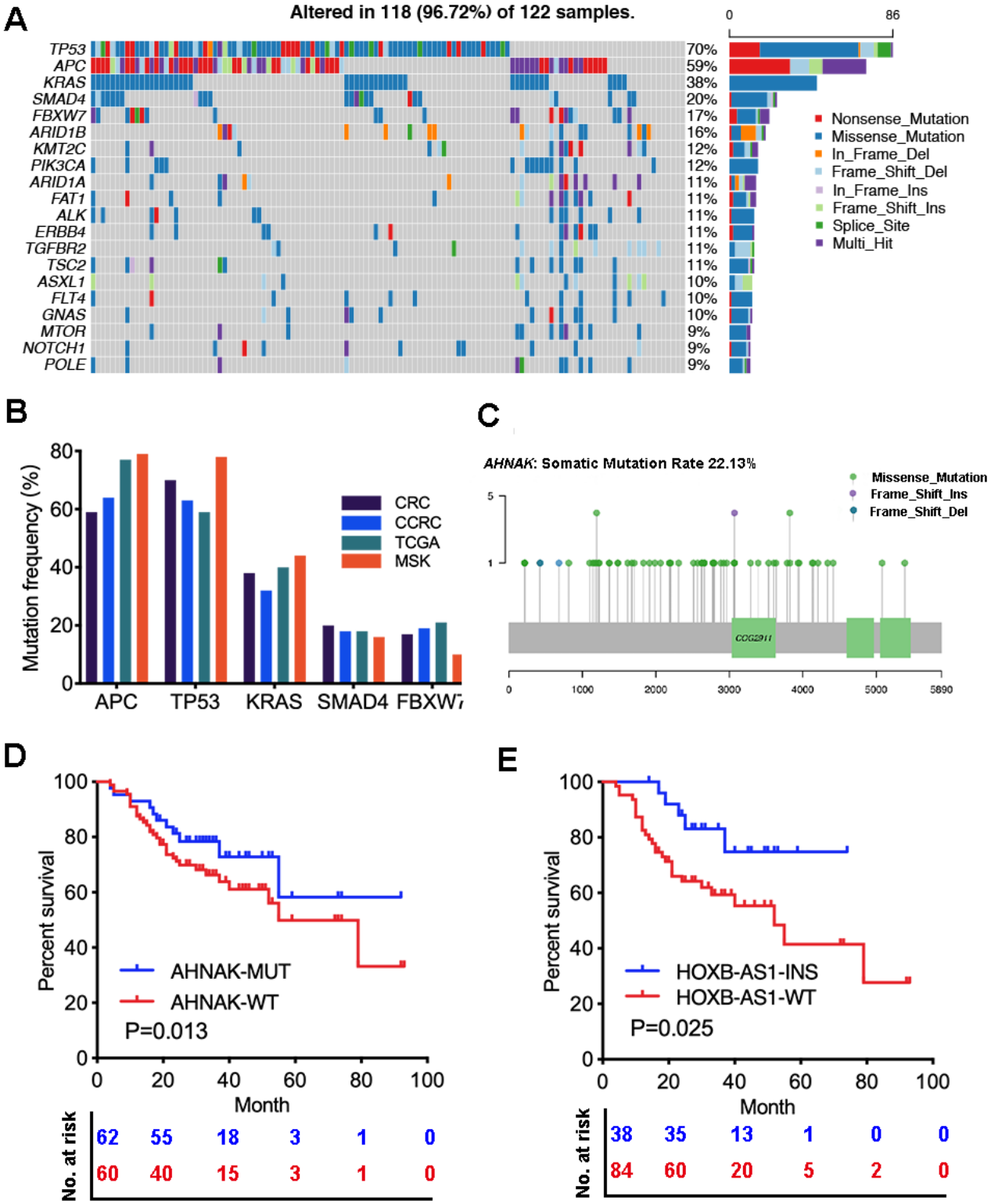


Figure 2

2Genetic landscape of CRC and their predictive role for prognosis. (A) Genetic profile of all 122 CRC patients. Bar plot at right indicates the distribution and compositions of mutation types in each gene. (B) Comparison of frequently mutated genes between CRC, CCRC, TCGA and MSK cohorts [24]. (C) Summary of AHNAK mutations. (D) Kaplan-Meier analysisfor AHNAK mutations of 122 patients. (E) Kaplan-Meier analysis of patients according to HOXB-AS1 copy number alteration.

