Mutations in Homologous Recombination Repair Genes in Colorectal Cancer Are Associated With Favorable Response to Immune Checkpoint Inhibitors

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Research Article

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Abstract

Background: A small proportion of patients with metastatic colorectal cancer (CRC) who might respond well to immune checkpoint inhibitors (ICIs) can be identified based on the biomarker of high microsatellite instability. Genes involved in homologous recombination repair (HRR) have been linked to response to such therapy.

Methods: HRR mutations in CRC were explored by analyzing genomic data from patients in The Cancer Genome Atlas (TCGA) (n=377), from a Chinese cohort (n=6106) and from our hospital in China (n=50). The impact of HRR mutations on prognosis of CRC patients was explored in the cohort from TCGA. A cohort of patients treated with ICIs from Memorial Sloan Kettering Cancer Center (MSKCC) (n=110), and two patients from our hospital were studied to characterize the impact of the HRR mutation in prognosis of CRC treated with ICIs.

Results: HRR genes were mutated in all the cohorts examined, with frequencies ranging from 22.44% in our hospital to 52.78% in TCGA. Frequencies of HRR mutations were 43.69-50.47% among patients showing microsatellite stability. HRR mutations were associated with higher tumor mutational burden and high microsatellite instability. HRR mutations correlated with higher neoantigen and increased CD8+ T cell infiltration in the cohort from TCGA. HRR mutations were associated with significantly better overall survival in patients treated with ICIs of MSKCC cohort, particularly among patients with microsatellite stability.

Conclusion: HRR mutations may be associated with stronger response to ICIs in CRC patients with microsatellite-stable, and may be useful for identifying CRC patients likely to benefit from immunotherapy.

Key Message

- The present study explored the landscape of HHR mutations in patients with metastatic CRC in an effort to establish their usefulness as biomarkers to predict response to ICI.
- HRR mutation might reflect an intrinsic pathological property of tumors, which in turn might help define the tumor immune microenvironment and sensitivity to ICI.
- HRR mutations were associated with significantly better overall survival in patients treated with ICIs of MSKCC cohort, particularly among patients with microsatellite stability.

Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death in the world, accounting for approximately 900,000 deaths annually [1–3]. Adjuvant treatments such as chemo- and radiotherapy have improved prognosis for patients with resectable tumors, but 40% of

patients with stage II or III disease suffer recurrence within 5 years after surgery, and 20% of patients with resectable tumors develop metastases. In addition, about a quarter of patients are diagnosed when the disease is already at an advanced stage and has metastasized [4,5].

CRC shows considerable heterogeneity, which affects the disease course, response to treatment and prognosis. About 15-20% of CRC cases involve defective DNA mismatch repair, resulting in microsatellite instability [6–8]. This subtype of CRC generally responds well to immune checkpoint inhibitors (ICI) such as antibodies against cytotoxic T lymphocyte-associated protein 4 and programmed cell death 1 (PD-1) [9,10]. Unfortunately, only about 5% of cases of metastatic CRC display microsatellite instability [11]. Thus, further work is needed to identify additional biomarkers that would help identify which CRC patients are more likely to benefit from ICI.

Some biomarkers have been proposed, but they have not held up well to validation studies. For example, expression of PD-L1 can predict response to ICI for patients with non-small cell lung cancer, but not for patients with metastatic CRC [12–16]. In one phase III clinical study, PD-L1 expression was not associated with overall or progression-free survival of patients with microsatellite-stable metastatic CRC [17]. High tumor mutational burden (TMB) has been associated with response to ICI in several cancers [18], but whether the same holds for metastatic CRC remains controversial. While one study has suggested that TMB can help predict response to durvalumab plus tremelimumab [19], other studies have failed to detect associations between TMB and prognosis after treatment [19,20].

Potentially more promising biomarkers of ICI response among patients with metastatic CRC are mutations in genes involved in homologous recombination repair (HRR), including the POLE gene encoding DNA polymerase ε, the POLD1 gene encoding DNA polymerase δ1 (POLD1), and the genes encoding BRCA 1 and 2 [21]. For example, POLE mutations contribute substantially to TMB in metastatic CRC [22], even leading to a ‘hypermutator’ phenotype with TMB >100 mutations per Mb [23], which in turn can affect response to ICI [24]. A study of nearly 48,000 CRC patients found POLE and POLD1 mutations to predict response to ICI treatment [25]. HRR mutations can even interact with mutations in genes involved in DNA damage repair, which may help explain how HRR mutations are associated with treatment response [26].

The present study explored the landscape of HHR mutations in patients with metastatic CRC in an effort to establish their usefulness as biomarkers to predict response to ICI.

Material And Methods

Clinical cohorts and study design

We downloaded genomic, transcriptomic and clinical data from The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/) and UCSC XENA (https://xenabrowser.net/). The resulting cohort of 377

patients is henceforth referred to as the “TCGA cohort”. We also included genomic data from 6106 Chinese CRC patients that had been collected by 3D Medicines (Shanghai, China) (henceforth “CN cohort”) and 50 Chinese CRC patients from our own hospital (henceforth “HL cohort”). In these cohorts, we compared patients with HRR mutations and those without mutations in terms of TMB, neoantigen levels and levels of tumor-infiltrating lymphocytes. We also compared the TCGA cohort with the CN and HL cohorts in order to explore ethnic differences in HRR mutations.

