State-of-the-art strategies for targeting the DNA damage response in cancer

*Patrick G. Pilié[1,8], Chad Tang[2,3,8], Gordon B. Mills[4,5] and Timothy A. Yap[3,5,6,7]**

Abstract | Genomic instability is a key hallmark of cancer that arises owing to defects in the DNA damage response (DDR) and/or increased replication stress. These alterations promote the clonal evolution of cancer cells via the accumulation of driver aberrations, including gene copy-number changes, rearrangements and mutations; however, these same defects also create vulnerabilities that are relatively specific to cancer cells, which could potentially be exploited to increase the therapeutic index of anticancer treatments and thereby improve patient outcomes. The discovery that *BRCA*-mutant cancer cells are exquisitely sensitive to inhibition of poly(ADP-ribose) polymerase has ushered in a new era of research on biomarker-driven synthetic lethal treatment strategies for different cancers. The therapeutic landscape of antitumour agents targeting the DDR has rapidly expanded to include inhibitors of other key mediators of DNA repair and replication, such as ATM, ATR, CHK1 and CHK2, DNA-PK and WEE1. Efforts to optimize these therapies are ongoing across a range of cancers, involving the development of predictive biomarker assays of responsiveness (beyond *BRCA* mutations), assessment of the mechanisms underlying intrinsic and acquired resistance, and evaluation of rational, tolerable combinations with standard-of-care treatments (such as chemotherapeutics and radiation), novel molecularly targeted agents and immune-checkpoint inhibitors. In this Review, we discuss the current status of anticancer therapies targeting the DDR.

DNA damage occurs constantly in cells owing to exogenous and endogenous stressors, and cells have consequently evolved a complex, coordinated DNA damage response (DDR) that encompasses numerous interdependent signalling pathways and machineries. The importance of the DDR in maintaining cell viability and preventing neoplasia is underscored by the additional integral roles of these pathways in regulating the cell cycle, chromatin remodelling, metabolism, immunogenicity and apoptosis[1,2]. For example, the detection of DNA damage results in the activation of checkpoints that enforce cell cycle arrest to provide the time necessary for DNA repair before cell division; DDR pathways are also closely linked with the apoptotic machinery to enable the elimination of cells with unrepaired DNA damage. Thus, the DDR pathways ultimately enable cell survival in the face of genomic instability and replicative stress, or direct irreparably damaged cells to undergo senescence or programmed death. Genomic instability is a key hallmark of cancer[3] and arises as a result of the high rate of cell division and the related rapid accumulation of aberrations on a background of the compromised DDR processes that contribute to cancer initiation and progression. Hence, defects in DDR genes have multiple roles in the promotion of cancer cell growth via accrual of driver mutations, generation of tumour heterogeneity and evasion of apoptosis[4]. Cells are programmed to constitutively respond to DNA damage, whereby the repair pathways used are dependent on the specific type of damage detected and repair machineries available (FIG. 1). DNA damage most commonly manifests as single-strand breaks (SSBs), although double-strand breaks (DSB) are more lethal to cells and require rapid countermeasures to ensure cell survival. Thus, most contemporary DDR-directed therapies target the signalling and repair mechanisms associated with DSBs, increase replication stress and thereby the frequency of DSBs, or inhibit cell cycle checkpoints that facilitate DSB repair (FIG. 1). Defects in certain high-fidelity DDR machineries, including DSB repair processes involving homologous recombination (HR), increase genomic instability and lead to a greater reliance on compensatory — and often error-prone — DDR and survival pathways[2,5,6]. These vulnerabilities have been exploited in anticancer therapy through the use of DNA-damaging radiation and chemotherapies and, more recently, with the rapid development of potent and selective molecularly targeted agents against key components of different DDR pathways (herein termed DDR inhibitors) (FIG. 1). However, the development of analytically and clinically

*e-mail: tyap@mdanderson.org

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Key points

- The DNA damage response (DDR) involves a complex network of genes responsible for sensing and responding to specific types of DNA damage, encompassing specific machineries mediating DNA repair, cell cycle regulation, replication stress responses and apoptosis.
- Defects in the DDR give rise to genomic instability in cells, aiding in cancer initiation and progression via mutation accumulation, but also providing targetable vulnerabilities relatively specific to cancer cells that can be exploited for clinical benefit with the use of DDR inhibitors.
- Targeting BRCA1/2-deficient cancers using poly(ADP-ribose) polymerase (PARP) inhibitors is the archetype of synthetic lethality, but now the therapeutic landscape of DDR inhibitors is rapidly expanding; bridging preclinical data on each of these agents to the clinical setting is vital to inform appropriate biomarkers and timing for their use.
- Preclinical and clinical data on DDR inhibitors indicate that biomarkers of response and resistance extend beyond BRCA1/2 to provide a more inclusive and functionally informed approach to patient selection.
- Preclinical and clinical research with PARP inhibition has revealed multiple resistance mechanisms across a variety of cancer subtypes, highlighting the need for functional biomarkers and sequential or combination treatment strategies.
- While impressive clinical responses can be seen rarely with the use of single-agent DDR inhibitors, a multitude of biologically informed combination treatment strategies using a backbone of DDR inhibitors are under development to extend their use to larger populations, while minimizing overlapping toxicities.

