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**Emerging Role of DNA Polymerase Epsilon Non-Exonuclease Domain Mutations in Colorectal Cancer: From Sequence Variants to Clinical Implications**

POLE Non-Exonuclease Domain Mutations in CRC

Jia-Ju Xu, Chun-Xiao Ni, Jia-Ju Xu

## **Abstract**

This editorial highlights the emerging significance of non-exonuclease domain mutations (non-EDMs) in DNA polymerase epsilon (POLE) in colorectal cancer (CRC), inspired by the recent study by Taskiran *et al.* published in the *World Journal of Gastroenterology*. Their study revealed an exceptionally high frequency of POLE mutations (53.65%) in a Turkish CRC cohort, primarily attributed to a specific frameshift variant (p.V1446fs\*3) with undetermined pathogenic significance. Notably, the non-EDMs showed significant co-occurrence with mutations in critical genes, such as MLH3, MSH3, KRAS, PIK3CA, and BRAF, implying a potential synergistic interaction between impaired DNA repair mechanisms and activation of oncogenic pathways. Although POLE-mutant tumors rarely display high microsatellite instability (MSI-H), their hypermutator phenotype may make them more responsive to immunotherapy. This commentary underscores the need for functional assays and validation through multi-center studies to establish the pathogenicity and clinical relevance of non-EDMs. Furthermore, it advocates for the incorporation of comprehensive POLE sequencing, including non-EDM regions, into standard molecular subtyping frameworks for CRC to refine personalized treatment strategies.

**Key Words:** Polymerase epsilon; Colorectal cancer; Non-exonuclease domain mutations; Co-mutation; Tumor mutational burden

**Core Tip:** Building on Taskiran *et al.*'s findings of a high frequency of non-exonuclease domain mutations (non-EDMs) in polymerase epsilon (POLE), particularly the p.V1446fs\*3 variant, this editorial highlights their potential clinical significance in colorectal cancer. These non-EDMs, often co-occurring with mutations in DNA repair and oncogenic signaling genes, may define a distinct subgroup of tumors. The editorial advocates for functional validation and the integration of comprehensive POLE sequencing into molecular subtyping to unlock their potential as biomarkers for guiding immunotherapy and personalized treatment strategies.

## **INTRODUCTION**

**1** Colorectal cancer (CRC) is a malignancy with high global incidence and mortality rates<sup>[1, 2]</sup>. The significant genetic heterogeneity of CRC remains a central focus and challenge in oncology research. Within the complex molecular landscape of CRC, mutations affecting the DNA polymerase epsilon (POLE) gene, particularly those affecting its exonuclease domain (exonuclease domain mutations, EDMs), have gained increasing attention due to their association with hypermutator phenotypes and potential favorable responses to immune checkpoint inhibitors (ICIs) <sup>[3, 4]</sup>. Compared to EDMs, the biological role, clinical significance, and relationships with microsatellite instability (MSI) status and co-mutation profiles of POLE non-exonuclease domain mutations (non-EDMs) in CRC remain largely unexplored and need further studies<sup>[5]</sup>.

The recent work by Taskiran *et al.*, entitled “DNA polymerase epsilon-mutant colorectal cancers: Insights into non-exonuclease domain mutation variants, microsatellite instability status, and co-mutation profiles”<sup>[6]</sup>, which was published in the *World Journal of Gastroenterology*, directly addresses this unmet need. Their retrospective analysis of 356 Turkish patients with CRC identified 191 patients with POLE mutations, enabling a systematic characterization of these mutations. The study specifically focused on non-EDMs, detailing their distribution, correlation with MSI status, and co-mutation landscapes. The research expands our understanding of the POLE mutation spectrum, effectively redirecting attention from the established EDMs to the overlooked non-EDMs. It also highlights the high frequency of non-EDMs in populations such as the Turkish cohort and emphasizes their potential as biomarkers and active players in tumorigenesis.