To explore the prognostic value of HRR mutations, we extracted targeted sequencing data and clinical characteristics of 110 patients (n=110) who received ICI at the Memorial Sloan Kettering Cancer Center (henceforth “MSKCC cohort”) [27]. We compared survival between patients with or without HRR mutations.

Collection and analysis of data on the HL cohort were approved by the Ethics Committee of Guangxi Medical University Cancer Hospital. The requirement for informed consent was waived because patients, at the time of treatment, consented for their anonymized medical data to be analyzed and published for research purposes. Collection and analysis of the CN cohort were approved by 3D Medicines, and the data were transferred from 3D Medicines to us under a specific agreement to preserve patient anonymity.

Next-generation sequencing

Libraries generated from CN and HL cohorts were loaded onto a NovaSeq 6000 platform (Illumina, California, USA) for 100 bp paired-end sequencing to a mean sequencing depth of 1000X. Raw data from paired samples of tumors and normal tissue were mapped to the reference human genome hg19 using the Burrows-Wheeler Aligner (version 0.7.12) [28]. PCR duplicate reads were removed using Picard (version 1.130), and sequence metrics were collected using SAMtools (version 1.1.19). Variants were called only in the targeted regions. Somatic single nucleotide variants (SNVs) were detected using an R package developed in-house, which detects variants based on a binomial test. Local realignment was performed to detect insertions and deletions (indels). Variants were then filtered by their unique supporting read depth, strand bias, and base quality as described [29]. All variants were then filtered using an automated false-positive filtering pipeline to ensure sensitivity and specificity at an allele frequency of ≥ 5%. Single nucleotide polymorphisms (SNPs) and indels were annotated using ANNOVAR against the following databases: dbSNP (version 138), 1000Genomes and ESP6500 (population frequency > 0.015). Only missense, stopgain, frameshift and non-frameshift indel mutations were kept. Copy number variations (CNVs) and gene rearrangements were detected as described [29].

Definition of HRR mutations, TMB and high microsatellite instability

HRR mutations were defined based on the known pathway [30] as any of the following mutations in 66 genes (Supplemental Table 1): TRUNC, including frameshift deletions, frameshift insertions, nonsense, nonstop, splice region, or splice site mutations; INFRAME, including inframe indels; or MISSENSE mutations. Patients were divided into those with at least one mutation in at least one HRR gene (HRRmut) or those with entirely wild-type HRR genes (HRRwt).

TMB was defined as the number of non-synonymous somatic SNVs and indels per megabase in the coding regions examined, excluding driver mutations. All SNVs and indels in the coding region of targeted genes were considered, including missense, silent, stop gain, stop loss, in-frame, and frameshift mutations. Microsatellite instability was assessed at 100 microsatellite loci, and the top 30 loci with the best coverage were used to calculate a microsatellite instability score for each assay. The distribution of reading counts across various repeat lengths was evaluated at each microsatellite locus using an R package developed in-house. Samples scoring at least 0.4 for microsatellite instability were considered to show high instability; otherwise, they were considered to show stability.

Statistical analysis

Data were analyzed using R (version 3.6.0). Survival was analyzed using Kaplan-Meier curves, which were compared to one another using a log-rank test. Associations between genomic determinants were assessed using the chi-squared test or Fisher's exact test. Inter-group differences in normally distributed data were assessed for significance using Student's *t* test; otherwise, the Mann-Whitney U test was used. Differences associated with $P < 0.05$ were considered statistically significant.

Results

Landscape of HRR mutations in the TCGA, CN and HL cohorts

Various characteristics between HRRmut and HRRwt subgroups were compared across the TCGA, CN, and HL cohorts (Supplementary Table 2). Mutations in five genes were identified in the TCGA-HRRmut subgroup (Figure 1A): APC, at a frequency of 72.36%; TIN, 71.36%; TP53, 56.78%; SYNE1, 44.22%; and CBSCN, 43.22%. The overall frequency of HRR mutations in the TCGA cohort was 52.78%. The two Chinese cohorts showed similar frequencies of HRR mutations in the genes (Figure 1B-C) TP53 (CN, 78.93%; HL, 86.36%), APC (67.77%, 86.36%), and KRAS (47.04%, 68.18%). The two cohorts also showed similar overall frequencies of HRR mutations (34.03%, 22.44%), which were lower than in the TCGA cohort. The types of mutations in the most frequently mutated genes differed across the three cohorts (Figure 1D). For example, POLD1 mutations in the TCGA-HRRmut subgroup were multi_hit, synonymous, missense_mutation, and frame_shift_del, whereas more than half of POLD1 mutations in the CN-HRRmut subgroup occurred in the 5'-untranslated region of the gene. Truncating SNVs and frameshifts in POLD1 are associated with underexpression of functional protein, which may contribute to high microsatellite instability in CRC [31].

In the TCGA-HRRmut subgroup, the most frequently mutated HRR gene was BRCA2 (18.09%), followed by SLX4 (17.09%), HFM1 (16.08%), POLQ (15.58%), POLD1 (11.56%) and TP53BP1 (11.56%) (Figure 2A). In contrast, the most frequently mutated HRR genes in the CN-HRRmut subgroup were POLD1 (44.73%), BRCA2 (35.93%), BRCA1 (33.96%), BLM (32.28%) and BRIP1 (29.92%) (Figure 2B). Similarly, POLD1 and BRCA2 were the most frequently mutated HRR genes in the HL cohort, but their mutation frequencies

were lower (18-23%) (Figure 2C). Interestingly, the HL-HRRmut and TCGA-HRRmut subgroups showed similar mutation frequencies for several genes (Figure 2D): BRCA2 (18.18% vs 18.09%), SLX4 (18.18% vs 17.09%), PPP4R4 (9.09% vs 9.05%), and PALB2 (9.09% vs 6.03%).

