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Cancer Diagnostics and Molecular Pathology

Genomic Alterations of *NTRK*, *POLE*, *ERBB2*, and Microsatellite Instability Status in Chinese Patients with Colorectal Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Colorectal cancer • DNA polymerase ε • Tropomyosin receptor kinase • *ERBB2* amplification • Tumor mutational burden

Abstract

Background. The increasing molecular characterization of colorectal cancers (CRCs) has spurred the need to look beyond *RAS*, *BRAF*, and microsatellite instability (MSI). Genomic alterations, including *ERBB2* amplifications and mutations, *POLE* mutations, MSI, and *NTRK1–3* fusions, have emerged as targets for matched therapies. We sought to study a clinically annotated Chinese cohort of CRC subjected to genomic profiling to explore relative target frequencies.

Methods. Tumor and matched whole blood were collected from 609 Chinese patients with CRC. Extracted DNA was analyzed for all classes of genomic alterations across 450 cancer-related genes, including single-nucleotide variations (SNVs), short and long insertions and deletions (indels), copy number variations, and gene rearrangements. Next-generation sequencing–based computational algorithms also determined tumor mutational burden and MSI status.

Results. Alterations in *TP53* (76%), *APC* (72%), and *KRAS* (46%) were common in Chinese patients with CRC. For the first time, the prevalence of *NTRK* gene fusion was observed to be around 7% in the MSI-high CRC cohort. Across the cohort, MSI was found in 9%, *ERBB2* amplification in 3%, and *POLE* pathogenic mutation in 1.5% of patients. Such results mostly parallel frequencies observed in Western patients. However, *POLE* existed at a higher frequency and was associated with large tumor T-cell infiltration.

Conclusion. Comparing to the Western counterparts, *POLE* mutations were increased in our cohort. The prevalence of *NTRK* gene fusion was around 7% in the MSI-high CRC cohort. Increased adoption of molecular profiling in Asian patients is essential for the improvement of therapeutic outcomes. ***The Oncologist*** 2020;25:e1671–e1680

Implications for Practice: The increasing use of genomic profiling assays in colorectal cancer (CRC) has allowed for the identification of a higher number of patient subsets benefiting from matched therapies. With an increase in the number of therapies, assays simultaneously evaluating all candidate biomarkers are critical. The results of this study provide an early support for the feasibility and utility of genomic profiling in Chinese patients with CRC.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies in China, accounting for 376,000 new cases and 191,000 deaths in 2015 alone [1]. Despite remarkable progress in biological understanding, currently, chemotherapy

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remains the evidence-based standard for first-line therapy in advanced disease. Regimens consisting of 5-fluorouracil, irinotecan, or oxaliplatin with biologic agents targeting epidermal growth factor receptor (EGFR) or vascular endothelial growth factor remain the global standard. With current therapies, the median overall survival (OS) of patients with metastatic CRC (mCRC) in the past few decades has improved to over 30 months [2]. In spite of significant improvements in mCRC treatment, the most effective treatment for a patient is relatively empiric, mainly based on clinical considerations. Standard biomarkers, including *RAS*/*BRAF* and microsatellite status, both inform prognosis and predict benefit, or lack thereof, from biologic agents [3]. The emergence of precision medicine has identified genomic variants such as *NTRK* gene fusion, microsatellite instability (MSI), *HER2* amplification, and *POLE* pathogenic mutation as potential agonistic biomarkers for immune or targeted therapies.

The recent approval of MSI and mismatch repair deficiency (dMMR) as a biomarker for immunotherapy further reinforces the concept that precision treatment can significantly extend the survival of certain patients. Microsatellites are short tandem repeats of the same base or simple sequence scattering throughout both coding and noncoding regions in the genome. Microsatellite instable tumors are tightly associated with defects in the DNA mismatch repair system, which also causes elevated tumor mutational burden (TMB). A clinical trial of patients with MSI-high (MSI-H)/dMMR mCRC treated with nivolumab has shown a median OS of over 33 months. Additionally, a basket trial treating patients with various dMMR cancers with pembrolizumab has demonstrated a median OS of over 36 months [4, 5].

Initially identified in colorectal and papillary thyroid carcinomas in the 1980s, *NTRK* fusions have recently been attractive biomarkers for targeted therapies research, leading to two drugs approved by the U.S. Food and Drug Administration (FDA), larotrectinib and entrectinib. The *NTRK* genes (*NTRK1*, *NTRK2*, and *NTRK3*) encode the tropomyosin receptor kinase (TRK) family of proteins including TRKA, TRKB, and TRKC, respectively [6]. *NTRK* gene fusions are the most common mechanisms of oncogenic TRK activation through intrachromosomal or interchromosomal rearrangements. The chimeric oncoprotein is characterized by the NTRK kinase's constitutive activation through the ligand-independent dimmer of domains generated by partner genes. For instance, *ETV6-NTRK3* gene fusion has a prevalence of >90% in secretory breast carcinoma [7, 8], mammary analog secretory carcinoma [9], and infantile fibrosarcoma [10]. However, it can be detected in <1% of common cancers such as lung adenocarcinomas, head and neck squamous cell cancer, and colorectal cancer. So far, approximately 80 different 5' *NTRK* gene fusion partners have been identified in a broad range of human tumor types, many of which have not yet been systematically characterized [11, 12].

DNA polymerase ε (*POLE*) pathogenic mutation is another gene alteration causing extremely high TMB. The DNA polymerase proofreading is essential for ensuring the fidelity of DNA replication. Mutations in proofreading domains of *POLE* impair the correction of wrongly paired bases and the fidelity of DNA replication. Cancers with *POLE* proofreading variants have among the highest mutation burden. The frequency of clinically significant polymerase mutations is around 1% in the Western CRC population [13]. Importantly, previous reports have shown an extraordinary response to immune checkpoint inhibition in *POLE*-mutated endometrial adenocarcinoma [14], CRC [15], and glioblastoma [16] with high TMB. Although the frequency of *POLE* mutations is low, it may become increasingly important as the role of immune checkpoint inhibitors evolves in CRC.

