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Original Article

Characteristic Genomic and Clinicopathologic Landscape of DNA Polymerase Epsilon Mutant Colorectal Adenocarcinomas – A Retrospective Cohort Study

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Abstract

Background and objectives: DNA polymerase epsilon catalytic subunit A (*POLE*) gene plays a crucial role in DNA repair and chromosomal replication. Mutations in the *POLE* gene have been linked to cancer, particularly colorectal carcinoma (CRC). However, the genomic landscape and pathological significance of POLE mutant CRC remain underreported. This study aimed to characterize the clinicopathologic features and genomic landscape of CRC harboring *POLE* mutations and to investigate the implications of co-occurring genetic alterations. ***Methods:*** We identified thirty-four CRC cases with *POLE* mutations from our institution's database using the next-generation sequencing gene panels including 161-gene panel for the cases of 2016–2021 and the 505-gene panel for the case of 2022–2023. We collected clinicopathologic data (age, sex, tumor site, and grading) and conducted comprehensive next-generation sequencing. Survival outcomes were assessed by reviewing patients' medical records at the time of data collection, with survival status determined based on the most recent clinical follow-up available with overall survival as the primary endpoint and a median follow-up time of 20.5 months. Statistical analyses, including chi-squared testing and CoMutation plotting, were performed using Python. ***Results:*** The enrolled 34 patients had a median age of 60.5 years (range: 37–84); tumors were in the colon (26 cases, 77%) and rectum (8 cases, 23%), with a mismatch repair deficiency rate of 29%. Next-generation sequencing analysis of a 505-gene panel revealed that *POLE* mutations were predominantly missense (89%). The mutations were distributed across various domains: 11.4% in the exonuclease domain, 25.7% in the catalytic domain, 20% in an unknown functional domain, and 42.9% in a nonfunctional domain. The average number of genomic mutations per case was 12.1 ± 12.3. CoMutation analysis identified two subsets: genomic mutation high (>5 mutations, range 6–60 mutations, n = 22) and mutation low (. Notably, TP53 mutations occurred in 55% of cases, and defects in double-stranded DNA repair proteins occurred in 47% of cases. *POLE* mutant CRC with co-occurring DNA repair mutations exhibited a significantly higher total number of genomic mutations (19.9 ± 14.4, range 7–60 mutations; chi-squared = 5.1, *p*-value = 0.02). Although a survival comparison between TP53 wild-type and TP53 mutant subgroups of *POLE*-mutant CRC is not statistical significant (p = 0.37), it showed a trend toward better survival in the TP53 wild-type group. ***Conclusions:*** Our findings reveal unique genomic landscapes in *POLE* mutant CRC, particularly with co-occurring TP53 or double-stranded DNA repair mutations, which are critical in colorectal carcinogenesis. These tumors demonstrate increased genetic instability, highlighting potential for immunotherapy.

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Introduction

Colorectal adenocarcinoma (CRC) is the third most common cancer in the United States, with an estimated annual incidence of around 150,000 in the United States.[1] Although clinical stage, histologic grade, lymph node status, and other morphologic and clinical features are important in prognostication,[2] molecular genetic classification of CRC is becoming increasingly important in both prognostication and therapeutic decision-making.[3] As the price and ease of performance of standard next-generation sequencing methods become more accessible, these features will likely become diagnostic standards among the pathology community.

The initial molecular classification of CRCs from The Cancer Genome Atlas in 2013 proposed three molecular subtypes: hypermutated (microsatellite repair protein–deficient, microsatellite unstable), chromosomal instability (Wnt pathway dysregulation), and ultramutated (frequent C to A transversions, DNA polymerase epsilon, catalytic subunit A (*POLE*)

Keywords: DNA polymerase epsilon catalytic subunit gene; *POLE*; Mutation; Next-generation sequencing; NGS; Colon; Adenocarcinoma.

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or DNA polymerase delta 1, catalytic subunit (*POLD1*) *gene* mutations).[4] In 2015, the consensus classification scheme evolved with expression-level data to include four groups: CMS1/MSI immune, CMS2/Canonical, CMS3/Metabolic, and CMS4/Mesenchymal.[5] This classification scheme was built upon expression data and machine learning algorithms and eschews the classic ultramutant phenotype associated with *POLE gene* mutations. As is evidenced by classification schemes of endometrial adenocarcinoma, tumors with *POLE* mutations and an ultramutant phenotype have significantly better outcomes and respond well to treatment with immune checkpoint inhibitors.[6] Although the frequency of *POLE* mutations is far higher in endometrial adenocarcinoma (*POLE* mutations show a frequency of 1.0–2.6% in overall populations), there are data that suggest treatment with PD-1–blocking agents in *POLE* mutant CRCs shows a significant improvement in disease-free survival.[7] However, the characteristic genomic and clinicopathologic landscape of *POLE* mutant CRCs is not well studied.

