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Trends in Cancer

Review

Targeting the DNA Repair Enzyme Polymerase θ in Cancer Therapy

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Targeted cancer therapies represent a milestone towards personalized treatment as they function via inhibition of cancer-specific alterations. Polymerase θ (POLQ), an error-prone translesion polymerase, also involved in DNA double-strand break (DSB) repair, is often upregulated in cancer. POLQ is synthetic lethal with various DNA repair genes, including known cancer drivers such as *BRCA1/2*, making it essential in homologous recombination-deficient cancers. Thus, POLQ represents a promising target in cancer therapy and efforts for the development of POLQ inhibitors are actively underway with first clinical trials due to start in 2021. This review summarizes the journey of POLQ from a backup DNA repair enzyme to a promising therapeutic target for cancer treatment.

Highlights

POLQ is a versatile DNA repair enzyme that is central in TMEJ for the error-prone repair of DNA DSBs. POLQ also functions in other DNA repair pathways including base excision repair, interstrand crosslink repair, and DNA damage tolerance by translesion synthesis.

Cancer cells often acquire mutations in DNA repair genes, making them dependent on remaining DNA repair pathways. Dependence on TMEJ is characterized by an increased POLQ expression which is associated with poor patient prognosis.

Depletion of POLQ in POLQ-dependent cancers leads to synthetic lethality. This is well described for malignancies deficient in homologous recombination (e.g., due to mutations in *BRCA1* or *BRCA2*). Hence, the use of POLQ inhibitors might be a promising strategy for targeted cancer therapy.

POLQ: Exploiting a Cancer Vulnerability for Therapy

To increase efficiency and lower the burden of toxic side effects, a major goal of cancer therapy is to progress from a 'one-drug-fits-all' to an individualized treatment approach tailored to the tumor-specific molecular features. Two main targeted therapeutic strategies are currently utilized in cancer treatment, both exploiting cancer-specific vulnerabilities. In the first approach, therapeutic suppression of aberrantly upregulated oncogenes alleviates the growth advantage of cancer cells. The second approach is based on the phenomenon that genetic alterations acquired by tumor cells cause their dependency on other compensatory pathways, loss of which leads to **synthetic lethality** (see Glossary). Therefore, therapeutic inhibition of pathways that are synthetic lethal with a cancer-specific alteration evokes cellular death in tumor cells while leaving normal cells unharmed [1]. The recent advent of genome-wide genetic interaction studies has demonstrated the extensive number of synthetic lethal interactions in cancer, many of which can potentially be translated to targeted cancer therapies [2].

Cancer cells frequently acquire mutations in DNA repair genes and respond by rewiring their DNA repair network to utilize compensatory pathways for survival. Dependency on compensatory DNA repair pathways opens room for the development of cancer-specific small molecule inhibitors. A group of successful drugs that use this mode of action are poly(ADP-ribose) polymerase (PARP) inhibitors, approved for the treatment of BRCA-deficient cancers. The essentiality of PARP for cancer cells with loss-of-function mutations in BRCA1/2 is remarkable as such cancer cells are up to 1000 times more sensitive to PARP inhibitors than healthy cells [3,4]. Although challenges such as the acquisition of drug resistance need to be faced, the clinical success of inhibitory drugs targeting DNA repair enzymes is highly encouraging. In this context, the DNA-repair enzyme **polymerase θ** (POLQ) has received increasing attention. POLQ is upregulated in numerous cancers and its overexpression is associated with poor prognosis [5–9]. Moreover, synthetic lethal interactions between POLQ and multiple DNA repair genes, including factors involved in homologous recombination (such as BRCA1/2), have been identified [10–16]. For these reasons, POLQ inhibitors, currently in development in multiple biotech companies and laboratories, represent a promising cancer treatment strategy and are soon to be tested in clinical trials.

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In this review, we first focus on the unique protein structure that allows POLQ to fulfill its diverse roles. We further discuss conflicting evidence of whether POLQ suppresses or promotes genetic stability, given that it is an intrinsically error-prone DNA synthesis enzyme. Finally, we address why POLQ meets the criteria of a promising target in cancer therapy and summarize the state-of-the art in POLQ inhibitor development.

POLQ Structure and Function: A Versatile DNA Repair Enzyme with a Unique Domain Architecture

POLQ Is Central in POLQ-Mediated End Joining, a DNA Double-Strand Break Repair Pathway

POLQ is involved in the repair of DNA double-strand breaks (DSBs), the most cytotoxic type of DNA lesion. If unrepaired, DSBs can have deleterious consequences including genomic rearrangements and cell death. Therefore, a specialized network consisting of at least three pathways is responsible for their repair (Figure 1A). Most DSBs are repaired by **canonical nonhomologous end joining** (c-NHEJ), a pathway that directly religates DNA ends without extensive processing, by introducing small insertions and deletions at break sites [17]. In S and G2 phases of the cell cycle, when a sister chromatid is available, **homologous recombination** (HR) is favored as the only precise DSB repair pathway [18]. POLQ is involved in a third pathway [originally named alternative end joining (alt-EJ) or microhomology-mediated end joining (MMEJ)] that was later termed **polymerase theta-mediated end joining** (TMEJ) due to requirement of POLQ [19]. TMEJ is initiated by PARP1 recruitment to resected DNA-ends [20–22]. Upon activation by phosphorylated CtIP, 3′ overhangs are generated by helicases such as the MRE11–RAD50–NBS1 (MRN) complex. POLQ then binds to long single-stranded DNA (ssDNA) overhangs generated by 5′–3′ resection of DSBs and anneals sequences with 2–6 base pairs of microhomology to use them as primers for DNA synthesis [23–25]. The stabilized DNA ends are then ligated by LIG3–XRCC1 or LIG1 [26–28] (Figure 1A).

Repair by TMEJ is error prone and introduces characteristic sequence alterations, also called **mutational signatures** with two characteristic attributes. Firstly, since POLQ uses microhomologies for strand annealing and yet only a minority of DNA ends contain such regions, end resection is necessary to make microhomologies accessible. The end joining of resected DNA at microhomologous sequences may result in characteristic **microhomology-flanked deletions** [29]. Secondly, POLQ tends to abort template-dependent extension from an annealed microhomologous sequence and reanneal at secondary sequences. This results in short stretches of *de novo* DNA that resembles the sequence flanking the break, also called **templated insertions** [30]. Templated insertions can originate from the opposite strand (in *trans*) or from the same strand (in *cis*), when the protruding ssDNA snaps back on itself [24,31]. Most interestingly, templated insertions can be utilized to map genome-wide TMEJ activity and by doing so, TMEJ most likely contributes to a variety of loci mutated in human disorders, emphasizing TMEJ's role in the etiology of human diseases [30].

