POLE/POLD1 mutation and tumor immunotherapy

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Abstract

POLE and POLD1 encode the catalytic and proofreading subunits of DNA polymerase ε and polymerase δ, and play important roles in DNA replication and proofreading. POLE/POLD1 exonuclease domain mutations lead to loss of proofreading function, which causes the accumulation of mutant genes in cells. POLE/POLD1 mutations are not only closely related to tumor formation, but are also a potential molecular marker for predicting the efficacy of immunotherapy in pan-carcinomatous species. The association of POLE/POLD1 mutation, ultra-high mutation load, and good prognosis have recently become the focus of clinical research. This article reviews the function of POLE/POLD1, its relationship with deficient mismatch repair/high microsatellite instability, and the role of POLE/POLD1 mutation in the occurrence and development of various tumors.

Keywords: Immune checkpoint inhibitor, MSI, POLD1, POLE, Tumor, Tumor mutation burden

Background

The incidence rate and mortality rate of cancer is increasing worldwide. In 2020, 19.3 million new cancer cases and 10 million cancer deaths occurred worldwide [1]. Cancer is one of the most common causes of death worldwide, and cancer prevention and treatment have thus become a major public health concern. Traditional cancer treatment methods, including surgery, radiotherapy, and chemotherapy, have limitations. An improved understanding of the tumor microenvironment and tumor immune mechanisms in recent years led to the development of tumor immunotherapy. Immunotherapy, represented by immune checkpoint inhibitors (ICIs), has improved the long-term survival of patients with advanced solid tumors such as lung cancer, melanoma, colorectal cancer, and esophageal cancer. Among them, programmed death receptor 1 (PD-1) antibody is the most widely used. Studies have confirmed that compared with traditional chemotherapy and targeted therapy, sustained remission is possible even after discontinuing ICI treatment. The 5-year overall survival rate of patients with non-small cell lung cancer (NSCLC) treated with ICIs increased to 16% [2]. However, the efficacy of ICIs is not satisfactory. A clinical study involving multiple cancer immunotherapies showed that only 20–40% of patients responded to ICI [3]. Therefore, it is important to identify additional reliable molecular markers for predicting the outcome of ICI treatment, which would allow the identification of patients who would benefit from ICI treatment, thereby decreasing overtreatment.

Deficient mismatch repair (dMMR)/high microsatellite instability (MSI-H) was the first identified molecular marker for predicting the efficacy of ICIs [4]. In 2017, Le et al. conducted a clinical study of dMMR patients with 12 tumor types, which showed that the objective response rate (ORR) of anti-PD-1 treatment for dMMR advanced cancer was 53%, and 21% of patients achieved complete remission [5]. Although new detection techniques have been proposed to improve the sensitivity of microsatellite fragment detection, the proportion of dMMR/MSI-H is still low in current clinical practice [6]. High tumor mutation burden (TMB-H) is the second...
molecular marker for predicting the efficacy of ICIs in pan-cancer species. Several studies have shown that it is significantly related to the benefit of immunotherapy [7, 8]. In 2019, MSKCC released the largest study of TMB predicting the effect of immunotherapy so far, analyzed the data of 7,033 cancer patients of 10 cancer species, and confirmed that TMB-H was related to better overall survival (OS) after receiving immunotherapy. In addition, there are many biomarkers related to the efficacy of ICIs, such as PD-L1 expression, epigenetic changes, somatic copy number changes, and intestinal microflora [9–11]. However, these biomarkers have their own limitations; therefore, it is important to explore effective markers for predicting the efficacy of ICIs. POLE/POLD1 mutation is not only closely related to tumor formation, but also a potential molecular marker for predicting the efficacy of immunotherapy in pan-cancer species. In this article, the function of POLE/POLD1 and the effect of POLE/POLD1 mutation on tumorigenesis, prognosis, and the effect of immunotherapy are reviewed.