The *POLE* gene is located at Chr12q24.33 and encodes the protein DNA POLE with vital DNA proofreading functions.[8,9] The protein is composed of three known functional domains: an exonuclease domain comprising amino acids 268–471, a catalytic domain comprising amino acids 531–1,153, and a domain of unknown biological function (though mutations within this domain cause functional protein alterations) comprising amino acids 1,538–1,925.[10,11] In general, mutations in the exonuclease domain (268–471) have been most frequently associated with mismatch repair–proficient tumors showing hypermutant phenotypes.[9] However, the details of *POLE* mutation–associated molecular alterations in colorectal carcinoma are not fully investigated.

Very few large studies exist that examine the unique molecular and clinicopathologic landscape of *POLE* mutant adenocarcinomas. With the potential for more accurate prognostication and the hope for improved therapeutic options, here we present the molecular and clinicopathologic landscape of a large, single-institution cohort of CRCs with *POLE* mutations. We hypothesize that a more robust description of the unique molecular and clinicopathologic landscape of *POLE* mutant colorectal carcinomas will elucidate unique genetic features that can help with more accurate prognostication and treatment decision-making.

Materials and methods

Patient cohort

This retrospective study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki (as revised in 2024) and was approved by the Institutional Review Board (IRB) of Northwestern University School of Medicine (Approval No. STU00220535: Genomic Landscape of Colorectal Adenocarcinomas). The full ethical approval details have been described in our IRB documentation. This study was approved by the Northwestern Hospital IRB, and informed consent was obtained as part of standard presurgical intake. This study was a retrospective cohort study, and the inclusion criteria for this study were patients seen at Northwestern with a primary diagnosis of CRC who underwent routine next-generation sequencing testing as part of the standard clinical workup between 2019 and 2022 and for whom the studies revealed a *POLE* mutation, regardless of pathogenicity. No additional histologic, immunophenotypic, or molecular criteria were used in determining inclusion in this study. An internal database of all CRC cases between 2019 and 2022 that had undergone clinical next-generation sequencing studies was searched for cases that met the inclusion criteria, which identified 34 total cases (as outlined in Fig. 1). This group of cases represented all eligible patients who had been seen at Northwestern during the time frame in which the study was conducted.

Next-generation sequencing

161 gene panel: Twenty-eight of the 34 cases were sequenced using a 161-gene panel. In brief, DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) slides using the Covaris truXTRAC FFPE extraction kit (Covaris, LLC; Woburn, MA) as per the manufacturer's instructions. Sequencing was then performed using the Oncomine Comprehensive Assay (Thermo Fisher Scientific; Waltham, MA) according to the manufacturer's instructions. Sequencing data were mapped to the genome assembly GRCh37, and variants were filtered by in-house variant analysts using GenomOncology software (GenomOncology; Cleveland, OH).

505-gene panel: Six of the 34 cases were sequenced using a 505-gene panel. In brief, DNA was extracted from FFPE tissue using Purigen FFPE extraction (Bionano; San Diego, CA), per the manufacturer's instructions. Sequencing was then performed using the PGDx elio 505-gene comprehensive tumor panel (Personal Genome Diagnostics; Baltimore, MD), per the manufacturer's instructions. Sequencing data were mapped to the genome assembly GRCh37, and variants were filtered by in-house variant analysts using GenomOncology software (GenomOncology; Cleveland, OH).

p53 immunohistochemical staining

p53 protein accumulation was analyzed using an immunohistochemical approach. Immunohistochemical staining was carried out using an automated immunostainer (Leica Bond III; Leica Biosystems, Buffalo Grove, IL) and the Bond Refine Polymer™ biotin-free 3,3′-diaminobenzidine detection kit. For p53 immunostaining, the BOND Ready-To-Use primary antibody (clone DO7; Leica Biosystems, Newcastle upon Tyne, UK) was used in accordance with the manufacturer's instructions. Intense staining in tumor cells in ≥10% of tumor cells was reported as positive.

Clinical data

Clinicopathologic data were gathered from the electronic health records, including patient age at diagnosis, gender, primary tumor site (cecum, right colon, transverse colon, left colon, sigmoid colon, rectum), tumor differentiation (well, moderate, poor), presence of an associated cancer syndrome, and whether the tumor sample represented a primary or metastatic tumor. Presence of a cancer syndrome was determined based on clinical criteria, including previous germline testing. Primary survival endpoints were determined as patient death or the most recent follow-up as documented in the patient's chart. The data were collected from the patient's initial diagnosis to March 1, 2024.