DNA DSB repair pathways are tightly regulated. In the G1 phase of the cell cycle, during which a sister chromatid for HR is unavailable, association of the highly abundant Ku-heterodimer and 53BP1 with free DNA ends inhibits end resection, thereby channeling repair towards c-NHEJ [32]. In G2 and S phases of the cell cycle, however, 53BP1 is removed from DNA ends by phosphorylated CtIP in complex with BRCA1 and MRN, thereby shifting the balance to favor HR. Since TMEJ and HR both require resected DNA ends, they directly compete with each other for the same substrate. POLQ appears to displace RAD51, a key HR factor, from ssDNA via a proposed RAD51-binding domain [12] and may also counteract RPA, another HR factor [33]. Furthermore, depletion of HR

Glossary

5′ deoxyribose phosphate lyase activity: the catalytic activity of cleaving the ribose phosphate linkage 5′ to an abasic site. Since dRP-lyase activity is usually preceded by a DNA-lyase that cleaves the ribose-phosphate linkage 3′ to the abasic site, dRP-lyase activity results in removal of the 5′ deoxyribose-5-phosphate at the abasic site.
Base excision repair: a repair pathway that is responsible for removing small, non-helix distorting base lesions such as alkylated, deaminated or oxidized bases.
Canonical non-homologous end joining: a DNA DSB repair pathway which, in contrast to HR, does not depend on a homologous repair template and joins the broken DNA ends after minimal modification.
Homologous recombination: an umbrella term for several pathways dedicated to the accurate repair of DNA DSBs using a homologous chromosome segment as a template.
Microhomology-flanked deletion: a characteristic scar that is introduced by TMEJ in DNA DSB repair. Since POLQ anneals sequences with microhomologies to prime DNA synthesis, the break point is characterized by a stretch of microhomology while the sequence that was originally between the microhomologies is lost.
Mutational signature: combinations of mutation types originating from the same mutational process, which can be endogenous (e.g., lack of a certain DNA repair pathway) or exogenous (e.g., exposure to UV light).
One-ended DNA DSB: a DNA DSB that only has one 5′ end and one 3′ end. Such a break is generated when DNA replication encounters a DNA single-strand break followed by replication fork collapse, or when the replication fork stalls and a nuclease cleaves one arm.
Polymerase θ: a DNA repair enzyme that acts in numerous DNA-repair pathways, most importantly in TMEJ. The only eukaryotic polymerase known to date that also contains a helicase domain.
Polymerase theta-mediated end joining: a DNA DSB repair pathway that depends on the activity of POLQ. This leaves a particular mutational signature that is characterized by microhomology-flanked deletions and/or templated insertions.

