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Pol θ : emerging synthetic lethal partner in homologous recombination-deficient tumors

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The most remarkable finding in synthetic lethality (SL) is the hypersensitivity to PARP inhibitors (PARPis) of the tumors harboring defects in genes involved in homologous repair (HR) such as *BRCA1/2*. Despite initial responsiveness to PARPi, the penetrance of the synthetic lethal interactions between *BRCA1/2* genes and PARPi is incomplete. Thus, a significant proportion of HR-defective tumors experience intrinsic or acquired resistance, representing a key challenge of clinical research. An expanded concept of SL is opening new ways and includes novel forms of genetic interactions, investigating not only traditional SL of pairs genes but also SL between biological pathways that regulate the same essential survival cell function. In this context, recent research showed that HR and theta-mediated end-joining (TMEJ) pathways exhibit SL. DNA polymerase theta (Pol θ) is encoded by the *POLQ* gene and is a key component of the TMEJ, an essential backup pathway, intrinsically mutagenic, to repair resected double-strand breaks (DSBs) when the non-homologous end joining (NHEJ) and HR are impaired. Pol θ is broadly expressed in normal tissues, overexpressed in several cancers, and typically associated with poor outcomes and shorter relapse-free survival. Notably, HR-deficient tumor cells present the characteristic mutational signatures of the error-prone TMEJ pathway. According to this observation, the loss of HR proteins, such as *BRCA1* or *BRCA2*, contributes to increasing the TMEJ-specific genomic profile, suggesting synthetic lethal interactions between loss of the *POLQ* and HR genes, and resulting in the emerging interest for Pol θ as a potential therapeutic target in *BRCA1/2*-associated tumors.

This review summarizes the converging roles of the *POLQ* and HR genes in DNA DSB repair, the early-stage clinical trials using Pol θ inhibitor to treat HR-defective tumors and to overcome *BRCA*-reversion mutations responsible for therapeutic resistance, and the novel pleiotropic effects of Pol θ , paving the way for the development of unexplored synthetic lethality strategies.

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INTRODUCTION

The concept of synthetic lethality (SL) opened novel therapeutic avenues for targeting homologous recombination (HR) deficient tumors [1]. Several SL-based targeted therapies have been approved in clinical practice, starting from the simple genetic concept of the interaction between two genes, whose simultaneous perturbation causes cell death [2].

The therapeutic approach of synthetic lethality primarily focused on targeting DNA damage response (DDR) pathways. The synthetic lethal action of PARP-inhibitors (PARPis) in Breast cancer susceptibility gene 1 (*BRCA1*) and breast cancer susceptibility gene 2 (*BRCA2*)-deficient tumors provided key improvement in the clinical outcomes of patients with homologous recombination deficiency (HRD), mainly ovarian, breast and prostate cancer patients [3]. Currently, the biggest challenge of the research in these tumors is the SL-targeted drug resistance.

Building upon the crucial role of DDR pathway in SL, several potential synthetic lethal genes have been identified, such as Ataxia-Telangiectasia Mutated (*ATM*), Enhancer of Zeste Homolog-2 (*EZH2*), Ataxia Telangiectasia and Rad3-related (*ATR*), and Checkpoint Kinase 1 (*CHK1*) genes [4]. An expanded concept of SL is opening new ways and includes novel forms of synthetic lethal interactions, investigating not only traditional SL of pairs genes but also SL between two biological pathways that regulate the same essential survival cell function [4]. In this context, recent research showed that Homologous Recombination Repair (HRR) and theta-mediated end joining (TMEJ) pathways exhibit SL [5]. TMEJ, also known as alternative non-homologous end joining (alt-NHEJ) or microhomology-mediated end joining (MMEJ), is an essential backup pathway to repair resected double-strand breaks (DSBs) when the canonical repair pathways non-homologous end joining (NHEJ) and HR repair (HRR) are impaired [6].

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Table 1. DNA damages and corresponding DNA repair system.

Type Of damage	DNA repair mechanism	Description	Components involved	Phase Of cell cycle
Single base lesions	Base excision repair (BER)	Repairs lesions involving a single nucleotide base or damaged bases, such as those caused by oxidative or alkylating agents [84, 85]	CHK1, enzymes like glycosylase, AP endonuclease, DNA polymerase, and ligase	All phases
Nucleotide segment lesions	Nucleotide excision repair (NER)	Corrects lesions involving a damaged DNA region, such as those caused by UV radiation or chemical agents [85, 86]	Protein complexes like XP, XPF, XPG, TFIIH, and DNA polymerase	All phases
Base mismatches	Mismatch repair (MMR)	Detects and corrects base-pairing errors and small insertion/deletion loops [87, 88]	Proteins MSH (MutS), MLH (MutL), PMS, DNA polymerase, and ligase	All phases
Transcriptional lesions	Transcription-coupled repair (TCR)	Repair of lesions occurring during DNA transcription [89]	Transcription factor IH (TFIIH), CSB, and CSA proteins	Active Transcription
Double-strand breaks (DSBs)	Homologous recombination (HR)	Repair of double-strand breaks using an identical DNA sequence as a template for repair [90]	Proteins RAD51, BRCA, RAD52, RAD54, ATM, EZH2, CHK1 and MRN complex proteins	S and G2
	Canonical non-homologous end joining (C-NHEJ)	Joins double-strand ends of damaged DNA, often causing small insertions or deletions [91]	Proteins Ku70/Ku80, CHK1, DNA-PKcs, and ligase IV	All phases
	Alternative end joining (ALT-EJ)	Joins double-strand ends of damaged DNA imprecisely, often causing large insertions or deletions [92, 93]	Proteins ATM, PARP, XRCC1, MRE11, RAD50, and NBS1	All phases, peak in G2
	Microhomology-mediated end joining (MMEJ) or theta-mediated end joining (TMEJ)	Joins double-strand ends of damaged DNA using microhomologies present at the ends [92, 93]	Proteins ATM, PARP-1, CtIP, DNA polymerase θ (POL θ), XPF/ERCC1 and ligase III	All phases, peak in G2
	Single-strand annealing (SSA)	A mutagenic repair pathway, independent of RAD51, involves the annealing of complementary DNA fragments onto resected DNA, followed by the deletion of intervening DNA sequences [91]	Proteins RAD52, RPA, RAD54, RAD55, RAD57, XPF/ERCC1, and ligase I	S and G2

Activation of TMEJ involves several enzymes, including PARP-1, and the repair of DSBs is facilitated by specific DNA polymerase, primarily DNA polymerase theta (Pol θ) [7]. The depletion of the *POLQ* gene, coding for Pol θ , seems to be synthetically lethal when combined with *BRCA1/2* pathogenic variants (PVs). Thus, Pol θ recently emerged as a novel and potential therapeutic target in HRD tumors [8].

Although the genetic concept of LS seems to be simple, the DDR pathway is composed of intricate mechanisms and genes, making the identification of suitable targets challenging [9]. At the same time, SL interactions that work in one tumor type are sometimes ineffective in another. The findings in preclinical research only provide a potential mechanism to be explored, and whether the Pol θ -inhibitors may have clinical relevance in the *BRCA*-mutated tumors still needs confirmation in the ongoing clinical trials.

In this review, we focused on the expanding landscape of the SL-driven discovery framework, with a focus on Pol θ as a novel, targetable, vulnerability in HRD cancers.

FROM DNA DAMAGE TO UNFUNCTIONAL DNA REPAIR SYSTEMS: AN ITINERARY TOWARDS SYNTHETIC LETHALITY

Cell survival relies on genome integrity and accurate cell function. DNA repair pathways represent an intricate intersection of processes, where specific perturbations can potentially lead to genetic instability. Among DDR pathways, four key mechanisms of DSBs play a central role: HR, the NHEJ, the alt-NHEJ or MMEJ, and the single-strand annealing (SSA) [10] (Table 1). Cancer cells can exploit these DNA repair mechanisms to their advantage, predominantly upregulating alternative repair pathways to maintain the integrity of their DNA: this interchange of pathways can ultimately boost the cancer cells' survival [11].

This survival strategy is evident in the context of *BRCA1/2* mutated cancers, where the loss of one repair pathway is compensated by another, leading to tumoral resilience and progression [12].

However, this compensatory reliance also presents a therapeutic vulnerability, placing the key concept that underlies synthetic lethality. The clinical success of small-molecule inhibitors of PARP in *BRCA*-mutated cancers demonstrated the success of this approach [13, 14]. In 2005, the identification of synthetic lethal interaction between the loss of *BRCA1/2* and the PARP-inhibition, emerged as a new paradigm for the identification of synthetic lethal targets in cancer, going beyond the traditional concept of oncogene addiction, where targeted alterations are druggable oncogenes [14, 15].

BRCA1/2 function in HR, the repair pathway that utilizes the replicated sister chromatid as a template for precise DSB repair. PARPi effectively impedes the base excision repair (BER) mechanism of DNA single-strand break (SSB), with the consequent transition from SSB to DSB. In patients with *BRCA1/2* PV, cancer cells are incapable of completing DSB repair through the high-fidelity HRR, and the use of alternative repair mechanisms results in genomic instability, leading to hypersensitivity to PARP inhibition [16] (Fig. 1).

PARPis, including olaparib, niraparib, rucaparib, and talazoparib, have been approved by the FDA as maintenance therapy in ovarian, primary peritoneum, fallopian tube, breast, pancreatic, and prostate cancers that have *BRCA1/2* PVs or the HRD status, with differences in therapy indications across the tumor type [17–31]. Unfortunately, the clinical benefit of PARPi is limited by the emergence of acquired or inherent resistance mechanisms in approximately 40–70% of patients [17, 18] (Table 2). Furthermore, the recent expansion of approved PARPis, not restricted to patients carrying *BRCA1/2*-PVs, produced a strong clinical need to explore a new wave of genetic cancer targets for synthetic lethal interactions [19, 20] (Created with BioRender.com).