Functions of POLE/POLD1
DNA polymerase ε (Pol ε) and polymerase δ (Pol δ) both belong to the DNA polymerase B family, which have polymerase activity and 3′–5′ exonuclease activity. Pol ε and Pol δ can accurately select a base complementary to the template chain to extend the DNA chain and guide the synthesis of the DNA leading and lagging strands [12, 13]. In addition, Pol ε and Pol δ recognize and repair the mismatched bases through the proofreading activity of its exonuclease region [14]. Pol ε and Pol δ also function in nucleotide excision repair and double strand break repair [15, 16]. The exonuclease domains of POLE and POLD1 have the highest homology, with 23% homology and 37% similarity [17]. Therefore, most studies analyze the two genes at the same time. POLE, also known as POLEI, FILD, and CRCSI2, is located in human chromosome 12q24.3. Its encoding product POLE, the largest subunit of Pol ε, contains 2286 amino acids and has a molecular weight of 262 kDa. POLE encodes the catalytic and collation subunits of Pol ε. POLD1 is located in 19q13.3–q13.4 and is the encoding gene of the Pol δ catalytic subunit. Its molecular weight is 126 kDa, and it can encode 1,107 amino acid residues. Pol δ expression was consistent with its involvement in DNA replication, suggesting that Pol δ expression was related to the state of proliferation. The exonuclease domain of POLE/POLD1 recognizes and removes wrong bases generated during replication. Therefore, exonuclease domain mutation of POLE/POLD1 (POLE/POLD1-EDM) lead to the loss of proofreading function, resulting in the accumulation of mutated genes in cells. In addition, there are three auxiliary subunits in eukaryotes, POLE2, POLE3, and POLE4, which play important roles in cell cycle regulation [14].

In eukaryotes, an error occurs for every $10^9$ to $10^{10}$ nucleotides of DNA replication. POLE/POLD1 not only participates in DNA replication, but also plays a critical role in maintaining the fidelity of DNA replication [18]. POLE/POLD1-EDM causes a 10–100-fold increase in the mutation rate during DNA replication [19]. Albertson et al. introduced a mutation into the exonuclease domain of POLE by gene homologous recombination and compared the somatic mutation rates of homozygous mutant mice, heterozygous mutant mice, and wild-type mice in the exonuclease domain of POLE. The results showed that the POLE homozygous mutation increased the somatic mutation rate of the mouse genome and the incidence of sporadic tumors in mice. The main symptoms were small intestinal adenoma and adenocarcinoma, and the median survival was lower in POLE-mutant mice than in POLE wild-type mice. There was no difference in the survival rate between heterozygous mutant mice and wild-type mice, indicating that the POLE mutation had the greatest impact on the proofreading function after homozygous deletion [20]. POLE somatic or germline mutations are found in many tumors, including non-melanoma skin cancer, endometrial cancer (EC), colorectal cancer (CRC), melanoma, bladder cancer, esophageal cancer, and lung cancer [21]. Wang et al. analyzed 47,721 patients with different cancer types and showed that the incidence of POLE and POLD1 somatic mutations was 2.79% and 1.37%. POLE/POLD1 germline mutation is a predisposing factor for CRC, EC, ovarian cancer, and brain tumors [22].