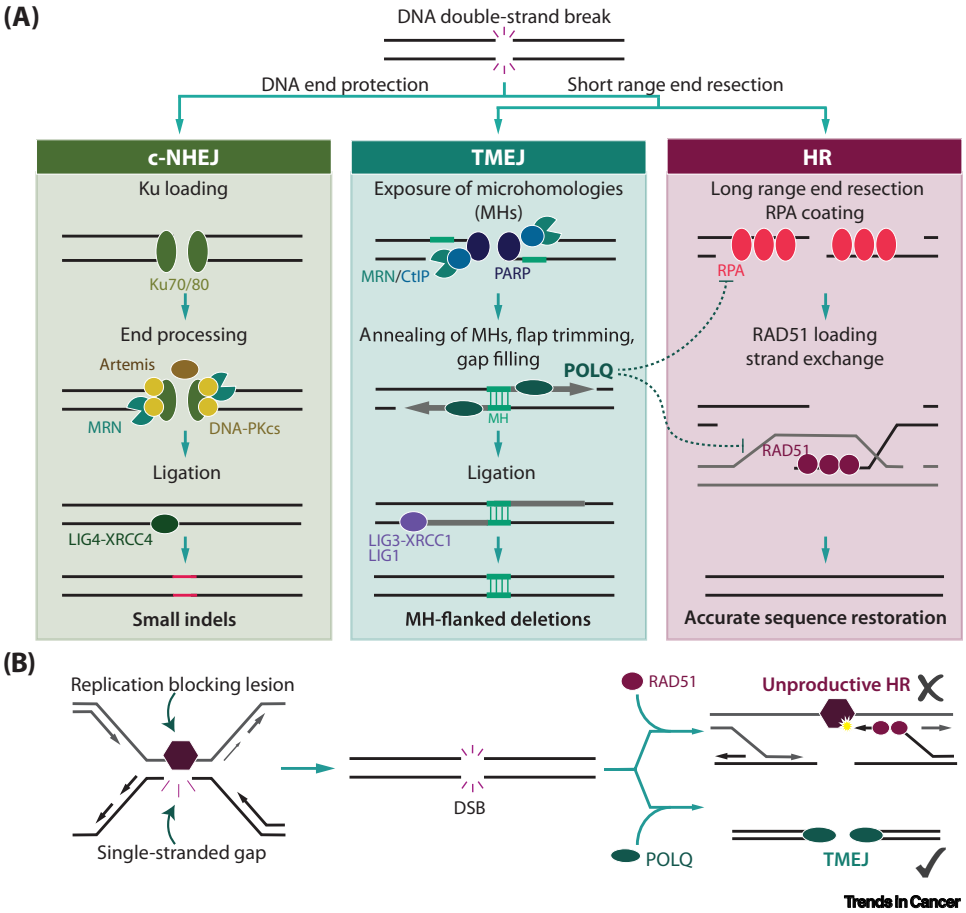

Figure 1. Roles of POLQ in DSB Repair. (A) DSBs can be repaired by three main pathways: c-NHEJ is characterized by DNA end protection by 53BP1 and Ku70/80 and DNA end processing by several factors including the MRN complex (MRE11–RAD50–NBS1) and Artemis. DNA-PKcs then recruits LIG4 with its scaffolding partner XRCC4 for ligation of processed ends. TMEJ and HR share the initial DNA end resection step. After recruitment of PARP, the MRN complex processes the DNA ends to generate 3′ overhangs. In TMEJ, POLQ anneals exposed sequences of microhomology, using them as a primer for DNA synthesis, followed by sealing of DNA ends by LIG3–XRCC1 or LIG1. In HR, the first short-range end-resection step is followed by long-range end resection and coating of 3′ single-stranded DNA with RPA. RAD51 then induces strand exchange using a homologous repair template for accurate restoration of the original DNA sequence. Competition between TMEJ and HR for resected DNA ends is highlighted by POLQ displacing RPA and RAD51 from ssDNA. (B) Certain types of DNA lesions depend on TMEJ for repair. Upon replication, replication-blocking lesions that are associated with regions of under-replicated DNA are converted into DSBs. Since the sister chromatid is unavailable as a repair template due to persistence of the replication blocking lesion, HR is unproductive, leaving TMEJ as the only remaining repair pathway available. Abbreviations: DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSB, DNA double-strand break; HR, homologous recombination; MH, microhomology; NHEJ, nonhomologous end joining; c-NHEJ, canonical NHEJ; PARP, poly(ADP-ribose)polymerase; POLQ, polymerase θ; TMEJ, polymerase theta-mediated end joining.

Synthetic lethality: the phenomenon that the combined loss of two genes causes cell death whereas the individual deficiency of either gene does not.
Templated insertion: a characteristic scar that is introduced by the action of TMEJ in DNA DSB repair. POLQ frequently aborts extension from one annealed sequence and reanneals at a secondary sequence to restart DNA synthesis, thereby generating small stretches that resemble the sequence around the DSB.
Terminal transferase: an enzyme that catalyzes the template-independent addition of nucleotides to the 3′ terminus of DNA.
Translesion synthesis polymerase: a specialized polymerase that can synthesize DNA opposite DNA lesions. The bypass of damaged DNA sites by translesion polymerases avoids stalling of replication forks.

proteins such as BRCA1, BRCA2, or RPA, increases the TMEJ-specific mutational signature suggesting that TMEJ factors are negatively regulated by HR factors [25].

Notably, due to its high mutagenicity, TMEJ has been considered merely a backup DNA repair pathway. However, it is becoming increasingly evident that TMEJ also functions in the presence of other DSB repair pathways and might be the only available pathway for specific types of DNA lesions [22]. Such lesions include collapsed replication forks with sister chromatids containing

replication-obstructing lesions (e.g., an interstrand crosslink) rendering them an unsuitable repair template for HR [34] (Figure 1B). In *Caenorhabditis elegans*, POLQ has been shown to be indispensable for repair of G4 quadruplex structures, thereby preventing genomic rearrangements at the expense of small deletions [19]. Future research is needed to assess the precise regulation of TMEJ and HR in order to identify the conditions and in particular the types of DNA lesions, that depend on TMEJ activity.

POLQ Is Involved in DNA Damage Tolerance and Repair Pathways beyond DSB Repair

Increasing evidence suggests that POLQ is involved in DNA damage tolerance and repair of lesions other than DSBs. POLQ can function as a **translesion synthesis polymerase** and thus incorporates nucleotides opposite apurinic/apyrimidinic sites, thymine glycols, and thymidine dimers [35–40]. In addition, POLQ has been shown to be important for replication and the repair of replication-associated lesions [41,42]. Depletion of POLQ results in decreased replication fork velocity and an increased amount of stalled replication forks upon treatment with hydroxyurea, a chemical used to induce replication fork stalling [12]. DNA single-strand breaks that are converted into DSBs upon encountering replication forks might also depend on repair by POLQ [11]. Furthermore, while POLQ appears to be essential for the repair of interstrand crosslinks (ICLs) in *Drosophila*, *Arabidopsis*, and *C. elegans* [43–45], most studies in mammalian systems demonstrate that POLQ is not required for this type of repair, potentially due to redundancy with other TLS polymerases [10,46]. A few exceptions have been reported: POLQ knockout (KO) mouse embryonic fibroblasts (MEFs) have been shown to be hypersensitive to mitomycin C, an ICL-inducing agent, and higher levels of micronuclei in response to mitomycin C were observed in POLQ mutant mice [15,47]. Finally, based on the presence of a weak **5′-deoxyribose phosphate lyase** activity in its polymerase domain, POLQ was suggested to act in **base excision repair** (BER) [48], although the extent of its involvement is a matter of debate [49–52]. In conclusion, POLQ is involved in multiple DNA repair pathways but deeper insights into both the variation between model organisms as well as the mechanistic function of POLQ in each pathway are lacking.

The Unique Domain Architecture of POLQ Enables Its Diverse Functions

POLQ encodes an A-family polymerase that contains both an N-terminal conserved superfamily 2 helicase domain and a C-terminal DNA polymerase domain, linked by an unstructured central region (Figure 2). As such, POLQ is the only eukaryotic polymerase known to date that contains a helicase domain. A coordinated interplay between all domains is necessary to allow for execution of POLQ activity [53] (Figure 2). The polymerase domain is responsible for DNA synthesis either using its **terminal transferase** or templated extension activity. Despite its low sequence conservation, the central domain appears to be important for regulating POLQ substrate selection. A mutant version of POLQ lacking its central domain can perform TMEJ on short ssDNA substrates (≤26 nucleotides) whereas full-length POLQ cannot [53]. Finally, the helicase domain contains both ATPase and helicase activity as well as proposed RAD51-binding motifs that may displace RAD51 from ssDNA in an ATPase-dependent manner [12,35,54–56]. This domain is required for performing TMEJ on longer ssDNA substrates since binding of the polymerase domain alone results in an unproductive snap-back mechanism [53]. In summary, POLQ contains a helicase domain capable of competing with HR for resected DNA-ends and a polymerase domain for strand annealing and extension, connected by a flexible central region.

POLQ and Genomic Stability: A Repair Enzyme That (De)stabilizes the Genome

Whether POLQ suppresses or promotes genomic instability is a matter of debate. Biochemical studies have shown that POLQ polymerase activity has low fidelity and its involvement in DSB repair frequently culminates in large deletions and templated insertions [29,30,57].