The amplification of the human epidermal growth factor receptor 2 (*HER2*) gene, a member of the EGFR family of tyrosine kinase receptors, is an established target in the treatment of patients with breast and gastric cancers. Recently, the HERACLES and MyPathway trials have shown that *HER2* gene amplification can serve as an effective biomarker for dual-targeted therapy with trastuzumab plus either lapatinib or pertuzumab in the treatment of refractory mCRC. Both trials demonstrated that, in treatment-refractory *HER2*-positive metastatic colorectal cancer, targeting *HER2* may represent a therapeutic option [17, 18].

To date, the examination of *NTRK*, *HER2*, and *POLE* in Asian patients has not been well represented. In order to overcome this gap in knowledge, we therefore sought to explore *NTRK*, *HER2*, and *POLE* in a cohort of Chinese patients with CRC.

Materials and Methods

Genomic Profiling, Mutation Calling, and MSI/TMB Calculation

Comprehensive genomic profiling was performed by next-generation sequencing with a panel of 450 cancer-related genes, as previously reported on samples collected from October 2016 to March 2019 [19]. More than 97% samples were from the 3A hospitals (top tier) across China; 24% of all samples were from the south, 26% from the north, 31% from the west, and 19% from the east. All patients provided written informed consent. To this end, both formalin fixed paraffin embedded (FFPE) tumor and paired peripheral blood samples from Chinese patients with CRC were used. In brief, DNA was extracted from unstained FFPE sections with a tumor content of no less than 20%. Subsequently, DNA was fragmented to ~250 base pairs by sonication. A library was constructed with KAPA Hyper Prep Kit (KAPA Biosystems; Roche, Basel, Switzerland). Then hybridization capture was performed with a custom panel containing about 24,000 individually synthesized 5'-biotinylated DNA probes. Genomic alterations, including single base substitution, short and long indels, copy number variations, and gene rearrangement and fusions, were assessed. Next-generation sequencing–based algorithms also determined the TMB and microsatellite status.

Immunohistochemistry

Immunohistochemistry (IHC) staining procedure was performed as previously described [20]. In brief, deparaffinization, rehydration, and target retrieval were performed. These steps were followed by incubation with the monoclonal PD-L1 (Clone 22C3; Dako, Agilent Technologies, Santa Clara, CA) and CD3 (Kit-0003, MXB Biotechnologies, Fuzhou, China) antibodies. Slides were then incubated with a ready-to-use

Table 1. Characteristics of patients with colorectal cancer

Total (*n* = 609)	*n* (%)
Gender	
Male[a]	372 (61)
Female[b]	237 (39)
Disease stage	
I	37 (6)
II	135 (22)
III	153 (25)
IV	282 (46)
Unknown	2 (/)
MMR/MSI	
dMMR/MSI-H	55 (9)
pMMR/MSS	554 (91)
KRAS	
Wild type	327 (54)
Mutant	282 (46)
BRAF	
Wild type	561 (91)
Mutant	48 (8)

[a]Median age: 61 years.
[b]Median age: 60 years.
Abbreviations: dMMR, mismatch repair deficiency; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, MSI-high; MSS, microsatellite stable; pMMR, mismatch repair proficiency.

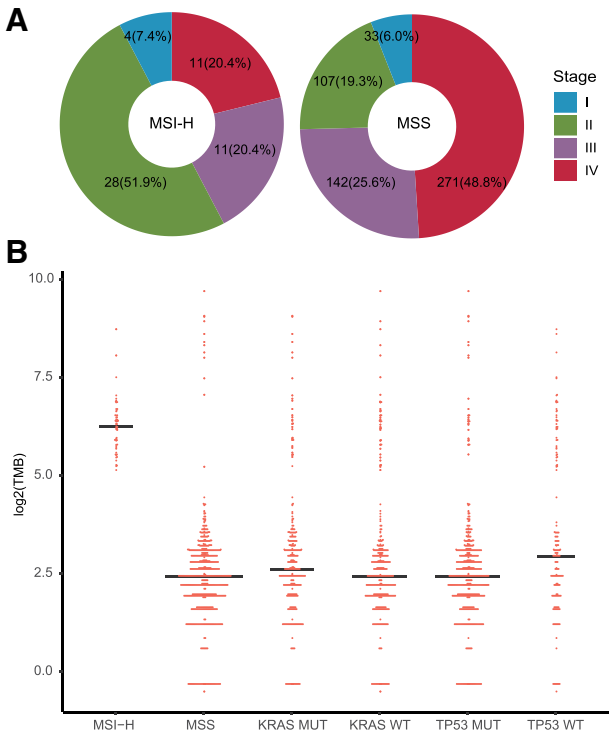

Figure 1. TMB's association with MSI, *TP53*, and *KRAS* mutations. **(A):** MSI-H colorectal cancers (CRCs) were found more commonly at early stage I or stages II and III (81%) versus the MSS CRC subcohort (51%). **(B):** Microsatellite statuses and TMB are shown for each tumor (n = 609). The MSI-H subcohort's median TMB (n = 55) is 59.4 mutations per megabase (mut/Mb), which is much higher than the MSS subcohort (n = 554) at 5.4 mut/Mb, with the exclusion of patients with *POLE* pathogenic mutation. *KRAS* mutations previously shown, causing elevated TMB in lung cancer, did not result in higher TMBs in CRC. Wild-type *TP53* was associated with a marginally higher TMB, mainly because of the relatively enriched MSI-H cases in this cohort.
Abbreviations: MSI, microsatellite instability; MSI-H, MSI-high; MSS, microsatellite stable; MUT, mutant; TMB, tumor mutational burden; WT, wild type.

visualization reagent consisting of horseradish peroxidase molecules coupled with a secondary antibody. The enzymatic conversion of the subsequently added chromogen, followed by the enhancer, resulted in the precipitation of a visible reaction product at the antigen's site. The specimens were then counterstained with hematoxylin and coverslipped. Results were interpreted using a light microscope. For the development of the finalized assay, the sensitivity was optimized with minimum nonspecific staining by adjusting both the primary antibody concentration and reagent incubation times.