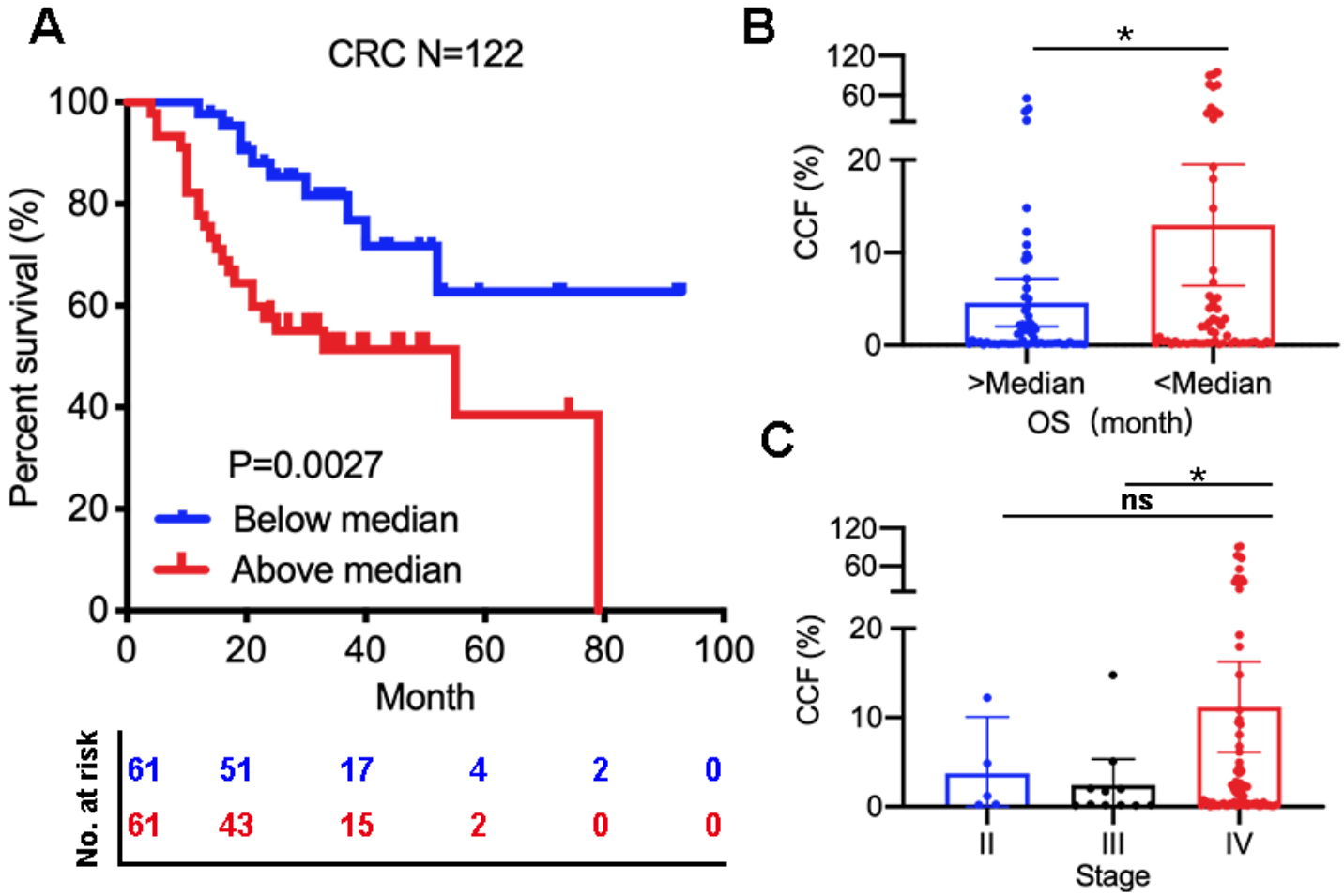


Figure 3

Baseline ctDNA level is associated with clinical outcomes in the total patients. (A) Kaplan-Meier analysis of all enrolled CRC patients grouped according to their baseline ctDNA levels (i.e. CCF values). (B) Box plot of baseline CCFs of patients with longer OS (above the median) or shorter OS (blew the median). (C) Box plot of baseline CCFs of patients with different clinical stages.