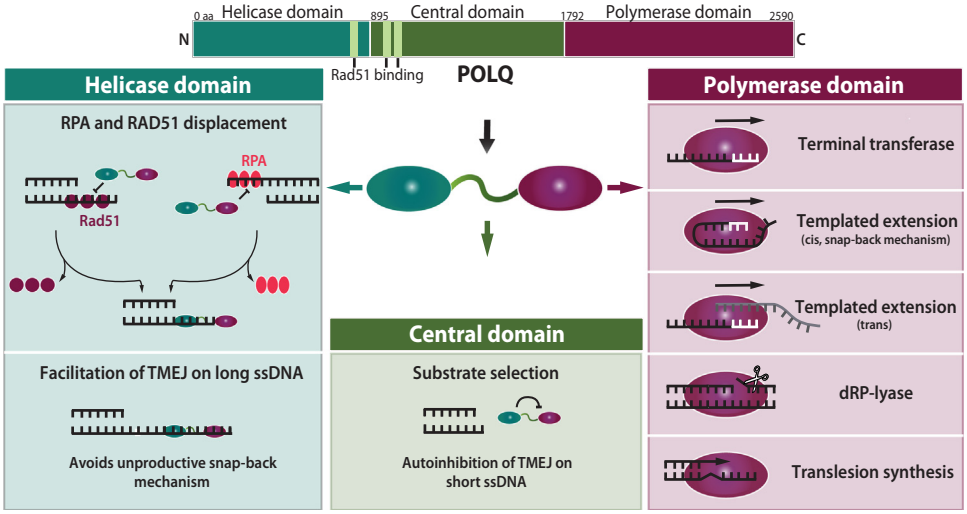

Figure 2. POLQ Has a Unique Domain Architecture Enabling Its Diverse Functions. POLQ consists of three domains: an N-terminal helicase domain that is linked to a C-terminal polymerase domain by an unstructured central region. Each domain fulfills specific functions that in combination contribute to the diverse functions of POLQ in DNA repair and damage tolerance pathways. The helicase domain (left panel) counteracts RPA and RAD51, thereby impeding repair by homologous recombination. In addition, helicase binding adjacent to the polymerase domain avoids an unproductive snap-back mechanism and therefore facilitates TMEJ on long ssDNA substrates. The central domain (middle panel) is vital for substrate selection as it autoinhibits POLQ activity on short ssDNA. The polymerase domain (right panel) of POLQ is a 'Swiss Army knife' in DNA repair: in TMEJ, it can function as a terminal transferase or catalyze templated extension from an annealed sequence using both the same strand snapped back on itself (in *cis*) or the other strand (in *trans*). In addition to double-strand break repair, the polymerase can function as a dRP-lyase in base excision repair and perform translesion synthesis opposite UV lesions. Abbreviations: dRP, 5′ deoxyribose phosphate; POLQ, polymerase θ; ssDNA, single stranded DNA; TMEJ, polymerase theta-mediated end joining.

Beyond *in vitro* systems, various studies performed in mouse and human systems have yielded conflicting findings both supporting and opposing its role as a guardian of genomic stability (Table 1). POLQ has been shown to protect genomic stability: its depletion increases DSB formation, exacerbates sensitivity to various genotoxic agents, and destabilizes replication forks [10,12,14,46,52,58]. Other studies, however, have reported that POLQ depletion decreases chromosomal translocations and UV-associated mutations and its overexpression increases DNA damage markers and impairs cell cycle progression [6,13,40]. Furthermore, POLQ overexpression in numerous cancer types including lung, bladder, ovarian, uterine, and breast cancer is associated with an increased mutation load and poor clinical outcome [5,6,12,59,60]. Along these lines, both a mutagenic effect of POLQ at the nucleotide level and a stabilizing effect at the chromosomal level have been described [40]. Here, POLQ was shown to be indispensable for mutagenic translesion synthesis opposite UV-induced lesions. However, upon UV exposure, POLQ-depleted cells acquired more chromosomal aberrations compared to wild-type (WT) cells, most likely as a consequence of reduced replication fork stability. Importantly, POLQ-deficient mice have an increased incidence of skin cancer, suggesting that POLQ promotes replication through UV-induced DNA lesions and therefore might prevent replication fork collapse. In the absence of translesion synthesis, unreplicated ssDNA might be converted into **one-ended double-strand breaks** and potentially chromosomal translocations, if not repaired properly [40]. This is in stark contrast to another study in which suppression of POLQ substantially decreased chromosomal translocations [13]. We hypothesize that the discrepancy between these two investigations originates from two main differences. (i) The protective effect of

Table 1. Does POLQ Promote or Suppress Genomic Stability?

POLQ status	Model system	Consequence	Refs
POLQ promotes genomic stability			
mut (S1932P, polymerase domain)	Mouse	- Increased spontaneous and radiation induced micronuclei in erythroblasts	[10,47]
KO	Mouse	- Sensitivity of clonal bone marrow stromal cells to IR and DSB-inducing agents (bleomycin, etoposide, ICRF-193 and camptothecin)	[46,51]
KO	Mouse embryonic fibroblasts	- Increased DSB formation - Reduced fork progression through UV lesions - Sensitivity to UV - Elevated sister chromatid exchanges - Elevated chromosomal aberrations - Elevated unreplicated ssDNA	[40]
KO	Mouse	- Increased incidence of skin cancer	[40]
KD	Human laryngeal cancer cell line (SQ20B), human cervical cancer cell line (HeLa)	- Sensitivity to IR - Increased IR-induced γH2AX foci	[52]
KO	Human bone osteosarcoma epithelial cells (U2OS)	- Sensitivity to cisplatin and IR	[14]
POLQ suppresses genomic stability			
Over-expression	Immortalized human lung fibroblasts (MRC5-SV)	- Accumulation in S-phase - Increased DNA damage markers (γH2AX, pCHK2) - Lower replication fork speed - Elevated chromosomal aberrations	[6]
KD	Mouse embryonic fibroblasts	- Decreased telomere fusions in the absence of the shelterin complex (Trf1/Trf2) and c-NHEJ factor Ku80	[13]
KD	Big blue mouse embryonic fibroblasts (BBMEFs)	- Reduced UV-induced mutations	[40]

HU, hydroxyurea; IR, ionizing radiation; KD, knockdown; mut, mutated.

POLQ most likely originates from its function in translesion synthesis which, although being intrinsically mutagenic, protects from cancer-driving chromosome rearrangements while the destabilizing effect likely stems from its role in TMEJ [13]. (ii) We speculate that the engagement of POLQ in the repair of one-ended and two-ended DSBs might differ substantially in its outcome.

