Article

*Discovery Medicine* 2024; 36(186): 1477–1485
https://doi.org/10.24976/Discov.Med.202436186.137

# Genomic Alterations and Clinical Characterization in Chinese Patients with Metastatic Colorectal Cancer

Xu-hui Zhang[1,†], Jie-qiong Zhou[2,†], Qing Wei[3], Jian Li[1], Ting Xu[1], Chun-mei Bai[4], Jian-feng Zhou[4,*], Xi-cheng Wang[1,*]

[1]Department of Gastrointestinal Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Peking University Cancer Hospital and Institute, 100142 Beijing, China
[2]Department of Gastroenterology, The Second Affiliated Hospital of Guangzhou Medical University, 510260 Guangzhou, Guangdong, China
[3]Department of Medical Oncology, The Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Institute of Basic Medicine and Cancer (IBMC), Chinese Academy of Sciences, 310005 Hangzhou, Zhejiang, China
[4]Department of Medical Oncology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 100730 Beijing, China
*Correspondence: ZhouJF@pumch.cn (Jian-feng Zhou); xicheng_wang@hotmail.com (Xi-cheng Wang)
†These authors contributed equally.
Published: 20 July 2024

**Background: Metastatic colorectal cancer (mCRC) is increasingly characterized by myriad genomic alterations beyond the well-known factors such as *RAS*, *BRAF*, and microsatellite instability (MSI). Novel genomic changes, including *ERBB2* amplifications, mutations, and gene fusions, are now recognized as potential targets for precision therapy. This study aims to explore the genomic landscape of a Chinese cohort with mCRC to identify potentially targetable genetic alterations for personalized treatment strategies.**

**Methods: A total of 500 mCRC patients in China were enrolled, based on which genomic profiling was performed using capture-based targeted sequencing across a panel of 520 genes on tumor tissues to identify prevalent genomic alterations. The mutations were analyzed by optimized proprietary algorithms. MSI and mismatch repair deficiency status were analyzed using the read-count-distribution approach. Besides, the overall survival (OS) related to these molecular changes was estimated.**

**Results: The cohort's genomic profiling revealed *TP53* mutations in 78%, *APC* in 60%, and *KRAS* in 47% of the patients. MSI-High status was confirmed in 5.8% of cases via a next-generation sequencing (NGS)-based algorithm. *ERBB2*/*HER2* amplifications were found in 12% (60/500) of patients, with potential therapeutic implications for those without concurrent *KRAS* mutations. A subset of patients (1.2%; 6/500) showed fusions and DNA damage response (DDR) gene mutations (except *TP53*) that could be targeted therapeutically. The *KRAS* (G12C) variant was detected in 14 patients (2.8%), and 61 (12.2%) had a *BRAF V600E* mutation. Notably, survival analysis showed no significant differences in OS between *KRAS* mutant loci and *NRAS* mutations ($p = 0.436$). However, *BRAF V600E* mutations were associated with a poorer prognosis than *BRAF* wild-type and non-*V600E* mutations (16.3 months vs. 29.5 and 31.1 months, respectively; $p < 0.001$).**

**Conclusions: This study validates the feasibility of using NGS to detect prognostic and therapeutically actionable genetic variants in Chinese mCRC patients, contributing to understanding the genomic variation within this population and highlighting the potential for personalized medicine in managing mCRC.**

**Keywords:** metastatic colorectal cancer; next-generation sequencing; genomic alternation; prognosis

## Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors in China. Although the burden of CRC has recently decreased in some European and northern American countries, incidence and mortality rates have increased in China [1]. Chemotherapy remains the standard first-line treatment for metastatic CRC (mCRC) and can be improved with monoclonal antibodies targeting epidermal growth factor receptor (EGFR) or vascular endothelial growth factor (VEGF) [2]. The pathogenesis of CRC is complex and influenced by multiple factors, including an individual's genetic background and dietary factors. Chinese patients have different eating habits and hereditary characteristics than Western patients [3].

In recent years, testing genes of specific types of cancer has become a standard practice in medical oncology because somatic mutations, gene expression changes, and epigenetic modifications mark cancer. Next-generation sequencing (NGS) technologies have provided potential therapeutic targets and have become the standard technique for genetic diagnostics and research [4]. Microsatellite insta-

bility (MSI) and mismatch repair deficiency (dMMR) further support the notion that precision therapy can significantly prolong the survival of patients with specific genomic alterations [5]. Results from Keynote-177 showed that Pembrolizumab significantly improved the prognosis of patients with MSI-High/dMMR mCRC compared to first-line chemotherapy combined with targeted therapy [6,7].

As a member of the ERBB family of receptor tyrosine kinases, the EGFR plays a central role in signal transduction pathways controlling various cellular processes. Activation of EGFR leads to increased signaling through the *RAS-RAF* and *PIK3CA* pathways, which are required for proliferation and invasion [8]. *KRAS*, *NRAS*, and *BRAF* (*RAS/BRAF*) gene mutations have been demonstrated as essential predictive biomarkers for patients' response to monoclonal antibodies that target EGFR [9]. Lately, there has been surprising progress in *KRAS* (G12C) inhibitors such as *AMG510* (sotorasib) and *MRTX849* (adagrasib), which have demonstrated encouraging efficacy in clinical trials [10]. These drugs may have the ability to abolish the activity of all mutant *RAS* isoforms. In addition, with the development of anti-*HER2* treatments, such as combined targeted therapy and antibody-conjugated drugs (ADC), *ERBB2* and *ERBB3* should also be examined for improving the treatment of CRC [11,12].

Dysregulation of *MET* signaling can lead to clinically relevant oncogenesis in several cancer types [13]. Identifying tumors that are genomically addicted to *MET* is essential to the clinical development of related drugs for these tumors. There is increasing evidence that mutations in the DNA damage response (DDR) pathway are promising targets for cancer [14]. Although preclinical studies have identified DDR alterations as potential targets in mCRCs, systematic and comprehensive testing has lagged, and clinical development has raised doubts [15]. In most types of cancers, gene fusions occur at a low frequency. Genomic translocations that activate receptor tyrosine kinases (RTKs) play a critical role in tumorigenesis across a wide range of malignancies, including CRC, and may be novel targets for therapeutic intervention [16,17].