Correlation between POLE/POLD1 mutation and TMB and MSI
DNA mismatch repair and DNA polymerase proofreading are two main mechanisms to ensure the fidelity of genome replication. These two mechanisms can occur independently or simultaneously. The most common mutation in MSI-H tumors is frameshift deletion mutation, whereas the most common mutation in POLE-mutant tumors is missense mutation [23]. Tumors with dMMR/MSI-H or POLE mutations usually show high TMB [23]. MSI-H is mainly limited to tumors in the range of 10–100 mut/Mb, whereas the TMB of POLE/POLD1 mutations can exceed 100 mut/Mb, also known as ultra-high mutation, which is associated with microsatellite stability (MSS). The loss of mismatch repair ability combined with the loss of replication polymerase proofreading ability can produce defects of full replication repair, resulting in ultra-high mutation with MSS (Fig. 1) [24]. However, not all POLE/POLD1 mutations are MSS. The
Cancer Genome Atlas (TCGA) considers POLE mutation and MSI as two important indicators of molecular typing, and many studies have confirmed the above views. In a study of EC, POLE mutation and MSI, as two independent indicators, were classified into ultra-high mutation phenotype and strong mutation phenotype, respectively (Fig. 2). In CRC, Carethers et al. classified POLE-mutant tumors and MSI tumors as strongly mutated phenotypes [25]. Church et al. found that MSI and POLE mutation did not occur in the same tumor at the same time. The incidence of MSI in EC is 18.5%, whereas all ECs with POLE mutations present with MSS [26]. Meng et al. performed immunohistochemical staining of four mismatch repair proteins (MLH1, PMS2, MSH2, and MSH6) and showed that only one of the eight cases of POLE-mutant EC had the MSH6 deficiency, whereas 46.6% of wild-type POLE EC had dMMR [27]. Some studies have shown opposite results. A study of 544 cases of EC with POLE mutations showed that the proportion of MSS and MSI was similar, and MSI lacking MLH1 methylation was the most common type. This suggests that POLE could be used as a candidate mutant gene for screening for Lynch Syndrome [28]. In 2020, Mo et al. found that CRC with POLE-EDMs was more prone to MSI-H, and all patients had high TMB, with an average of 200.8 mut/Mb [29]. In 2019, proteomic studies showed that colon cancer with MSI-H was mainly enriched in mismatch repair pathways and POLE and BRAF mutations [30].

For tumors with both POLE mutation and MSI-H, the sequence of POLE mutation and dMMR is a research hotspot. Most MSI cases are caused by methylation of the MLH1 promoter. If dMMR precedes POLE mutation, similar proportions of MLH1 methylation can be expected in MSI patients of the POLE-wild-type and POLE-mutant type. However, wild-type POLE patients account for more than 80% of MLH1-silenced patients, and mutant POLE patients account for less than 50% of MLH1-silenced patients, providing opposing evidence for the MSI priority model [31]. Temko et al. explored the timing of POLE mutations during cell carcinogenesis and found that POLE mutations could also be detected in precursors of POLE-mutated tumors [32]. In addition, mutation of the tumor suppressor gene PTEN is considered to be an early event in the pathogenesis of EC. In the analysis of pathogenic PTEN mutations in EC, researchers found that PTEN mutations were more prevalent in POLE-mutated tumors than in POLE-wild-type tumors with pMMR or dMMR [33]. In addition, because dMMR may occur later than other mutation processes, it can be wrongly identified as MSS at an early stage. Therefore, the pathogenic somatic POLE mutation is considered an early or even initial mutation, and its accompanying mutant phenotype determines the unique carcinogenic pathway. Repeat assessment of microsatellite status at a later stage of the disease may yield different results from those obtained initially, which may provide a new direction for clinical diagnosis and treatment. Hwang et al. observed that the deficiency of MSH6 and MSH2 preceeds POLE mutations. These authors suggested that POLE mutation was a secondary mutation to dMMR,
leading to a significant increase of TMB in tumors with concurrent dMMR and POLE mutations through their effects on POLE function [23]. To further clarify the interaction between POLE mutations and MSI and their effect on tumorigenesis, He et al. divided tumors into different categories according to the clone ratio of POLE mutations. They found that POLE-mutated tumors were more prone to MSI-H than wild-type POLE tumors [34]. In conclusion, current evidence supports that POLE mutations are a driver of dMMR in tumors with both POLE mutations and MSI-H, ultimately leading to the occurrence of MSI-H.

Pathogenic and non-pathogenic mutations of POLE/POLD1
Most current studies indicate that POLE mutation is related to ultra-high mutation load. However, some studies do not support this theory. Hwang et al. analyzed the POLE-mutant phenotype and found that tumors with a large number of POLE mutations do not show an abnormally high TMB [23]. These authors further explored the associated immune microenvironment and found that many POLE mutations may be non-functional mutations that fail to initiate an immune response [23].