POLQ is evolutionary conserved in metazoans and plants, illustrating its importance in genome stability. Despite its error-prone activity, repair by POLQ is often the safer option compared to other processes that act in its absence and potentially result in gross genomic aberrations. Understanding the role of POLQ in maintaining genome stability requires more in-depth studies and will provide more insight into whether POLQ-activity in various DNA repair and damage tolerance pathways is driving or protecting from tumorigenic progression.

POLQ and Cancer: A Novel Candidate for Targeted Cancer Therapy

POLQ Is Overexpressed in Cancer, Associated with a Characteristic Mutational Signature and Poor Prognosis

The overexpression of POLQ in a variety of malignancies, including those of colon, rectum, lung, stomach, breast, ovary and head and neck, sparked the interest in POLQ as a novel cancer target [5,6,8,9,12,61]. In breast and lung cancer, POLQ upregulation is linked to poor prognosis and shorter relapse-free survival of patients [7,9]; therefore, POLQ is included in a gene panel whose expression is used to predict cancer aggressiveness [62,63].

Whether POLQ overexpression is causative for cancer progression or occurs as a protective mechanism in genomically unstable cancer cells remains elusive. Since TMEJ activity is known to generate genomic translocations, it is intuitive to assume that POLQ upregulation contributes to carcinogenesis. Nonetheless, several arguments support a model in which POLQ expression is upregulated just after malignant transformation [12,64]. Based on several studies, we discuss two potential mechanisms that explain cancer-related POLQ upregulation. (i) The proliferative advantage of cells with elevated POLQ expression within the tumor might lead to their expansion in a Darwinian model. This model is supported by findings that high POLQ expression allows cancer cells to tolerate increased replication stress and might therefore increase tumor fitness [65]. (ii) POLQ expression might also be induced by a specific signaling mechanism. The depletion of HR genes was shown to increase POLQ expression and this could be reversed by complementation of HR factors, proposing a negative regulation of POLQ expression by the HR pathway [12]. As a direct link between HR deficiency and POLQ overexpression, depletion of BRCA1/2 is thought to upregulate FANCD2 which recruits POLQ to DNA lesions [64]. However, this is difficult to reconcile with the observation that FANCD2 and POLQ share a synthetic lethal relationship and further work is needed to clarify this interaction [12,66]. Furthermore, the tumor suppressor p53 influences POLQ expression, as shown by an up to 20-fold higher POLQ expression levels in *TP53* mutated cells compared to WT cells [67].

Another piece of evidence supporting HR-directed POLQ upregulation comes from the analysis of cancer genomes. POLQ-mediated repair translates into a particular mutational signature, which is increased in frequency in breast, ovarian, and pancreatic cancers, all associated with HR deficiency [68,69]. In addition, templated insertions, another feature of TMEJ activity, are more prevalent in genomes of breast cancer patients with *BRCA1/2* germline mutations [70]. Thus, it seems plausible that cancer cells lacking an intact HR pathway use POLQ-dependent repair as a compensatory mechanism to maintain genome stability.

POLQ Is Synthetic Lethal with Genes Frequently Mutated in Cancer

Depletion of POLQ in an HR-deficient background has been shown to impair cell viability, proposing a synthetic lethal relationship between POLQ and HR factors [10–13,66,71] (Table 2, depicting genes with a validated synthetic lethal or synthetic sick relationship with POLQ). Yet, the exact mechanism of the synthetic lethality between POLQ and HR factors is poorly characterized. We postulate two models to explain this; one focusing more on the role of POLQ in TMEJ (the pathway model) and one on its effect on RAD51 (the RAD51 model) (Figure 3). In the pathway model, an HR-deficient cancer relies on POLQ due to its activity in TMEJ. Continuous proliferation of cancer cells causes chronic replication stress and therefore an increased load of DSBs when collapsed replication forks are not resolved. While such DSBs would be repaired by HR in healthy cells, HR-deficient cancer cells depend on TMEJ for their repair. The observation that inhibitors of LIG3 and LIG1, both acting in TMEJ, synergize with PARP inhibitors in human breast cancer cell lines, supports this model [72]. In the RAD51 model, an HR-deficient cancer cell relies on POLQ due to its antirecombinase activity. Upon depletion of POLQ, the increased RAD51 activity in HR-deficient cells is cytotoxic by an unknown mechanism [12,71]. This model is supported by a series of sophisticated complementation studies in HR-deficient cells, where re-expression of POLQ lacking its RAD51-binding domain does not rescue cellular survival in POLQ-depleted, HR-deficient cancer cells to the extent of WT POLQ cells [12]. Furthermore, loss of RAD51 in a POLQ- and HR-deficient setting rescues cellular survival, suggesting that increased RAD51 activity is toxic to HR-deficient cells [12,71]. Yet, colony-formation assays of BRCA1-depleted cells lacking the RAD51-interaction domain of POLQ have shown that the interaction with RAD51 is dispensable for HR-deficient cells [33]. Both models potentially