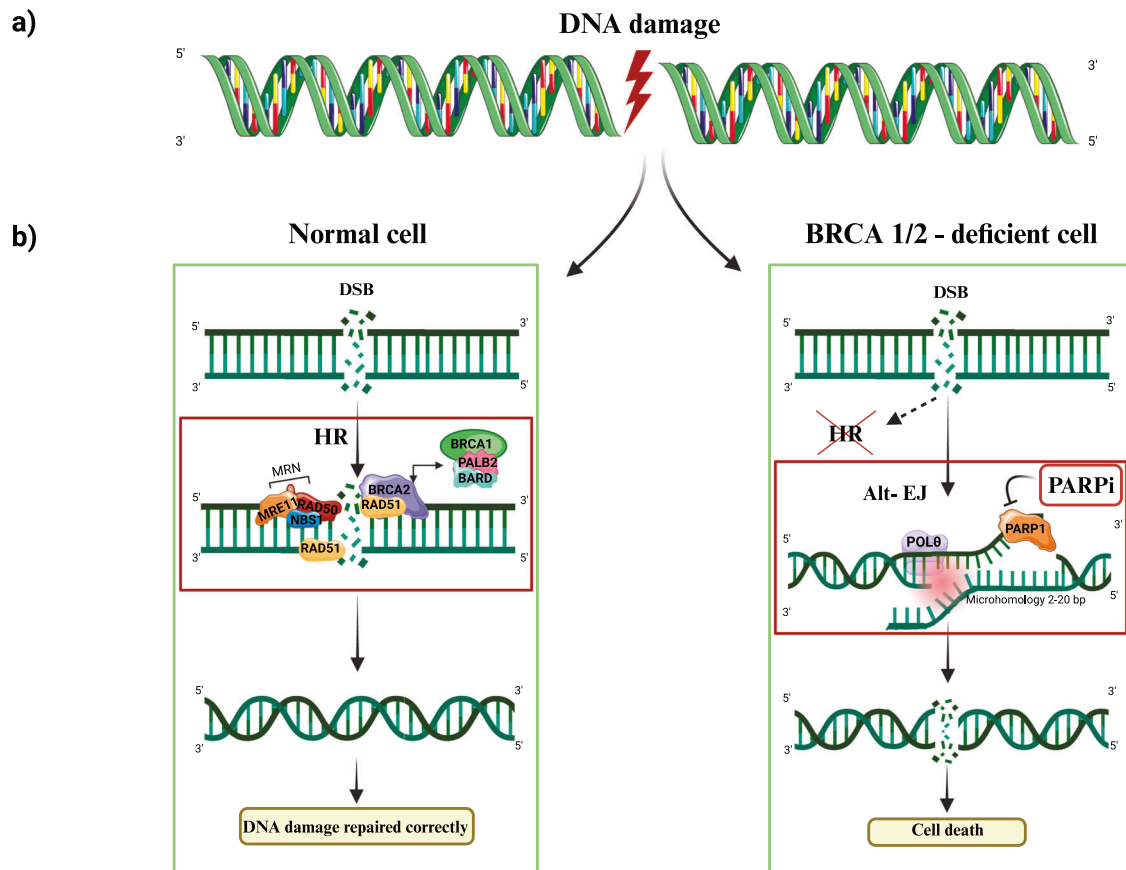


Fig. 1 PARP inhibition and synthetic lethality in *BRCA*-deficient cells. **a** In the *BRCA*-proficient cells the HR repair system is physiologically involved in DNA repair, with DNA damage correctly repaired; **b** In the *BRCA*-deficient cells, the absence of efficient homologous recombination, along with the PARP-inhibition, leads to the use of base excision repair (BER) as compensatory pathway, resulting in genomic instability and cell death.

EXPANDING THE SYNTHETIC LETHALITY PARADIGM: BEYOND PARP INHIBITION IN *BRCA* AND *P53*-DEFICIENT TUMORS

Clinically, the penetrance of the synthetic lethal interactions between *BRCA1/2* genes and PARPi is incomplete. Thus, some HRD patients, especially those with advanced tumors, despite the presence of a *BRCA* PV, showed either de novo or acquired PARPi-resistance [32]. Acquired resistance occurs through multiple mechanisms: from reverse *BRCA1/2* mutations, in which the molecules' function is reacquired and the HR repair system restored, to non-reverse mutations occurring in other aside pathways, like epigenetic modifications and restorations of ADPrbosylations, better and efficient drug efflux, ultimately causing the resistance to PARPi [32]. This reversion and non-reversion resistance, not only underscores the extreme adaptability of cancer cells but also highlights the urgency for novel synthetic lethal interaction identification [21]. Furthermore, as our understanding of the complexity of cancer-cell DNA repair networks continues to evolve, numerous gene pairs are being identified as further potential candidates for synthetic lethal therapy.

In this context, several molecules involved in different shelter systems, such as Ataxia-Telangiectasia Mutated (*ATM*), Enhancer of Zeste Homolog-2 (*EZH2*), Ataxia Telangiectasia and Rad3-related (*ATR*), and Checkpoint Kinase 1 (*CHK1*), have been considered to overcome PARP resistance in *BRCA*-mutated cancers as well as in other tumors [22].

Among these molecules, *EZH2* and *ATM*, still involved in the HR repair system, emerged. *EZH2*, is an enzymatic catalytic subunit of polycomb repressive complex 2 (PRC2) that modulates target gene expression via trimethylation of Lys-27 in histone 3 (H3K27me3) [23]. In *BRCA*-deficient cancers, *EZH2* is often found to be overexpressed,

which makes it a potential tumor target [23]. Research by Ratz et al. has explored the benefits of combining *EZH2* inhibitors, like the GSK126, with chemotherapy agents such as cisplatin, to improve cancer cells' death, using cell lines derived from *BRCA1*-deficient and -proficient mouse mammary tumors [23]. The effect of *EZH2* was also maximized by *ATM*, a crucial molecule for DSB repairs, whose inhibition was already found effective in *BRCA1*-deficient cells [23, 24]. *ATM* is also involved in the MRN complex, a key HR player. *ATM* recruitment and activation lead to its target phosphorylation, blocking the cell cycle and initiating DNA repair [25]. Studies have shown that simultaneous inhibition of *EZH2* and *ATM* in *BRCA*-deficient cells amplifies DNA damage, leading to increased apoptosis and significantly reduced tumor growth. This effect is particularly notable when using GSK126 (*EZH2*-inhibitor) in combination with AZD1390 (*ATM* inhibitor), compared to their use alone. Interestingly, cells with functional *BRCA* genes do not exhibit the same level of cytotoxicity, suggesting an effectiveness of *EZH2/ATM* inhibition specific to the presence of *BRCA1* mutation context [26].

Despite these advances, the lack of clear and significant data on these molecules is still predominant and affects their use in clinical practice to overcome PARPi resistance.

Several studies have highlighted another potential synthetic lethal interaction between *ATR* and *CHK1* within the broader context of *p53*-mutated cancers. Tumors harboring mutant *p53* (mutp53) often are resistant to conventional cancer therapies, tend to progress more rapidly, and generally, have poorer prognosis. Given that mutations in *p53* enhance cancer metastasis and proliferation, targeting this signaling pathway might represent a new strategic approach [27]. Tumor cells with *p53* mutations are particularly vulnerable to cell cycle arrest in the intra-S and G2 phases, a

Table 2. Overview of the main approval trials of PARPis: primary results, setting of use and subgroup analyses of patients who experienced disease progression during the treatment.

Type of Cancer	Parpi	Patients	Clinical trial	Type of analyses	Results	Not responders (%)
mPaC	Olaparib	gBRCAm mPaC after platinum-based CT	POLO NCT02184195 [94, 95]	BRACAnalyses CDx Test (Myriad Genetic Laboratories)	mPFS: 7.4 vs 3.8 mo (HR 0.53 95% CI, 0.35–0.82; $P = 0.004$) mOS: 19.0 vs 19.2 mo (HR 0.83 95% CI, 0.56–1.22; $P = 0.3487$) SPFS: 13.2 vs 9.2 mo (HR 0.76 95% CI, 0.46–1.23)	61% (55/90)
mCRPC	Olaparib	HRD + mCRPC progressed on or after former treatments	PROfound NCT02987543 [96]	FoundationOne CDx next-generation sequencing test	mPFS (BRCAm or ATMm, 245 pts): 7.4 vs 3.6 mo (HR 0.34 95% CI, 0.25–0.47; $P < 0.001$) mPFS (overall population, 87 pts): 5.8 vs 3.5 mo (HR 0.49 95% CI, 0.38–0.63; $P < 0.001$) mOS (BRCAm or ATMm, 245 pts): 18.5 vs 15.1 mo (HR 0.64 95% CI, 0.43–0.97; $P = 0.02$)	50% (129/256)
eBC	Olaparib	gBRCAm Her-2 negative, eBC	OlympiA NCT02032823 [97, 98]	BRACAnalyses CDx Test (Myriad Genetic Laboratories)	IDFS: 82.7% vs 75.4% (HR 0.63 95% CI, 0.50–0.78) mOS: 89.8% vs 86.4% (95%CI, 0.1–6.8%) DDFS: 86.5% vs 79.1% (95% CI, 3.6–11.3%)	4.5% (41/911)
mBC	Olaparib	gBRCAm Her-2 negative, mBC	OlympiAD NCT02000622 [99, 100]	BRACAnalyses CDx Test (Myriad Genetic Laboratories)	mPFS: 7.0 vs 4.2 mo (HR 0.58 95% CI, 0.43–0.8; $P < 0.001$) mOS: 19.3 vs 17.2 mo (HR 0.89 95% CI, 0.66–1.23; $P = 0.513$)	74% (152/205)
mBC	Talazoparib	gBRCAm Her-2 negative, advanced or metastatic BC	EMBRACA NCT01945775 [101]	BRACAnalyses CDx Test (Myriad Genetic Laboratories)	mPFS: 8.6 vs 5.6 mo (HR 0.54 95% CI, 0.4–0.7; $P < 0.0001$) mOS: 19.3 vs 19.5 mo (HR 0.8 95% CI, 0.67–1.07; $P = 0.17$)	69% (197/287)
HGSOC	Niraparib	HGSOC after 1 st line platinum-based CT regardless HRD status	PRIMA NCT02655016 [102]	Myriad Genetics myChoice HRD	mPFS (overall population, 733): 13.8 vs 8.2 mo (HR 0.66 95% CI, 0.56–0.79; $P < 0.001$) mPFS (HRD +, 373): 24.5 vs 11.2 mo (HR 0.52 95% CI, 0.40–0.68; $P < 0.001$) mPFS (BRCAm, 223): 31.5 vs 11.5 mo (HR 0.45 95% CI, 0.32–0.64) mPFS (BRCA wt, HRD +, 150): 19.4 vs 10.4 mo (HR 0.66 95% CI, 0.44–1.00) mPFS (HRp, 360): 8.4 vs 5.4 mo (HR 0.65 95% CI, 0.49–0.87)	69% (281/405)
HGSOC	Olaparib	HRD + HGSOc in combination with bevacizumab after 1 st line platinum-based CT	PAOLA 1 NCT02477644 [103, 104]	Myriad Genetics myChoice HRD	mPFS (Overall population): 22.1 vs 16.6 mo (HR 0.59 95% CI, 0.49–0.72; $P < 0.001$) mPFS (HRD+): 37.2 vs 17.7 mo (HR 0.43 95% CI, 0.28–0.66) mPFS (BRCA wt, HRD+): 28.1 vs 16.6 mo (HR 0.43 95% CI, 0.28–0.66) mPFS (HRp): 16.9 vs 16.0 mo (HR 1.00 95% CI, 0.72–1.17) mOS (overall population): 56.5 vs 51.6 mo (HR 0.92 95% CI, 0.76–1.12; $P = 0.4118$)	37% (197/535)
HGSOC	Niraparib	Recurrent platinum-sensitive HGSOc after (≥2) platinum-based CT regardless HRD status	NOVA NCT01847274 [105, 106]	Myriad Genetics myChoice HRD	mPFS (BRCAm, 203): 21 vs 5.5 mo (HR 0.27 95% CI, 0.17–0.41; $P < 0.0001$) mPFS (BRCA wt, 350): 9.3 vs 3.9 mo (HR 0.45 95% CI, 0.34–0.60; $P < 0.0001$)	BRCAm 46% (63/136) BRCA wt 55% (128/234)