T-cell infiltration of tumors was assessed by semiquantitative estimation of the density of CD3-positive cells as previously described: (1) no, or sporadic cells; (2) moderate numbers of cells; (3) abundant occurrence of cells; and (4) highly abundant occurrence of cells [21]. PD-L1 expression in CRC is determined by using combined positive score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100 [22].

Results

Characteristics of Patients with Colorectal Cancer

In the present study, a cohort of 609 patients with CRC comprising 372 men (median age, 61 years) and 237 women (median age, 60 years) had their FFPE tumor samples and paired blood controls undergo NGS comprehensive genome profiling with a 450-gene panel. The majority (46%) of the patients were stage IV, followed by 25% stage III, 22% stage II, and 6% stage I. Nine percent of the patients (55/609) were MSI-H determined by either polymerase chain reaction (PCR)/IHC- or NGS-based algorithm or both. Forty-six percent of the patients carried variants in the *KRAS* gene, whereas only 8% carried *BRAF* mutations (Table 1). MSI-H CRCs were found far less commonly at late stage IV (20%) versus microsatellite stable (MSS) CRCs (49%; Fig. 1A).

The Genomic Alteration Landscape of Colorectal Cancer

The median TMB of MSI-H CRC samples at 59.4 (range, 35–264.9) mutations per megabase (mut/Mb) was significantly higher than the MSS subgroup's 5.4 (range, 0.7–37.1) mut/Mb, with the exclusion of pathogenic *POLE* mutation samples ($p < .0001$; Fig. 1B) [23].

Genomic profiling of the 609 Chinese patients with CRC revealed a typical CRC genomic alteration landscape with the most frequent mutations observed in *TP53* (76%), *APC* (72%), and *KRAS* (46%). However, these results had a lower frequency compared with a similar study on FFPE samples in a

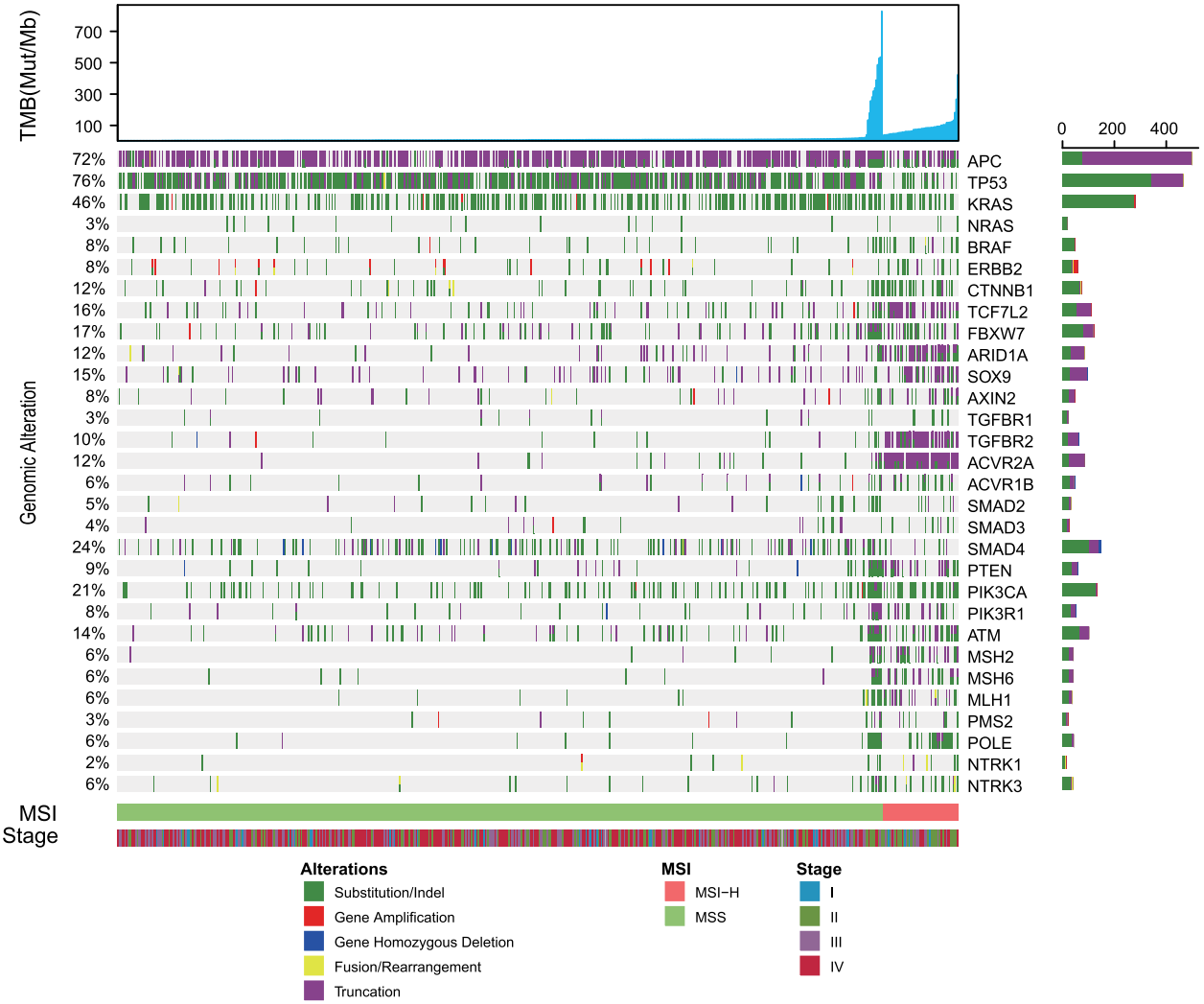

Figure 2. Landscape of colorectal cancer genomic alterations. A total of 609 patients with colorectal cancer were classified into two groups based on their microsatellite status. The remaining pathologic and genomic information was plotted accordingly. In each subcohort, patients were arranged by their TMB values in ascending order, as follows: individuals with extremely high TMB at the MSS section carrying *POLE* pathogenic mutations; patients in MSI segment displaying overall elevated mutational burden. The bottom half of the figure shows that MSS colorectal cancer's genomic alteration was *TP53*, *APC*, and *KRAS*, whereas the high frequency mutated genes in the MSI counterpart were *ACVR2A*, *ACVR1B*, *TGFBR2*, *CTNNB1*, *ARID1A*, and mismatch repair–related genes (i.e., *MSH6*, *MSH2*, and *MLH1*).
Abbreviations: MSI, microsatellite instability; MSI-H, MSI-high; MSS, microsatellite stable; mut/Mb, mutations per megabase; TMB, tumor mutational burden.