Table 2. Validated POLQ Synthetic Lethal and Synthetic Sick Genes

POLQ synthetic lethal gene	Model system	Depletion of POLQ	Depletion of synthetic lethal gene	Double depletion phenotype reported	Refs
ATM	Mouse	LOF mutation (S1932P, polymerase domain)	LOF mutation	- Neonatal lethality - Growth retardation - Enhanced genomic instability	[10]
	Human ovarian cancer cell line (A2780)	KD	Inhibitor Ku55933	- Reduced cellular viability	[12]
ATR	Human bone osteosarcoma epithelial cells (U2OS)	KO	KD, inhibitor VE822	- Enhanced genomic instability - Reduced cellular viability	[11]
	Human breast cancer cell lines (BT-474, MDA-MB-436)	KO	Inhibitor VE822	- Reduced cellular viability	[11]
BRCA1	Mouse embryonic fibroblasts	KD	Cre-mediated KO	- Enhanced genomic instability - Reduced clonogenicity	[13]
	Human breast cancer cell lines (MCF7, HCC1937)	KD	LOF mutation	- Reduced clonogenicity	[13]
	Human breast cancer cell line (MDA-MB-436)	KD	LOF mutation	- Hypersensitivity to PARP-inhibitor rucaparib	[12]
	Human colon cancer cell line (HCT-116)	Inhibition	KD	- Reduced cellular viability	https://ir.ideayabio.com/news-events/presentations
	Genetically engineered mouse model	Inhibition	KO	- Reduced tumor growth	[71]
	Retinal pigmented epithelium cell line (RPE-1)	Inhibition	KO	- Reduced cellular viability - Hypersensitivity to PARP-inhibitor rucaparib	[71]
BRCA2	Mouse embryonic fibroblasts	KD	Cre-mediated KO	- Enhanced genomic instability - Reduced clonogenicity	[13]
	Human Fanconi anemia cell line (VU423)	KD	LOF mutation	- Increased chromosomal aberrations in response to MMC - Hypersensitivity to PARP inhibitor rucaparib	[12]
	Human lung cancer cell line (A549)	KD	KD	- Hypersensitivity to cisplatin and PARP-inhibitor BMN673	[66]
	Human colon cancer cell line (HCT-116)	Inhibition	KD	- Reduced cellular viability	https://ir.ideayabio.com/news-events/presentations
	Retinal pigmented epithelium cell line (RPE-1)	Inhibition	KO	- Reduced cellular viability	[71]
FANCD2	Xenotransplants of human ovarian cancer cell line (A2780)	KD	KD	- Hypersensitivity to cisplatin, MMC and PARP-inhibitor ABT-888 - Decreased tumor volume	[12]
	Mouse	KO	LOF mutation	- Most double mutants die neonatally - Congenital malformation - Premature death	[12]
	Mouse embryonic fibroblasts	KO	LOF mutation	- Hypersensitivity to PARP-inhibitor rucaparib	[12]
	Human lung cancer cell	KD	KD	- Hypersensitivity to cisplatin	[66]

(continued on next page)

Table 2. (continued)

POLQ synthetic lethal gene	Model system	Depletion of POLQ	Depletion of synthetic lethal gene	Double depletion phenotype reported	Refs
	line (A549)			and PARP-inhibitor BMN673	
Ku70	Mouse embryonic fibroblasts	KO	LOF mutation	- Reduced clonogenicity - Proliferative defect	[34]
RAD51C	Patient derived xenograft	Inhibition	Loss of expression	- Reduced tumor growth - Hypersensitivity to PARP-inhibitor olaparib	[71]
RAD52	Human bone osteosarcoma epithelial cells (U2OS)	LOF mutation (exon 16)	LOF mutation	- Reduced rate of replication fork progression	[14]
TP53BP1	Mouse embryonic fibroblasts	KO	LOF mutation	- Proliferative defect - Impaired cell cycle progression - Accumulation of non-productive HR-intermediates in S-phase	[15]

LOF, loss-of-function.

contribute to the synthetic lethal interaction between HR and POLQ: RAD51 channels repair pathway choice towards HR while POLQ counteracts this process by antagonizing RAD51. If HR is nonfunctional, diversion of pathway choice by RAD51 is detrimental and needs to be suppressed by POLQ. In the absence of RAD51, however, pathway choice is no longer diverted towards nonfunctional HR, making POLQ's antirecombinase activity dispensable.

Surprisingly, increasing evidence suggests that loss of POLQ can also be detrimental in the presence of a functional HR pathway, suggesting that HR is not able to fully compensate for TMEJ activity [15,65]. Poor c-NHEJ substrates that are also excluded from repair by HR; for example, collapsed replication forks with damaged sister chromatids might depend on TMEJ activity (Figure 1B). In line with this, high POLQ expression levels have been shown to protect from replication stress in the presence of functional HR, as shown by hypersensitivity to replication fork stalling agents upon POLQ depletion [11,65]. This holds promise for the use of POLQ inhibitors in HR-proficient cancers, particularly in combination with other drugs that exacerbate replication stress (e.g., ATR or topoisomerase inhibitors) [11].

In addition, POLQ has synthetic lethal interactions with genes beyond the HR pathway. A DNA damage response (DDR) focused CRISPR KO screen in POLQ-deficient MEFs, revealed that a surprisingly high number (45%) of the 309 analyzed murine DDR genes were synthetic lethal with POLQ [15]. The identified and validated POLQ-synthetic lethal genes function in numerous DDR pathways, including ICL repair, highlighted by hypersensitivity of POLQ KO MEFs to mitomycin C (MMC). Although it should be kept in mind that mouse cells are more prone to using TMEJ compared to human cells [73], this study positions POLQ at the center of a dense network of compensatory interactions that can be actively explored for expanding the set of cancers with POLQ dependency. In fact, some 30% of breast cancer cases in the Cancer Genome Atlas harbor mutations in POLQ synthetic lethal genes identified in this study, thereby significantly expanding the subset of POLQ-dependent cancers [15].

Development of POLQ Inhibitors

As POLQ activity is essential in HR-deficient cells, inhibition of POLQ is a promising cancer treatment strategy. The availability of crystal structures for both the helicase and the polymerase domain has been instrumental in the design of potent inhibitors [56,74]. Since both POLQ

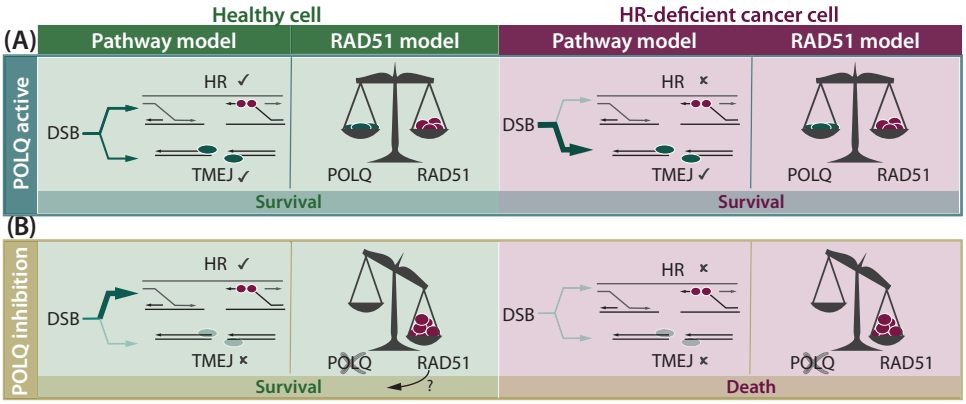

Figure 3. Two Models Explain POLQ Synthetic Lethality in HR-Deficient Cancers. (A) In the presence of functional POLQ, healthy cells can repair end-resected DSBs using both HR and TMEJ while HR-deficient cancer cells depend on POLQ for repair. (B) Upon POLQ inhibition, two models explain the hypersensitivity of HR-deficient cells: according to the pathway model, HR-deficient cancer cells have no remaining pathway for repair of end-resected DSBs resulting in cellular death. The RAD51 model, in contrast, suggests that increased RAD51 levels, caused by loss of RAD51 suppression by POLQ, drive synthetic lethality. It is unclear, however, why increased RAD51 levels are tolerated in the presence of HR. Abbreviations: DSB, DNA double-strand break; HR, homologous recombination; POLQ, polymerase θ; TMEJ, polymerase theta-mediated end-joining.