In this study, we aimed to explore the genomic landscape of mCRC in a Chinese clinical cohort and identify potentially targetable genetic alterations that could inform personalized treatment strategies, focusing on genomic changes beyond well-known factors such as *RAS*, *BRAF*, and MSI. We present this article in accordance with the STROBE reporting checklist.

## Methods

### *Patients and Sample Collection*

This retrospective study involved a retrospective clinical analysis of patients treated at the Peking University Cancer Hospital and the Peking Union Medical College Hospital from January 2013 to December 2020. The patients included in this study were those with confirmed mCRC, determined through routine clinical practices such as histopathological and radiological examinations. The inclusion criteria were (1) underwent curative surgery for mCRC, (2) with CRC tissues collected for pathological examination, (3) complete clinical data. The exclusion criteria were (1) combination of other malignant tumors, (2) received preoperative adjuvant radiotherapy or chemotherapy, (3) suffered from other intestinal diseases such as inflammatory bowel disease. Archived formalin-fixed, paraffin-embedded (FFPE) tumor tissues were collected from 500 patients. Clinical information and follow-up data were retrieved using electronic medical records, charts, and telephone interviews. The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of Peking University Cancer Hospital (2022KT133). Written informed consent was obtained from all patients.

### *Sequencing Panel*

A panel of 520 cancer-related genes was sequenced in cancer tissues using NGS, comprising 1.64 megabases of the human genome (OncoScreen Plus, Burning Rock Biotech, Guangzhou, China).

### *DNA Extraction, Mutation Calling*

As recommended by the manufacturer, genomic DNA was extracted from FFPE samples using the QIAamp DNA FFPE Tissue Kit (Cat. #56404, Qiagen, Carlsbad, CA, USA). To analyze the sequencing data, proprietary algorithms optimized to detect somatic and germline alterations and distinguish artifacts from true mutations were developed.

### *Assessment of MSI*

MSI and dMMR status were analyzed using the read-count-distribution approach. The tumor sample was classified as MSI-High (MSI-H) if over 40% of the marker loci were length-unstable, microsatellite stable (MSS) if the percentage of length-unstable loci was $<$15%, or MSI-Low (MSI-L) if the percentage was between 15% and 40%.

### *Comparative Genomic Analysis*

To contextualize our findings within the global landscape of mCRC, we compared the genomic profiles of our cohort with those from established databases. Specifically, we utilized publicly available data from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/TCGA) and the Memorial Sloan Kettering Cancer Center (MSKCC) cohorts (https://www.cbioportal.org/study/summary?id=msk_impact_2017), which provide comprehensive genomic profiling data on mCRC patients accessed through their respective public portals. Our analysis in-

**Table 1. Risk factors associated with prognosis of Chinese patients with metastatic CRC by non-parametric Cox regression analysis.**

| Variables | Numbers | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| | | HR (95% CI) | *p* | HR (95% CI) | *p* |
| Age | | | | | |
| ≥50 | 263 (52.6%) | | | | |
| <50 | 237 (47.4%) | 0.983 (0.778–1.242) | 0.885 | | |
| Gender | | | | | |
| Male | 290 (58.0%) | | | | |
| Female | 210 (42.0%) | 1.313 (1.039–1.659) | 0.02 | 1.252 (0.990–1.585) | 0.061 |
| Hereditary history | | | | | |
| Negative | 377 (75.4%) | | | | |
| Positive | 123 (24.6%) | 0.909 (0.696–1.186) | 0.481 | | |
| Histology | | | | | |
| Adenocarcinoma | 444 (88.8%) | | | | |
| SRCC | 4 (0.8%) | 2.421 (0. 774–7.575) | 0.129 | | |
| Mucinous | 52 (10.4%) | 1.044 (0.722–1.510) | 0.82 | | |
| Differentiation | | | | | |
| High | 19 (3.8%) | | | | |
| Moderate | 353 (70.6%) | 0.904 (0.477–1.711) | 0.756 | | |
| Low | 116 (23.2%) | 1.512 (0.783–2.918) | 0.218 | | |
| Unknown | 12 (2.4%) | | | | |
| Peritoneal metastasis | | | | | |
| Negative | 364 (72.8%) | | | | |
| Positive | 136 (27.2%) | 1.720 (1.338–2.211) | <0.001 | 1.413 (1.087–1.836) | 0.01 |
| Liver metastasis | | | | | |
| Negative | 215 (43.0%) | | | | |
| Positive | 285 (57.0%) | 0.836 (0.662–1.056) | 0.133 | | |
| Lung metastasis | | | | | |
| Negative | 362 (72.4%) | | | | |
| Positive | 138 (27.6%) | 0.810 (0.625–1.051) | 0.112 | | |
| Location | | | | | |
| Right | 173 (34.6%) | | | | |
| Left | 196 (39.2%) | 0.405 (0.305–0.538) | <0.001 | 0.439 (0.328–0.586) | <0.001 |
| Rectum | 131 (26.2%) | 0.334 (0.241–0.462) | <0.001 | 0.379 (0.270–0.533) | <0.001 |

Abbreviations: CRC, colorectal cancer; SRCC, signet-ring-cell carcinoma; HR, hazard ratio.

volved extracting the frequency of mutations in genes commonly altered in CRC, such as *TP53*, *APC*, and *KRAS*, from the TCGA and MSKCC datasets to evaluate the distinctiveness of the genomic alterations in our Chinese cohort relative to international datasets, facilitating a broader understanding of the molecular underpinnings of mCRC across different populations.

### *Statistical Analysis*

The SPSS 26.0 (SPSS Inc, Chicago, IL, USA) software was used for data analysis. The patients' demographical and clinicopathological characteristics and family history were analyzed using descriptive statistics. Categorical data were analyzed using Fisher's exact tests and Chi-square tests. Risk factors associated with prognosis were analyzed by non-parametric Cox regression analysis. Kaplan-Meier was used to estimate and plot overall survival, and log-rank tests were used to assess significance levels. A $p$ value $< 0.05$ was considered statistically significant.