Statistical analysis

Survival statistics were performed using Kaplan–Meier survival curves, and other statistical correlations were performed using chi-squared testing without adjustments for confounders. All statistical analyses were performed using either R v4.4.2 or Python v3.13. Chi-squared tests and Kaplan–Meier survival analyses were performed using the corresponding R packages (base R stats package and survminer package, respectively), while co-mutation plots were created using the CoMut Python library.[12] Statistical significance was defined as $p < 0.05$. Statistical datapoints are provided as

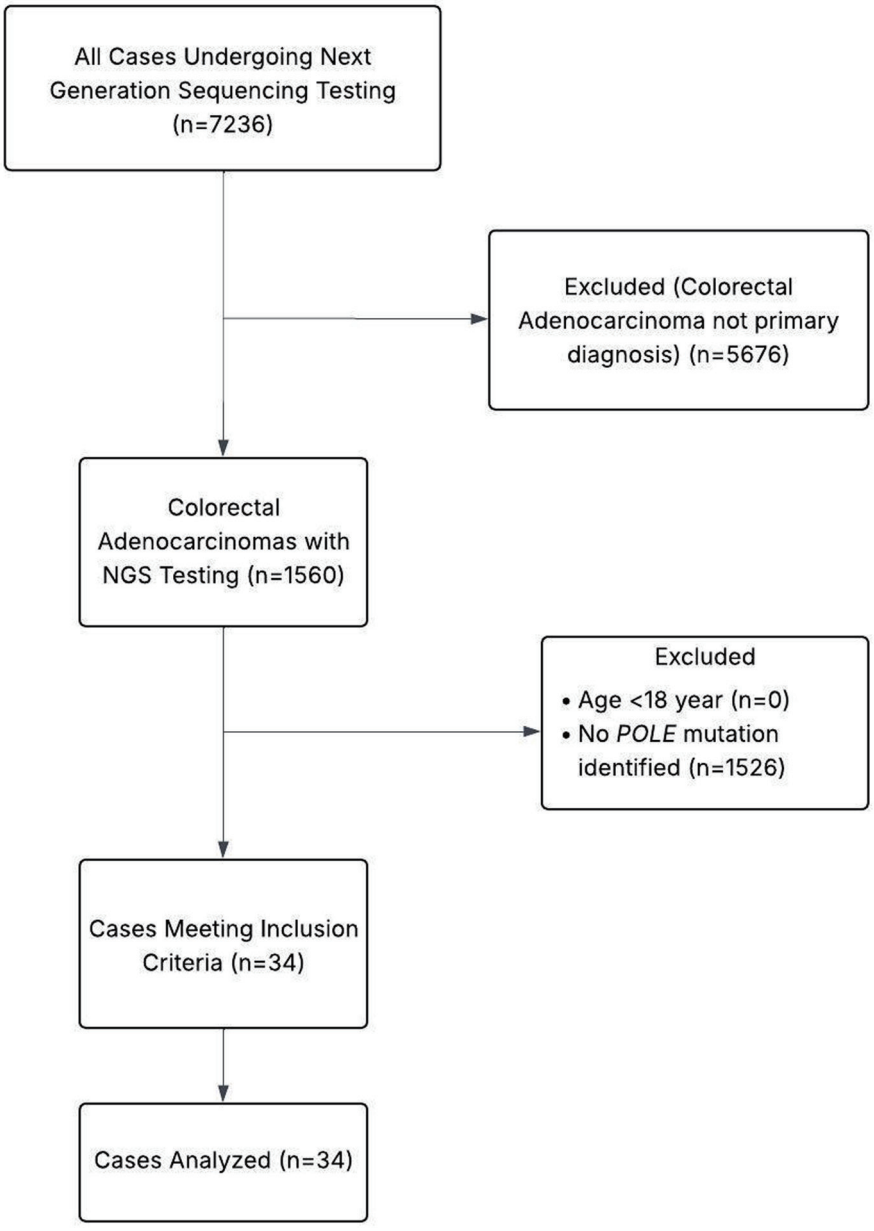

Fig. 1. Inclusion flow diagram of the study cohort. NGS, next generation sequencing; *POLE*, DNA polymerase epsilon, catalytic subunit A.

mean ± SD throughout, unless otherwise specified.

Results

Patient demographics and clinicopathologic features

Of a total of 484 CRCs with next-generation sequencing (NGS) analysis, 34 patients (14%) with *POLE*-mutant CRC were identified. The patient demographics and select clinicopathologic features are summarized in Table 1. The 34 patients with *POLE*-mutant CRC had a mean age of 60.6 ± 12.6 years (range: 37–84 years) and a male-to-female ratio of 1.6 (21 male patients and 13 female patients). Two tumors were diagnosed as well differentiated, 26 as moderately differentiated, and six as poorly differentiated. The vast majority of tumors in our cohort were primary (27 cases; 79%). The anatomic locations of the tumors were as follows: four cecal, 10 right colon, one transverse colon, three left colon, four sigmoid colon, 11 rectal, and one in a non-specified colonic site (Fig. 2a). Among the patients in our cohort, six had clinically documented cancer predisposition syndromes (one with sessile serrated polyposis syndrome and five with Lynch syndrome). Due to the relatively short time frame, no patients were lost to follow-up. The survival analysis is reported in Section 3.7 and Figure 2b.