domains contain druggable sites, it remains elusive which domain is the preferred target [56]. BRCA1-depleted cells carrying inactivating mutations in the helicase or the polymerase domain show compromised growth compared to that of WT POLQ cells, suggesting that both enzymatic activities are essential in an HR-deficient background [33]. Complementation of POLQ lacking its helicase domain in HR-deficient cancers does not rescue viability to the extent of WT POLQ, proposing the helicase domain as an effective target site [12]. However, the helicase domain proved indispensable for translesion synthesis opposite UV lesions, which protects from the development of skin cancer in mice [40]. Further experiments are required to confirm this in human cells. The polymerase domain, however, is required to perform most of the TMEJ functions *in vitro*, arguing that targeting this domain will interfere with most of POLQ mediated functions in DSB repair [24]. In addition, both the polymerase and lyase enzymatic activities reside in a single nucleophilic residue within the polymerase domain, representing an enzymatic Achilles heel that might serve as an attractive target site given that the absence of both translesion synthesis as well as TMEJ activity of POLQ is not toxic to human cells [75].

To date, at least three independent biotech companies have invested into the development of POLQ inhibitors, starting with first clinical trials in 2021: IDEAYA Biosciences (San Francisco, USA), REPARE Therapeutics (Montreal, Canada) and Artios Pharma (Cambridge, UK). IDEAYA Biosciences introduced inhibitors with <10 nM potency directed against both helicase and polymerase domains while Artios Pharma's lead POLQ inhibitor program focuses on molecules targeting polymerase activity with another helicase inhibitor program in progress (https://ir.ideayabio.com/news-events/presentations). Numerous other companies have included POLQ in their pre-clinical research focused on synthetic lethality-based drug discovery. Recently, the antibiotic novobiocin has been identified to function as an inhibitor of POLQ helicase activity in an *in vitro* screen and as such being suppressive on HR-deficient cancer cell viability and tumor growth [71]. While treatment with this compound shows promising results both *in vitro* and *in vivo*, further work is required to investigate potential off-target effects, especially considering the high required drug doses.

Potential Use of POLQ Inhibitors in the Clinics

To ensure the clinical success of POLQ inhibitors, it is important to identify patient groups that would benefit from such an approach and synergistic drug combinations to potentiate the antiproliferative effect. Since POLQ dependency is best described in the context of HR deficiency, first clinical trials will most likely include patients with HR-deficient solid tumors (https://ir.ideayabio.com/news-events/presentations). Based on experimental data obtained *in vitro* and *in vivo*, patients harboring cancer-specific alterations in genes beyond the HR pathway might benefit from POLQ inhibitor treatment (Table 2). However, further research is needed to expand the repertoire of targetable synthetic lethal interactions of POLQ.

Novel POLQ inhibitors can be used as single agents or in combination with either classical chemotherapeutics or DNA repair inhibitors (Table 2) [12,66]. Despite the revolutionary efficacy of PARP inhibitors for the treatment of *BRCA*-mutated tumors, the clinical trial objective response rate is rarely above 50% and acquisition of drug resistance has been observed in most patients [76,77]. Depletion of POLQ via shRNA was shown to further sensitize HR-deficient cells to PARP inhibitors and combining PARP inhibition with POLQ inhibition also elevated antiproliferative effects as compared to each treatment alone (Table 2) [12,71] (https://ir.ideayabio.com/news-events/presentations). *BRCA* mutations in PARP-inhibitor-resistant cells often display a TMEJ-specific mutational signature, hence it is possible that POLQ might contribute to the acquisition of PARP inhibitor resistance [78]. In addition, resistance to PARP inhibitors can occur via loss of *53BP1*, a gene shown to be synthetic lethal with POLQ, thereby rendering these cells dependent on POLQ [15,79]. Therefore, using POLQ inhibitors in combination with PARP inhibitors, or as a second-line therapy, might prolong drug response and delay resistance acquisition [71,80]. It remains to be elucidated whether POLQ inhibition would also be beneficial for the treatment of cells that acquire PARP inhibitor resistance via other mechanisms, such as loss of the Shieldin complex [81].

Apart from PARP inhibitors, other drugs could potentially synergize with POLQ inhibitors and thus may be utilized independent of the HR functional status. Due to the involvement of POLQ in the resolution of replication associated lesions, POLQ-deficient cells are hypersensitive to the accumulation of DNA lesions at replication forks [11]. Consequently, combining POLQ inhibitors with ATR or topoisomerase inhibitors might represent a novel cancer treatment strategy. POLQ inhibitors might also synergize with traditional genotoxic agents as POLQ overexpression was identified as a resistance mechanism upon exposure of lung cancer cells to cisplatin [66]. Furthermore, p53-deficient cells use NHEJ and TMEJ to cope with therapy-induced DSBs. Therefore, POLQ inhibition reduces cellular viability after neocarzinostatin (a radiomimetic drug) treatment, especially in combination with DNA-PK inhibitors to suppress NHEJ [67]. Thus, POLQ inhibition might represent a synergistic treatment strategy also in HR-competent cancers, in combination with replication stress-inducing agents or in *TP53*-mutant cancers in combination with genotoxic agents and with NHEJ inhibitors.