## Results

### *Characteristics of mCRC Patients*

Of the 500 mCRC patients, 290 were men, and 210 were women (median age, 58 years). Signet-ring cell cancer was rare, with a prevalence of 0.8%. Most of the patients (70.6%) had moderately differentiated tumors, and 123 (24.6%) patients had a positive family history. Distribution of tumor locations indicated that 39.2% of patients had left-sided colon cancer, 26.2% had rectal cancer, and the remaining 34.6% had right-sided colon cancer. Based on our findings, liver metastases were the most prevalent,

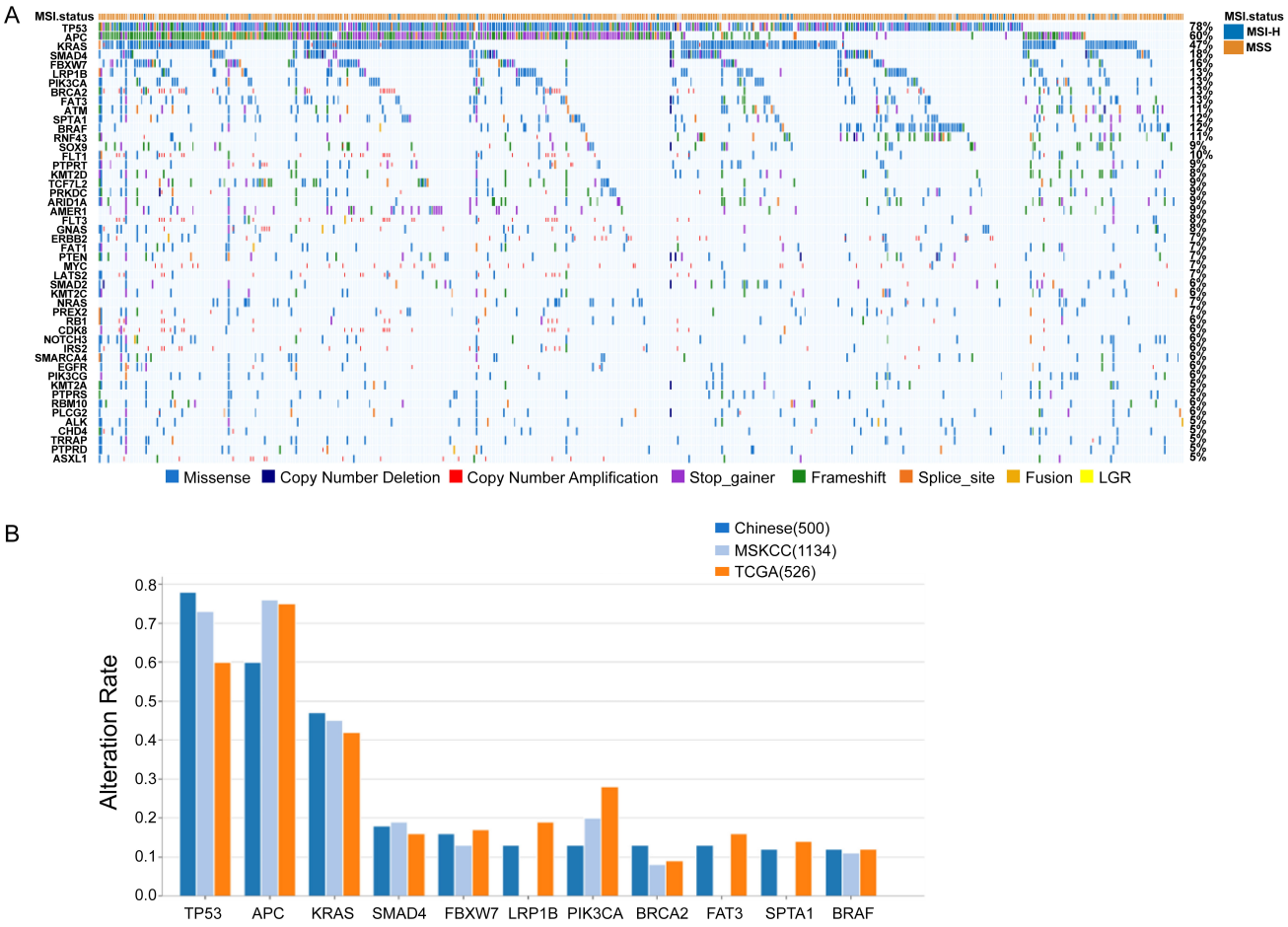



**Fig. 1. Overview of genomic alterations in metastatic colorectal cancer (mCRC).** (A) A comprehensive mutational profile for the overall cohort of 500 Chinese mCRC patients. (B) Comparative alteration rates in key CRC-related genes. This bar graph illustrates the alteration rates of critical CRC-related genes within the Chinese cohort compared to data extracted from the Memorial Sloan Kettering Cancer Center (MSKCC) and The Cancer Genome Atlas (TCGA) cohorts. The alteration rate is displayed on the Y-axis, representing the percentage of patients with alterations in each gene. Genes along the X-axis have been selected based on their established or emerging relevance to CRC pathogenesis and therapy.

affecting 57% of patients. Additionally, 27.6% of patients had lung metastases, and 27.2% exhibited peritoneal metastases. Interestingly, peritoneal metastasis and tumor location on the left colon or rectum were identified as independent prognostic factors (Table 1). As of the final follow-up on February 5, 2021, the median follow-up duration was 32.03 months.

### *Overview of Genomic Alterations in mCRC Patients*

An extensive analysis of 500 Chinese patients with mCRC identified *TP53*, *APC*, and *KRAS* as the most frequent mutations, observed in 78%, 60%, and 47% of patients, respectively (Fig. 1A). Notably, the frequency of *APC* mutations was lower compared to data from the MSKCC and TCGA cohorts. Among the EGFR pathway mutations, *KRAS* and *BRAF* were consistent in the three cohorts, while *PIK3CA* mutations were less common in the Chinese cohort (Fig. 1B).