Relationships between HRR mutations and TMB or microsatellite instability

In all three cohorts, HRR mutations were significantly more frequent among patients showing microsatellite instability than among those showing stability (Figure 3). Nevertheless, 43.69-50.47% of patients with microsatellite stability in the TCGA and CN cohorts had HRR mutations. This suggests that CRC patients with microsatellite stability may also benefit from ICI.

Mutation frequencies in some individual genes differed substantially even within each subgroup with microsatellite instability or stability. For example, POLD1 was mutated among patients with microsatellite stability much more often in the CN cohort (16%) than in the TCGA cohort (2%). In contrast, it was mutated similarly often among patients with microsatellite instability in the two cohorts (47% vs 39%).

In all three cohorts, the HRRmut subgroup showed much greater TMB than the HRRwt subgroup (Figure 3A-C). This suggests that HRR mutation might reflect an intrinsic pathological property of tumors, which in turn might help define the tumor immune microenvironment and sensitivity to ICI.

HRR mutations are associated with CRC tumor immune microenvironment but not prognosis

Given our results suggesting a link between HRR mutations and TMB in CRC, we examined whether HRR mutations might be associated with prognosis. Surprisingly, HRR mutation did not significantly influence overall survival in the TCGA cohort (Figure 4A), regardless of whether patients showed microsatellite instability or stability (Figure 4B-C).

In contrast, HRR mutation was significantly associated with higher neoantigen load in the TCGA cohort (Figure 4D). Among patients with microsatellite stability, CD8+ T cells were significantly more abundant in HRRmut tumors than in HRRwt tumors (Figure 4E). Given that high tumor infiltration by CD8+ T cells can sensitize to ICI [32–35], our results implied that HRR mutations might predict response to ICI among patients with metastatic CRC.

HRR mutations can predict response to ICI in CRC patients

To test this possibility, we compared post-ICI survival between HRRmut or HRRwt patients in the MSKCC cohort. HRR mutation was associated with significantly better overall survival in the entire cohort (HR 0.28, 95%CI 0.14-0.59, P = 0.0004; Figure 5A), as well as specifically in the subgroup of patients with microsatellite stability (HR 0.31, 95% CI 0.09-1.01, P = 0.0407; Figure 5B). In contrast, TMB was not significantly associated with overall survival (Figure 5C).

We recently treated at our hospital two patients with metastatic CRC whose different disease courses provide additional, if anecdotal, support to the idea that HRR mutations can predict response to ICI. Patient A was a 71-year-old Chinese man with a confirmed diagnosis of stage IV colorectal cancer with microsatellite stability as well as multiple unretractable pulmonary and hepatic metastases. His disease progressed continuously while on standard chemotherapy. Patient B, a 47-year-old Chinese man with stage IV colorectal cancer with microsatellite stability, also failed to respond to standard chemotherapy. Both patients had an Eastern Cooperative Oncology Group (ECOG) score of 1 and reported no personal or family history of malignancy, heart disease, diabetes, hereditary disease or contagious disease.

Patient A received five cycles of sintilimab combined with anlotinib, and computed tomography revealed significant shrinkage of the hepatic metastases as well as stable metastases in the lung and celiac lymph node for at least 15 months (Figure 6A). Patient B, in contrast, did not respond to 2month treatment with sintilimab and regorafenib (Figure 6B). Next-generation sequencing revealed that patient A had a mutation in the HRR gene PARP1 (c.1895C>T at 29.8%), while no HRR mutations were detected in patient B (Supplementary Table 3). The serum levels of two patients are shown in Supplementary Table 4. These two cases justify larger studies to explore HRR mutations for predicting CRC patient response to ICI.

Discussion

High microsatellite instability in CRC tumors is already used to screen patients to identify those more likely to benefit from ICI, but this phenotype is found in only about 5% of patients with metastatic CRC. This highlights the need to identify additional markers that might predict good response to immunotherapy. CRC tumors that show microsatellite stability but have a hypermutation phenotype, often involving POLE mutations, tend to respond strongly to ICI, which has been attributed to strong tumor infiltration by immune cells and high neoantigen burden [36]. Tumors with microsatellite stability and high TMB may also respond well [22]. The present results suggest that HRR mutations may be useful for predicting response, which could improve the screening of CRC patients for ICI.

More than 70% of CRC cases appear to develop first through mutation in APC, followed by mutations in KRAS, PIK3CA, SMAD4 and TP53, as well as loss of heterozygosity of chromosome 18. Such tumors usually show high CIN expression, microsatellite stability, and negligible CpG island methylation. Consistent with this pathway, APC, KRAS and TP53 were very often mutated in our TCGA, CN and HL cohorts (Figure 1), implying that these mutations co-occurred with HRR mutations. HRR mutations may result in chromosomal instability through deletions, frameshifts, aneuploidy and chromosome aberrations [37,38]. Research on other cancers has shown that HRR mutations in the same genes as in our cohorts, such as BRCA2 and POLD1 (Figure 2), can influence response to immunotherapy. In patients with hereditary breast and ovarian cancer [39], BRCA2 mutations may be associated with stronger response to immunotherapy in clinical trials [40,41]. Mutations in POLD1 or POLE have been linked to stronger response to ICI in endometrial and non-small cell lung cancers [42,43]. Consistent with our findings,

mutations in POLD1 or POLE may be useful for identifying patients with CRC who respond to ICI even though they lack high microsatellite instability [44]. We found significantly longer survival among HRRmut patients than HRRwt patients after ICI therapy (Figure 4), supporting the idea that HRR mutations can recognize CRC patients with microsatellite stability who may respond well to ICI.