POLE mutational profile

Among the 34 cases in the cohort, 42 *POLE* mutations were

Table 1. The patient demographics and select clinicopathologic features

ID	Age	Gender	Primary tumor site	Tumor differentiation	Primary vs. Met	TMB	MMR status (IHC)	Negative MMR By IHC
1	55	M	Right colon	Moderate	Metastasis	Not tested	Not performed	Not performed
2	55	M	Rectum	Moderate	Metastasis	Not tested	Not performed	Not performed
3	66	M	Rectum	Poor	Primary	1	MMR deficient	PMS2 absent
4	57	M	Sigmoid colon	Moderate	Primary	Not tested	Not performed	Not performed
5	76	M	Right colon	Poor	Primary	High	Not performed	Not performed
6	62	F	Cecum	Moderate	Primary	Not tested	MMR deficient	PMS2, MSH6
7	60	M	Right colon	Well	Primary	Not tested	Intact	Intact
8	84	M	Transverse colon	Moderate	Metastasis	Not tested	Intact	Intact
9	48	F	Cecum	Poor	Primary	Not tested	MMR deficient	MLH1, PMS2
10	57	M	Cecum	Moderate	Primary	Not tested	Intact	Intact
11	72	M	Right colon	Moderate	Primary	Not tested	MMR deficient	MLH1, PMS2
12	72	M	Right colon	Moderate	Primary	Not tested	MMR deficient	MLH1, PMS2
13	37	F	Rectum	Moderate	Primary	125	Intact	Intact
14	59	F	Rectum	Moderate	Primary	Not tested	Intact	Intact
15	43	M	Rectum	Moderate	Primary	52.5	MMR deficient	PMS2
16	54	M	Sigmoid colon	Moderate	Primary	Not tested	Intact	Intact
17	42	M	Left colon	Moderate	Primary	17.22	Intact	Intact
18	54	M	Left colon	Moderate	Primary	Not tested	Intact	Intact
19	51	M	Sigmoid colon	Moderate	Primary	Not tested	Intact	Intact
20	62	F	Colon	Moderate	Metastasis	Not tested	Not performed	Not performed
21	81	M	Rectum	Moderate	Metastasis	Not tested	Intact	Intact
22	53	M	Sigmoid colon	Moderate	Primary	Not tested	Intact	Intact
23	77	F	Rectum	Moderate	Primary	Not tested	Intact	Intact
24	66	F	Right colon	Poor	Primary	Not tested	MMR deficient	MLH1, PMS2
25	81	M	Right colon	Moderate	Primary	Not tested	MMR deficient	MLH1, PMS2
26	77	F	Left colon	Moderate	Primary	Not tested	MMR deficient	MLH1, PMS2
27	63	M	Right colon	Moderate	Primary	Not tested	Intact	Intact
28	60	M	Right colon	Poor	Primary	Not tested	Intact	Intact
29	39	F	Rectum	Moderate	Metastasis	2	Not performed	Not performed
30	39	F	Cecum	Moderate	Primary	43	MMR deficient	MSH2, MSH6
31	68	F	Right colon	Well	Primary	13.8	Intact	Intact
32	69	M	Rectum	Moderate	Metastasis	Not tested	Intact	Intact
33	61	F	Rectum	Poor	Primary	Not tested	Intact	Intact
34	61	F	Rectum	Moderate	Primary	Not tested	Intact	Intact

IHC, immunohistochemistry; MMR, DNA mismatch repair; TMB, tumor mutation burden.

identified (six tumors harbored two unique *POLE* mutations, and one harbored three unique *POLE* mutations). These mutations were predominantly non-synonymous missense mutations (37/42), with rarer splice site (3/42), frameshift insertion (1/42), and frameshift deletion (1/42) mutations identified. The mutations were mapped onto the known functional domains of the POLE protein and showed 22 mutations outside of known functional domains, five in the exonuclease domain (residues 268–471), nine in the catalytic domain (residues 531–1,153), and six in the domain of unknown function (residues 1,538–1,925) (Fig. 3a).