### *Targetable Genes*

In our study, 29 patients, constituting 5.8% of the cohort, were identified as having MSI-H status using the NGS-based algorithm. Among the patients examined, 12% (60/500) displayed amplifications in the *ERBB2/HER2* gene, with 18 *KRAS* wild type potentially benefiting from HER2-targeted therapy (Fig. 2A). On the other hand, *EGFR*, *FGFR1* and *MET* gene amplifications were exclusively observed in patients with MSS colorectal cancer, occurring in 2% (10/500), 2% (10/500), and 3% (15/500) of cases, respectively. All *EGFR*, *FGFR1*, and *MET* amplification cases were found to be *HER2* non-amplified and mutually exclusive. Importantly, *EGFR* amplification exhibited a statistically significant association with RAS/BRAF wild-type status. Furthermore, amplification was the predominant gene alteration observed in *FGFR1* and *MET* genes. Fusion events were detected in 1.2% of mCRC patients (6/500). Specifically, *ALK* (2/500, 0.4%) and *ROS1*

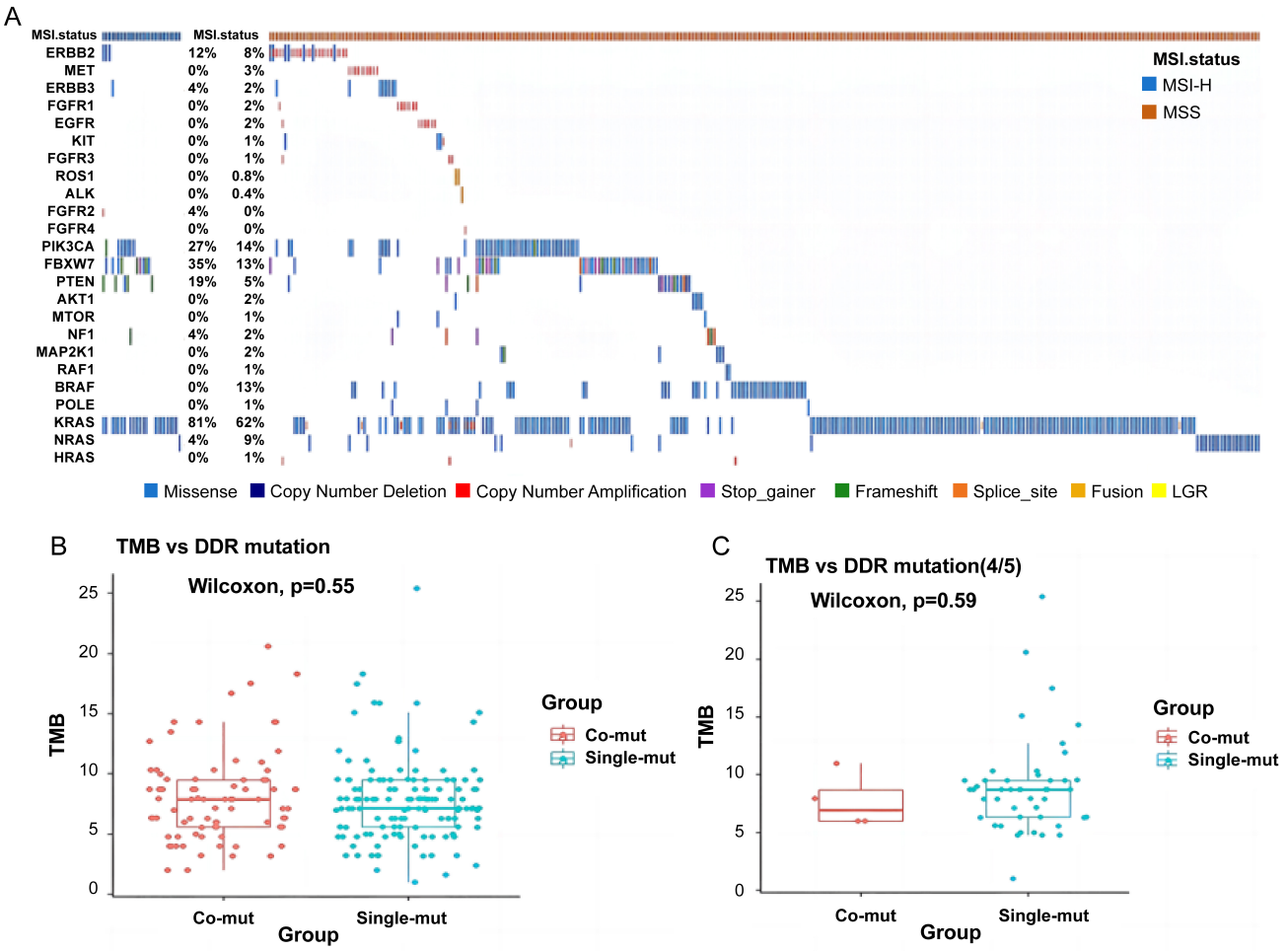



**Fig. 2. Analysis of targetable genomic alterations in Chinese mCRC patients.** (A) Mutation prevalence and microsatellite status correlation. This figure ranks targetable gene mutations by importance via a heatmap, which shows the correlation between specific gene mutations and microsatellite instability status (MSI-H for high instability and microsatellite stable (MSS) for stable). The color coding within the heatmap indicates the type of mutation, such as missense, copy number deletions or amplifications, stop gained, frameshift, splice site, or fusion events. The top bar indicates the presence of MSI-H status, providing an immediate visual correlation between MSI status and mutation patterns. (B) Tumor mutational burden (TMB) versus DNA damage response (DDR) gene co-mutations. This scatter plot compares the TMB levels in patients with co-mutations versus those with single mutations in DDR genes, excluding *TP53*. Each point represents a patient, with the spread of points indicating the variability in TMB among the patients. (C) TMB versus category 4-5 DDR gene co-mutations. This category includes the most deleterious mutations and illustrates whether a significant difference in TMB is associated with the severity of DDR mutations. MSI, microsatellite instability; MSI-H, MSI-High.