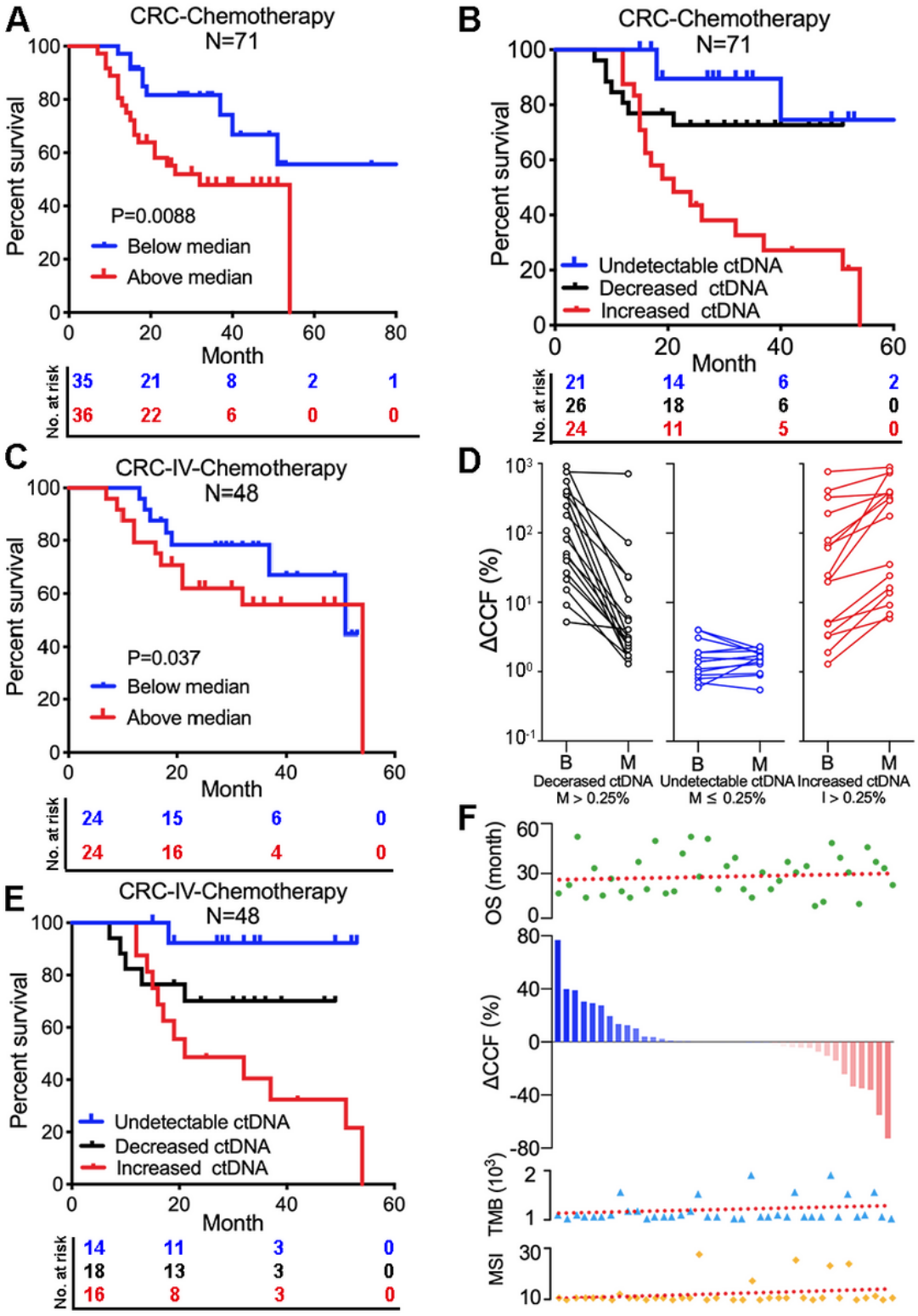
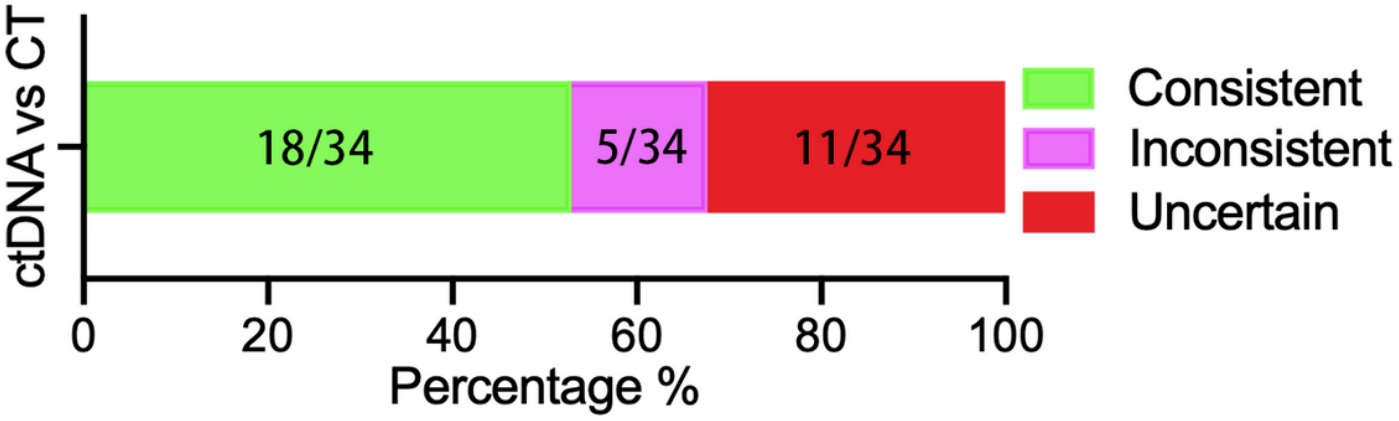