HRR mutations may influence response to ICI via their association with TMB: such mutations were associated with higher TMB in all our cohorts, regardless of whether tumors showed microsatellite instability or stability (Figure 3). The higher TMB may translate to presentation of more altered proteins as neoantigens, which induce antitumoral immune responses. In other words, the higher TMB may primarily alter the tumor immune microenvironment, which might explain why neither TMB nor HRR mutation was associated with overall survival (Figure 3B-C). Indeed, HRRmut tumors contained greater neoantigen load and more abundant $CD8^+$ T cells than HRRwt tumors (Figure 5). In patients with early-stage CRC, increased tumor infiltration by CD8+ PD-1^+ T cell is associated with better response to ICI [45]. Interestingly, microsatellite instability in CRC is associated with greater tumor infiltration by CD68+ macrophages, CD8+ cytotoxic lymphocytes and CD45+ RO^+ T memory cells, which may help explain the better survival of these patients than those with microsatellite stability [46]. These considerations imply that the tumor immune microenvironment may strongly influence response to immunotherapy, and our findings indicate that HRR mutations can influence that microenvironment.

In anecdotal support of our proposal that HRR mutations can help predict response to ICI, we describe here two patients at our hospital with metastatic CRC, one of whom had HRR mutations and the other did not, and who responded quite differently to immunotherapy (Figure 6). Our findings justify systematic studies, preferably with larger samples from multiple centers, to validate and optimize HRR mutations as predictors of response to immunotherapy in patients with metastatic CRC.

Declarations

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Conflict of interest disclosures

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statements

The datasets analysed during the current study are available in the The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/) and UCSC XENA (https://xenabrowser.net/) repository.

Author contributions

Yan Lin, Shanshan Luo, Rong Liang and Yongqiang Li designed and coordinated the study, and prepared the manuscript. Min Luo, Yumei Zhang, Xuerou Lu, Qian Li provided assistance in the design of the study and participated in manuscript preparation. Mingzhi Xie, Yu Huang and Xiaoli Liao participated in data gathering. All authors have read and approved the content of the manuscript.