(4/500, 0.8%) gene fusions were observed exclusively in MSS tumors, whereas no NTRK fusions were identified (Fig. 2A).

Additionally, we analyzed genes related to the DNA damage response (DDR) pathway. There was no significant difference between the distribution of tumor mutational burden (TMB) in patients with co-mutations and those with single mutations ($p$ = 0.55) (Fig. 2B). Furthermore, for the category of DDR genes with 4–5 mutations, excluding *TP53*, there was no significant difference between the co-mutations and single mutations in TMB associated with the severity of DDR mutations ($p$ = 0.59) (Fig. 2C).

### *ERBB2/HER2 and HER3 Mutations*

Given the favorable response of *HER2*-mutated lung cancer to *DS8201* [18], we also analyzed *HER2* mutation loci in CRC patients and observed *ERBB2* and *ERBB3* mutations in 1.8% (9/500) of CRC patients each (Fig. 3A,B). The main mutation loci of *ERBB2/HER2* mutations were *p.V842I*, *p.G776V*, *p.L775S*, *p.S310F*, *p.T862A* and *p.V777L* (Fig. 3A). The main mutation loci of *ERBB3/HER2* mutations were *p.A232T*, *p.A232V*, *p.G284R*, *p.V104L* and *p.V104M* (Fig. 3B).

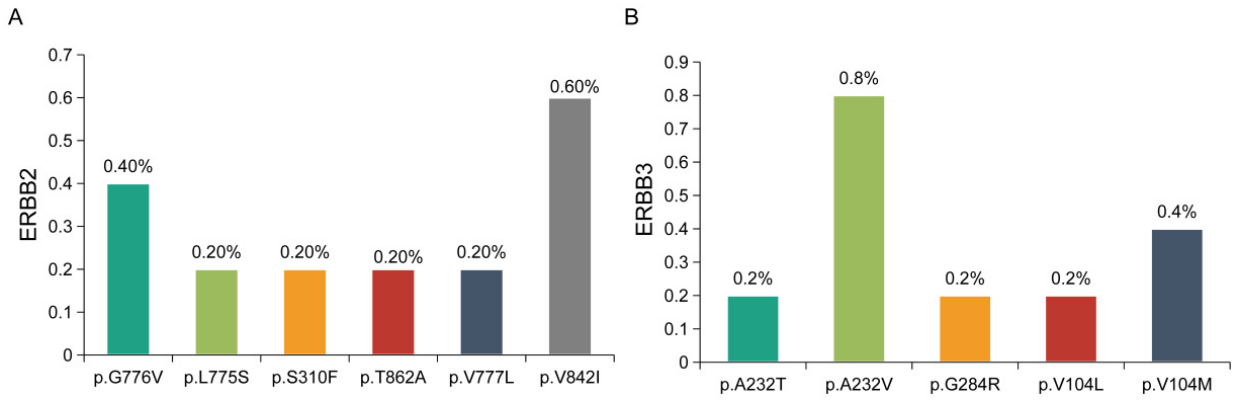
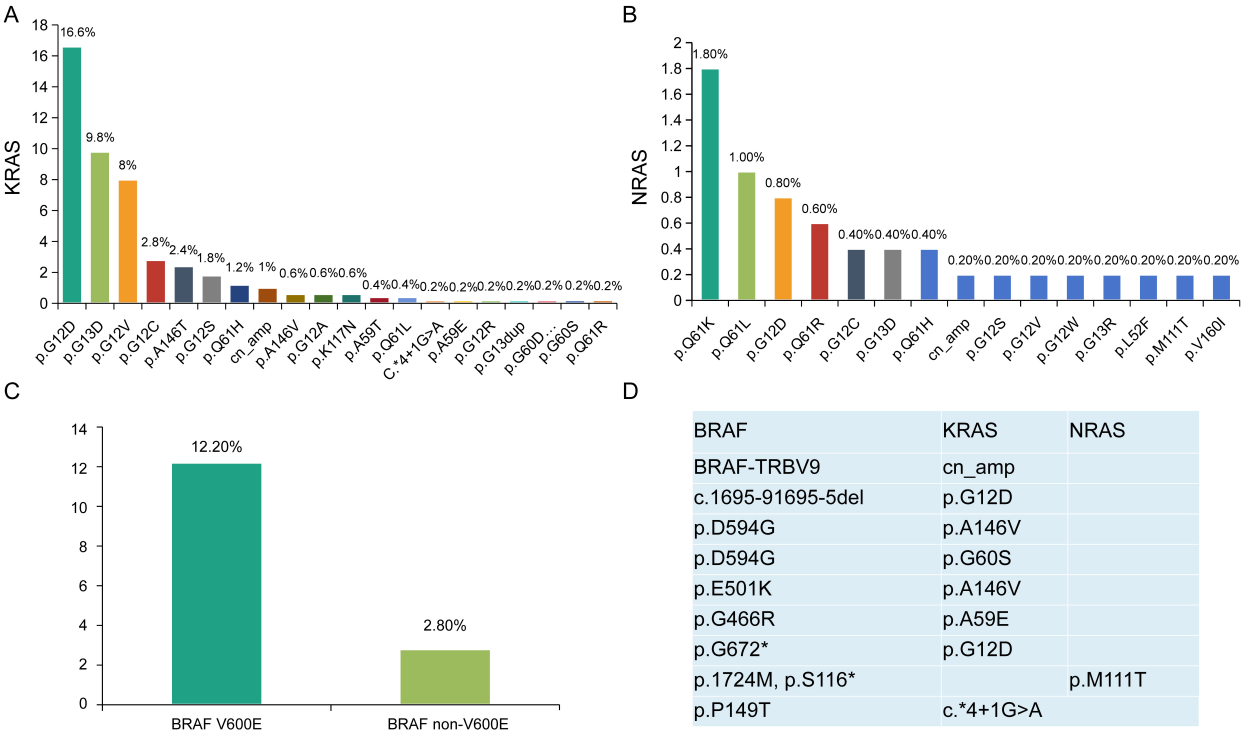