The location of POLE mutations may be the dominant factor determining tumor phenotype and clinical outcome. POLE-EDMs can cause loss of proofreading function, resulting in the accumulation of mutated genes in cells. However, not all mutation sites in the exonuclease domain are pathogenic. The five most common mutation sites are P286R, V411L, S297F, A456P, and S459F, and are known as hotspot mutations [35]. To clarify the mutation site that best defines the pathogenic mutation of POLE, Castillo et al. analyzed 530 EC cases from TCGA including 82 cases with POLE mutations, of which 59 were in the exonuclease domain and 23 were outside the exonuclease domain. The results showed that EC cases with one of the five hotspot mutations had a characteristic genome sequence that differed from that of wild-type POLE EC with MSI-H or MSS. The researchers developed a practical scoring system by measuring the proportion of TMB, C > A, T > G, C > G, and used index scales as the scoring criteria (C > A over 20% = 1, T > G over 4% = 1, indels below 5% = 1, C > G below 0.6% = 1, TMB over 100 mut/Mb = 1, recurrent variant in EC=1). To define the threshold of pathogenicity, the POLE scoring system was applied to cases with POLE hotspot mutation, POLE non-hot spot mutation, and POLE-wild-type in TCGA. Of the 41 cases with POLE hotspot mutation, 38 had a score > 5, and the remaining 3 cases had V411L mutation with a score of 4, whereas the POLE-EDM scores of non-hotspot mutations were lower. Therefore, a score ≥ 4 was used to define the pathogenicity of POLE mutations, a score ≤ 2 was classified as non-pathogenic mutations, and a score of 2–4 was defined as uncertain mutations [35].

In addition, a non-exonuclease domain mutation of POLE/POLD1 has been found in 3–4% of CRCs and ECs [17]. Although there is not enough evidence to support its pathogenic role, a small number of studies have confirmed the pathogenic mutation of POLE outside the exonuclease domain. Some studies have confirmed that the V1368M mutation of POLE destroyed the function or structure of the protein and was considered as a pathogenic mutation [36]. Garmezzy et al. also found pathogenic missense or frameshift mutations outside the exonuclease domain of POLD1 [37].
The relationship between the POLE/POLD1 mutation and common tumors

The POLE/POLD1 mutation and endometrial carcinoma

As early as 2013, TCGA classified POLE-mutant EC as a special molecular type and showed that POLE-mutant status is present in 10% of EC patients [38]. The somatic mutation rate of POLE is approximately 6.9%, and it usually shows MSS. Roberts et al. detected 30 POLE mutations in 535 cases of EC (5.6%) [39]. Church et al. found that 48 of 788 cases of EC (6.1%) had POLE mutations [40]. In FIG03 EC, the mutation rate of POLE is 15–22% [18, 26, 38]. In addition, common gene mutations in EC such as PTEN, PIK3R1, PIK3CA, FBXW7, and KRAS have a high mutation rate in this type of EC [38]. Castillo et al. found five hotspot mutations in a data analysis of ECs, which confirmed that mutations at different loci of POLE can have different functional effects [34]. The researchers also explored the complex relationship between POLE hotspot mutation and dMMR/MSI-H in EC. EC with both POLE hotspot mutation and MSI-H is rare, occurring only in 4.3% of cases. However, the associated TMB is high (median TMB is 339.0 mut/MB), which is consistent with that of cases with both POLE hotspot mutation and MSS. The median TMB of tumors with POLE non-hotspot mutation and MSI is 207.1 mut/MB. The median TMB of EC with POLE non-exonuclease domain mutation and MSI is 48.5 mut/MB [34]. Therefore, different POLE mutation sites can lead to different degrees of tumor mutation load. In another study of 173 cases of EC, 13 cases had POLE mutations, including 6 cases of P286R mutation and 2 cases of V411L mutation [25]. V411L is usually independent of the catalytic site, and its mutation may lead to tumorigenesis through secondary changes in the configuration of the DNA binding domain. P286R is a stronger missense mutation than V411L, which further indicates that mutations at different sites of POLE may lead to different somatic mutation profiles [40]. The POLD1 mutation is very rare in EC. Briggs et al. showed that women with POLD1 S478N mutation had a significantly increased risk of EC [17].