Figure 4

Trend of ctDNA fingerprints level change is associated with prognosis in CRC patients after chemotherapy. (A) Kaplan-Meier analysis of chemo-treated CRC patients grouped according to their baseline CCF. (B) Kaplan-Meier analysis of chemo-treated CRC patients grouped according to their baseline and trend of CCF: an "Undetectable" group with undetectable ctDNA at both the baseline and post-therapy samplings (CCF < 0.25%), a "Decreased" group with the median of post-therapy samplings is

lower than the baseline, and an "Increased" group with the median of post-therapy samplings is higher than the baseline. (C) Kaplan-Meier analysis of advanced (stage IV) CRC patients according to their baseline CCF. (D) Comparison of the median of post-therapy samplings vs. baseline CCFs of advanced CRC patients after chemotherapy in the three groups of patients as defined in B. (E) Kaplan-Meier analysis of advanced CRC patients after chemotherapy in the discovery group stratified by “Undetectable ctDNA”, “Decreased ctDNA” and “Increased ctDNA”. (F) Waterfall plot of patients CCF and their matched OS, TMB and MSI. Green dot represents OS, Blue triangle and yellow rhombus represent TMB and MSI respectively, and red dotted lines represent the trendlines of each of the parameters (ΔCCF R2=0.773, TMB R2=0.029, MSI R2=0.033).



clinical detected \ ctDNA monitored (No.)	Negative ctDNA	Decreased ctDNA	Increased ctDNA
PD/SD→SD/PR→SD/PR	Consistent (5)	Consistent (7)	Inconsistent (3)
PR/SD→SD/PD→SD/PD	Inconsistent (1)	Inconsistent (1)	Consistent (6)
PD/SD→SD/PR→SD/PD	Uncertain (4)	Uncertain (7)	Uncertain (0)

Figure 5

Trend of ctDNA fingerprints level is associate with clinical disease progression. The relationship of ctDNA fingerprints level (ctDNA) and clinical response as evaluated by CT imaging (CT) is displayed. Top panel: horizontal bar graph of the distribution of “Consistent" patients (green), who had consistent evaluation by either CCF change or clinical CT test (i.e., CCF increase correlates with progression disease by CT, or CCF decrease correlates with disease remission by CT); “Inconsistent” patients (purple), who had different evaluations by CCF change and clinical CT test; and "Uncertain" patients (red), who had either clinically unstable disease. Bottom panel: Contingency table of patient numbers in different CT evaluation and CCF change trends.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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