**Fig. 3. Distribution of *ERBB2*/*HER2* and *ERBB3*/*HER3* mutations in Chinese patients with mCRC.** (A,B) The pie chart shows the prevalence of various *ERBB2*/*HER2* mutations (A) and *ERBB3*/*HER3* mutations (B) in our Chinese cohort with mCRC, with each color representing a different mutation and the size of the segment reflecting its frequency in the patient cohort.




| BRAF | KRAS | NRAS |
|---|---|---|
| BRAF-TRBV9 | cn_amp | |
| c.1695-91695-5del | p.G12D | |
| p.D594G | p.A146V | |
| p.D594G | p.G60S | |
| p.E501K | p.A146V | |
| p.G466R | p.A59E | |
| p.G672* | p.G12D | |
| p.1724M, p.S116* | | p.M111T |
| p.P149T | c.*4+1G>A | |

**Fig. 4. *KRAS*, *NRAS*, and *BRAF* mutations in mCRC Chinese patients.** (A) *KRAS* mutation distribution. This bar graph shows the *KRAS* mutation types identified in the cohort, highlighting the most prevalent mutations with larger segments. (B) *NRAS* mutation distribution. The bar graph categorizes the different *NRAS* mutations in the patient group. (C) *BRAF V600E* and non-*V600E* mutations. The bar graph differentiates between the proportions of the common *BRAF V600E* mutations and other non-*V600E* variants. (D) *RAS* and *BRAF* co-mutations. The table lists specific co-mutation combinations found within the *RAS* and *BRAF* genes, providing insights into the complexity of mutational interactions. c.*4+1G>A indicates a mutation at the 4th nucleotide position after the end of the coding sequence, from G to A. * indicates the termination of the coding sequence, and +1 indicates the first nucleotide at the 3' end of the termination codon.

## *RAS/BRAF Variants*

Further analysis showed that 47% of patients in the cohort harbored *KRAS* mutations, and the distribution of *NRAS* mutations was 7% (Fig. 4A,B). *KRAS G12D* was the most common mutation in 83 (16.6%) cases, followed by *KRAS G13D* (n = 49 cases). In addition, 14 (2.8%) patients were found to carry the *KRAS G12C* variant (Fig. 4A), *BRAF V600E* mutation was detected in 61 (12.2%) patients (Fig. 4C), and *RAS* and *BRAF* co-mutations occurred in patients with nonpathogenic mutations in the *BRAF* gene (Fig. 4D).

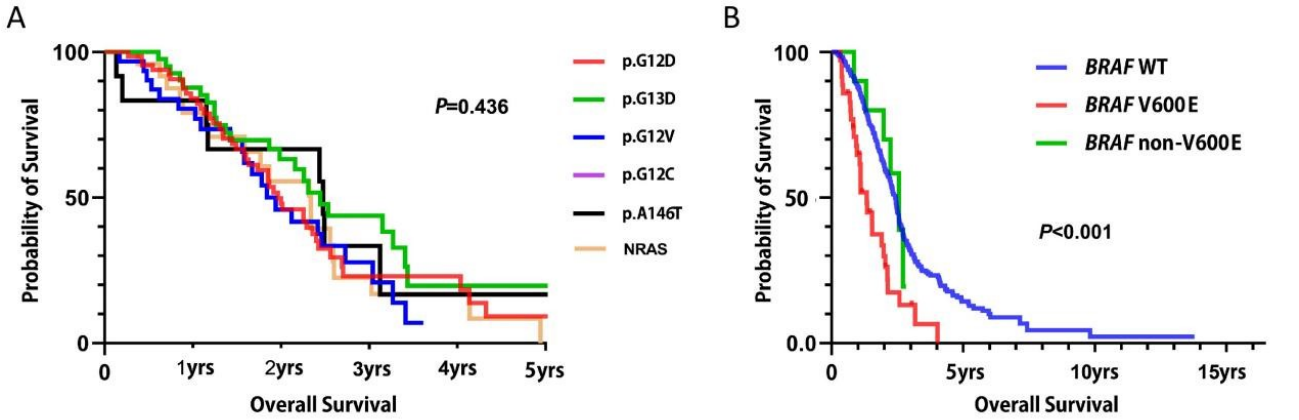



**Fig. 5. Survival analysis by mutational status in Chinese mCRC patients.** (A) Kaplan-Meier curves show the overall survival for patients with various *KRAS* mutations (different colors represent different mutation loci) and patients with *NRAS* mutations (shown in orange). (B) The graph compares the overall survival of patients with the *BRAF* wild type (WT, in blue), *BRAF V600E* mutations (in red), and *BRAF* non-*V600E* mutations (in green).

Regarding prognosis, survival differences were not observed between different *KRAS* mutant loci and *NRAS* mutations ($p$ = 0.436) (Fig. 5A). However, *BRAF* mutations, particularly the *BRAF V600E* variant, had an adverse impact on survival (*BRAF V600E* vs. *BRAF* wild type vs. BRAF non-*V600E*: 16.3 months vs. 29.5 months vs. 31.1 months; $p < 0.001$) (Fig. 5B).

## Discussion

To our knowledge, this is the most extensive report highlighting the potential targetable genes in Chinese patients. Additionally, this study described the clinicopathologic characteristics and prognosis of these patients. It is currently possible to screen patients for EGFR antibodies utilizing biomarkers available for CRCs, such as *RAS/RAF*. Newer biomarkers, including *HER2*, DDR, MSI-H, and *c-Met*, are becoming increasingly significant. Therefore, it is imperative to simultaneously identify multiple candidate biomarkers to spare patients from potentially harmful procedures. Moreover, genomic analysis will be necessary to expand the percentage of patients with CRC who may benefit from precision medicine. This dataset contributes to the existing literature and generates interesting hypotheses.