This editorial aims to provide an in-depth analysis and forward-looking perspective based on the key findings by Taskiran *et al.*, integrating current knowledge on POLE mutations in CRC. We also discuss the potential functional impact of POLE non-EDMs, explain their clinical relevance, and illuminate future research directions, inspiring new viewpoints for precision medicine in CRC.

### **Revising the POLE Gene in CRC: From Classical EDMs to Neglected Non-EDMs**

The POLE protein primarily comprises two core functional domains: The C-terminal 3'→5' exonuclease domain (EDM, responsible for proofreading function) and the N-terminal DNA polymerase domain (non-EDM, responsible for DNA synthesis) [7]. Highly expressed during the S phase of the cell cycle, POLE is crucial for maintaining genomic stability and fidelity. "Hotspot" mutations within the EDM (typically referring to the exon 9 to 14 region), such as P286R, V411 L, and S459F, impair the proofreading function of the polymerase and lead to the accumulation of errors during DNA replication, triggering an ultra-hypermuted phenotype[8]. Studies have shown that compared to common hypermutated and non-hypermuted tumors, POLE-class tumors possess a higher number of nonsynonymous single-nucleotide variants (SNVs) and elevated expression levels of PD-1, PD-L1, and CD8A. Patients in this category are generally younger at disease onset, a finding corroborated by contemporary molecular profiling studies of early-onset CRC cohorts[9]. All early-onset cases carry the P286R mutation, a feature associated with POLE EDM variants like P286R[10]. These hypermutated tumors typically exhibit an exceptionally high tumor mutational burden (TMB), generating a large number of neoantigens, which may enhance sensitivity to ICIs[11]. Despite the low overall incidence of POLE EDMs in CRC (approximately 1%-2% in previous studies[12-15]), their clinical significance is considerable. Recent analyses have shown that POLE-mutant CRCs, including those with non-EDMs, are characterized by distinct clinicopathological features, most notably a high TMB and younger age, and can occur in both right- and left-sided colons[16]. More importantly, several studies and clinical cases have indicated that patients with metastatic CRC with pathogenic POLE EDMs may benefit significantly from ICIs, regardless of the MSI status[17-19]. A global study also showed that compared to patients with deficient mismatch repair (dMMR)/MSI-H mCRC, patients with mCRC and POLE/POLD1 proofreading deficiency (POLE/D1pd) achieve a superior objective response rate (ORR) and

progression-free survival (PFS) after receiving ICIs<sup>[20]</sup>. These findings establish POLE EDMs as highly promising predictive biomarkers for immunotherapy in CRC.

Compared to the well-characterized EDMs, there are relatively few studies on POLE non-EDMs, and their functional and clinical significance remain largely unknown. Non-EDMs are distributed within the polymerase domain or other regions of the POLE gene and may indirectly affect DNA replication fidelity by modulating the stability of the POLE protein and its interactions with other proteins and controlling the activity of DNA polymerase or the overall function of the replication complex<sup>[21]</sup>. Although previous studies have reported the presence of POLE non-EDMs in CRC, their precise role remains unclear<sup>[5, 17]</sup>. Notably, some of the previous studies have found that a synergistic effect occurs when POLE EDMs coexist with specific non-EDMs (*e.g.*, AA 1906, 1826-7), resulting in a significantly higher TMB than either variant type alone<sup>[5]</sup>. The study by Taskiran *et al.* reported the opposite results. Their analysis of a Turkish CRC cohort revealed an overall POLE mutation frequency of 53.65% (191/356), substantially higher than the previously reported range of 1%-12.3%. However, further analysis showed that this high prevalence was primarily driven by a specific non-EDM variant-exon 34 c.4337\_4338delTG p.V1446fs\*3, which was detected in 182 patients (95.29% of POLE-mutant cases). After excluding this high-frequency variant, the POLE mutation rate dropped to 2.53%, aligning with the mutation rates reported in other studies <sup>[12-15]</sup>. This finding highlights the need for careful interpretation of the p.V1446fs\*3 variant, whose high frequency suggests potential population-specific effects or functional relevance, despite conflicting classifications of the pathogenesis. The high prevalence of the p.V1446fs\*3 variant in this cohort underscores a pressing need for its functional characterization. Future studies should employ *in vitro* assays to assess its impact on DNA polymerase fidelity and replication rates, and utilize cellular models (*e.g.*, CRISPR-edited organoids or isogenic cell lines) to determine its effects on mutation rates, genomic stability, and cellular proliferation. *In vivo* models should also evaluate their tumorigenic potential and effects on the tumor microenvironment.