Table 2. continued

Type of Cancer	Parpi	Patients	Clinical trial	Type of analyses	Results	Not responders (%)
HGSOC	Rucaparib	Recurrent platinum-sensitive HGSOC after (≥2) platinum-based CT regardless HRD status	ARIEL 3 NCT01968213 [107]	Foundation Medicine T5 NGS	mPFS (overall population): 10.8 vs 5.4 mo (HR 0.36 95% CI, 0.30–0.45; $P < 0.0001$) mPFS (BRCAm, 196): 16.6 vs 5.4 mo (HR 0.23 95% CI, 0.16–0.34; $P < 0.0001$) mPFS (HRD +, 354): 13.6 vs 5.4 mo (HR 0.32 95% CI, 0.24–0.42; $P < 0.0001$)	38% (215/564)
HGSOC	Olaparib	BRCAm HGSOC after platinum-based CT	SOLO 1 NCT01844986 [108, 109]	Myriad Genetics myChoice HRD	mPFS : 5.6 vs 13.8 mo (HR 0.33 95% CI, 0.25–0.43) OS a 7 y : 67 vs 46.5%	20% (51/260)
HGSOC	Rucaparib	Recurrent platinum-sensitive HGSOC after (≥1) platinum-based CT regardless HRD status	ARIEL 2 (part 1) NCT01891344 [110]	Foundation Medicine T5 NGS	mPFS (BRCAm, 40): 12 mo (95% CI, 9.0–14.75) mPFS (BRCA wt, HRD +, 82): 5.7 mo (95% CI, 5.3–7.6) mPFS (HRp, 70): 5.2 mo (95% CI, 9.0–14.75)	BRCAm 60% (24/40) BRCA wt, HRD + 67% (55/82) HRp 81% (57/70)

mPaC metastatic pancreatic cancer, **gBRCAm** germline mutation in *BRCA 1/2* genes, **CT** chemotherapy, **PFS** progression-free survival, **MO** months, **HR** hazard ratio, **OS** overall survival, **SPFS** second progression-free survival, **mCRPC** metastatic castration-resistant prostate cancer, **HRD** homologous recombination deficiency, **pts** patients, **eBC** early breast cancer, **ADJ** adjuvant setting, **IDFS** invasive disease-free survival, **DDFS** distant disease-free survival, **mBC** metastatic breast cancer, **HGSOC** high-grade serous ovarian cancer, **HRp** homologous recombination proficient.

vulnerability also attributed to checkpoint regulators like *ATR*. By phosphorylating *CHK1*, *ATR* manages the cell cycle and DNA damage response by recognizing specific single-stranded DNA locations [28]. *ATR/CHK1* inhibitors, able to block such response, could potentiate the effects of cytotoxic drugs or radiation therapy, increasing tumor cells' death in *p53*-deficient scenarios. *ATR* inhibitors, for instance, stall replication forks and cause chromosomal breakage, sensitizing tumors to DNA-damaging therapies [29]. This is especially effective in cancers experiencing replication stress or with deficient DNA repair mechanisms, such as triple-negative breast cancer, castration-resistant prostate cancer, and other cancers with *BRCA1/BRCA2* mutations [30, 31, 33–35]. Furthermore, they can resensitize tumor cells to PARP inhibitors when resistance develops, such as in the presence of mutations that overcome *BRCA1*-mediated DNA repair pathways [32, 36, 37]. Similarly, *CHK1* inhibitors target cells under replication stress, amplifying the effects of chemotherapeutic agents like gemcitabine, cisplatin, and camptothecin, enhancing damage from PARP inhibitors [38, 39]. When used with immune checkpoint inhibitors, such as the anti-PD-L1 therapy, they showed synergistic anti-tumor responses, as demonstrated in mouse models of small-cell lung cancer [40]. Currently, clinical trials involving these inhibitors are investigating the optimal timing and dosage for using these inhibitors in combination with both conventional chemotherapies and new therapeutic agents to minimize side effects and maximize treatment efficacy.

Other synthetic lethal interactors of *BRCA* genes are explored, represented by proteins with physiological functions in DDR pathways, including Fanconi anemia complementation group D2 (FANCD2), radiation sensitive 52 (RAD52), apurinic/apyrimidinic endodeoxyribonuclease 2 (APE2), and Flap structure-specific endonuclease 1 (FEN1) [41]. Among these molecules, Polθ represents an attractive target.

POLθ AND BRCA: CONVERGING ROLES IN DNA DSB REPAIR

Deficiency in one DNA repair pathway in cancer cells leads to genetic instability and hyper-dependence on compensatory pathways. This dependency represents a key tumor vulnerability that can be exploited to target cancer cells [22].

Polθ is a DNA polymerase, encoded by the *POLQ* gene, involved in numerous DNA repair pathways, including TMEJ, a backup pathway for DNA repair when NHEJ, HR, or BER are compromised [42]. TMEJ is intrinsically mutagenic. Resected DNA DSBs, which cannot be repaired through HR in *BRCA1/2*-mutant cancer cells, enter the Polθ-dependent TMEJ, introducing typical sequence alterations represented by the microhomology-flanked deletions and the templated insertions.

Notably, HRR-deficient tumor cells present the characteristic mutational signatures caused by the high mutagenicity of the driving force of the error-prone TMEJ pathway [43]. According to this observation, the loss of HRR proteins, such as *BRCA1* or *BRCA2*, contributes to increasing the TMEJ-specific genomic profile, suggesting synthetic lethal interactions between loss of the *POLQ* gene and HRR genes like *BRCA1/2*, *ATM*, and *FANCD2*, and resulting in the emerging interest for Polθ as a potential therapeutic target in *BRCA1/2*-associated tumors.

Interestingly, the similarity between genomic scar signatures of HRD in *BRCA*-associated tumors and TMEJ genomic scar pattern indicates the hyperactive TMEJ as a mechanism to investigate a potential driver of genomic scar in many cancer types, maybe involved in the lack of full correlation between HRD genomic signatures and functional HRR assays [44, 45].

Moreover, using a CRISPR genetic screen, a recent study identified 140 synthetic lethal genes with Polθ. In this study, 29.7% of breast cancers in the TCGA cohort having genetic alterations in at least one of these 140 Polθ synthetic lethal genes and genomic scar pattern of TMEJ hyperactivity,

underlining the potential benefit to target Polθ to improve cancer patients clinical outcomes [41, 44]. Consistent with these results, Polθ-inhibition enhances cell death in HRD cells exposed to cisplatin or PARPi [46]. Therefore, depletion of Polθ using Polθ inhibitor in combination with or after a PARPi or platinum salts has been proposed as a new strategy to treat HRD tumors and to overcome *BRCA*-gene reversion mutations responsible for therapeutic resistance [22].

POLθ: THE SYNTHETIC LETHAL INTERACTIONS WITH OTHER DDR-RELATED GENES AND THE EFFECT ON INNATE IMMUNITY ACTIVATION

Although Polθ inhibition is emerging as a strategy to improve the synthetic lethality and cell death in *BRCA*-mutated tumors, Polθ showed synthetic lethal interactions also with other DNA repair-related genes that control DSB repair and HR.

The first example is the Tp53BP1. *BRCA1* functions in HR promoting DNA end resection through inhibition of proteins that inhibit this pathway, such as the Tp53BP1 [47]. Thus, Tp53BP1 loss-of-function enhances DNA end resection, induces end-joining pathway hyperactivation, and Polθ-dependent survival [48]. Defects in the 53BP1/Shieldin DNA repair complex are known sources of PARPi resistance [22]. Therefore, Polθi not only has clinical potential as a therapeutic strategy in *BRCA*-associated tumors but could also be used to target PARPi resistance [22].

A recent study showed that the type of HR gene mutation had an impact on DNA end resection, and represents a key determinant of TMEJ pathway addiction and the specific response to Polθi. In this study, human isogenic *PALB2*- and *BRCA2*-deficient cells, were more sensitive to Polθi compared to *BRCA1*-deficient cells, with clinical implications for future therapy of mutation-dependent Polθ vulnerabilities [48].

Another study demonstrated that synthetic lethality with Polθ loss required mammalian *RAD52* [49]. *RAD52* is involved in the HR repair pathway by promoting numerous recombination-mediated repair and replication mechanisms and showed a critical role in cells lacking *BRCA1/2* or *PALB2* genes. Interestingly, in 53BP1-mutated and *BRCA2*-deficient cells, *RAD52* influenced Polθ inhibition. *RAD52* provided toxicity by suppressing DNA synthesis and promoting DNA resections, chromosomal breaks, and micronucleus formation, with subsequent cell death. Instead, a *BRCA1*-mutated context did not produce similar results. These observations underline the importance of the patient's genetic background during the treatments for the resistance onset [49, 50]. The mechanistic basis associated with a genotype-dependent influence of Polθ inhibitor mechanisms remains to be clarified.

Some recent studies explored the potential innate immunity activation mediated by Polθ depletion through the cyclic GMP-AMP synthase–stimulator of interferon genes (cGAS-STING) signaling pathway [51]. Polθ inhibition causes the accumulation of cytosolic DNA and activates the cGAS-STING signaling pathway in *BRCA2*-mutated pancreatic cancers. This enhances the expression of Type I interferon (IFN), the transcription and secretion of several inflammatory cytokines and chemokines, thus increasing the intratumoral T cell infiltration [52]. The preliminary evidence on Polθi and STING agonists synergy represents a potential therapeutic strategy to combine DDR inhibitors and immunotherapy in the population of HR-deficient pancreatic adenocarcinoma [51, 52].