Overall mutational profile

A co-mutation plot showing the overall molecular landscape of all tumors within our cohort is shown in Figure 4a, organized by increasing number of mutations per case (analogous to tumor mutational burden). Aside from the cohort-defining mutations in *POLE*, our cohort showed frequent *TP53* mutations (19/34 cases; ~56%), in line with frequencies reported in the literature of 40–50%,[13] as well as frequent *KRAS* mu-

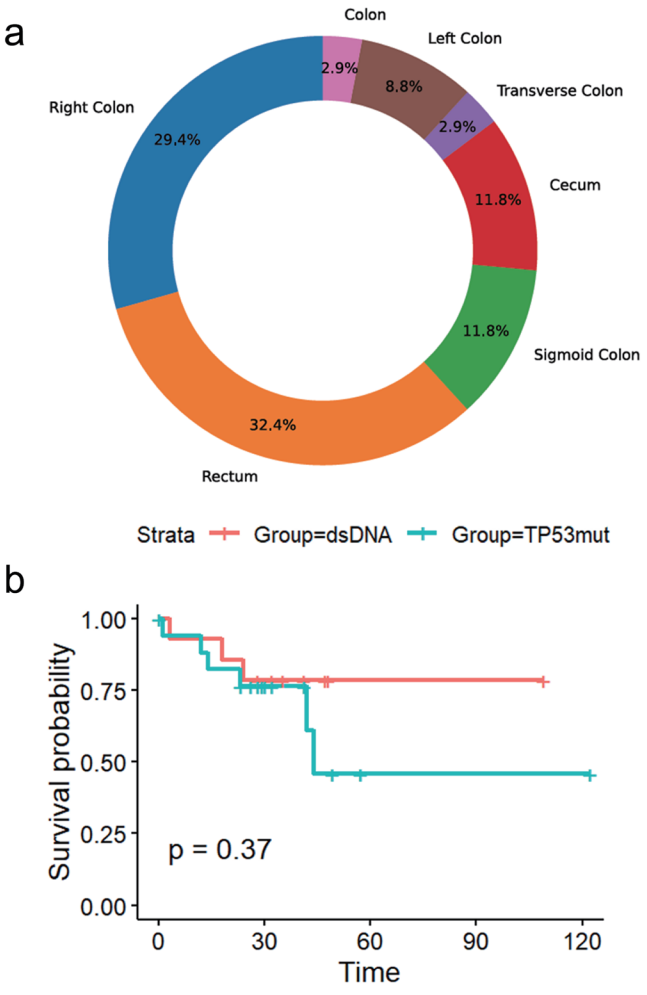

Fig. 2. CRC anatomic distribution and survival analysis. (a) Anatomic distribution of colorectal adenocarcinoma in the cohort patients. (b) Kaplan-Meier survival analysis showing *TP53* wild type (red line) vs *TP53* mutant (blue line). CRC, colorectal carcinoma; dsDNA, double-stranded DNA.

tations (13/34 cases; 38%), also in line with frequencies reported in the literature of 30–40%.[14]

We selected a subset of genes, including those that are key in double-stranded DNA break repair (*SLX4, FANCI, FANCD2, FANCA, RAD50, RAD51, BRCA1, BRCA2*), *KRAS, MSH2*, and *TP53*, and created a co-mutation plot highlighting these mutations (Fig. 4b), given their frequencies both in our cohort and in CRC as a whole. The co-mutation plot reveals two discrete subsets of colorectal carcinomas with *POLE* mutations: one subset characterized by a low number of total mutations per case (<5 mutations, n = 12), frequent *TP53* alterations (10/12; 83%), and occasional cases without *TP53* alterations but with other colorectal carcinoma drivers (*KRAS* and *MSH2*); and another subset characterized by a high mutational burden (>5 mutations per case, n = 22), with predominantly wild-type *TP53* and frequent alterations in double-stranded DNA repair proteins (*BRCA* family, Fanconi anemia family, *RAD* family, and *SLX4*), which better represents the classic ultramutant phenotype.

DNA mismatch repair (MMR) status

MMR status, as assessed by immunohistochemical staining, is included in Table 1. Overall, MMR testing by immunohistochemistry (IHC) was performed on 28 of the 34 total cases at the time of initial workup. Of the 28 cases on which testing was performed, 10 showed MMR deficiency, defined as loss of at least one MMR-associated protein by IHC staining (35.7%). The remaining cases (64.3%) showed intact staining for the MMR-associated proteins by IHC.