The study also identified various other non-EDMs, including p.D612N, p.R1909, p.N518fs\*10, p.Q1774, p.Q911\*, p.A1885T, and p.L1171fs\*6. Additionally, two EDM variants, namely p.Y458F and p.Y468N, were detected, each accounting for 0.52% of cases. p.Y458F has been confirmed as a pathogenic variant, whereas the significance of p.Y468N remains uncertain. Consistent with previous studies, these findings suggest a more complex and comprehensive perspective of the POLE mutational landscape in CRC and underscore the importance of systematically screening the entire POLE coding region, beyond classical EDMs<sup>[22]</sup>.

In summary, Taskiran *et al.* not only revealed the high frequency of POLE non-EDMs in specific populations but also challenged the traditional understanding of POLE mutations in CRC.

### **Role of Mutations, Microsatellite Instability, and Co-mutation Profiles in CRC**

Studies have confirmed that MSI is frequently observed in non-EDM high-TMB tumors, whereas EDM tumors display a distinct pattern of MSI<sup>[5]</sup>. Taskiran *et al.* not only delineated the distribution profile of POLE mutations in a Turkish CRC cohort but also provided valuable insights into the complex relationships between these mutations, MSI status, and co-occurring somatic mutations, explaining how POLE mutations shape the molecular landscape and clinical behavior of CRC. In their study, the vast majority (87.96%) of patients with CRC and POLE mutations exhibited proficient mismatch repair (pMMR; *i.e.*, MSI-Low [MSI-L] or microsatellite stable [MSS]), while only 12.04% of patients with CRC and POLE mutations revealed dMMR (MSI-High [MSI-H]). This finding indicates that non-EDMs represent the predominant form of POLE mutations in the Turkish population, occurring more frequently in MSS/MSI-L tumors. This observation partly aligns with previous studies suggesting that POLE EDMs are primarily found in MSS tumors and may confer a hypermutated phenotype, potentially enhancing the response to immunotherapy, similar to that observed in MSI-H tumors<sup>[23]</sup>. Consistent with prior studies<sup>[24]</sup>, the strong association between POLE mutations and MSI-L/MSS status reported by Taskiran *et al.* suggests the need for re-

evaluating the interaction between different POLE mutation types (EDMs vs. non-EDMs) and the MMR system. This association is particularly driven by the high frequency of non-EDMs in this context.

The observation that most POLE-mutant tumors in this cohort were MSS/MSI-L strongly suggests that predominant non-EDMs (especially the high-frequency p.V1446fs\*3 variant) are not sufficient to drive the ultra-hypermuted phenotype classically associated with pathogenic EDMs. It is crucial to clarify that the MSI status and TMB are distinct biomarkers for immunotherapy, albeit sometimes overlapping. The response to immunotherapy in MSS/MSI-L tumors relies on whether the specific POLE mutation confers a sufficiently high TMB, not on MSI-H. Taskiran *et al.* did not report TMB data, which is a limitation. Previous studies have reported a spectrum of functional consequences for non-EDMs. For instance, some studies indicated that certain non-EDMs are associated with an intermediate or high TMB<sup>[25]</sup>, potentially activating immune responses in an MSS background<sup>[24]</sup>. In contrast, other non-EDMs, like the majority of those reported by Taskiran *et al.*, may not drive hypermutation as strongly as EDMs, and their effects may be contingent on the MMR context<sup>[24, 26]</sup>. A study found that most non-EDMs were not associated with the characteristic genomic alterations of pathogenic POLE mutations<sup>[26]</sup>. Therefore, the potential for immunotherapy responsiveness is likely not a class effect of all non-EDMs but relies on the specific mutation and its functional impact, which must be empirically determined through TMB measurement and functional studies.