Another two molecules showing synthetic lethal interactions with Polθ are *FANCD2* and *APE2*. *FANCD2* acts in the HR, but also in the Fanconi anemia pathway, which repairs the inter-strand DNA crosslinks [53]. *FANCD2* also facilitates the Polθ recruitment at the DNA damage sites during TMEJ activation. Preclinical and clinical studies demonstrated a synthetic lethality relationship between Polθ and *FANCD2* in several cancers, such as esophageal

squamous cell carcinoma [51]. Double knockdown of *POLQ* and *FANCD2* resulted in hypersensitivity to cisplatin in lung cancer cell lines [54], and hypersensitivity to PARP inhibitors in *FANCD2*-deficient ovarian carcinoma cells while, in vivo, the double knockdown reduced the tumor volumes of xenotransplants of human ovarian tumors [46].

Recent discoveries have highlighted how *APE2* nuclease is a key effector of MMEJ repair and it is partly related to the Polθ-mediated repair pathway. The role of *APE2* in MMEJ contributes to the addition of HR-deficient cells to *APE2*, and this feature could be explored for the treatment of HRD tumors [55].

Another interesting scenario includes Polθ as an adjuvant of radiotherapy. ART558 and, even better, the more recent and stable, ART899 made different tumor cells more susceptible to radiotherapy. The radiosensitivity is favored in S-phase and hypoxic conditions, characteristics predominant in tumor cells, often limiting chemotherapy response and generating resistance development. Evaluation of Polθ in combination with radiotherapy is now under investigation in a clinical trial (NCT04991480). A clinical confirmation would open a new promising therapeutic front, more precise and with less off-target effects [56].

Data provided so far only proved Polθ's potential. Despite its intricate nature and the research at a preliminary level, this enzyme could represent a promising target for innovative therapeutic strategy (Fig. 2).

POLθ: THE ROLE IN CANCER PROGNOSIS AND CLINICAL OUTCOMES

Polθ is emerging as a prognostic marker, as well as how potential therapeutic target in *BRCA*-associated tumors. Polθ begins to show broader implications in tumor biology and cancer patient clinical outcomes [57].

Overexpression of Polθ has been consistently reported across a wide spectrum of cancers, including breast, ovarian, lung, gastric, colorectal, and prostate cancers (Table 3) [57–61]. Conversely, in non-tumor cells, it is expressed at a low level or completely not expressed [62–64]. The overexpression was associated with aggressive tumor behavior and adverse clinical prognosis, highlighted by shorter relapse-free survival and overall survival [65].

As previously highlighted, the expression of Polθ is characteristically linked to distinct mutational signatures, predominantly in cancers with HRD, suggesting Polθ upregulation as a compensatory survival mechanism for HR-deficient cancer cells. Intriguingly, Polθ expression is not solely beneficial to HR-deficient cancer cells but appears to offer HR-proficient cells an enhanced ability to survive DNA replication stress, thereby contributing to genomic stability. This multifaceted role of Polθ underlines its prognostic relevance, with overexpression being a marker of poor outcomes in diverse cancer types [66]. In breast cancer, high levels of Polθ are linked with estrogen receptor (ER) negative tumors and higher tumor grades, which are known markers of poor prognosis [58, 67]. Recent studies suggest that Polθ overexpression is associated with an intrinsic mechanism of resistance to genotoxic treatments and radiotherapy. Higgins GS et al. showed that the depletion of Polθ resulted in a marked radio-sensitization in tumor cells contrasting with minimal effects on normal tissue cells, indicating a potential for tumor-specific therapeutic exploitation [58]. Further inquiry into the therapeutic potential of Polθ-inhibition revealed its role in the response to chemotherapeutic agents that generate replicative stress. Studies in ovarian cancer revealed a strong association between Polθ expression and platinum chemotherapy resistance. Lower expression levels of Polθ have been associated with increased chemo-sensitivity, indicating its role as a negative predictor of chemotherapy response [59]. In prostate cancer, Polθ overexpression has been associated with the development of castration-resistant prostate cancer (CRPC) and resistance to

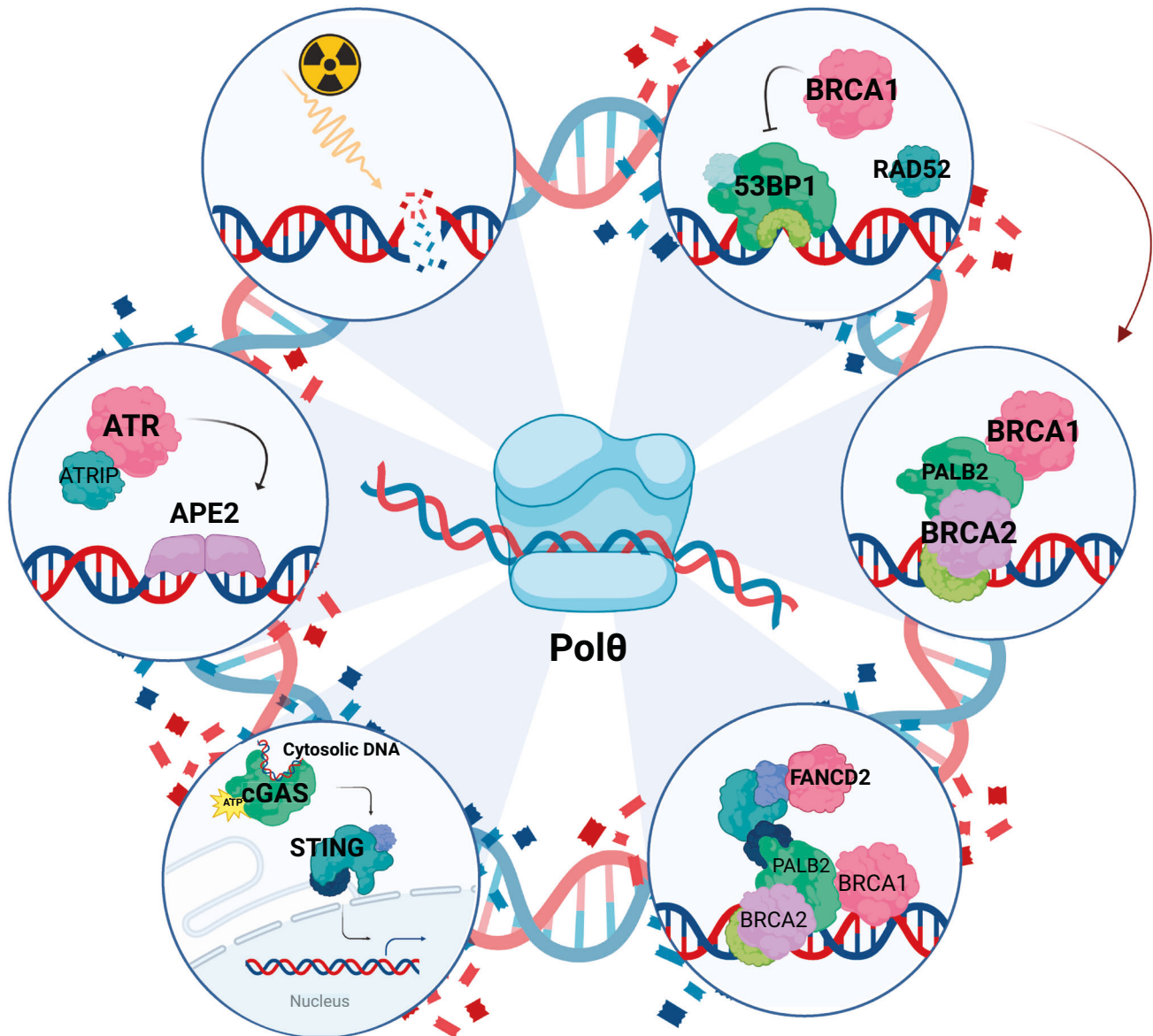


Fig. 2 Pleiotropic Polθ influence of different repair mechanism systems and molecular pathways. Beyond *BRCA*-mutated tumors, Polθ showed synthetic lethal interactions also with other DNA repair-related genes that control DSB repair and HR, such as 53BP1, RAD52, PALB2, and FANCD2. Furthermore, the effect of the cGAS-STING immunological and inflammatory pathway, on the MMR repair system through APE2, and the synergy with radiotherapy, was recently highlighted (Created with BioRender.com).

olaparib. This resistance identifies Polθ as a critical determinant of treatment outcomes and highlights the enzyme as a candidate for targeted therapy. Targeting Polθ could enhance the effectiveness of treatments like docetaxel and olaparib, making prostate cancer cells more responsive to standard therapy [57, 68].

Recently, several studies have explored the mutational status of other functionally related HR genes, beyond *BRCA1/2* and the established HR-associated genes such as *ATM*, *CHEK2*, and *PALB2*. Particularly, the study of D. R. Principe et al. in a large pan-cancer cohort, showed high rates of mutations in many HR-associated genes unexplored in the predictive setting of PARP inhibition, including *POLQ*, and frequently associated with poor clinical outcomes in tumors like cutaneous melanomas adenocarcinoma and squamous lung carcinoma, gastric, esophageal and head and neck cancers [69]. Therefore, Polθ's overexpression and/or *POLQ* mutation across various malignancies and its correlation with poor clinical outcomes position it as a significant prognostic marker. It plays a pivotal role in cancer cell survival under stress, particularly

in HR-deficient contexts, making it an attractive target for therapeutic intervention. The current focus on Polθ inhibitors and their capacity to resensitize cancer cells to standard treatments opens new avenues for cancer therapy, warranting further exploration into their clinical applicability across both HR-proficient and HR-deficient tumors. The therapeutic manipulation of Polθ expression and function, therefore, holds substantial promise for improving the prognosis of cancer patients and overcoming resistance to conventional therapies.

POLθ-INHIBITORS: A CHEMICAL OVERVIEW

The potential to exploit the synthetic lethal effects of the *POLQ*/HR genes has been investigated through novel small-molecule inhibitors.

From a structural point of view, Polθ is a multifunctional protein divided into two domains linked by an undefined central region: a DNA polymerase domain (Polθ-pol, residues 1819–2590, Fig. 3a)

Table 3. Overview of POLQ expression across various cancer types and its correlation with molecular, pathological and prognostic features.