POLE-mutant EC is a unique clinical entity with a high immune response and good clinical prognosis. TCGA reported that patients with POLE-mutant EC had a younger age of onset and lower recurrence and mortality rates [38]. Billingsley et al. found that among 534 EC patients, 30 (5.6%) had POLE mutations and one recurred, with a recurrence rate of 3.4%, whereas the recurrence rate of POLE-wild-type patients was 17% [27]. Similarly, Church et al. showed that the recurrence and mortality rates of POLE-mutant EC patients were significantly lower than those of POLE-wild-type patients [40]. POLE-mutant ECs have a young age of onset (< 60 years old) and are mainly found in stage I EC patients. The mutation characteristics are different from those of MSI and are related to a good clinical prognosis [39]. EC with POLE hotspot mutation has a good prognosis and can achieve better progression-free survival. It can be used as a prognostic molecular marker to guide the treatment of patients with grade 3 EC [41, 42].

The POLE/POLD1 mutation and colorectal cancer

CRC is one of the most common malignant tumors of the digestive system. Zhu et al. confirmed that POLE mutations were associated with increased risk of gastrointestinal tumors, and POLE germline mutation might be an effective molecular marker for predicting survival and metastasis [43]. The incidence of somatic mutations in the exonuclease domain of POLE in CRC is approximately 3%, which is higher than that of germline mutations [44]. In a study of 6517 CRC patients, Glaire et al. found that POLE somatic mutations are more common in men, the right colon, and early stage patients, and are associated with a good prognosis [45]. Kane et al. found that the POLE P286R somatic mutation occurs in human CRC. The site of P286R is close to motif I, which is located in amino acids 271–285 and is necessary for the exonuclease function, as well as being next to the DNA binding site. Therefore, the P286R mutation affects the binding of the exonuclease domain to DNA and the proofreading function [46]. Palles et al. detected the L424V and POLE S478N germline mutations in multiple colorectal adenoma and CRC [47]. L424V is a conserved site in the exonuclease domain of the DNA polymerase B family. The germline mutation can reduce the fidelity of replication related polymerase proofreading and generate base substitutions, resulting in an increase in mutation rate and eventually leading to tumor occurrence [47]. Therefore, germline mutations can easily develop into multiple colorectal adenomas and CRC. The POLD1 serine 478 is highly conserved in eukaryotes. The characteristics of colorectal tumors with POLD1 S478N mutation are basically similar to those with the POLE L424V mutation [47]. Although the incidence of germline mutations in exonuclease domain of POLE in familial colorectal adenoma or CRC is only 0.1–0.25%, inheritance of POLE germline mutations significantly increases the risk of CRC [48, 49]. Rohlin et al. found POLE Asn363Lys germline mutation in another hereditary tumor family. This family not only has inherited CRC, but also a high risk of EC, ovarian cancer, brain tumors, and other extraintestinal tumors. This site plays an important role in the binding of the exonuclease to substrates and is considered to be more oncogenic than the L424V mutation, thereby potentially leading to a variety of tumors [50].

There are few studies addressing the relation between POLE mutation and the prognosis of CRC patients. In
2016, Domingo et al. showed that mutant POLE CRC had a younger age of onset than wild-type POLE CRC, which was an independent prognostic factor relative to BRAF and KRAS mutations [45]. Other studies showed no significant difference in prognosis between POLE-mutant and POLE-wild-type CRC patients. However, the mortality of POLE-mutant patients who reach stage 3 or above and who are treated with adjuvant or palliative chemotherapy is significantly higher than that of POLE-wild-type CRC patients [51]. The effect of POLE mutation on the prognosis of CRC patients remains unclear, and further research is needed.