In this study involving 500 Chinese patients with mCRC, we comprehensively analyzed genetic alterations and clinicopathological characteristics. We found that mCRC predominantly affected individuals of median age 58 years, with a rare occurrence of signet-ring cell cancer. Most patients had moderately differentiated tumors, and a significant proportion had a positive family history of cancer. Tumor distribution showed varying locations within the colon and rectum. Liver metastases were the most common, and peritoneal metastasis and tumor location on the left colon or rectum were identified as independent prognostic factors. Genomic analysis revealed *TP53*, *APC*, and *KRAS* as the most prevalent mutations, occurring in 78%, 60%, and 47% of patients, respectively, with distinct mutation profiles compared to Western cohorts. Notably, *HER2* mutations were identified in a subset of patients, which may have clinical implications, particularly for the 12% of patients with *ERBB2/HER2* amplifications who could benefit from *HER2*-targeted therapy. Furthermore, *EGFR*, *FGFR1*, and *MET* amplifications were detected exclusively in patients with MSS CRC, emphasizing the importance of molecular subtyping. Additionally, the adverse prognostic impact of *BRAF V600E* mutations was observed, suggesting its relevance as a prognostic marker. These findings shed light on the genetic landscape of mCRC in the Chinese population and have potential implications for personalized treatment strategies, highlighting the importance of precision medicine in managing this complex disease.

The most common variants of CRC genomic alterations in this genomic analysis are *TP53*, *APC*, and *KRAS*. In addition, 5.8% of patients were determined as MSI-H by an NGS-based algorithm. It was found that TMB was increased in individuals with co-mutations in the homologous recombination repair pathway (HRR-MMR) and the homologous recombination repair pathway (HRR-BER) (defined as co-mut+). Most patients with CRC harboring *ERBB2/HER2* amplifications had *KRAS* wild type, which suggests that these patients may be suitable candidates for *HER2*-targeted therapy (e.g., trastuzumab + lapatinib/pertuzumab/tucatinib/T-Dxd) [18–20]. It is necessary to comprehensively examine ERBB2 mutations in CRC due to the clinical availability of several *ERBB2* kinase inhibitors. Despite the growing importance and therapeutic relevance of *ERBB2* amplifications, little is known about *ERBB2/ERBB3* mutations in CRC. In Western countries, *ERBB2* (V842I) and *ERBB3* (V104M) are the most

common mutant loci in mCRC [21]. Our study reported that ERBB2 (V842I) and ERBB3 (A232V) were the most common in China.

The EGFR pathway is still the most important component of initiation and progression in CRC [22]. Many drugs have been developed for EGFR blocking. *KRAS* has long been considered "undruggable" despite its dominant cancer-driver function in tumorigenesis until the development of inhibitors targeting the *KRAS G12C* allele. Although specific inhibitors for *KRAS G12C* have shown significant activity in patients, there are currently no approved inhibitors for *NRAS*, *HRAS*, or non-G12C *KRAS* variants. *G12D* and *G12V* are the two most common CRC subtypes of *KRAS* mutations [23]. However, in the Chinese cohort, *G13C* rates ranked second. Also, the rate of *KRAS G12C* was much lower than that in Western countries. A pooled analysis of five randomized trials, including 1239 patients with mCRC, concluded that patients harboring the *KRAS* G12C mutation, had lower overall survival than nonmutated tumors [24]. In our cohort, we did not detect the same phenomenon, which may be due to the smaller sample size.

*BRAF V600E* mutations were detected in 61 (12.2%) patients, comparable to Western countries. In a past study, combined therapy with *BRAF* and *EGFR* inhibitors prolonged overall survival for patients with *BRAF V600E* mutations to 9.3 months [25]. However, we noticed in the Chinese cohort that this subtype of patients had more prolonged survival, with a median overall survival of 16.3 months. *BRAF V600E* mutations have been demonstrated as an important biomarker for poor prognosis in CRC. Nowadays, with the benefit of a triplet cytotoxic regimen and targeted therapies combination, this subtype's survival has been prolonged [26,27].

In this study, we mainly focus on the genomic alterations in mCRC in a Chinese cohort and acknowledge the limitations of not including a comparative analysis with non-metastatic CRC. Comparative studies are crucial for a more comprehensive understanding of CRC's mutational landscape at different stages. Thus, further research is necessary to compare these findings with non-mCRC, especially in the Chinese population. This would enhance our findings' comprehensibility and contribute significantly to the broader understanding of CRC's genomic variations and their implications in personalized medicine, as well as CRC's mutational landscape at different metastasis organs.

## Conclusions

In conclusion, this study confirms the feasibility of using NGS in identifying prognostic and therapeutically targetable genetic variants in Chinese mCRC patients. The comprehensive analysis of genomic variation within this population contributes to understanding mCRC and underscores the potential for personalized medicine in managing this disease.

## Availability of Data and Materials

Datasets used in this article are available from the corresponding authors upon reasonable request.

## Author Contributions

XHZ, JQZ, JFZ and XCW designed the research study. XHZ, JQZ, JFZ and XCW performed the research. QW, JL, TX and CMB provided help and advice on experiments. QW, JL, TX and CMB analyzed the data. All authors were involved in drafting and critical revision of the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

The study protocol was conducted in accordance with the Declaration of Helsinki. All procedures were approved by the Medical Ethics Committee of Peking University Cancer Hospital (2022KT133). Written informed consent was obtained from all patients.

## Acknowledgment

Not applicable.

## Funding

This research received no external funding.

## Conflict of Interest

The authors declare no conflict of interest.

## References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021; 71: 209–249.

[2] Biller LH, Schrag D. Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. JAMA. 2021; 325: 669–685.

[3] Barrera M, Jr, Castro FG, Strycker LA, Toobert DJ. Cultural adaptations of behavioral health interventions: a progress report. Journal of Consulting and Clinical Psychology. 2013; 81: 196–205.

[4] Mosele F, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP, *et al*. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. Annals of Oncology: Official Journal of the European Society for Medical Oncology. 2020; 31: 1491–1505.

[5] Lizardo DY, Kuang C, Hao S, Yu J, Huang Y, Zhang L. Immunotherapy efficacy on mismatch repair-deficient colorectal cancer: From bench to bedside. Biochimica et Biophysica Acta. Reviews on Cancer. 2020; 1874: 188447.