Cancer type	Setting	POLQ expression	Methods	Molecular, pathological, and prognostic features
Breast Cancer [58, 67]	All stages, untreated patients	High	RT-PCR	-Triple-negative BC -High tumor grades -Poor OS
	Early-stage breast cancer	High	Microarray gene expression analysis	-Poor OS -Worse RFS
Ovarian Cancer [59]	All stages, untreated patients	Low	qRT-PCR, Western Blot	-Better response to platinum-based chemotherapy -Median OS and DFS
Pancreatic Cancer [52, 60]	Cell lines and genetically engineered mouse models (GEMMs)	Low	qPCR, Western Blot	-cGAS-STING activation and decreased tumor growth -Higher survival rates, regardless of KRAS status
Prostate Cancer [57, 68]	Metastatic CRPC	High	RT-PCR, qPCR, Western Blot	-Risk factor for cancer recurrence -Development of CRPC -Poor response to docetaxel or irradiation treatment -Poor OS
	HRD prostate cancer	High	RT-PCR	-Olaparib resistance -Shorter PFS -Higher immune score
Esophageal Cancer [51]	All stages	Low	RNA-seq, qPCR	-cGAS-STING activation -Upregulation of ISGs -Increased sensitivity to genotoxic agents
Lung Cancer [111, 112]	All stages	High	qRT-PCR, RNA-seq, IHC	-Advanced pathologic stage -TP53-mutated and ALK-mutated status -Increased TMB -Poor OS
			IHC, Western Blot	-Advanced pathologic stage -EGFR-WT status -Increased TMB

BC breast cancer, **CRPC** colorectal cancer, **CRPC** castration-resistant prostate cancer, **DFS** disease-free survival, **IHC** immunochemistry, **ISGs** interferon-stimulated genes, **OS** overall survival, **PFS** progression-free survival, **qPCR** quantitative PCR, **qRT-PCR** quantitative reverse transcription polymerase chain reaction, **RNA-seq** RNA sequencing, **RFS** relapse-free survival, **TMB** tumor mutational burden.

and a helicase-like domain (Pol θ -hel, residues 32–899; Fig. 3a). These domains are essential in the TMEJ process, while the central part plays a regulatory role [70, 71]. In this light, selective inhibition of Pol θ -pol and/or Pol θ -hel represents a promising efficient strategy in anticancer selective treatment development [22, 72, 73]. In the last decade, several studies led to the identification of different Pol θ -hel and/or Pol θ -pol inhibitors, and, given the increasing interest at a clinical level, new more powerful, and selective Pol θ inhibitors are still being investigated and here highlighted.

Pol θ -polymerase Inhibitors

Among the Pol θ inhibitors landscape, DNA nucleotide derivatives, first analyzed by Temple University in 2017, are considered the ancient ones. These size-expanded nucleotides recognized by Pol θ as their substrates inhibit the polymerase activity. Differently from the physiological ones, these synthetic derivatives present a 4, 5, or 6-membered additional ring modified with different substituents. The incorporation of these two consecutive deoxyribonucleoside monophosphates led to an effective Pol θ inhibition, probably due to a polymerase active site distortion [74, 75]. Figure 3b-1 shows the generalized 2D-chemical structure of such size-expanded nucleotides.

Other classes of Pol θ -pol inhibitors, presenting a completely different chemical structure, were heteroarylmethylene and acetamido derivatives, both consisted of two (hetero)aromatic groups (Ar₁ and Ar₂) separated by four atoms (N/C-C-C-X). In some

derivatives, the atoms N/C-C are part of a heteroaromatic ring (A₃), while in the acetamido derivative, the atoms N-C are part of a tertiary amide. The distance between the two aromatic groups (Ar₁ and Ar₂) plays a pivotal role in their activity and effectiveness [76, 77]. Figure 3b-2 highlights the 2D-chemical compound structures, which exhibit high and significant affinity, with an IC₅₀ in the range of 0.2–10 μ M.

The evolution of these inhibitors arrived in 2021 when Ideaya and collaborators synthesized cyclic acetamido derivatives, in which a nitrogen heterocycle, blocking the stereochemistry of the molecule, was added. Among this new category, compound ART558 (Fig. 3b-3) was extensively investigated, showing the best potency with an IC₅₀ of 7.9 nM, 3 orders of magnitude lower [22, 78]. At the same level ART812 (Fig. 3b-3), in which additional hydroxyl and carbonyl groups increased the metabolic stability. ART812 exhibited good oral bioavailability in both mouse and rat models, as well as moderate-to-high exposure after a single oral dose, a relevant feature for a clinical application. In vivo pharmacodynamic studies determined ART812 target engagement and a twofold increase induction of micronuclei in reticulocytes, a marker of DNA damage, after 4-day treatment at the maximum tolerated dose of 150 mg/kg BID, a result analogous to Pol θ loss in knockout mice.

Concurrently, a new series of Pol θ -pol inhibitors are reported, such as the RP-6685 (Fig. 3b-3), with an IC₅₀ of 5.8 nM. Structurally, RP-6685 still presents two linked aromatics groups, fundamental

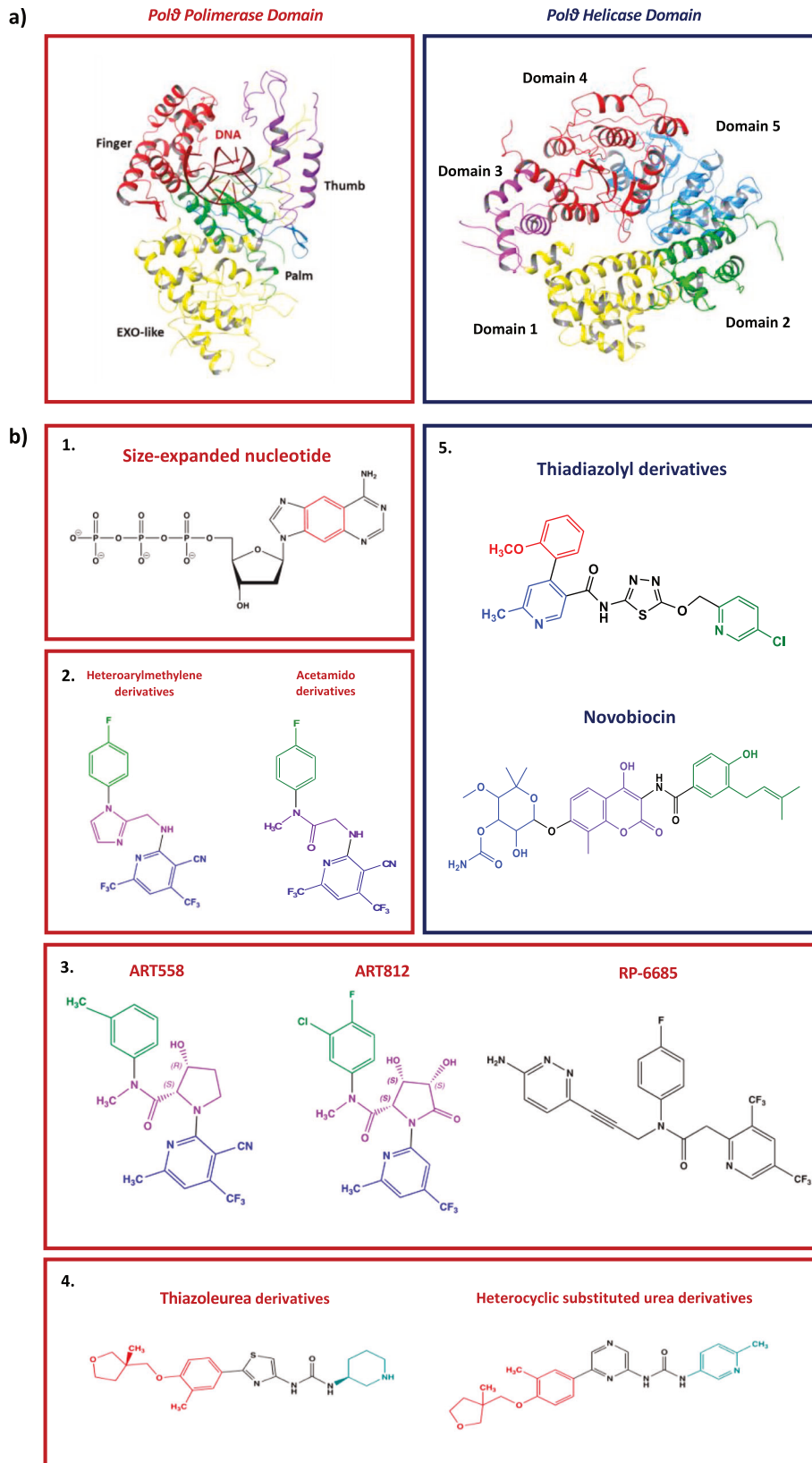


Fig. 3 Pol θ structure and Pol θ -chemical inhibitors. **a** 3D structure of the Pol θ -helicase and polymerase domains (PDB code 5AGA; PDB code 6XBU); **b** 2D-Chemical structures of Pol θ -hel inhibitors: size-expanded nucleotides [1]; heteroaryl-methylene and acetamido derivatives [2]; ART558, ART812 and RP-6685 [3]; thiazoleurea and heterocyclic substituted urea derivatives [4]. 2D-Chemical structures of Pol θ -pol inhibitors: thiadiazole derivatives and novobiocin [5].

Table 4. Summary of ongoing clinical trials on Polθ inhibitors.

Clinical Trial ID	Phase	Agent	Combination	Enrollment (estimated)	Start Date	Completion date (estimated)
NCT05898399	I/II	ART6043	Monotherapy, Olaparib, Talazoparib	250	06/23	12/26
NCT04991480	I/II	ART4215	Monotherapy, Talazoparib, Niraparib	390	09/23	01/26
NCT06077877	I/II	GSK4524101	Monotherapy, Niraparib	135	10/23	11/25
NCT05687110	I	Novobiocin	Monotherapy	30	07/23	05/24

for inhibitors' activity. In this compound, the usual linker N-C(=O)-C-X was shortened by deleting the X atom.

A very high affinity was also reported with thiazoleurea and heterocyclic substituted urea derivatives. These derivatives, structurally different, are characterized by the presence of a central urea moiety connected to thiazole/pyrazine [79, 80]. Such compounds (Fig. 3b-4) with a Polθ IC₅₀ of 7 nM and 2 nM, respectively, lead to a strong and effective affinity.

Polθ-helicase inhibitors

To enhance Polθ effective inhibition, many efforts, even though not comparable to the Polθ-pol subunit, have been made also in Polθ-hel inhibitors research. Among them, thiadiazolyl derivatives, characterized by the presence of a central *N*-thiadiazolyl acetoamide group with a methoxyl side, stand out. Here, two aromatic groups are linked to the acetoamide methoxy moieties. Figure 3b-5 shows an example of such compounds, which possess an IC₅₀ < 200 nM [81, 82].