The POLE/POLD1 mutation and non-small cell lung cancer
Song et al. analyzed 319 NSCLC patients and found that 2.8% of patients had POLE mutations [52]. Tobacco smoke, ultraviolet radiation, and alkylating agents are associated with high mutation load in NSCLC patients. Ultraviolet-related characteristics are almost only found in the squamous cell carcinoma subtype [20]. Min et al. reported that the V1446fs frameshift mutation of POLE was the site associated with the highest incidence of NSCLC (56.8%) [53]. In a study of the Chinese population, P286R and F699Vfs*11 were identified as the hotspot mutations of POLE in lung cancer, whereas the hotspot mutations recorded in the COSMIC database are P286R and V411L. P286R is the most common mutation in both the Chinese population and the COSMIC database, whereas F699Vfs*11 may be the most frequent mutation in the Chinese population. F699Vfs*11 is a frameshift mutation in the DNA polymerase type B ε subfamily catalytic domain that leads to the insertion of a termination codon, resulting in truncated, immature, or nonfunctional proteins. According to its location and type, F699Vfs*11 is generally considered a destructive mutation that may result in the destruction of a key polymerase domain and damage the function of the entire protein [54]. Due to the limited number of POLD1 mutations, no hot spot changes of POLD1 was found. The current analysis showed that the POLE mutation may be through the MMR, TGF-β, and RTK/RAS/RAF signaling pathways affecting tumor development, while the POLD1 mutation may affect tumor development through the MMR signaling pathway [55].

The expression of TMB, PD-L1, and CD8+ tumor infiltrating lymphocyte is higher in POLE-mutant NSCLC patients than in POLE-wild-type patients. Therefore, POLE mutations may be candidate biomarkers for immunotherapy response in NSCLC patients [52]. However, there are important clinical differences between lung squamous cell carcinoma and lung adenocarcinoma. Liu et al. analyzed data of 513 patients with adenocarcinoma and 497 patients with squamous cell carcinoma from TCGA cohort, and the results suggested that POLE mutation is an effective biomarker of improved OS in patients with lung squamous cell carcinoma, whereas it has no effect on OS in patients with lung adenocarcinoma [56]. Further analysis of the lung adenocarcinoma population showed that patients with both PD-L1 overexpression and POLE mutation have significantly better OS [57]. Data from previous studies suggest that DNA repair status and TMB of lung adenocarcinoma could be used as independent biomarkers. Zhang et al. also confirmed that high expression of POLD1 was associated with poor prognosis of lung adenocarcinoma [58]. In a study of lung squamous cell carcinoma, Chae et al. confirmed that mutations in the DNA repair pathway are associated with high TMB [59]. However, these two subtypes are often grouped together in immunotherapy research, and few studies have explored their potential differences in the response to ICIs. The association between DNA repair pathway mutations and the treatment of different subtypes of NSCLC needs to be confirmed in a large number of basic studies.

POLE2, the second largest subunit of the Pol ε family, is involved in various cell functions, and POLE2 mutations can affect cell repair, replication, and cell cycle regulation. Li et al. suggested that POLE2 promotes NSCLC proliferation and growth. This study verified the oncogenic effect of POLE2 on NSCLC using biological tests [57]. A study found that β-elemene inhibited the proliferation of lung cancer cells, and gene chip assays were used to detect β-elemene treated A549 cells. The results showed that intracellular POLE2 was the most significantly down-regulated gene, and deletion of POLE2 inhibited proliferation and colony formation in A549 and NCI-H1299 cells, thus inducing apoptosis of tumor cells. Silencing of POLE2 inhibited the growth, proliferation, and apoptosis of tumor cells in vitro [57].