Concluding Remarks and Future Perspectives

Recent work has highlighted the potential of POLQ as a novel target in the treatment of HR-deficient cancers and potentially also other cancer types. However, understanding the synthetic lethal environment of POLQ and its implications for cancer therapy represents an ongoing and important challenge for experimental and computational research (see Outstanding Questions). Learning about the individual contribution of each domain to POLQ function will not only provide more insight into POLQ biology but also aid potent inhibitor design, while minimizing toxic side effects. Furthermore, a topic of particular controversy is the effect of POLQ on genomic stability. It is unclear whether POLQ has a destabilizing effect on the genome due to its intrinsic

Outstanding Questions

Is TMEJ dispensable in cells that are proficient in other DSB repair pathways (such as c-NHEJ and HR) and if not, which types of DNA lesions depend on TMEJ?

Since both HR and TMEJ compete for resected DNA ends through several inhibitory interactions, which factors ultimately dictate pathway outcome?

What are the conditions that determine whether POLQ has a protective or a detrimental outcome on genome stability?

Does the repair outcome of TMEJ depend on whether it acts on one-ended DSBs (e.g., replication stress associated lesions) or two-ended DSBs (e.g., induced by endonucleases such as Cas9)?

Does POLQ overexpression act as a protective mechanism against cancer-associated genomic instability or is its overexpression causative for cancer progression?

Which mechanisms upregulate POLQ expression? Are POLQ overexpressing cancer cells selected in a Darwinian manner or do specific regulatory signaling mechanisms exist (or both)?

Which mechanisms cause cellular death upon depletion of POLQ in an HR-deficient background? Do HR-deficient cancers depend on POLQ due to its role in TMEJ (the pathway model; Figure 3) or due to its inhibitory effect on Rad51 (the RAD51 model; Figure 3)?

Does POLQ share synthetic lethal interactions with other genes other than those that function in HR that could be exploited for cancer therapy?

Which POLQ domain is the better drug target? Is it the polymerase domain which is nearly self-sufficient for most TMEJ functions or is it the helicase domain which contains important RAD51-inhibition binding sites?

Based on the observation that certain DNA lesions depend on TMEJ for repair, does inhibition of POLQ have toxic outcomes in healthy cells?

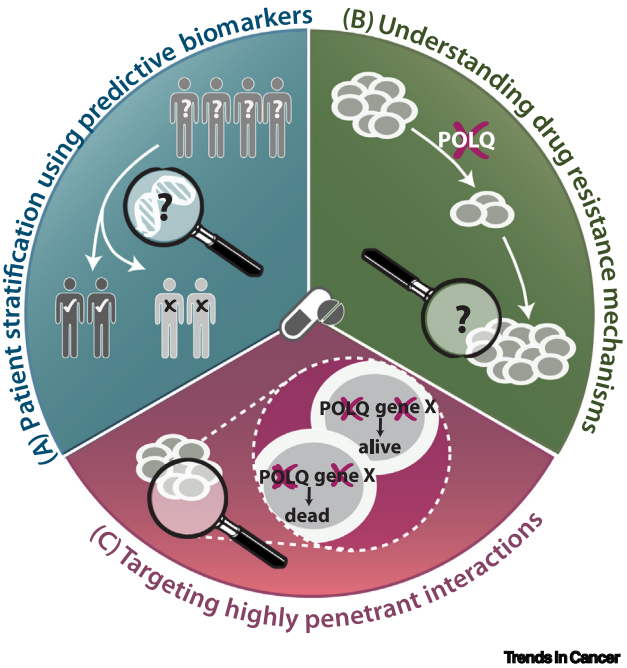

Figure 4. Clinical Challenges Associated with POLQ Inhibitors. To ensure clinical success of POLQ inhibitors and their safe and efficient applicability, three major areas of knowledge must be expanded. (A) Identification of patient biomarkers that predict outcomes of POLQ inhibition in particular tumor types. (B) Understanding of how cancer cells acquire resistance to POLQ inhibitors and how to address emerging resistance. (C) Identification of highly penetrant synthetic lethal interaction partners of POLQ to achieve sufficient efficiency despite tumor heterogeneity. Abbreviations: POLQ, polymerase θ.

What are predictive biomarkers that are indicative of the response to POLQ inhibition in cancer treatment? Can simple and scalable assays be developed to identify such biomarkers?

Which rational drug combinations with POLQ inhibitors can be used to prolong drug response and delay or prevent acquisition of treatment resistance?

What are the mechanisms of resistance to POLQ inhibitors and what are the second line therapies that could re-establish a treatment response?

mutagenicity or whether pathways involving POLQ are the only feasible repair option for numerous DNA lesions, including collapsed replication forks and G4 quadruplex structures.

Given that potent and specific POLQ inhibitors are moving into the clinics, a number of challenges remain and here much can be learnt from PARP inhibitors as approved drugs for DNA repair-targeted cancer therapy (Figure 4). A major challenge is the identification of predictive biomarkers, such as a common genomic or transcriptional signature, characterizing tumors that would respond to POLQ inhibition. Beyond using expensive next-generation sequencing strategies, the introduction of routine assays will simplify patient stratification. For PARP inhibitors, diagnostic tools have been introduced that quantify genomic signatures indicative of PARP inhibitor sensitivity.

Another hurdle common to most drugs used in cancer therapy is the acquisition of drug resistance. Apart from pharmacological resistance mechanisms shared between many drugs, such as upregulation of P-glycoprotein pumps, administration of POLQ inhibitors will most likely select for specific resistance mechanisms [82]. An anticipated resistance mechanism will be the restoration of HR, for example, by reversal mutations in BRCA, as has also been described for PARP inhibitors [78,83]. The identification of resistance mechanisms, as well as drug combinations for the treatment of resistant tumors, is key to adjusting cancer therapy in a timely manner.

For POLQ inhibitors being utilized as highly efficient chemotherapeutics, it is important to target highly penetrant synthetic lethal interactions; for example, using POLQ inhibitors in patients with mutations in genes that share a strong and highly penetrant synthetic lethal interaction with POLQ. Ideally, the synthetic lethal relationship between POLQ and the cancer-specific alteration (e.g., *BRCA2* mutation) is penetrant to an extent that tumor heterogeneity does not reduce the cancer-specific POLQ dependency. It remains to be seen whether the interactions between POLQ and its described synthetic lethal partners, such as HR factors, fulfil those criteria [84].

Finally, it is key to highlight that as small-molecule inhibitors targeting DNA repair enzymes, such as POLQ inhibitors, are emerging, a deeper mechanistic understanding of the rewiring of the DNA repair network will expand the repertoire of actionable therapeutic strategies to improve cancer treatment.

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