TP53 mutational profile

Among the 19 cases that harbored *TP53* alterations, 22 individual alterations were identified, comprising 18 missense mutations, two frameshift deletions, one frameshift insertion, and one pathogenic splice site mutation. When mapped to the functional domains of the p53 protein, all alterations were located in the DNA-binding domain (Fig. 3b), composed of exons 4–9, which is the most frequently pathogenically altered region of the p53 protein.[15] Of the 22 mutations, nine were located in common hotspots (residues R175, G245, R248, R249, R273, and R282), and the remainder represented pathogenic changes in the protein.

p53 immunohistochemical status

Due to the prevalence of *TP53* mutations and the unique molecular profile seemingly delineated by *TP53* mutational status, additional immunohistochemical analysis for p53 protein expression was performed on cases that had sufficient remaining tumor in the block to allow for immunohistochemi-

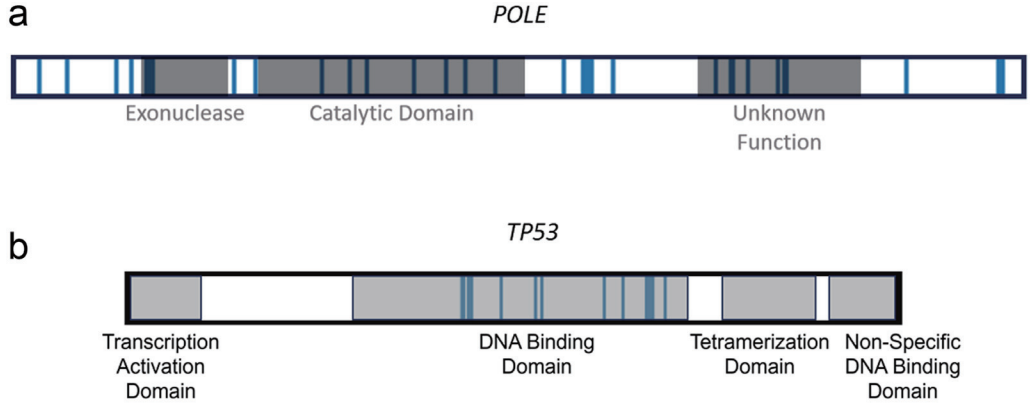

Fig. 3. *POLE* and *p53* genes. (a) Location of *POLE* mutations within the protein (vertical blue line indicates a mutation). (b) Location of p53 mutations within the protein (vertical blue line indicates a mutation). *POLE*, DNA polymerase epsilon, catalytic subunit A.