Still, in this scenario, the 2-oxo-2H-chromene, naphthalene, and quinoline derivatives are of particular interest [83]. Among the synthesized compounds, Novobiocin (NVB) (Fig. 3b-5), an antibiotic derived from streptomyces, is the only one already studied. In vivo studies have shown that the use of NVB reduces tumor growth in genetically modified mice with a *BRCA1* deficiency, and also increases the survival of tumor-bearing mice [72]. Currently, a clinical trial with NVB is now ongoing.

Over the last decade, chemical research on Polθ-inhibitors has progressed beyond the initial discovery phase, identifying always new promising candidates as potential anticancer drugs.

POLθ-INHIBITORS IN THE CLINICAL SETTING: AN OVERVIEW OF THE ONGOING PHASE I/II TRIALS

Breakthroughs on Polθ, already led to the same phase I/II clinical trials. These trials are ongoing and will play the role of critical milestones in bringing Polθ inhibitors from preclinical promises to clinical applications, addressing essential aspects such as efficacy, safety, and potential benefits for cancer patients (Table 4).

The NCT05898399 is a Phase I/II trial enrolling up to 250 patients with advanced or metastatic cancer. It explores the combination of ART6043 with olaparib or talazoparib, both oral PARPi. The study was designed in four different parts: part A1 (ART6043 as monotherapy), part A2 and A3 (ART6043 in combination with olaparib or talazoparib, respectively), and part B (ART6043 in combination with a PARPi or a PARPi alone). Participants are treated with these drugs in 21-day cycles. Primary outcome measures included the number of participants with dose-limiting toxicities (DLTs) and progression-free survival (PFS). Secondary outcome measures focus on the number of participants with adverse events, best overall response (BOR), objective response rate (ORR), disease control rate (DCR), duration of response (DOR), change in tumor size, and change in cancer antigen 125 (CA-125) levels.

The NCT04991480 is a Phase I/II trial evaluating the efficacy of drug ART4215 in 390 estimated patients with advanced or

metastatic solid tumors, specifically focusing on safe and recommended doses, either alone and in combination with talazoparib or niraparib. This study aims to understand the side effects and effectiveness of ART4215 in these settings. The trial includes various experimental parts, such as part B2 for participants with solid cancers showing sensitivity to Polθ inhibition and part B3 for participants with *HER2*-negative *BRCA* breast cancer patients. Primary outcome measures include the number of participants with DLTs and PFS, while secondary outcomes focus on BOR, ORR, DCR, DOR, and changes in tumor size.

The NCT06077877 was a Phase I/II study designed to evaluate GSK4524101 administration, alone or in combination with niraparib, for the treatment of 135 patients with advanced solid tumors. It is structured in two parts: part 1 focuses on the food effect of GSK4524101 and part 2 combines GSK4524101 with niraparib. The primary outcome measures for part 1 include the number of participants with DLTs, treatment-emergent adverse events (AEs), serious adverse events (SAEs), and the percentage of participants who receive all planned doses. In part 2, the confirmed ORR is the key outcome. Secondary outcomes involve pharmacokinetic parameters like Area Under Curve (AUC) and maximum Concentration (C_{max}) of GSK4364973, a metabolite of GSK4524101, and plasma concentration of niraparib.

Finally, the NCT05687110 (Phase I, National Cancer Institute, NCI) trial, was designed to evaluate the safe and optimal novobiocin dose in up to 30 estimated patients with solid tumors characterized by DNA repair gene alteration. Participants are receiving novobiocin sodium and are undergoing procedures like tumor biopsy, biospecimen collection, and medical imaging scans. The primary outcome measure is the maximum tolerated dose (MTD) and recommended phase 2 dose for continuous Novobiocin administration. Secondary outcome measures include plasma concentrations of Novobiocin and biological effectiveness, defined as an increase in *RAD51*-foci positive cells. Other outcome measures focus on Polθ mRNA levels and *ATM* immunohistochemistry (IHC).

Each of the trials encounters typical challenges of early-phase research, such as difficulties in patient recruitment and the necessity for accurate patient selection. Additionally, potential side effects and the determination of the optimal dosage for clinical use are still in need of careful evaluation. These challenges underscore the complexities of translating preclinical promising molecular targets into effective clinical therapies, emphasizing the need for cautious optimism.

Emerging data from preclinical studies are providing hopeful signs that these ongoing trials may confirm. The current trials on Polθ inhibitors, particularly in combination with PARPi such as olaparib, niraparib, and talazoparib, highlight an emerging trend in cancer treatment. This strategy aims to discover synergistic effects that could improve the overall effectiveness of these therapies. Notably, the use of Polθ inhibitors has the potential to be effective, in a clinical setting, not just in treating cancers with *BRCA*-gene defects but also in overcoming resistance to PARPi, which often occurs due to reversion mutations [22, 72].

Looking forward, the outcomes of these trials are anticipated to significantly shape the future of Polθ inhibitor research. If

successful, they could pave the way for expanded investigations into Polθ across various cancer types, validating their clinical utility. Furthermore, positive results may encourage the exploration of combination therapies, refining patient selection criteria, and potentially positioning Polθ as a standard component in cancer treatment protocols.

CONCLUSION

The most remarkable finding in synthetic lethality is the hypersensitivity to PARPis and the benefit in clinical outcomes of HRD-associated cancer patients, mainly ovarian, breast, and prostate cancer patients. Approximately 50% of HRD tumors show innate or acquired drug resistance to PARPis, and overcoming the resistance mechanism is one of the major challenges of clinical research in the *BRCA* and other non-*BRCA*, HR-associated, tumors.

Polθ is largely explored as a drug target and potential synthetic lethal partner of a novel form of SL. Exploiting the central role of Polθ in TMEJ, and the emerging link between TMEJ, Polθ, and *BRCA*-gene reversion mutations, Polθ inhibitors seem to have clinical potential not only in targeting HRD-defective tumors but also to prevent or delay the onset of PARPi resistance.

Encouragingly, although the clinical trials exploring these Polθ/HR-genes interaction are only at an early stage, preliminary reports demonstrated that the inhibition of Polθ is a promising cancer treatment strategy, and identify Polθ as emerging target for rational combinations with PARP inhibitors.

In addition, Polθ showed synthetically lethal interactions with other DDR genes, such as *RAD52* controlling in *SSA*, the *ATM*, *ATR*, and *FANCD2* genes working in the HR pathway, and *TP53BP1* involved NHEJ, expanding the landscape for future clinical applications. Other pleiotropic effects, like the improved radiosensitization of *p53*-mutated tumor cells, and the new role in the tumor immune environment through the cGAS-STING signaling pathway activation, pave the way for the development of unexplored synthetic lethality strategies. Further work is needed to optimize the therapeutic window, bridging the genetic and molecular insights to oncological clinical practice.