In terms of concomitant mutations, Taskiran *et al.* revealed specific co-mutation patterns in POLE-mutant CRC. MLH3 (72.25%) and MSH3 (72.25%) were the genes most frequently co-mutated with POLE mutations (especially non-EDMs), followed by KRAS (41.36%), PIK3CA (14.66%), BRAF (6.81%), *etc.* Among these, the functional relevance of co-mutations in MMR genes (MLH3/MSH3) and their potential synergistic effects with POLE deserve particular attention. The high co-occurrence of POLE mutations with MLH3/MSH3 mutations suggests a potential "double-hit" or synergistic effect, where decreased DNA replication fidelity (due to POLE mutation) and impaired

DNA mismatch repair capacity (due to MLH3/MSH3 mutations) act together. This may further increase genomic instability and elevate TMB, potentially affecting the biological behavior of the tumor and its response to treatment. One study indicated that the co-occurrence of POLE EDMs with mutations in MMR genes, including MLH3 and MSH3, may be associated with higher TMB and an MSI-H phenotype<sup>[27]</sup>. Other studies have indicated that the POLE mutation spectrum is modulated by the properties of the mutant allele, its abundance, and MMR status, suggesting an interactive mechanism between POLE and the MMR system<sup>[28]</sup>. Furthermore, studies have confirmed the association of MSH4 mutations with the MSI-H phenotype<sup>[29]</sup>, supporting the notion that mutations in non-core MMR genes can also lead to microsatellite instability.

Equally critical are co-mutations in oncogenic drivers (KRAS, PIK3CA, and BRAF), which directly affect signaling pathways and therapeutic targeting. KRAS, PIK3CA, and BRAF are common oncogenic drivers of CRC. They regulate the MAPK and PI3K/AKT signaling pathways, respectively, which play central roles in cell proliferation, differentiation, survival, and metabolism<sup>[30, 31]</sup>. Studies have confirmed that significantly more MSI-High or TMB-High samples are found in subgroups with RAS/BRAF alterations<sup>[32]</sup>. The co-occurrence of POLE mutations with mutations in these key signaling genes suggests that POLE-mutant tumors might be driven by both DNA repair defects and the activation of oncogenic signaling pathways. This complex molecular background not only affects tumor aggressiveness and metastatic potential but also has complex implications for responses to targeted therapy and immunotherapy. For instance, KRAS mutations typically predict resistance to EGFR inhibitors, while the BRAF V600E mutation is associated with poor prognosis. Here, specific combinations (*e.g.*, BRAF inhibitor + EGFR inhibitor ± MEK inhibitor) have shown clinical application potential<sup>[33]</sup>. PIK3CA mutations may suggest sensitivity to PI3K/AKT/mTOR pathway inhibitors. Therefore, understanding the presence of these concomitant mutations in the context of the POLE mutation is crucial for developing individualized treatment strategies.

Finally, other notable co-mutations (*e.g.*, TP53) add further complexity to the molecular landscape. The co-mutation analysis by Taskiran *et al.* revealed mutations in several other genes beyond the core MMR and signaling pathways, including PMS2, MSH6, ERCC5, TCF7 L2, and the critical tumor suppressor TP53. For instance, inactivation of TP53 is a well-established driver that can accelerate tumor progression<sup>[34]</sup>. To succinctly summarize the key co-mutation patterns and their implications, we have compiled them in Table 1. Collectively, the combination of multiple gene mutations detailed in Table 1 suggests that POLE-mutant CRC is not driven by a single genetic event but is regulated by a complex molecular network, where defects in DNA replication and repair (POLE, MMR genes) may establish genomic instability, which then cooperates with activated oncogenic pathways (KRAS, PIK3CA, BRAF) and loss of tumor suppressors (TP53) to drive tumorigenesis and shape clinical behavior. Insights from tumor evolution studies indicate that defects in DNA replication and repair (*e.g.*, POLE and MMR genes) may establish a permissive genomic landscape that precedes and facilitates the acquisition of driver alterations in signaling pathways<sup>[35]</sup>. Future work involving functional experiments and larger clinical cohorts is needed to decipher the interplay between these co-mutation events and their overall impact on clinical outcomes.