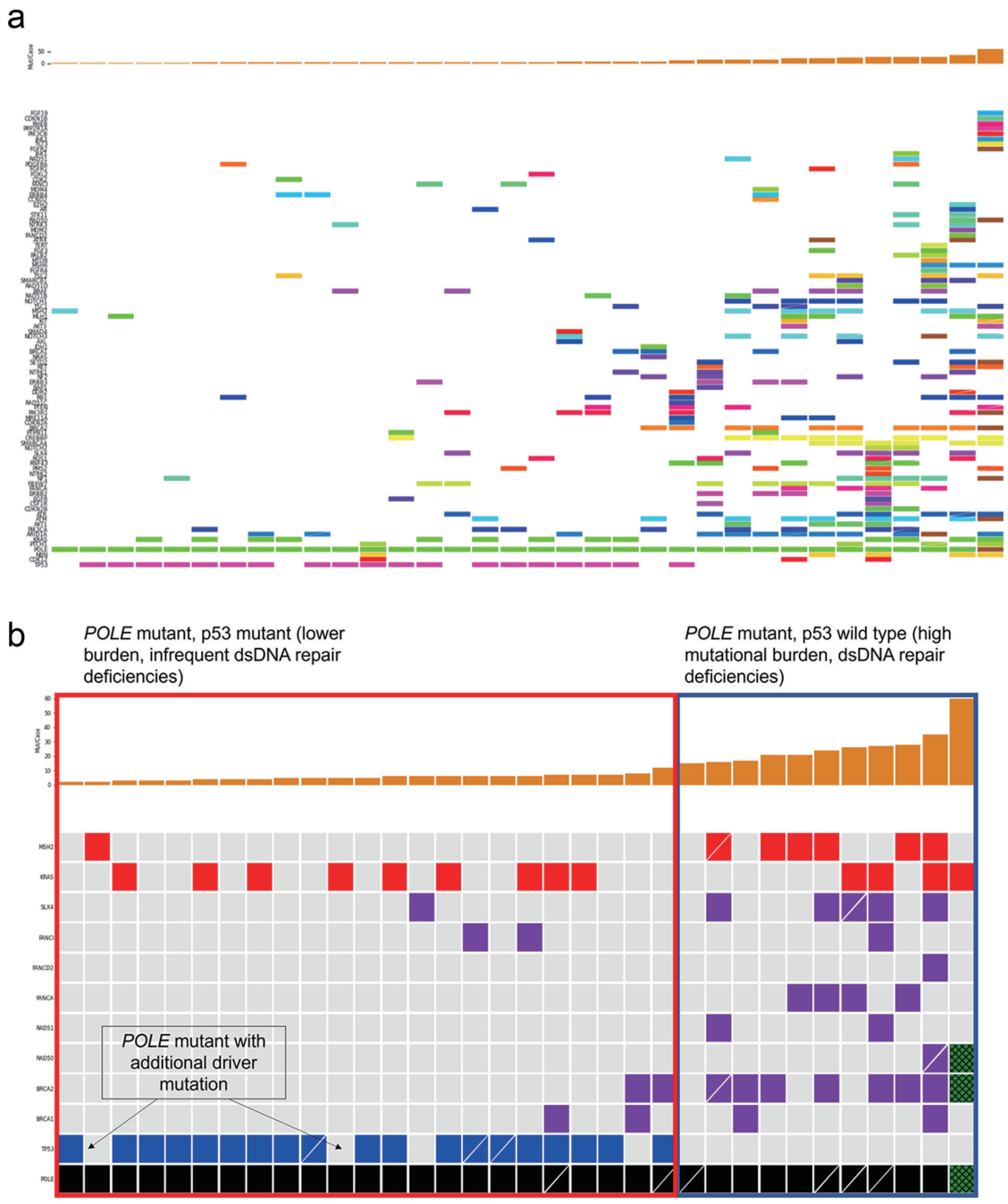

Fig. 4. Co-mutation plot and a subset genes. (a) Overall co-mutation plot showing pathogenic mutations across cases organized by increasing number of mutations per case. (b) Co-mutation plot focusing on a subset of genes (color-filled square: mutated; two color-filled triangles: two mutations; green hatch-filled square: three or more mutations). dsDNA, double-stranded DNA; *POLE*, DNA polymerase epsilon, catalytic subunit A.

cal staining as well as any future diagnostic tissue needs. In total, immunohistochemical staining was performed on four of the 19 mutant cases. All four cases showed mutant immunohistochemical staining, with three missense mutations showing diffuse overexpression and one frameshift deletion showing a null staining pattern. These findings are consistent with previous publications linking immunohistochemical staining patterns with mutation type in *TP53* within gastrointestinal tumors.[16]

Survival analysis

Given the impact that co-occurring *POLE* mutations with *TP53* mutations have on overall outcomes of endometrial adenocarcinomas, with the presence of *POLE* mutations showing a protective effect on overall survival,[17] a survival analysis was performed between groups of *POLE* mutations with or without TP53 mutations within our cohort (Fig. 2b). Due to the small sample size (n = 34), the difference did not reach statistical significance (p = 0.37); however, the

survival curve shows a pattern that closely resembles that of the well-documented endometrial adenocarcinoma survival curve. With a larger cohort, this outcome difference will likely reach statistical significance.

Discussion

The effect of *POLE* mutations on prognosis has been well documented in both endometrial and CRCs.[6,10,17,18] To date, however, the data on the unique molecular and clinicopathologic landscape of colorectal carcinoma with *POLE* mutations have lagged behind the understanding of endometrial adenocarcinoma, likely due to the lower frequency of *POLE* mutations in colorectal carcinoma.

With NGS analysis of a 505-gene panel, 34 POLE mutant CRCs exhibited the characteristic genomic landscape: 1) POLE mutations were predominantly missense mutations (89%), with 11.4% occurring in the exonuclease domain, 25.7% in the catalytic domain, 20% in the unknown functional domain, and 42.9% in the non-functional domain. 2) There was an average number of genomic mutations per case of 12.1 ± 12.3. The CoMutation plot revealed two distinct subsets of POLE mutant CRC (genomic mutation high [>5 mutations] and mutation low). 3) Unique co-occurring genetic alterations in POLE mutant CRC included TP53 mutations (19/34, 55%) and defects in double-stranded DNA repair proteins (BRCA family, Fanconi anemia family, RAD family, and SLX4) (16/34, 47%). 4) POLE mutant CRC with co-occurring double-stranded DNA repair protein mutations displayed a significantly higher total number of genomic mutations (19.9 ± 14.4, chi-squared = 5.1, p-value = 0.02).

Overall, NGS testing showed predominantly missense mutations located throughout the protein in the catalytic domain (25.7%), exonuclease domain (11.4%), unknown functional domain (20%), and non-functional domain (42.9%). The CoMutation plot revealed two discrete subgroups within the population: those with increased mutational burden (>5 mutations), which typically showed alterations within the double-stranded DNA repair protein pathway, and those with lower mutational burden, which often showed co-occurring *TP53* mutations. Our data suggest that there are at least two discrete subsets of *POLE* mutant CRCs: those that demonstrate high mutational burden and associated double-stranded DNA repair protein alterations (ultramutant phenotype), and those with low mutational burden, frequent *TP53* alterations, and worse survival.

One subset of *POLE* mutant colorectal carcinomas is driven by *POLE* mutations and subsequently accumulates large numbers of mutations and DNA repair protein alterations (high tumor mutation burden), with potential implications for immunotherapy.