REFERENCES

- Russo A, Incorvaia L, Malapelle U, Del Re M, Capoluongo E, Vincenzi B, et al. The tumor-agnostic treatment for patients with solid tumors: a position paper on behalf of the AIOM- SIAPEC/IAP-SIBioC-SIF Italian Scientific Societies. *Crit Rev Oncol Hematol*. 2021;165:103436.
- O'Neil NJ, Bailey ML, Hieter P. Synthetic lethality and cancer. *Nat Rev Genet*. 2017;18:613–23.
- Gori S, Barberis M, Bella MA, Buttitta F, Capoluongo E, Carrera P, et al. Recommendations for the implementation of *BRCA* testing in ovarian cancer patients and their relatives. *Crit Rev Oncol Hematol*. 2019;140:67–72.
- Li S, Topatana W, Juengpanich S, Cao J, Hu J, Zhang B, et al. Development of synthetic lethality in cancer: molecular and cellular classification. *Signal Transduct Target Ther*. 2020;5:241.
- Bhamidipati D, Haro-Silerio JI, Yap TA, Ngoi N. PARP inhibitors: enhancing efficacy through rational combinations. *Br J Cancer*. 2023;129:904–16.
- Wyatt DW, Feng W, Conlin MP, Yousefzadeh MJ, Roberts SA, Mieczkowski P, et al. Essential roles for polymerase θ-mediated end joining in the repair of chromosome breaks. *Mol Cell*. 2016;63:662–73.
- Mateos-Gomez PA, Gong F, Nair N, Miller KM, Lazzarini-Denchi E, Sfeir A. Mammalian polymerase θ promotes alternative NHEJ and suppresses recombination. *Nature*. 2015;518:254–7.
- Schrepf A, Bernardo S, Arasa Verge EA, Ramirez Otero MA, Wilson J, Kirchhofer D, et al. POLθ processes ssDNA gaps and promotes replication fork progression in *BRCA1*-deficient cells. *Cell Rep* 2022;41:111716.
- Russo A, Incorvaia L, Capoluongo E, Tagliaferri P, Galvano A, Del Re M, et al. The challenge of the molecular tumor board empowerment in clinical oncology practice: a position paper on behalf of the AIOM- SIAPEC/IAP-SIBioC-SIC-SIF-SIGU-SIRM Italian Scientific Societies. *Crit Rev Oncol Hematol*. 2022;169:103567.
- Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. *Mol Cell*. 2010;40:179–204.
- Lee JK, Choi YL, Kwon M, Park PJ. Mechanisms and consequences of cancer genome instability: lessons from genome sequencing studies. *Annu Rev Pathol*. 2016;11:283–312.
- Ceccaldi R, Rondinelli B, D'Andrea AD. Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol*. 2016;26:52–64.
- Curtin NJ, Szabo C. Poly(ADP-ribose) polymerase inhibition: past, present and future. *Nat Rev Drug Discov*. 2020;19:711–36.
- Russo A, Incorvaia L, Capoluongo E, Tagliaferri P, Gori S, Cortesi L, et al. Implementation of preventive and predictive *BRCA* testing in patients with breast, ovarian, pancreatic, and prostate cancer: a position paper of Italian Scientific Societies. *ESMO Open*. 2022;7:100459.
- Ryan CJ, Mehta I, Kebabci N, Adams DJ. Targeting synthetic lethal paralogs in cancer. *Trends Cancer*. 2023;9:397–409.
- Li H, Liu ZY, Wu N, Chen YC, Cheng Q, Wang J. PARP inhibitor resistance: the underlying mechanisms and clinical implications. *Mol Cancer*. 2020;19:107.
- Kim D, Nam HJ. PARP inhibitors: clinical limitations and recent attempts to overcome them. *Int J Mol Sci*. 2022;23:8412.
- Incorvaia L, Passiglia F, Rizzo S, Galvano A, Listi A, Barraco N, et al. "Back to a false normality": new intriguing mechanisms of resistance to PARP inhibitors. *Oncotarget*. 2017;8:23891–904.
- Bono M, Fanale D, Incorvaia L, Cancelliere D, Fiorino A, Calò V, et al. Impact of deleterious variants in other genes beyond *BRCA1/2* detected in breast/ovarian and pancreatic cancer patients by NGS-based multi-gene panel testing: looking over the hedge. *ESMO Open*. 2021;6:100235.
- Fanale D, Incorvaia L, Filorizzo C, Bono M, Fiorino A, Calò V, et al. Detection of germline mutations in a cohort of 139 patients with bilateral breast cancer by multi-gene panel testing: impact of pathogenic variants in other genes beyond *BRCA1/2*. *Cancers*. 2020;12:2415.
- Groelly FJ, Fawkes M, Dagg RA, Blackford AN, Tarsounas M. Targeting DNA damage response pathways in cancer. *Nat Rev Cancer*. 2023;23:78–94.
- Zatreanu D, Robinson HMR, Alkhatib O, Boursier M, Finch H, Geo L, et al. Polθ inhibitors elicit *BRCA*-gene synthetic lethality and target PARP inhibitor resistance. *Nat Commun*. 2021;12:3636.
- Simon JA, Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutat Res*. 2008;647:21–9.
- Puppe J, Opdam M, Schouten PC, Józwiak K, Lips E, Severson T, et al. EZH2 is overexpressed in *BRCA1*-like breast tumors and predictive for sensitivity to high-dose platinum-based chemotherapy. *Clin Cancer Res*. 2019;25:4351–62.
- Hu M, Zhou M, Bao X, Pan D, Jiao M, Liu X, et al. ATM inhibition enhances cancer immunotherapy by promoting mtDNA leakage and cGAS/STING activation. *J Clin Invest*. 2021;131:e139333.
- Ratz L, Brambillasca C, Bartke L, Huetzen MA, Goergens J, Leidecker O, et al. Combined inhibition of EZH2 and ATM is synthetic lethal in *BRCA1*-deficient breast cancer. *Breast Cancer Res*. 2022;24:41.
- Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol*. 2010;2:a001008.
- Cimprich KA, Cortez D. ATR: an essential regulator of genome integrity. *Nat Rev Mol Cell Biol*. 2008;9:616–27.
- Toledo LI, Murga M, Zur R, Soria R, Rodriguez A, Martinez S, et al. A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. *Nat Struct Mol Biol*. 2011;18:721–7.
- Tu X, Kahila MM, Zhou Q, Yu J, Kalari KR, Wang L, et al. ATR inhibition is a promising radiosensitizing strategy for triple-negative breast cancer. *Mol Cancer Ther*. 2018;17:2462–72.
- Tang Z, Pilié PG, Geng C, Manyam GC, Yang G, Park S, et al. ATR inhibition induces CDK1-SPOP signaling and enhances anti-PD-L1 cytotoxicity in prostate cancer. *Clin Cancer Res*. 2021;27:4898–909.
- Yazinski SA, Comaills V, Buisson R, Genois MM, Nguyen HD, Ho CK, et al. ATR inhibition disrupts rewired homologous recombination and fork protection pathways in PARP inhibitor-resistant *BRCA*-deficient cancer cells. *Genes Dev*. 2017;31:318–32.
- Thomas A, Takahashi N, Rajapakse VN, Zhang X, Sun Y, Ceribelli M, et al. Therapeutic targeting of ATR yields durable regressions in small cell lung cancers with high replication stress. *Cancer Cell*. 2021;39:566–79.e7.
- El Touny LH, Hose C, Connelly J, Harris E, Monks A, Dull AB, et al. ATR inhibition reverses the resistance of homologous recombination deficient MGMT. *Oncotarget*. 2021;12:2114–30.
- Wallez Y, Dunlop CR, Johnson TI, Koh SB, Fornari C, Yates JWT, et al. The ATR inhibitor AZD6738 synergizes with gemcitabine. *Mol Cancer Ther*. 2018;17:1670–82.
- Nam AR, Yoon J, Jin MH, Bang JH, Oh KS, Seo HR, et al. ATR inhibition amplifies antitumor effects of olaparib in biliary tract cancer. *Cancer Lett*. 2021;516:38–47.
- Murai J, Feng Y, Yu GK, Ru Y, Tang SW, Shen Y, et al. Resistance to PARP inhibitors by SFLN11 inactivation can be overcome by ATR inhibition. *Oncotarget*. 2016;7:76534–50.

38. Massey AJ, Stephens P, Rawlinson R, McGurk L, Plummer R, Curtin NJ. mTORC1 and DNA-PKcs as novel molecular determinants of sensitivity to Chk1 inhibition. *Mol Oncol*. 2016;10:101–12.
39. Ma CX, Cai S, Li S, Ryan CE, Guo Z, Schaiff WT, et al. Targeting Chk1 in p53-deficient triple-negative breast cancer is therapeutically beneficial in human-mouse tumor models. *J Clin Invest*. 2012;122:1541–52.
40. Sen T, Rodriguez BL, Chen L, Corte CMD, Morikawa N, Fujimoto J, et al. Targeting DNA damage response promotes antitumor immunity through STING-mediated T-cell activation in small cell lung cancer. *Cancer Discov*. 2019;9:646–61.
41. Patel PS, Algouneh A, Hakem R. Exploiting synthetic lethality to target BRCA1/2-deficient tumors: where we stand. *Oncogene*. 2021;40:3001–14.
42. Truong LN, Li Y, Shi LZ, Hwang PY, He J, Wang H, et al. Microhomology-mediated end joining and homologous recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. *Proc Natl Acad Sci USA*. 2013;110:7720–5.
43. Schrepf A, Slysokova J, Loizou JI. Targeting the DNA repair enzyme polymerase θ in cancer therapy. *Trends Cancer*. 2021;7:98–111.
44. Feng W, Simpson DA, Carvajal-Garcia J, Price BA, Kumar RJ, Mose LE, et al. Genetic determinants of cellular addiction to DNA polymerase theta. *Nat Commun*. 2019;10:4286.
45. Incorvaia L, Perez A, Marchetti C, Brando C, Gristina V, Cancelliere D, et al. Theranostic biomarkers and PARP-inhibitors effectiveness in patients with non-BRCA associated homologous recombination deficient tumors: still looking through a dirty glass window? *Cancer Treat Rev*. 2023;121:102650.
46. Ceccaldi R, Liu JC, Amunugama R, Hajdu I, Primack B, Petalcorin MI, et al. Homologous-recombination-deficient tumours are dependent on Pol θ -mediated repair. *Nature*. 2015;518:258–62.
47. Bunting SF, Callén E, Wong N, Chen HT, Polato F, Gunn A, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell*. 2010;141:243–54.
48. Kraiss JJ, Glass DJ, Chudoba I, Wang Y, Feng W, Simpson D, et al. Genetic separation of Brca1 functions reveal mutation-dependent Pol θ vulnerabilities. *Nat Commun*. 2023;14:7714.
49. Ronson GE, Starowicz K, Anthony EJ, Piberger AL, Clarke LC, Garvin AJ, et al. Mechanisms of synthetic lethality between BRCA1/2 and 53BP1 deficiencies and DNA polymerase theta targeting. *Nat Commun*. 2023;14:7834.
50. Belan O, Sebald M, Adamowicz M, Anand R, Vancevska A, Neves J, et al. POLQ seals post-replicative ssDNA gaps to maintain genome stability in BRCA-deficient cancer cells. *Mol Cell*. 2022;82:4664–80.e9.
51. Li J, Ko JM, Dai W, Yu VZ, Ng HY, Hoffmann JS, et al. Depletion of DNA polymerase theta inhibits tumor growth and promotes genome instability through the cGAS-STING-ISR pathway in esophageal squamous cell carcinoma. *Cancers*. 2021;13:3204.
52. Oh G, Wang A, Wang L, Li J, Werba G, Weissinger D, et al. POLQ inhibition elicits an immune response in homologous recombination-deficient pancreatic adenocarcinoma via cGAS-STING signaling. *J Clin Invest*. 2023;133:e165934.
53. Moldovan GL, D'Andrea AD. How the fanconi anemia pathway guards the genome. *Annu Rev Genet*. 2009;43:223–49.
54. Dai CH, Chen P, Li J, Lan T, Chen YC, Qian H, et al. Co-inhibition of pol θ and HR genes efficiently synergize with cisplatin to suppress cisplatin-resistant lung cancer cells survival. *Oncotarget*. 2016;7:65157–70.
55. Fleury H, MacEachern MK, Stiefel CM, Anand R, Sempeck C, Nebenfuhr B, et al. The APE2 nuclease is essential for DNA double-strand break repair by microhomology-mediated end joining. *Mol Cell*. 2023;83:1429–45.e8.
56. Rodriguez-Berriguete G, Ranzani M, Prevo R, Puliyadi R, Machado N, Bolland HR, et al. Small-molecule Pol θ inhibitors provide safe and effective tumor radiosensitization in preclinical models. *Clin Cancer Res*. 2023;29:1631–42.
57. Kuei CH, Lin HY, Lin MH, Lee HH, Lin CH, Lee WJ, et al. DNA polymerase theta repression enhances the docetaxel responsiveness in metastatic castration-resistant prostate cancer. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866:165954.
58. Higgins GS, Harris AL, Prevo R, Helleday T, McKenna WG, Buffa FM. Overexpression of POLQ confers a poor prognosis in early breast cancer patients. *Oncotarget*. 2010;1:175–84.
59. Long J, Zhu JY, Liu YB, Fu K, Tian Y, Li PY, et al. Helicase POLQ-like (HELQ) as a novel indicator of platinum-based chemoresistance for epithelial ovarian cancer. *Gynecol Oncol*. 2018;149:341–9.
60. Smolinska A, Singer K, Golchert J, Smyczynska U, Fendler W, Sandler M, et al. DNA polymerase theta plays a critical role in pancreatic cancer development and metastasis. *Cancers*. 2022;14:4077.
61. Allera-Moreau C, Rouquette I, Lepage B, Oumouhou N, Walschaerts M, Leconte E, et al. DNA replication stress response involving PLK1, CDC6, POLQ, RAD51 and CLASPIN upregulation prognoses the outcome of early/mid-stage non-small cell lung cancer patients. *Oncogenesis*. 2012;1:e30.
62. Kawamura K, Bahar R, Seimiya M, Chiyo M, Wada A, Okada S, et al. DNA polymerase theta is preferentially expressed in lymphoid tissues and upregulated in human cancers. *Int J Cancer*. 2004;109:9–16.
63. Pillaire MJ, Selves J, Gordien K, Gourraud PA, Gentil C, Danjoux M, et al. A 'DNA replication' signature of progression and negative outcome in colorectal cancer. *Oncogene*. 2010;29:876–87.
64. Pismataro MC, Astolfi A, Barreca ML, Pacetti M, Schenone S, Bandiera T, et al. Small molecules targeting DNA polymerase theta (POL θ) as promising synthetic lethal agents for precision cancer therapy. *J Med Chem*. 2023;66:6498–522.
65. Wood RD, Doublie S. DNA polymerase θ (POLQ), double-strand break repair, and cancer. *DNA Repair*. 2016;44:22–32.
66. Goulet de Rugy T, Bashkurov M, Datti A, Betous R, Guitton-Sert L, Cazaux C, et al. Excess Pol θ functions in response to replicative stress in homologous recombination-proficient cancer cells. *Biol Open*. 2016;5:1485–92.
67. Lemée F, Bergoglio V, Fernandez-Vidal A, Machado-Silva A, Pillaire MJ, Bieth A, et al. DNA polymerase theta up-regulation is associated with poor survival in breast cancer, perturbs DNA replication, and promotes genetic instability. *Proc Natl Acad Sci USA*. 2010;107:13390–5.
68. Nappi L, Mazurek S, Khazamipour N, Janfaza S, Jia A, Ozcan G, et al. 21P polymerase theta inhibition in homologous recombination-deficient prostate cancer. *ESMO Open*. 2024;9.
69. Principe DR, Narbutis M, Koch R, Rana A. Frequency and prognostic value of mutations associated with the homologous recombination DNA repair pathway in a large pan cancer cohort. *Sci Rep*. 2020;10:20223.
70. Seki M, Marini F, Wood RD, POLQ (Pol theta), a DNA polymerase and DNA-dependent ATPase in human cells. *Nucleic Acids Res*. 2003;31:6117–26.
71. Black SJ, Ozdemir AY, Kashkina E, Kent T, Rusanov T, Ristic D, et al. Molecular basis of microhomology-mediated end-joining by purified full-length Pol θ . *Nat Commun*. 2019;10:4423.
72. Zhou J, Gelot C, Pantelidou C, Li A, Yücel H, Davis RE, et al. A first-in-class polymerase theta inhibitor selectively targets homologous-recombination-deficient tumors. *Nat Cancer*. 2021;2:598–610.
73. Bubenik M, Mader P, Mochirian P, Vallée F, Clark J, Truchon JF, et al. Identification of RP-6685, an orally bioavailable compound that inhibits the DNA polymerase activity of Pol θ . *J Med Chem*. 2022;65:13198–215.
74. Kent T, Rusanov TD, Hoang TM, Velema WA, Krueger AT, Copeland WC, et al. DNA polymerase θ specializes in incorporating synthetic expanded-size (xDNA) nucleotides. *Nucleic Acids Res*. 2016;44:9381–92.
75. Pomerantz RT, Kool ET. Compositions and methods of treatment using expanded-size DNA analogs. *World Patent WO2018035410*. 2018.
76. Beck HP, Dillon M, Jones B, Martinez LP. Heteroarylmethylene derivatives as DNA polymerase theta inhibitors. *World Patent WO2020160213*. 2020.
77. Beck HP, Jones BT, Martinez LP. Acetamido-amino and acetamido-sulfur derivatives as DNA polymerase theta inhibitors. *World Patent WO2022026548*. 2022.
78. Stockley ML, Ferdinand A, Benedetti G, Blencowe P, Boyd SM, Calder M, et al. Discovery, characterization, and structure-based optimization of small-molecule in vitro and in vivo probes for human DNA polymerase theta. *J Med Chem*. 2022;65:13879–91.
79. Blencowe P, Charles M, Ekwuru T, MacDonald E, McCarron H, Rigoreau L. Thiazoleureas as anticancer agents. *World Patent WO2020030924*. 2020.
80. Blencowe P, Charles M, Cridland A, Ekwuru T, Heald R, MacDonald E, et al. Heterocyclic substituted ureas, for use against cancer. *World Patent WO2020030925*. 2020.
81. Barsanti PA, Beck HP, Fleury M, Jones BT, McSpadden ED, Pei Z, et al. Substituted thiadiazolyl derivatives as DNA polymerase theta inhibitors. *World Patent WO2022118210*. 2022.
82. Barsanti PA, Beck HP, Fleury M, Knox JE, McSpadden ED, Jones BT, et al. Thiadiazolyl derivatives as DNA polymerase theta inhibitors. *World Patent WO2020243459*. 2020.
83. D'Andrea A, Blagg BSJ, Davis RE, Zhou J. Compounds and methods for treating cancer. *World Patent WO2021046220*. 2021.
84. Caldecott KW. Single-strand break repair and genetic disease. *Nat Rev Genet*. 2008;9:619–31.
85. Krokan HE, Bjørås M. Base excision repair. *Cold Spring Harb Perspect Biol*. 2013;5:a012583.
86. Caldecott KW. DNA single-strand break repair. *Exp Cell Res*. 2014;329:2–8.
87. Jiricny J. Postreplicative mismatch repair. *Cold Spring Harb Perspect Biol*. 2013;5:a012633.
88. Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol*. 2006;7:335–46.
89. Hanawalt PC, Spivak G. Transcription-coupled DNA repair: two decades of progress and surprises. *Nat Rev Mol Cell Biol*. 2008;9:958–70.
90. Jeggo PA, Löbrich M. DNA double-strand breaks: their cellular and clinical impact? *Oncogene*. 2007;26:7717–9.