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Figures

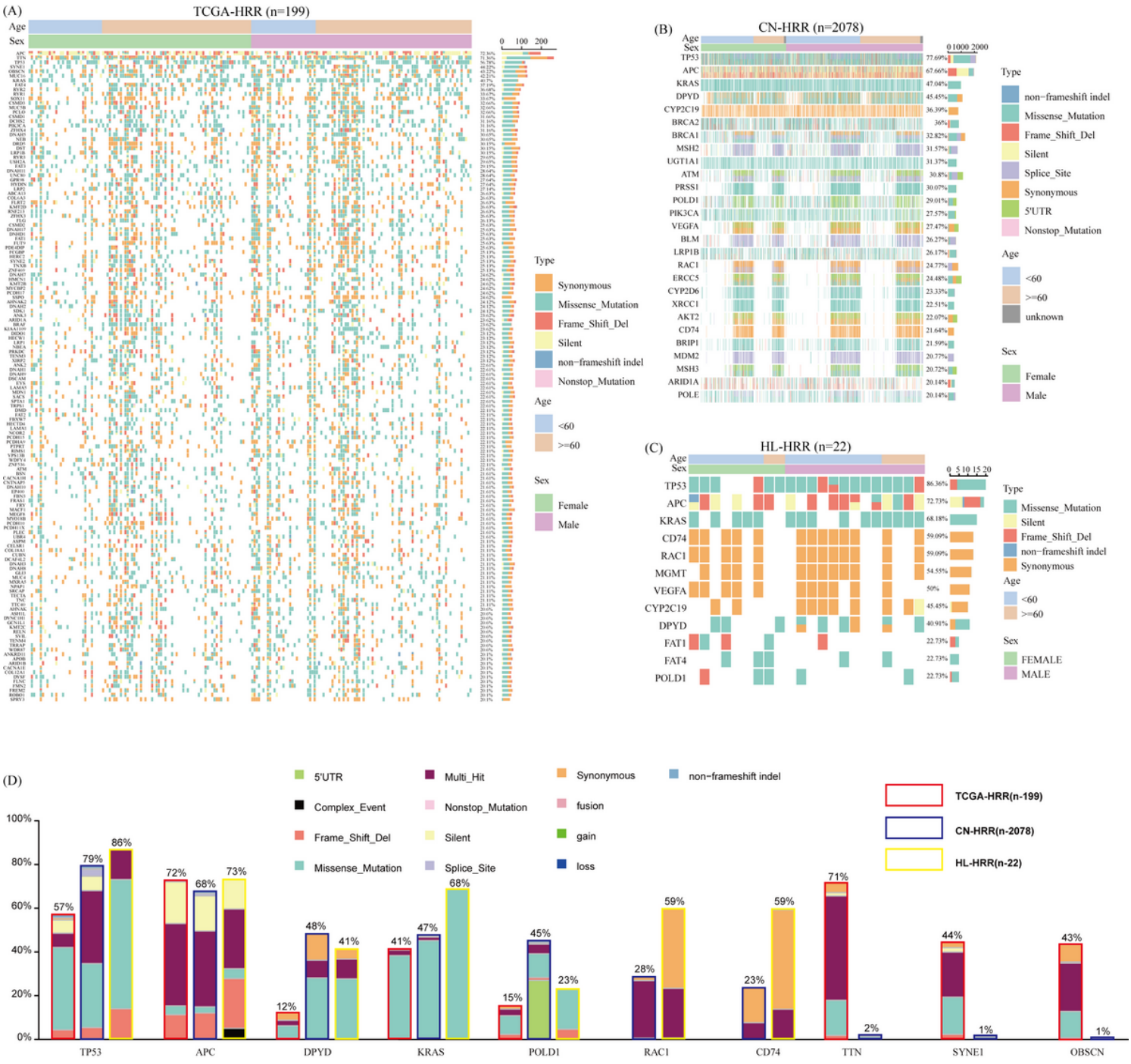

Figure 1

Genetic profiling of colorectal cancer patients with HRR mutations, including (A) TCGA CRC cohort, (B) CN cohort, and (C) HL cohort. (D) Mutation characteristics of high frequency mutated genes were compared in the three groups.

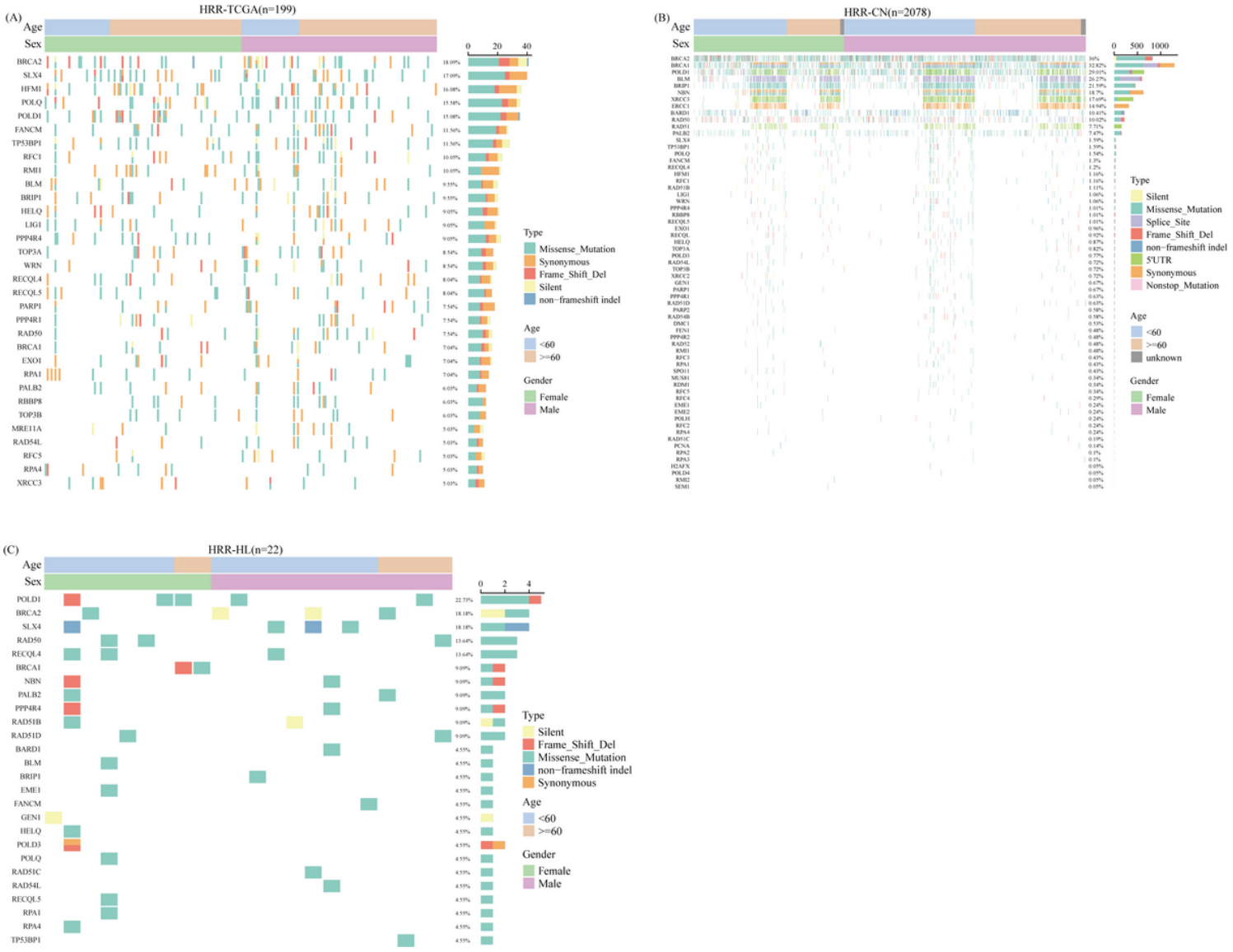

Figure 2

HRR genetic profiling of colorectal cancer patients with HRR mutations, including (A) TCGA CRC cohort, (B) CN cohort, and (C) HL cohort.