U.S. cohort [24, 25]. Recurrent somatic mutations in genes including *ACVR2A*, *ACVR1B*, *TGFBR2*, *CTNNB1*, *ARID1A*, and mismatch repair–related genes (*MSH6*, *MSH2*, and *MLH1*) were observed more frequently in MSI-H tumors, whereas *APC* and *TP53* were less frequently alternated than they were in MSS CRC (Fig. 2). Fifteen patients with MSI-H CRC carried germline pathogenic mutations in mismatch repair–related genes, such as frameshift in *MSH2* (Q170Rfs*4), truncations in *MLH1* (R226* and E609*), SNVs in *MLH1* (R265C and G67R), and splice site mutation in *MLH1* (c.1038+1G>T). Although mutated *TP53* or *KRAS* has been reported associated with higher TMB in lung cancer, neither of these mutations had elevated TMB in patients with CRC (Fig. 1B).

The signal pathway analysis in 609 patients with CRC revealed the presence of dysregulations in well-defined pathways (i.e., WNT, MAPK, PI3K, TGF-β, and P53 pathways). In line with prior reports, the latter were related to cell proliferation, genomic stability, and apoptosis [25, 26]. We found that the WNT signaling pathway was altered in 84% of MSS CRCs and 98% of MSI-H CRCs (p = .002) because of the higher incidence of activating mutations of *CTNNB1* and deletions in *TCF7L2*, *SOX9*, *ARID1A*, and *FBXW7*. Interestingly, *APC*'s mutation in the WNT signaling pathway was frequently found in both MSS (73%) and MSI-H (64%) CRCs. Such observations suggest that, in both cohorts, *APC* mutation was the dominant alteration affecting this pathway. It has been shown that, in CRC, the TGF-β signaling pathway is also extensively deregulated. Genomic alterations in the TGF-β pathway's genes, including *TGFBR1*, *TGFBR2*, *ACVR2A*, *ACVR1B*, *SMAD2*, *SMAD3*, and *SMAD4*, were found in 37% of the MSS and 96% of the MSI-H CRCs (p < .00001). Additionally, genes such as *TGFBR2* and *ACVR2A*, containing

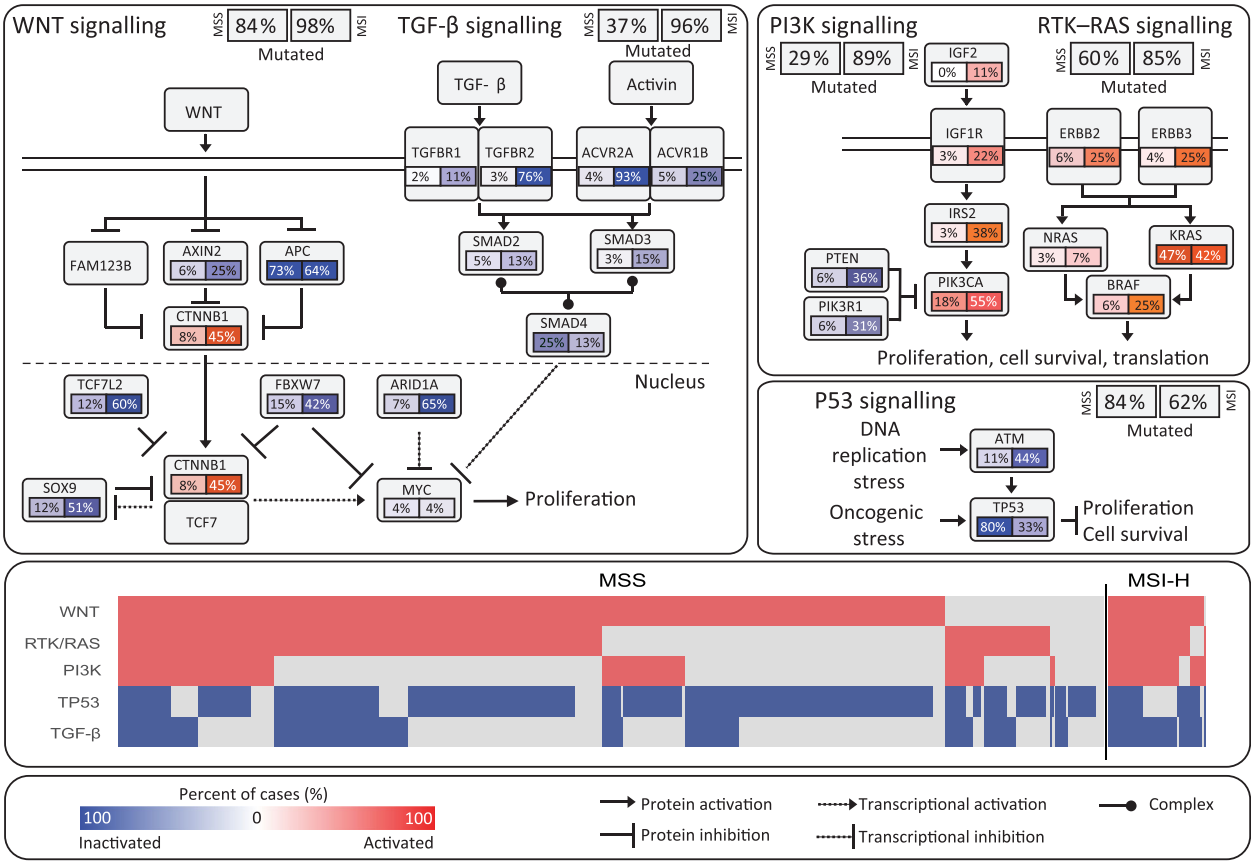

Figure 3. Frequently deregulated signaling pathways in colorectal cancer (CRC). MSI-H and MSS CRC samples with confirmed genetic variants in related signal pathways such as WNT, TP53, RAS, and TGF-β were analyzed separately. Alteration frequencies of activated (red) and inactivated (blue) alterations are expressed as a percentage of all cases in each cohort. Samples with alterations in five pathways—WNT, RTK/RAS, PIK3K, TP53, and TGF-β—are plotted in the middle panel.
Abbreviation: MSI, microsatellite instability; MSI-H, MSI-high; MSS, microsatellite stable.