91. Mladenov E, Mladenova V, Stuschke M, Iliakis G. New facets of DNA double strand break repair: radiation dose as key determinant of HR versus c-NHEJ engagement. *Int J Mol Sci.* 2023;24:14956.
92. Perrault R, Wang H, Wang M, Rosidi B, Iliakis G. Backup pathways of NHEJ are suppressed by DNA-PK. *J Cell Biochem.* 2004;92:781–94.
93. Wang H, Perrault AR, Takeda Y, Qin W, Iliakis G. Biochemical evidence for Ku-independent backup pathways of NHEJ. *Nucleic Acids Res.* 2003;31:5377–88.
94. Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, et al. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. *N. Engl J Med.* 2019;381:317–27.
95. Kindler HL, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, et al. Overall survival results from the POLO trial: a phase III study of active maintenance olaparib versus placebo for germline BRCA-mutated metastatic pancreatic cancer. *J Clin Oncol.* 2022;40:3929–39.
96. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for metastatic castration-Resistant Prostate Cancer. *N. Engl J Med.* 2020;382:2091–102.
97. Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, et al. Adjuvant olaparib for patients with BRCA1- or BRCA2-mutated breast cancer. *N. Engl J Med.* 2021;384:2394–405.
98. Geyer CE, Garber JE, Gelber RD, Yothers G, Taboada M, Ross L, et al. Overall survival in the OlympiA phase III trial of adjuvant olaparib in patients with germline pathogenic variants in BRCA1/2 and high-risk, early breast cancer. *Ann Oncol.* 2022;33:1250–68.
99. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *N. Engl J Med.* 2017;377:523–33.
100. Robson ME, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. OlympiAD extended follow-up for overall survival and safety: olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. *Eur J Cancer.* 2023;184:39–47.
101. Litton JK, Hurvitz SA, Mina LA, Rugo HS, Lee KH, Gonçalves A, et al. Talazoparib versus chemotherapy in patients with germline BRCA1/2-mutated HER2-negative advanced breast cancer: final overall survival results from the EMBRACA trial. *Ann Oncol.* 2020;31:1526–35.
102. González-Martín A, Pothuri B, Vergote I, Graybill W, Lorusso D, McCormick CC, et al. Progression-free survival and safety at 3.5years of follow-up: results from the randomised phase 3 PRIMA/ENGOT-OV26/GOG-3012 trial of niraparib maintenance treatment in patients with newly diagnosed ovarian cancer. *Eur J Cancer.* 2023;189:112908.
103. Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *N Engl J Med.* 2019;381:2416–28.
104. Ray-Coquard I, Leary A, Pignata S, Cropet C, González-Martín A, Marth C, et al. Olaparib plus bevacizumab first-line maintenance in ovarian cancer: final overall survival results from the PAOLA-1/ENGOT-ov25 trial. *Ann Oncol.* 2023;34:681–92.
105. Del Campo JM, Matulonis UA, Malander S, Provencher D, Mahner S, Follana P, et al. Niraparib maintenance therapy in patients with recurrent ovarian cancer after a partial response to the last platinum-based chemotherapy in the ENGOT-OV16/NOVA trial. *J Clin Oncol.* 2019;37:2968–73.
106. Mirza MR, Benigno B, Dørum A, Mahner S, Bessette P, Barceló IB, et al. Long-term safety in patients with recurrent ovarian cancer treated with niraparib versus placebo: results from the phase III ENGOT-OV16/NOVA trial. *Gynecol Oncol.* 2020;159:442–8.
107. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* 2017;390:1949–61.
108. Banerjee S, Moore KN, Colombo N, Scambia G, Kim BG, Oaknin A, et al. Maintenance olaparib for patients with newly diagnosed advanced ovarian cancer and a BRCA mutation (SOLO1/GOG 3004): 5-year follow-up of a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* 2021;22:1721–31.
109. DiSilvestro P, Banerjee S, Colombo N, Scambia G, Kim BG, Oaknin A, et al. Overall survival with maintenance olaparib at a 7-year follow-up in patients with newly diagnosed advanced ovarian cancer and a BRCA mutation: the SOLO1/GOG 3004 trial. *J Clin Oncol.* 2023;41:609–17.
110. Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H, Sun J, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol.* 2017;18:75–87.
111. Rao X, Xing B, Wu Z, Bin Y, Chen Y, Xu Y, et al. Targeting polymerase θ impairs tumorigenesis and enhances radiosensitivity in lung adenocarcinoma. *Cancer Sci.* 2023;114:1943–57.
112. Shinmura K, Kato H, Kawanishi Y, Yoshimura K, Tsuchiya K, Takahara Y, et al. POLQ overexpression is associated with an increased somatic mutation load and PLK4 overexpression in lung adenocarcinoma. *Cancers.* 2019;11:722.

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AUTHOR CONTRIBUTIONS

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COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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