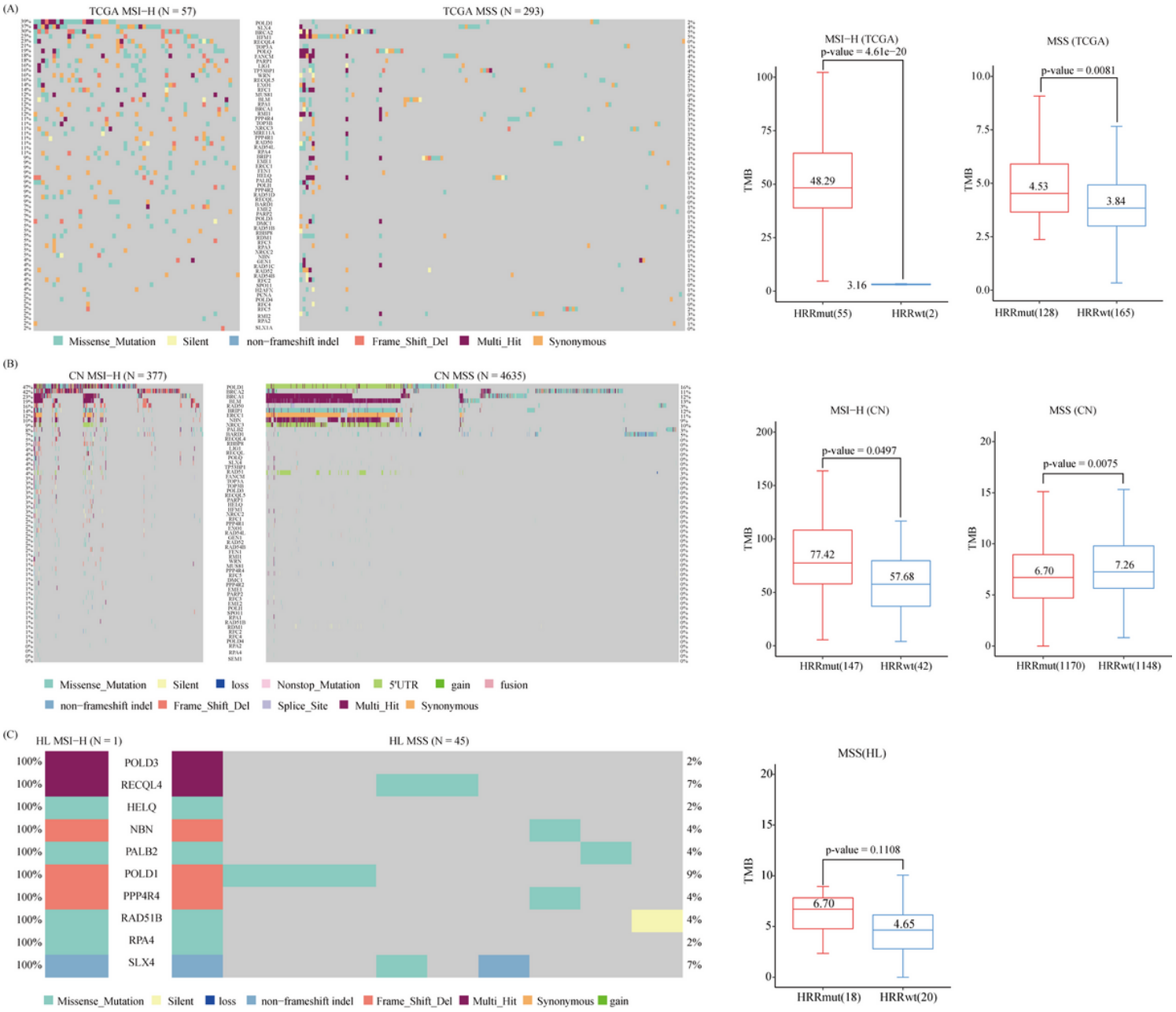

Figure 3

(A) HRR genetic profiling for MSI-H and MSS group in TCGA CRC cohort; TMB level of MSI-H and MSS for HRRmut and HRRwt group in TCGA patients. (B) HRR genetic profiling for MSI-H and MSS group in CN cohort; TMB level of MSI-H and MSS for HRRmut and HRRwt group in CN cohort. (C) HRR genetic profiling for MSI-H and MSS group in HL cohort; TMB level of MSS for HRRmut and HRRwt group in HL cohort.