The POLE/POLD1 mutation and other solid tumors
Studies show that POLE2 is highly expressed in patients with esophageal squamous cell carcinoma (ESCC). In vivo and in vitro experiments show that POLE2 down-regulation inhibits cancer cell proliferation and migration and promotes apoptosis. High expression of POLE2 predicts tumor deterioration and poor prognosis in patients with ESCC, suggesting that POLE2 is a potential therapeutic target for preventing or delaying the progression of ESCC [60]. In addition, gastric adenocarcinoma patients with POLE/POLD1 mutations usually show adaptive immune resistance to the tumor microenvironment, loss of mismatch repair protein, increased PD-L1 expression, and higher TMB, suggesting that the
**POLE/POLD1** mutation could be used as a biomarker to improve the clinical efficacy of precision medicine in gastric adenocarcinoma patients [61].

**POLE S297F** somatic mutation is detected in ovarian cancer. Unlike the common P286R and V411L mutations, S297F interacts with site 275 of the exonuclease catalytic region and affects the structure of the active site [62]. Detection of **POLE** hotspot mutations (P286R and V411L) in 251 samples of different subtypes of ovarian cancer in China showed that the **POLE** S297F mutation is more common in ovarian endometrioid carcinoma than other types of mutations, supporting that the **POLE** S297F mutation may actively participate in the pathogenesis of ovarian endometrioid carcinoma [62]. In 2018, Bhangoo et al. reported that the detection of **POLE** mutation and TMB could improve the therapeutic potential of ICIs in patients with refractory uterine sarcoma [63]. In addition, familial nonmuscular invasive bladder cancer caused by a G178R germline mutation of **POLD1** has also been reported, which has enriched the newly discovered genetic mutation pattern of this disease [64].

**The POLE/POLD1 mutation, immunotherapy and prognosis**

A high TMB is considered a molecular marker of the response to immunotherapy. Its mechanism is related to increased tumor immunogenicity and T cell activity caused by a high TMB. At present, there are few studies on the correlation between **POLD1** and the efficacy of ICI, and most of them focused on **POLE** mutations. Pathogenic mutations of **POLE** are closely related to a high TMB. In a correlation analysis of **POLE-EDM** and immune-related gene expression in TCGA [54, 65], Mo et al. found that **POLE-EDM** was positively correlated with significant CD8+ cytotoxic T lymphocyte (CTL) infiltration, and cytotoxic T cell effect markers were significantly upregulated. Further studies showed that CD8+CTL, CD45RO+memory immune cells (MIC), and CD8+CD45RO+MIC were significantly more abundant in **POLE-EDM**s than in **POLE-wild-type** and **POLE** non-exonuclease type. Further combined with MSI status, CD8+CTL, CD45RO+MIC, and CD8+CD45RO+MIC in MSI-H tumors were lower than those in **POLE-EDM** tumors, but higher than those in MSS. This suggest that regardless of MSI status, **POLE-EDM** tumors have a stronger T lymphocyte infiltration ability and a stronger antitumor activity [28].

To determine the effect of **POLE/POLD1** mutation on immunotherapy efficacy, Wang et al. collected and analyzed gene detection data of 47,721 patients with different types of cancer. Multivariate Cox regression analysis after adjustment for MSI status and tumor type showed that **POLE/POLD1** is an independent risk factor for immunotherapy benefit [21]. Other studies showed that the expression of immune checkpoint related proteins such as PD-1 and cytotoxic T lymphocyte associated antigen-4 (CTLA-4) is increased in **POLE**-mutated EC, and the number of tumor-infiltrating T cells is high. One possible underlying mechanism is that **POLE** mutations lead to tumor hypermutation and increase the expression of tumor neoantigens [66, 67]. In 2015, Howitt et al. showed that the tumor neoantigen produced by EC with **POLE** mutation is 15-fold higher than that of MSI and more than 100-fold higher than that of MSS [68].