A notable limitation of the study by Taskiran *et al.*, which compared POLE mutation subtypes, is the limited number of EDM cases (only 2), hindering a meaningful comparison of MSI status distribution or co-mutation patterns between EDMs and non-EDMs. Therefore, future studies with larger sample sizes, particularly those including sufficient EDM cases, are needed to clarify the precise relationship between POLE mutation types and MSI status.

### **The Potential and Challenges of POLE Non-exonucleases Domain Mutations in The Precision Diagnosis and Treatment of CRC**

Taskiran *et al.* provided an in-depth analysis of POLE mutations in a Turkish CRC cohort, with a particular focus on understudied non-EDMs. This work advances the

field of precision oncology in CRC by highlighting novel research avenues. A key implication is that POLE non-EDMs, especially variants like p.V1446fs\*3, may be present at remarkably high frequencies in specific populations. It is important to note that a high TMB, which can be driven by DNA replication repair deficiencies, has been established as a predictive biomarker for response to immunotherapy independent of the MSI status. This provides a mechanistic rationale for exploring immunotherapy in MSS/MSI-L CRCs harboring specific, functionally impactful POLE mutations. These mutations often co-occur with specific MSI statuses (predominantly MSI-L or MSS) and distinct co-mutation profiles involving genes such as MLH3, MSH3, KRAS, PIK3CA, and BRAF. Collectively, these findings make POLE non-EDMs potential biomarkers with an active role in the pathogenesis of CRC, moving beyond the concept of mere "bystander" mutations.

This perspective is gaining formal recognition in the field. A recent comprehensive review on predictive biomarkers for immunotherapy in CRC explicitly categorized POLE/POLD1 mutations alongside dMMR as one of the few currently established biomarkers<sup>[36]</sup>. From a biomarker perspective, if validated, POLE non-EDMs that are correlated with specific clinicopathological features, prognosis, or treatment responses (such as to immunotherapy), particularly variants with functional impact, hold significant clinical value. While POLE EDMs are well-established biomarkers for high TMB and sensitivity to ICIs<sup>[17, 20]</sup>, it remains crucial to determine whether non-EDMs confer similar or distinct immunomodulatory effects. Emerging clinical evidence supports this potential. For instance, a recent study on neoadjuvant immunotherapy in locally advanced CRC reported that patients with POLE mutations achieved pathological complete response or clinical complete response<sup>[37]</sup>. Though preliminary, this finding provides direct clinical support for the immunogenicity of POLE-mutant tumors, including those with non-EDMs. In the study conducted by Taskiran *et al.*, most POLE-mutant cases were pMMR/MSI-L, a subgroup that typically responds well to conventional chemotherapy but gains limited benefit from ICI monotherapy. Using POLE non-EDMs to identify an ICI-benefitting subset in this population can markedly

expand the application of immunotherapy in CRC. Moreover, the frequent co-occurrence of POLE mutations, including non-EDMs, with driver mutations in genes such as KRAS, PIK3CA, and BRAF strengthens the rationale for combination therapies. The combination of ICIs may target neoantigens arising from POLE-driven genomic instability, with targeted agents against coexisting driver alterations. This strategy may yield synergistic antitumor effects and help overcome treatment resistance. However, it is crucial to emphasize that clinical evidence supporting the use of non-EDMs to guide therapy, particularly immunotherapy, is currently limited and largely hypothetical. Therefore, our conclusions regarding personalized treatment must be tempered. Prospective clinical trials that stratify patients based on specific, functionally validated POLE non-EDMs are unequivocally needed to definitively establish their predictive value for treatment outcomes, especially for immunotherapy.