microsatellite sites in their coding regions, were mutated at a very high frequency in the MSI-H group compared with the MSS group (76% vs. 3%, $p < .00001$ and 93% vs. 4%, $p < .00001$, respectively). Furthermore, we evaluated the TP53 pathway, which is potentially targetable by a newly developed drug (AZD1775), displaying promising clinical efficacy in head and neck squamous cell carcinoma [27]. Of note, alterations in TP53 and ATM, a kinase that phosphorylates and activates TP53 after DNA damage, was found in 62% of MSI-H and 84% of MSS CRCs (Fig. 3). Additionally, the *KRAS* G12C variant was discovered in 16 (2.6%) patients. Importantly, this variant is a potential target for a small molecule such as AMG 510 [28].

High Incidence of *NTRK* Gene Fusion in Patients with MSI-H CRC

NTRK fusions occur at low frequencies in most types of cancers. CRC is among the tumor types that have been shown to harbor *NTRK* fusions with frequency around 1% [12]. Consistently with previous reports, in the present study, six *NTRK* gene fusion cases were identified of 609 samples. However, we observed that the prevalence of *NTRK* fusion was around 7% in MSI-H CRCs (4/55), which was 20-fold higher than in the MSS CRC (0.36%) subcohort ($p = .0008$). The four *NTRK* fusions in the MSI-H cohort are well-characterized functional chimeric *TPR* (e21)–*NTRK1* (e11), *TPM3* (e7)–*NTRK1* (e9), and two cases of *ETV6* (e5)–*NTRK3* (e15). The two *NTRK* fusions in the MSS cohort were *LMNA* (e4)–*NTRK1* (e10) and *TPM3* (e7)–*NTRK1* (e9). The *NTRK* fusions were more frequent in older patients (median age, 72 years; range, 57–76 years vs. 60 years; range, 17–96 years; $p = .03$). *NTRK* fusions have been known as critical drivers for cancer development. With this in mind, we further analyzed the genomic alterations of six CRC cases with *NTRK* fusions to evaluate the co-occurrence of driver gene mutation. We observed the inactivation of tumor suppressor genes such as *APC*, *TP53*, *ACVR2A*, and *TGFBR2*. However, the activating variants of oncogenes (e.g., *KRAS* and *BRAF*) were not identified in patients with either MSS or MSI-H CRC with *NTRK* fusion (Fig. 4). In addition, except for three MSI-H CRCs with *NTRK* fusions that were at stage II, the rest of tumor samples with presumably productive fusions were at either stage III or stage IV (Table 2). The gene rearrangement events were significantly more prevalent in the MSI-H (6/55, 10.9%) than in the MSS (6/554, 1.1%) cohort ($p = .0003$).

High Incidence of *POLE* Mutation in Chinese Patients with CRC

We identified a high incidence (6%, 39/609) of Chinese patients with CRC harboring *POLE* somatic mutations either