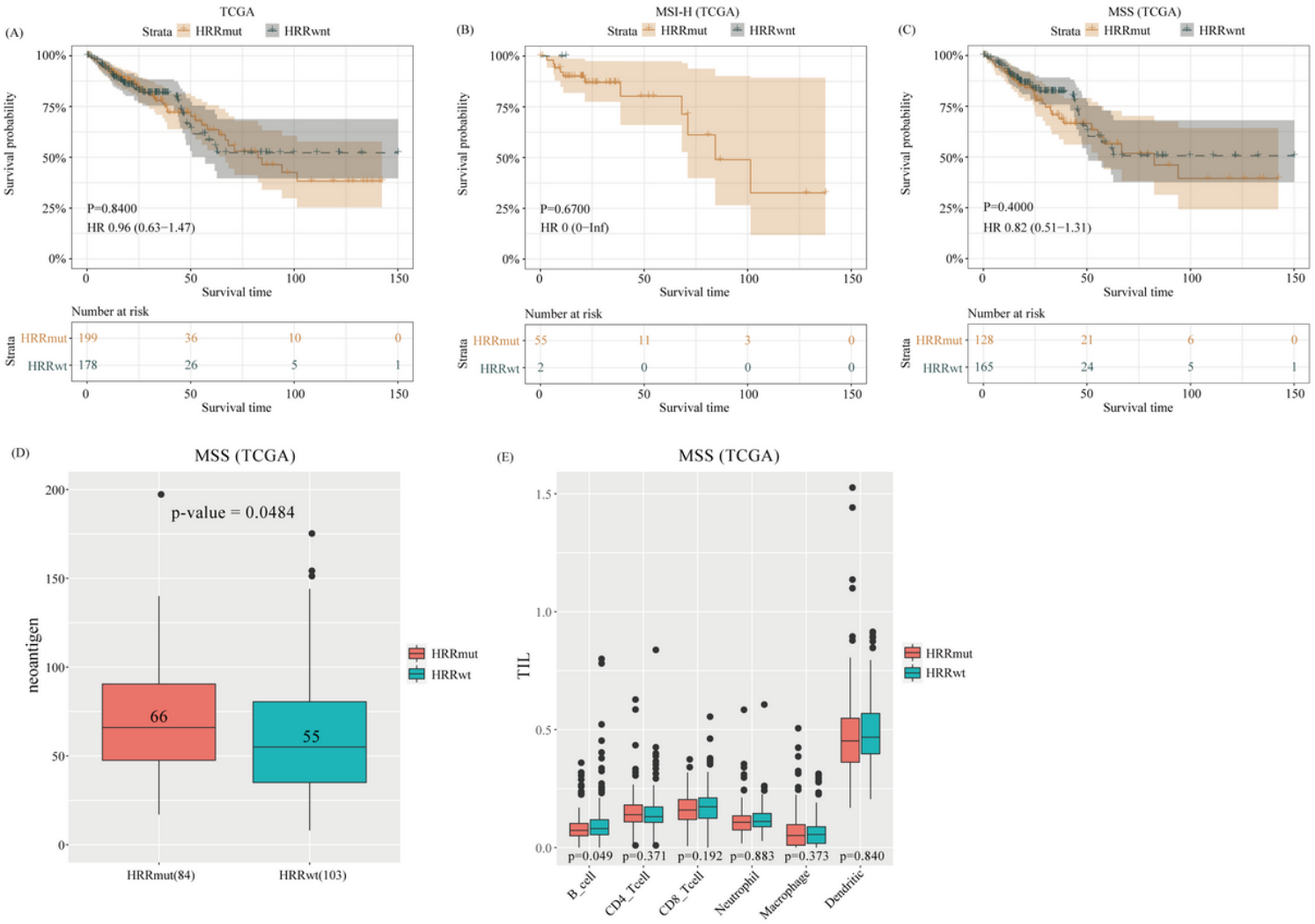

Figure 4

Kaplan-Meier curves of overall survival in the (A) total, (B, C) MSI-H and MSS patients of TCGA for HRRmut and HRRwt groups. (D) Neoantigen level in MSS patients of TCGA for HRRmut and HRRwt groups. (E) Immune cell infiltration level in MSS patients of TCGA for HRRmut and HRRwt groups.

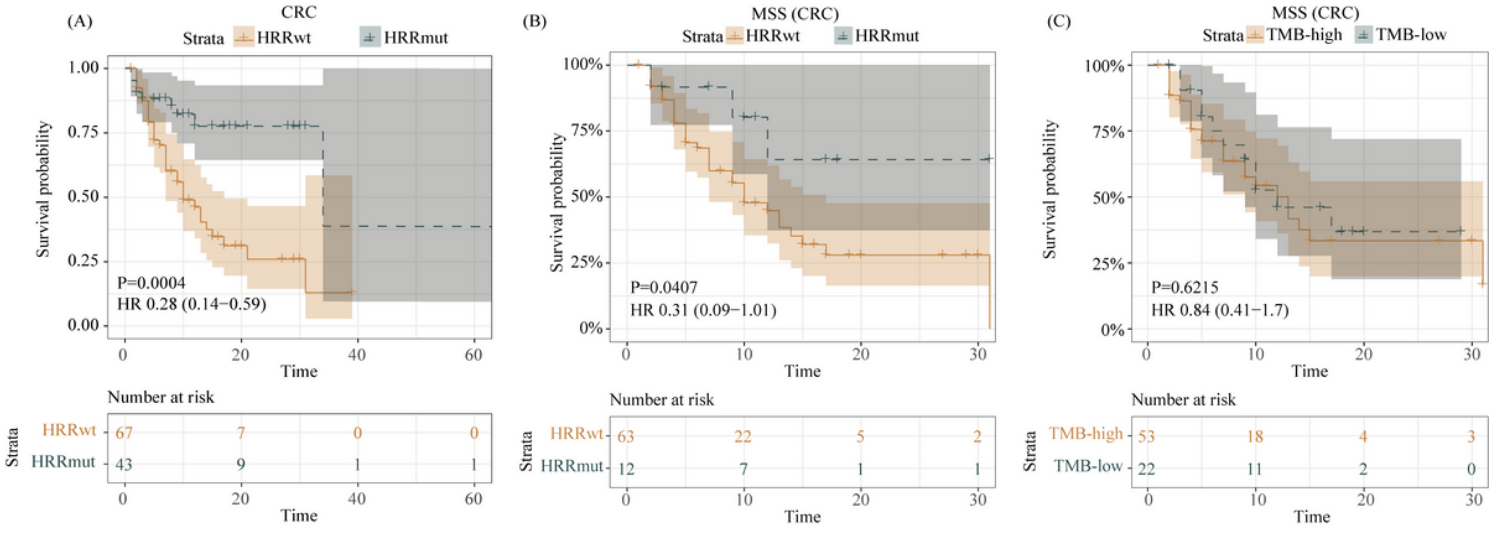

Figure 5

Kaplan-Meier curves of overall survival in the MSKCC CRC immunotherapy cohort for HRRmut and HRRwt groups, (A) total CRC population (MSS+MSI-H), (B) MSS CRC. (C) Kaplan-Meier curves of overall survival in the MSKCC CRC immunotherapy cohort for TMB-high and TMB-low.