**POLE-EDM** has a positive effect on prognosis. Studies show that only a small proportion of somatic mutations in tumor cells are driver mutations, resulting in the inability of cancer cells to complete normal differentiation, thus becoming malignant proliferating cells characterized by uncontrolled growth and division. In addition, most of the mutations are passenger mutations. In the past, passenger mutations were thought to be unrelated to the occurrence and progression of tumors. However, in recent years, studies have shown that accumulated passenger mutations can destroy tumor cells, slow down their growth, and even promote cell death through different mechanisms, such as the production of toxic proteins, induction of immune responses, or loss of tumor cell function. Loss of the proofreading function of **POLE**-mutant tumors leads to a large number of wrong bases produced during replication. Passenger mutations that accumulate in cells may lead to the slow growth and even death of tumor cells with **POLE** mutation, which may underlie the favorable prognosis of **POLE**-mutant tumors. Some studies have explored the prognosis of patients with **POLE** mutation treated with ICIs. Rizvi et al. reported that four NSCLC patients with **POLE** mutation achieved a progression free survival of 8–14 months after treatment with pembrolizumab, suggesting that the efficacy of anti-PD-1 therapy is higher in NSCLC with **POLE** mutation [69]. Min et al. also showed that the level of infiltrating T cells in **POLE**-mutated NSCLC is increased, resulting in a favorable prognosis [53]. The KEYNOTE-028 study reported that a **POLE**-mutated patient with advanced EC achieved partial remission after 8 weeks of treatment with pembrolizumab and achieved continuous remission for more than 14 months [70]. Other studies have found that patients with **POLE** mutations outside the exonuclease domain could still have a lasting response to ICI, through the analysis of multiple large genome data sets. An analysis of 1,278 patients with advanced cancer with low/moderate TMB treated with ICIs showed that the occurrence of **POLE** missense mutations outside the exonuclease domain was associated with better overall survival [36]. Therefore, although the **POLE** mutations outside the exonuclease
domain do not lead to high mutations, they may enhance immunogenicity through a mechanism independent of new antigens.

Conclusions

**POLE/POLD1** is involved in the development of multiple tumors. Patients with **POLE/POLD1**-mutated tumors are characterized by a young age and good prognosis, suggesting the potential of **POLE/POLD1** mutation as a new indicator of prognosis. **POLE/POLD1** mutation is closely related to a high mutation load, increased neoantigens, and increased intracellular immune cell infiltration in tumors. However, whether it is a potential biomarker to predict the efficacy of immunotherapy, needs more clinical data. Relevant clinical studies are ongoing (NCT05103969, NCT03810339). But, the incidences of **POLE/POLD1** mutations are low and their locations differ, which may be a dominant factor in its clinical limitations. In addition, studies on **POLE/POLD1** mutations have not been extended to all tumor types, and there are relatively few studies on the morphological and immunohistochemical expression rates of **POLE/POLD1**-mutated tumors. Furthermore, the differences in **POLE/POLD1** mutation frequency among different regions and races need to be further verified. Further multi-center prospective clinical studies are needed to identify new diagnostic and treatment methods for **POLE/POLD1**-mutant tumors.

**Abbreviations**

ICI: Immune checkpoint inhibitors; PD-1: Programmed death receptor 1; NSCLC: Non-small cell lung cancer; dMMR: Deficient mismatch repair; MSI-H: High microsatellite instability; ORR: Objective response rate; TMB-H: High tumor mutation burden; OS: Overall survival; Pol ε: Polymerase ε; Pol δ: Polymerase δ; Exonuclease domain mutation of **POLD1**-EDM: Exonuclease domain mutation of **POLD1**; **POLE**/**POLD1**-mutant tumors.

**Acknowledgements**

Not applicable.

**Authors’ contributions**

XTM and LY designed the article form and wrote the manuscript. XL and KO consulted and browsed the literature. LD and LY revised the manuscript. All authors read and approved the final manuscript.

**Funding**

Not applicable.

**Availability of data and materials**

The references supporting the conclusions of this article is included within the article.

**Declarations**

**Ethics approval and consent to participate**

The authors declare that they have no competing interests.

**Consent for publication**

Not applicable.

**Competing interests**

Not applicable.

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Received: 6 May 2022 Accepted: 17 June 2022 Published online: 02 July 2022

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