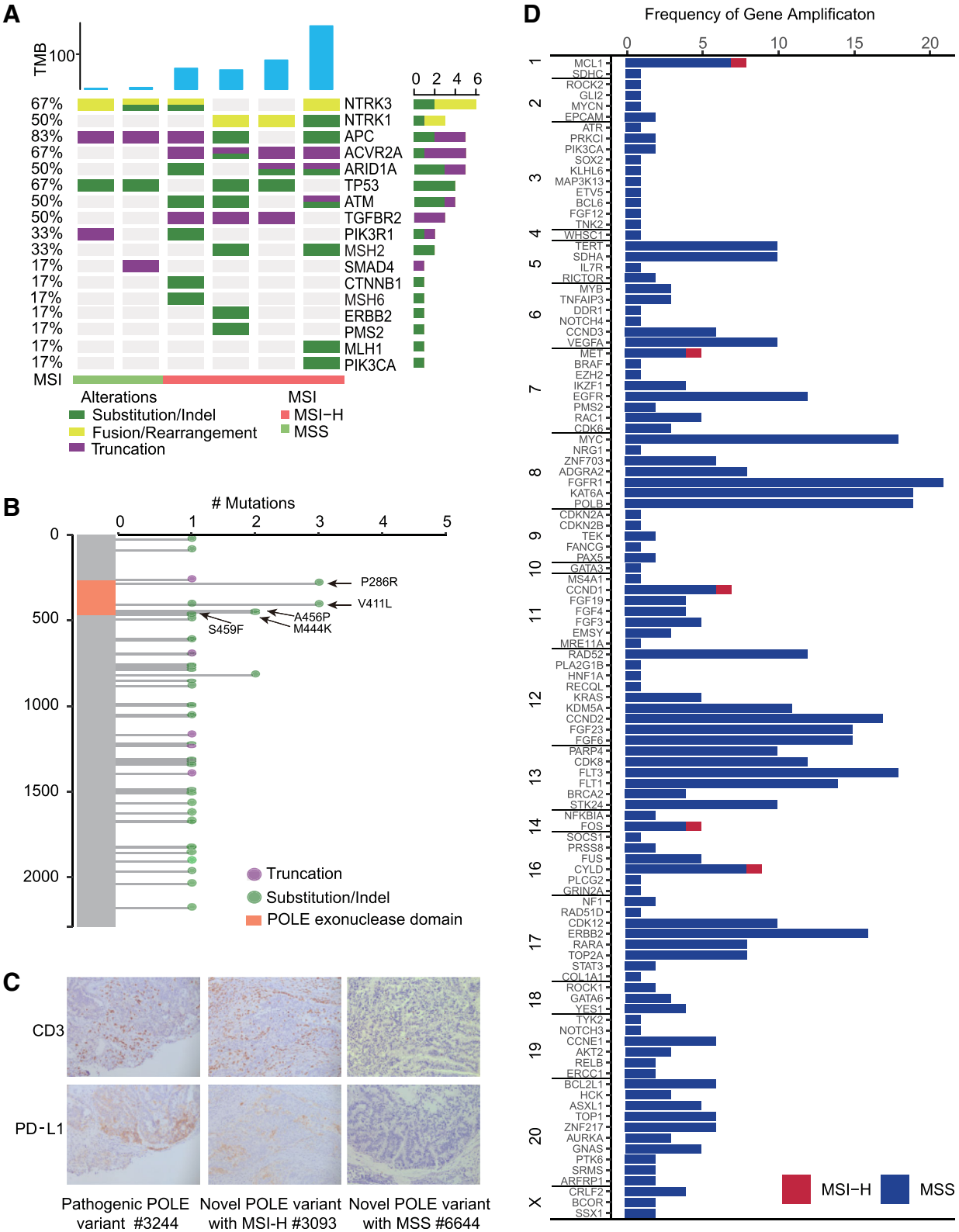

Figure 4. Variants of *NTRK* and *POLE*. **(A):** Genomic profiling of *NTRK* gene fusion–positive colorectal cancer (CRC). The *NTRK*-positive cancer lacks other activating gene variants and is enriched in MSI-H CRC. **(B):** The mutations in the DNA polymerase proofreading exonuclease domain encoded by exons 9 to 14 (residues 268–471), causing exceptional high mutational burden, are designated as pathogenic. The remaining mutations in the nonproofreading region appear to be benign. **(C):** Immunohistochemical analysis of CD3 and PD-L1 for CRC carrying *POLE* pathogenic mutation. A highly abundant infiltration of CD3-positive lymphocytes was observed in all five CRC samples carrying *POLE* pathogenic mutation. Additionally, a positive staining for PD-L1 was found in #3634 (TPS, 5%) and #3244 (TPS, 60%). **(D):** The red and blue bars represent gene amplification events occurring in MSI-H and MSS CRC, respectively. The event of gene amplification occurred much less frequently in the MSI-H (5 events in 55 patients) than in the MSS (668 events in 554 patients) cohort.
Abbreviations: MSI, microsatellite instability; MSI-H, MSI-high; MSS, microsatellite stable; TMB, tumor mutational burden; TPS, tumor proportion score.

Table 2. Gene fusion events that were predicted to generate functional chimeric proteins

ID	Gene pair	MSI	TMB	Stage	*KRAS*/*BRAF*	Novel
4080	*FFP-SEPT14*	MSS	10.1	III	N/A	Reported
5172	*ERBB2-GRB7*	MSS	8	III	N/A	Reported
6444	*LMNA-NTRK1*	MSS	6.2	IV	N/A	Reported
4089	*MAP3K9-ROS1*	MSS	6.1	IV	N/A	Novel
1959	*STARD3-ERBB2*	MSS	3	III	N/A	Novel
8050	*TPM3-NTRK1*	MSS	8.5	III	N/A	Reported
2105	*CUL1-BRAF*	MSI-H	82.8	III	*BRAF*	Reported
3943	*ETV6-NTRK3*	MSI-H	59.4	III	N/A	Reported
8115	*ETV6-NTRK3*	MSI-H	180.3	II	N/A	Reported
9942	*FGFR2-PIBF1*	MSI-H	73.5	III	N/A	Novel
7363	*TPM3-NTRK1*	MSI-H	82.8	II	N/A	Reported
3950	*TPR-NTRK1*	MSI-H	54.9	II	N/A	Reported

Twelve gene fusion events in this cohort that were predicted to generate functional chimeric proteins are listed in this table. Three of them, including *MAP3K9-ROS1*, *STARD3-ERBB2*, and *FGFR2-PIBF1*, had not been reported previously.
Abbreviations: MSI, microsatellite instability; MSI-H, MSI-high; MSS, microsatellite stable; N/A, not altered; TMB, tumor mutational burden.

confirmed by the COSMIC and MKSCC database or novel (Fig. 2) [29]. Despite the high frequency of somatic mutations, we did not find any germline pathogenic *POLE* variants. Specifically, we observed only one benign splice site mutation (c.2865-4delT) in our cohort. The recurrent variants within the DNA polymerase proofreading exonuclease domain encoded by exons 9 to 14 (residues 268–471) are known to cause an exceptionally high mutational burden in CRC [30]. As a consequence, they were designated as pathogenic mutations. In the present cohort, the frequency of *POLE* pathogenic mutations, including ten cases of V411L, P286R, M444K, A456P, and a dual mutation S459F/Y468C, was 1.8% (11/609), which is higher than 1% in the European counterpart (Fig. 4B) [13]. The genome of patients carrying recurrent *POLE* pathogenic mutations was hypermutated with extremely high TMB compared with the whole cohort (median 361 vs. 5 mut/Mb). Of note, all of them were MSS (Table 3). Interestingly, four *POLE* pathogenically mutated CRCs carried *MSH2*/*MSH6*/*PSM2*-truncated variants, however, with a stable microsatellite. We therefore assumed that these truncated variants were monoallelic, and the combination of *POLE* pathogenic mutation and the dMMR might be detrimental to the cancer cells. Moreover, 17 novel somatic variants of uncertain significance (VUS) of *POLE* were found in 13 patients (13/609, 2%), and all of these variants were located at the regions out of the DNA polymerase proofreading exonuclease domain (Table 3). Of note, of these individuals, 11 were associated with MSI-H status. The general association of these novel VUS mutations with MSI-H status might indicate the collateral damage caused by the deficiency of a mismatch repair system. And the high TMB scores of these individuals, except for one with novel somatic *POLE* variants, resembled the feature of MSI-H rather than the *POLE* pathogenic mutation [31]. The sample (#9104) with TMB at 421 mut/Mb carried three mutations at exon 31 (T1322A), exon 43 (E1968G), and exon 23 (P886S), which may cause the defect in proofreading function by altering the tertiary structure of *POLE*. We subsequently performed anti–PD-L1 and CD3 IHC staining on 12 available CRC tissue samples including seven pathogenic *POLE* mutations, three VUS *POLE* mutations with MSI-H status, and two VUS *POLE* mutations with MSS status. We found the presence of abundant/high abundant CD3-positive lymphocytes infiltration in six of seven CRC tumor samples with pathogenic *POLE* mutations and all three MSI-H CRC samples with *POLE* mutations but not in MSS CRC samples with *POLE* mutations. Additionally, elevated PD-L1 expression was observed in six of seven CRC tumor samples with pathogenic *POLE* mutations and in three cases was particularly high (ID: 3634, CPS, 25; ID: 4371, CPS, 30; and ID: 3244, CPS, 80; Fig. 4C). In additional, the three MSI-H CRC samples with *POLE* mutations also displayed elevated PD-L1 expression, but not the MSS CRC samples with *POLE* mutations (Table 3).