Another subset is driven by alternative genetic alterations, probably due to *POLE* mutation and functional deficiency leading to *TP53* and *KRAS* gene mutations, which are the dominant driver mutations of carcinogenesis. Our analysis of the molecular mutation profile and immunohistochemical mutant protein accumulation of TP53 further indicates that all cases with p53 mutations in our cohort mapped to functional domains of the p53 protein, indicating functional alteration. The prognosis of the second subset is likely driven not by the presence of *POLE* mutations, but by the driver mutation that initiated carcinogenesis. However, we suspect that the presence of the *POLE* mutation has a protective effect, much like the analogous endometrial cohort. This is likely due to the theorized effect of *POLE* mutations increasing immune recognition of tumors and subsequent host immune response.[19] The spectrum of mutations seen in *TP53* is analogous to the alterations observed in standard CRCs. Pathogenic alterations in *TP53* are seen frequently across the spectrum of CRCs and in all types of malignancies.[20]

Immunohistochemical assessment of MMR status showed an overall MMR deficiency frequency of 35.7%, which is higher than the published general frequency of MMR deficiency in CRC of 14.2%.[21] The implication of this frequency is unclear, as it is known that increasing tumor grade is somewhat associated with increased MMR deficiency rates,[22] and a large number of tumors in this cohort were moderate to high grade. However, drawing conclusions based on this cohort is difficult due to the small cohort size and the lack of confirmatory molecular genetic testing for MSI status.

This study has several limitations. First, although the cohort of 34 cases represents one of the larger single-institution series of *POLE*-mutant colorectal adenocarcinomas reported to date, the relatively small sample size and limited follow-up duration restrict the statistical power of survival analyses. Future multi-institutional studies with larger cohorts and longer longitudinal follow-up will be essential to more definitively assess survival outcomes and prognostic implications.

Second, comprehensive assessment of true tumor mutational burden was not feasible in all cases due to the limited genomic coverage of earlier next-generation sequencing panels. Future studies utilizing uniform, large-panel or whole-exome sequencing platforms will allow for standardized tumor mutational burden calculation and more precise correlation with *POLE* mutation status and immune-related biomarkers.

Third, this analysis was performed at a single large academic medical center, which may limit generalizability across diverse demographic and geographic populations. Validation in multi-center and population-based cohorts will be important to confirm the reproducibility of these findings and their broader applicability.

Finally, *POLE* mutations were included irrespective of established pathogenicity, encompassing pathogenic variants, likely pathogenic variants, and variants of uncertain significance. While this inclusive approach allowed for comprehensive genomic characterization, it may have influenced subgroup stratification and mutation burden analyses, as not all POLE variants are known to confer an ultramutated phenotype. Future studies incorporating functional annotation, exonuclease-domain–specific classification, and emerging pathogenicity frameworks will be critical to refine the biological and clinical relevance of individual POLE variants.

Future studies with larger, multi-institutional cohorts, standardized sequencing platforms, longer follow-up, and refined pathogenicity classification of *POLE* variants will be necessary to validate and extend these findings.

Conclusions

POLE mutant adenocarcinoma represents a distinct molecular and clinicopathologic entity with two subgroups. One subgroup is characterized by traditional colorectal carcinoma driver mutations and secondary *POLE* mutations with outcomes that reflect more traditional colorectal carcinoma, and the other is driven by *POLE* mutations with a corresponding ultramutant phenotype and better outcomes. Further studies of these two subgroups may allow improved prognostication of patients with *POLE* mutant colorectal carcinoma and may support the use of immunotherapy for those with driver *POLE* mutations. Furthermore, these data suggest that the classification of *POLE* mutant colorectal carcinoma is incomplete

and requires further investigation to fully understand the impact of *POLE* mutations.

Acknowledgments

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Conflict of interest

Dr. Guang-Yu Yang is a member of the editorial board for the *Journal of Clinical and Translational Pathology.* Other authors declared no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

Author contributions

Study concept and design (GYY, ZCF, DE), acquisition of data (GYY, DE, JN, JG, JJ, LJ), analysis and interpretation of data (GYY, ZCF, DE), drafting of manuscript (GYY, ZCF, DE), revision of manuscript (GYY, DE), and study supervision (GYY). All authors have approved the final version and publication of the manuscript.

Ethical statement

This retrospective study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki (as revised in 2024) and was approved by the Institutional Review Board (IRB) of Northwestern University School of Medicine (Approval No. STU00220535: Genomic Landscape of Colorectal Adenocarcinomas). The full ethical approval details have been described in our IRB documentation. This study was approved by the Northwestern Hospital IRB, and informed consent was obtained as part of standard pre-surgical intake. The study is a retrospective cohort study.

Data sharing statement

The dataset used in support of the findings of this study is available from the corresponding author at guangyu.yang@vcuhealth.org upon request.

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