However, several challenges impede the translation of POLE non-EDMs, as biomarkers, into clinical practice. Specifically, functional validation is necessary to distinguish driver mutations from passive bystanders. Although Taskiran reported a high frequency of non-EDMs, it did not provide direct evidence of their functional impact. Future studies should adopt a multi-locus molecular profiling approach, as exemplified by a recent study of a POLD1 variant carrier. In that work, sequencing multiple independent tumors and polyps from affected individuals consistently revealed hypermutation and specific mutational signatures, providing compelling evidence for the variant's pathogenicity<sup>[38]</sup>. This model underscores the need for applying similar and rigorous molecular analyses to prevalent non-EDMs like p.V1446fs\*3. The frameshift mutation p.V1446fs\*3 is expected to result in a truncated protein and loss of function; however, its precise molecular mechanisms and biological impacts necessitate further *in vitro* and *in vivo* functional experiments. Second, issues of population specificity and generalizability remain considerable, and the high frequency of p.V1446fs\*3 observed in this Turkish cohort may not be replicated in other ethnic or geographic populations. Large-scale, multi-center, prospective studies are needed to validate these findings and determine which non-EDMs represent genuine, recurrent

driver mutations. Third, standardized detection methods are essential. To ensure accurate identification of all types of POLE mutations, including non-EDMs, we recommend the use of high-quality, comprehensive next-generation sequencing (NGS) panels that cover the entire coding region of the POLE gene. Wet-lab protocols must ensure sufficiently high and uniform coverage depth to reliably call mutations<sup>[39]</sup>. Bioinformatic pipelines rely on rigorous benchmarking and calibration to ensure robust detection of SNVs, with particular emphasis on insertion/deletion (indel) mutations (*e.g.*, p.V1446fs\*3), which are known to be error-prone in standard variant-calling workflows<sup>[40, 41]</sup>. Integrating cost-effective, standardized POLE testing into existing CRC molecular diagnostic workflows represents an important future step. Notably, ongoing research into deep learning models that predict the MSI status and POLE mutations based on routine histological slides<sup>[42]</sup> offers a novel and promising screening approach. Finally, clinical interpretation and decision-making present a major hurdle. Even after confirming the functional and predictive value of non-EDMs, interpreting their significance in complex clinical contexts and formulating optimal treatment strategies necessitates extensive real-world data, potentially supported by multi-omics integration and AI-driven decision support systems.

Future studies on POLE non-EDMs should focus on several key areas. First, using molecular, cellular, and animal models, functional and mechanistic studies are needed to systematically evaluate how various non-EDM variants affect POLE protein function, cellular phenotype, and tumorigenesis. Second, large-scale clinical cohort studies that integrate comprehensive clinical, pathological, treatment response, and survival data from diverse patients with POLE-mutant CRC are pivotal to definitively establish the prognostic and predictive value of non-EDMs. Third, it is critical to explore novel therapeutic strategies by identifying synthetic lethal interactions for POLE-deficient tumors and optimizing combination regimens of ICIs with chemotherapy, targeted therapy, or radiotherapy. Finally, by determining their correlation with CRC risk, researchers should investigate the role of specific POLE mutation signatures in early detection and prevention, which may improve early warning and risk stratification.

Notably, the updated Chinese Society of Clinical Oncology (CSCO) guidelines have strengthened the recommendation for POLE testing, underscoring its growing clinical relevance<sup>[43]</sup>. This shift highlights the need for integrating comprehensive POLE sequencing, including non-EDM regions, into standard molecular diagnostics to guide personalized therapy in CRC.

### **CONCLUSION**

The study by Taskiran *et al.* opened a window into the complexity and potential significance of POLE non-EDMs in CRC. Despite the considerable challenges ahead, continued exploration in this field promises to advance precision diagnosis and treatment for patients with CRC. As the study itself emphasizes, functional validation and multi-center collaboration are essential in the next step.

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