ERBB2/*HER2* Amplification in Chinese Patients with CRC

About 3% (16/609) of the patients with CRC in this cohort harbored *ERBB2*/*HER2* amplifications, 14 of which were *KRAS* wild type. Therefore, the 16 patients with MSS CRC and *ERBB2* amplification may be candidates for *HER2*-targeted therapies (e.g., trastuzumab + lapatinib/pertuzumab/tucatinib) [32]. We also found that all (n = 10) of the *CDK12* gene amplifications, located close to *ERBB2* at 17q12, were concurrent with *ERBB2* amplification. Moreover, all 16 cases of *ERBB2* amplification occurred exclusively in MSS CRC (n = 554; Fig. 4D). Specifically, across our cohort, only 5 of 673 gene amplification events took place in MSI-H CRC (0.1 gene amplification per patient). Such a finding is significantly lower than in the MSS subcohort (1.2 gene amplification per patient; Fig. 4D). This observation likely reflects the types of mutations prone to accumulate in dMMR, with genomic rearrangements being less common through this mechanism. Additionally, 43 confirmed somatic *ERBB2* point mutations were detected in 35 patients with CRC (35/609, 6%), including 23 with MSS and 12 with MSI-H tumors.

Table 3. *POLE* genomic alterations in patients with colorectal cancer

ID	Age	Sex	Stage	MSS MSI-H	TMB	DNA change	AA change	Exon	CD3	PD-L1CPS
4371	30	M	I	MSS	336	857C>G	P286R	9	4	30
8186	42	F	II	MSS	825	857C>G	P286R	9	/	/
5892	23	M	II	MSS	525	857C>G	P286R	9	/	/
0698	32	F	IV	MSS	387	1231G>T	V411L	13	4	2
2231	47	M	IV	MSS	176	1231G>T	V411L	13	2	1
3021	68	M	II	MSS	132	1231G>T	V411L	13	3	4
9759	39	M	III	MSS	533	1331T>A	M444K	14	/	/
8381	43	M	II	MSS	279	1331T>A	M444K	14	3	5
3634	55	M	II	MSS	317	1366G>C	A456P	14	3	25
2525	36	M	II	MSS	254	1366G>C	A456P	14	/	/
3244	52	M	IV	MSS	484	1376C>T	S459F	14	4	80
						1403A>G	Y468C			
6644	62	M	II	MSS	10	89C>T	p.S30L	2	1	0
1388	39	M	IV	MSS	19	3652G>C	p.V1218L	30	1	0
7414	55	M	II	MSI-H	61	5021C>T	p.A1674V	38	/	/
0366	63	M	IV	MSI-H	78	2084T>A	p.F695Y	19	/	/
						5468G>A	p.R1823H	40		
4123	45	F	II	MSI-H	85	1225A>G	p.R409G	12	/	/
9942	71	F	III	MSI-H	74	2324G>T	p.W775L	21	/	/
2460	33	M	II	MSI-H	89	6119C>T	p.A2040V	44	/	/
2786	42	M	II	MSI-H	92	4005G>T	p.Q1335H	31	/	/
						4193_4194del	P.Y1398*	33		
3093	47	M	III	MSI-H	116	4027G>A	p.G1343S	32	4	30
1886	75	F	IV	MSI-H	116	4493C>T	p.A1498V	35	/	/
7281	55	F	II	MSI-H	100	5464T>A	p.Y1822N	40	4	8
0737	43	F	II	MSI-H	124	1823C>T	p.A608V	17	/	/
9104	50	M	IV	MSI-H	421	3964A>G	T1322A	31	3	1
						5903A>G	p.E1968G	43		
						2656C>T	p.P886S	23		

Patients with colorectal cancer harboring pathogenic (n = 11, with gray background) and novel (n = 13, with blank background) *POLE* somatic mutations were listed below. Patients with colorectal cancer (CRC) with pathogenic *POLE* mutations are younger than the entire CRC cohort (median 42 vs. 61 years), and the majority of them are male (82%, 9/11). The novel *POLE* variants were mainly associated with MSI-H status, which indicated collateral damage caused by the deficiency of mismatch repair system. The samples with pathogenic *POLE* mutations and novel *POLE* somatic mutations with MSI-H were associated with upregulated expression of PD-L1 and elevated density of tumor-infiltrating lymphocytes.

Abbreviations: AA, amino acids; CPS, combined positive score; F, female; M, male; MSI-H, MSI-high; MSS, microsatellite stable; TMB, tumor mutational burden.