[6] Andre T, Amonkar M, Norquist JM, Shiu KK, Kim TW, Jensen BV, *et al*. Health-related quality of life in patients with microsatellite instability-high or mismatch repair deficient metastatic colorectal cancer treated with first-line pembrolizumab versus chemotherapy (KEYNOTE-177): an open-label, randomised, phase 3 trial. The Lancet. Oncology. 2021; 22: 665–677.

[7] Diaz LA, Jr, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, *et al*. Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. The Lancet. Oncology. 2022; 23: 659–670.

[8] Khan K, Valeri N, Dearman C, Rao S, Watkins D, Starling N, *et al*. Targeting EGFR pathway in metastatic colorectal cancer- tumour heterogeniety and convergent evolution. Critical Reviews in Oncology/hematology. 2019; 143: 153–163.

[9] Yoshino T, Arnold D, Taniguchi H, Pentheroudakis G, Yamazaki K, Xu RH, *et al*. Pan-Asian adapted ESMO consensus guidelines for the management of patients with metastatic colorectal cancer: a JSMO-ESMO initiative endorsed by CSCO, KACO, MOS, SSO and TOS. Annals of Oncology: Official Journal of the European Society for Medical Oncology. 2018; 29: 44–70.

[10] Drosten M, Barbacid M. Targeting the MAPK Pathway in KRAS-Driven Tumors. Cancer Cell. 2020; 37: 543–550.

[11] Siena S, Di Bartolomeo M, Raghav K, Masuishi T, Loupakis F, Kawakami H, *et al*. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): a multicentre, open-label, phase 2 trial. The Lancet. Oncology. 2021; 22: 779–789.

[12] Strickler JH, Yoshino T, Graham RP, Siena S, Bekaii-Saab T. Diagnosis and Treatment of ERBB2-Positive Metastatic Colorectal Cancer: A Review. JAMA Oncology. 2022; 8: 760–769.

[13] Guo R, Luo J, Chang J, Rekhtman N, Arcila M, Drilon A. MET-dependent solid tumours - molecular diagnosis and targeted therapy. Nature Reviews. Clinical Oncology. 2020; 17: 569–587.

[14] Pilié PG, Tang C, Mills GB, Yap TA. State-of-the-art strategies for targeting the DNA damage response in cancer. Nature Reviews. Clinical Oncology. 2019; 16: 81–104.

[15] Mauri G, Arena S, Siena S, Bardelli A, Sartore-Bianchi A. The DNA damage response pathway as a land of therapeutic opportunities for colorectal cancer. Annals of Oncology: Official Journal of the European Society for Medical Oncology. 2020; 31: 1135–1147.

[16] Shaw AT, Hsu PP, Awad MM, Engelman JA. Tyrosine kinase gene rearrangements in epithelial malignancies. Nature Reviews. Cancer. 2013; 13: 772–787.

[17] Pietrantonio F, Di Nicolantonio F, Schrock AB, Lee J, Tejpar S, Sartore-Bianchi A, *et al*. ALK, ROS1, and NTRK Rearrangements in Metastatic Colorectal Cancer. Journal of the National Cancer Institute. 2017; 109: djx089.

[18] Sartore-Bianchi A, Trusolino L, Martino C, Bencardino K, Lonardi S, Bergamo F, *et al*. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. The Lancet. Oncology. 2016; 17: 738–746.

[19] Nakamura Y, Okamoto W, Kato T, Esaki T, Kato K, Komatsu Y, *et al*. Circulating tumor DNA-guided treatment with pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer: a phase 2 trial. Nature Medicine. 2021; 27: 1899–1903.

[20] Zuo WJ, Jiang YZ, Wang YJ, Xu XE, Hu X, Liu GY, *et al*. Dual Characteristics of Novel HER2 Kinase Domain Mutations in Response to HER2-Targeted Therapies in Human Breast Cancer. Clinical Cancer Research: an Official Journal of the American Association for Cancer Research. 2016; 22: 4859–4869.

[21] Loree JM, Bailey AM, Johnson AM, Yu Y, Wu W, Bristow CA, *et al*. Molecular Landscape of ERBB2/ERBB3 Mutated Colorectal Cancer. Journal of the National Cancer Institute. 2018; 110: 1409–1417.

[22] Martinelli E, Ciardiello D, Martini G, Troiani T, Cardone C, Vitiello PP, *et al*. Implementing anti-epidermal growth factor receptor (EGFR) therapy in metastatic colorectal cancer: challenges and future perspectives. Annals of Oncology: Official Journal of the European Society for Medical Oncology. 2020; 31: 30–40.

[23] Haigis KM. KRAS Alleles: The Devil Is in the Detail. Trends in Cancer. 2017; 3: 686–697.

[24] Modest DP, Ricard I, Heinemann V, Hegewisch-Becker S, Schmiegel W, Porschen R, *et al*. Outcome according to KRAS-, NRAS- and BRAF-mutation as well as KRAS mutation variants: pooled analysis of five randomized trials in metastatic colorectal cancer by the AIO colorectal cancer study group. Annals of Oncology: Official Journal of the European Society for Medical Oncology. 2016; 27: 1746–1753.

[25] Kopetz S, Guthrie KA, Morris VK, Lenz HJ, Magliocco AM, Maru D, *et al*. Randomized Trial of Irinotecan and Cetuximab With or Without Vemurafenib in BRAF-Mutant Metastatic Colorectal Cancer (SWOG S1406). Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 2021; 39: 285–294.

[26] Tabernero J, Grothey A, Van Cutsem E, Yaeger R, Wasan H, Yoshino T, *et al*. Encorafenib Plus Cetuximab as a New Standard of Care for Previously Treated *BRAF* V600E-Mutant Metastatic Colorectal Cancer: Updated Survival Results and Subgroup Analyses from the BEACON Study. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 2021; 39: 273–284.

[27] Cremolini C, Loupakis F, Antoniotti C, Lupi C, Sensi E, Lonardi S, *et al*. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. The Lancet. Oncology. 2015; 16: 1306–1315.