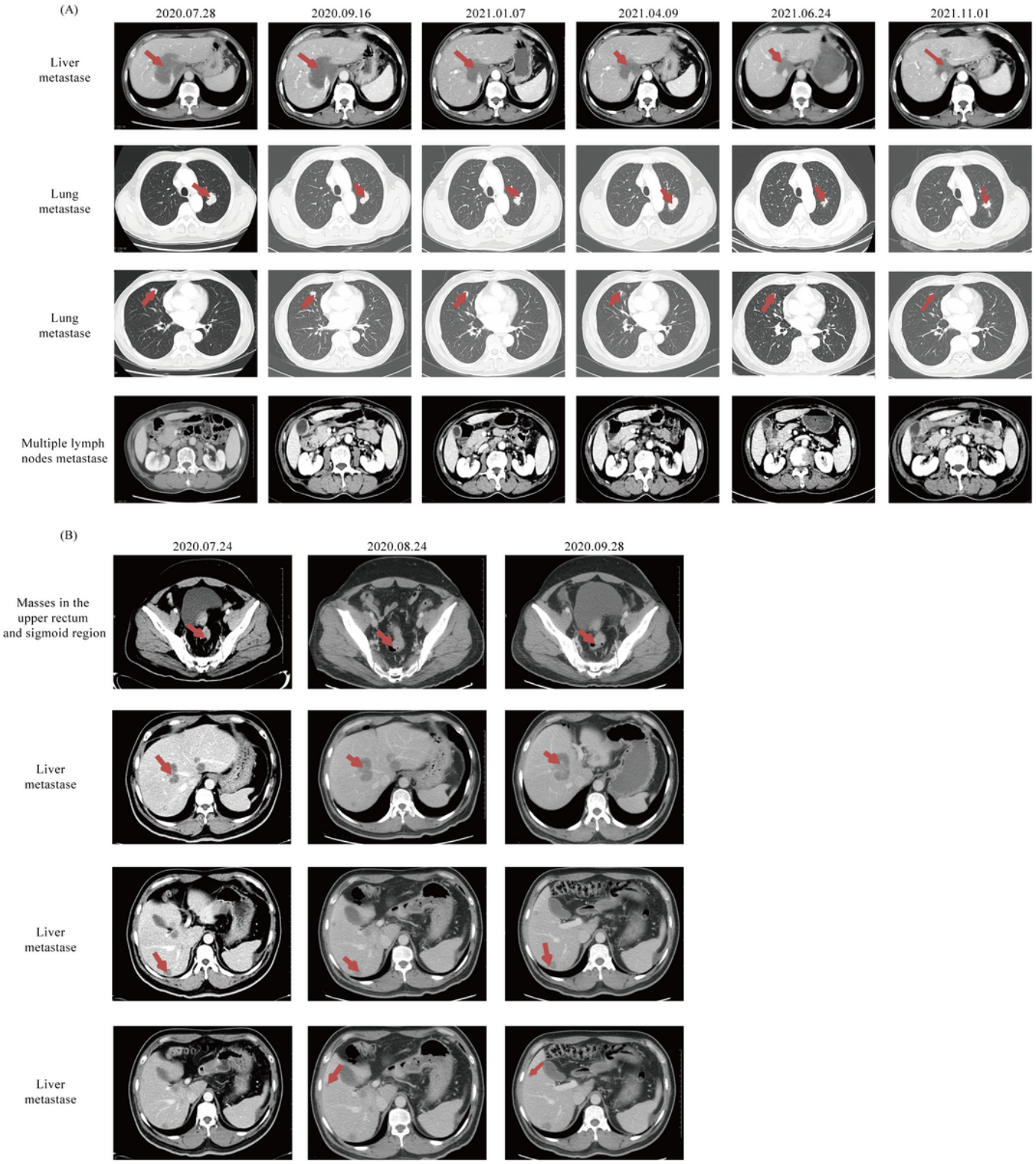

Figure 6

Radiological features of the patient before and after therapy. (A)The computed tomography scans of the patient before immunotherapy (2020.07.28), after approximately 2 months (2020.09.16), after approximately 6 months (2021.01.07), after approximately 9 months (2021.04.09), after approximately 11 months (2021.06.24), and after approximately 15 months (2021.11.01) of treatment with sintilimab combined with anlotinib. (B)The computed tomography scans of the patient before immunotherapy (2020.07.24), after 1 months (2020.08.24), and after 2 months (2020.09.28) of treatment with sintilimab combined with regorafenib.

Supplementary Files

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

- SupplementaryTable1.docx
- SupplementaryTable2.docx
- SupplementaryTable3.docx
- SupplementaryTable4.docx