Discussion

Here we show the genomic landscape of a Chinese cohort, mostly comprising patients with advanced CRC. We believe that this is the first report highlighting the high frequency of *NTRK* gene fusion in patients with MSI-H CRC, as well as the higher incidence of pathogenic *POLE* mutations in Chinese patients. Importantly, this study has immediate therapeutic implications for these patient subsets. Current CRC biomarkers, including *RAS*/*RAF*, can spare patients toxicities from therapies (i.e., EGFR inhibitors) that are not effective in this genomically defined group. Newer biomarkers, including *NTRK*, *HER2*, and *POLE*, are becoming increasingly important. Non-small cell lung cancer (NSCLC) remains the paradigm for precision medicine, and U.S. guidelines (from the National Comprehensive Cancer Center) now recommend broad panel-based testing to assess the multiple therapeutic targets in NSCLC. In CRC, there is an increasing need to simultaneously assess multiple candidate biomarkers and spare potentially repeated invasive procedures. Additionally, expanding the portion of patients with CRC who may benefit from immunotherapy approaches will require broad genomic profiling. Our data set adds to the current literature and makes several hypotheses generated by observations in Chinese patients.

MSI formerly served as a biomarker for FOLFOX-based adjuvant treatment, as observed in the ACCENT database containing clinical information from around 8,000 patients with colon cancer. Specifically, MSI-H patients at stage II

had a more favorable prognosis and could routinely be spared cytotoxic therapy, whereas patients at stage III were advised to receive adjuvant chemotherapy irrespective of MSI status [33]. Recently, MSI has been approved by the FDA as a pancancer biomarker for the PD-1 checkpoint inhibitor pembrolizumab, partly because of the extraordinary response of patients with MSI-H CRC to the therapy [4, 5]. Compared with a small number of mononucleotide MSI markers used by traditional PCR assays, NGS-based MSI detection aims to scan massive amounts of microsatellite loci more comprehensively [34, 35]. Of note, our NGS-based MSI determination algorithm, evaluating a large number of instability-prone microsatellite loci in the CRC genome, yields 100% concordance with either the classic PCR MSI or IHC mismatch repair assays or both. Additionally, the tight association between MSI-H status and TMB value reinforces the patient selection function of comprehensive genomic profiling for immunotherapy.

Around 1% of patients in this cohort carried *NTRK* gene fusions, which more frequently occurred in elderly patients (p = .03). *NTRK* gene fusion occurs at a high frequency in the MSI-H CRC cohort (7%). This finding is consistent with the previous studies reporting that *ALK*, *RET*, *ROS1*, and *NTRK* gene fusions are rare and associated with older age and MSI-H status [36–38]. The constitutive signal from the *NTRK* fusion protein seems critical for cancer development because this subset lacks common activating mutations in *KRAS* or *BRAF*. Furthermore, it has a lower frequency of *CTNNB1*- and *PIK3CA*-activating mutations. Therefore, it may provide this already treatable cohort an additional opportunity for highly effective therapy. Patients with MSI-H CRC resistant to anti–PD-1 immunotherapy may benefit from the treatment targeting *NTRK* fusion by larotrectinib and entrectinib.

Accumulating evidence from clinical trials has suggested that *ERBB2* amplification might be a potential biomarker for CRC treatment. The HERACLES trial observed a remarkable 30% objective response rate (ORR) to dual-targeted therapy (i.e., trastuzumab and lapatinib) in heavily pretreated metastatic CRC with *ERBB2* amplification but *KRAS* codon 12/13 wild type. The HERACLES trial result is also in line with data from the MyPathway basket trial, which observed 38% ORR in patients with refractory disease with the overexpression of *HER2* regardless of *KRAS* status. Together, the preclinical data from both trials supported inhibition of *HER2* with trastuzumab plus either lapatinib or pertuzumab as an effective treatment option. These results represent a breakthrough in CRC treatment, even though they apply only to about 3% of Chinese patients with MSS CRC. Although IHC and fluorescence in situ hybridization constitute the current standard for *HER2* amplification's assessment, the hybrid-based comprehensive genomic profiling is a surrogate for the evaluation of this genomic alteration, with a 98.4% overall concordance [39]. An advantage of genomic profiling is the ability to assess the copy number quantitatively, and subset analyses suggest that higher copy number may be associated with improved outcomes [17].

A key observation in our series is the finding that pathogenic *POLE* mutations exist in 1.8% of Chinese patients with CRC, which is higher than in the Western population. Our finding is preliminary and potentially confounded by the stage distribution. Similar to MSI, patients with *POLE*-mutant nonmetastatic disease have lower recurrence rates, as well as metastatic frequency, owing to enhanced immune surveillance [13]. Risk factors for *POLE*-mutant CRC are unknown. Future studies should focus on exploring the relationship between a traditional Chinese diet and how potential germline differences increase the risk of *POLE* mutation. Although the frequency of *POLE* pathogenic mutation is relatively low, the extraordinary response of patients with CRC and *POLE* pathogenic mutation to immune checkpoint inhibition makes it an attractive biomarker. CRC tumors with *POLE* mutations are constantly associated with extremely high TMB. However, a large proportion of these patients are MSS, and standard IHC or PCR alone would oversee them. Hence, the direct sequencing of *POLE* exons containing either functional domains or the indirect evaluation of TMB value of CRC tissues or both is the ideal way to discover mutations in this gene [31]. The significant mutual exclusion of *POLE* pathogenic mutation and dMMR indicates that the concurrent alteration of these two DNA proofreading and repair mechanisms might be detrimental to the cancer cell. Our data suggest that testing for *POLE* mutations in CRC holds the promise of identifying a subgroup of patients who are excellent candidates for immune checkpoint inhibitor therapy. The extensive CD3 infiltration seen in our *POLE*-mutant cases is consistent with the immunogenicity of these tumors [40, 41].

Conclusion

In summary, we confirm the feasibility and potential of identifying predictive and prognostic biomarkers in a cohort of Chinese patients with CRC by comprehensive genomic profiling. The observation that *POLE* mutations may exist at higher frequency requires further validation. If confirmed, this finding would expand the number of patients in China who may benefit from immune-based approaches.

Author Contributions

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Disclosures

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