validated assays to robustly assess predictive biomarkers of response and/or resistance to DDR inhibitors has lagged behind. In this Review, we provide an overview of the current landscape of DDR-directed therapies, focusing on the respective DDR pathways and replication stress responses pertaining to the most promising targets and emerging therapeutics. We also discuss putative predictive biomarkers of response, mechanisms of resistance and ongoing preclinical and clinical efforts to develop combinatorial strategies to optimize therapeutic targeting of the DDR.

PARP inhibitors

Similarities and differences

The poly(ADP-ribose) polymerase (PARP) family comprises a group of nuclear proteins that are activated upon binding to damaged DNA and have crucial roles in various aspects of the DDR (FIG. 1). The main function of these proteins is to detect SSBs and DSBs, recruit the DNA repair machinery and stabilize replication forks during repair[7]. The rationale for the antitumour activity of single-agent PARP inhibitors in selected HR-deficient (HRD) tumours, initially those with germline *BRCA1* or *BRCA2 (BRCA1/2)* mutations, is based on the concept of synthetic lethality, whereby the combination of a functional genetic defect in an HR-related gene and pharmacological inhibition of a compensatory DDR-pathway component, such as PARP, leads to insurmountable genomic instability, mitotic catastrophe and cell death[7–12]. PARP inhibitors are the best-studied class of DDR inhibitors, with robust preclinical and clinical data informing refinements in patient selection and treatment protocols (FIG. 2). With several PARP inhibitors already FDA approved or undergoing testing in late phase clinical trials as single agents in various disease and treatment settings (TABLE 1; Supplementary Table 1), investigators must be cognizant of the mechanisms underlying the anticancer activity of drugs within this class, including any pharmacological similarities and differences, and the respective clinical outcomes and toxicities reported to date. Importantly, a detailed understanding of the toxicities and how to mitigate them will be necessary to optimize the therapeutic index.

All PARP inhibitors currently used in the clinic have a similar capacity to inhibit the catalytic activity of PARP because they share a nicotinamide moiety that competes with NAD^+ for binding to this enzyme; however, differences do exist regarding the dose required to inhibit PARP activity and the relative selectivity for different PARP family members. Notwithstanding, the mechanism of cytotoxicity of PARP inhibitors goes beyond simply abrogating catalytic activity. When PARP activity is inhibited, unrepaired SSBs and stalled replication forks accumulate, owing to ‘trapping’ of PARP in a complex with the DNA strand. During S phase of the cell cycle, these unrepaired SSBs convert to DSBs, which are lethal to HRD cancer cells[7]. Indeed, PARP–DNA complexes are markedly more damaging to the genomic integrity of cells than unbound SSBs alone and activate various pathways outside of HR-dependent DNA repair, including cell cycle checkpoints and post-replication repair[13]. The concept of PARP trapping was first described more than two decades ago[14,15] (FIG. 2), although only within the current decade was DDR demonstrated to be stunted to a greater degree in PARP1-inhibited cells, in which PARP1 remains associated with DNA, than in cells completely lacking PARP1 (REFS[14,15]). Notably, the PARP-trapping abilities of the five most-studied PARP inhibitors — niraparib, rucaparib, talazoparib, olaparib and veliparib — vary markedly, in contrast with their capacities to inhibit protein poly ADP-ribosylation (PARylation), which have a much narrower range. For example, talazoparib, the PARP inhibitor with the greatest PARP-trapping ability observed preclinically, has cytotoxic potency in the nanomolar range, whereas veliparib results in less PARP trapping and is inactive at 100 mM (REF.[13]). Furthermore, the maximum tolerated dose (MTD) of these PARP inhibitors mirrors their respective PARP-trapping ability, rather than their capacity to inhibit PARP catalytic activity[16,17].

Apart from veliparib, the four other aforementioned PARP inhibitors seem to have generally similar

Author addresses

[1]Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
[2]Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
[3]Department of Investigational Cancer Therapeutics (Phase I Program), The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
[4]Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
[5]Khalifa Institute for Personalized Cancer Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
[6]The Institute for Applied Cancer Science, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
[7]Department of Thoracic/Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
[8]These authors contributed equally: Patrick G. Pilié and Chad Tang.

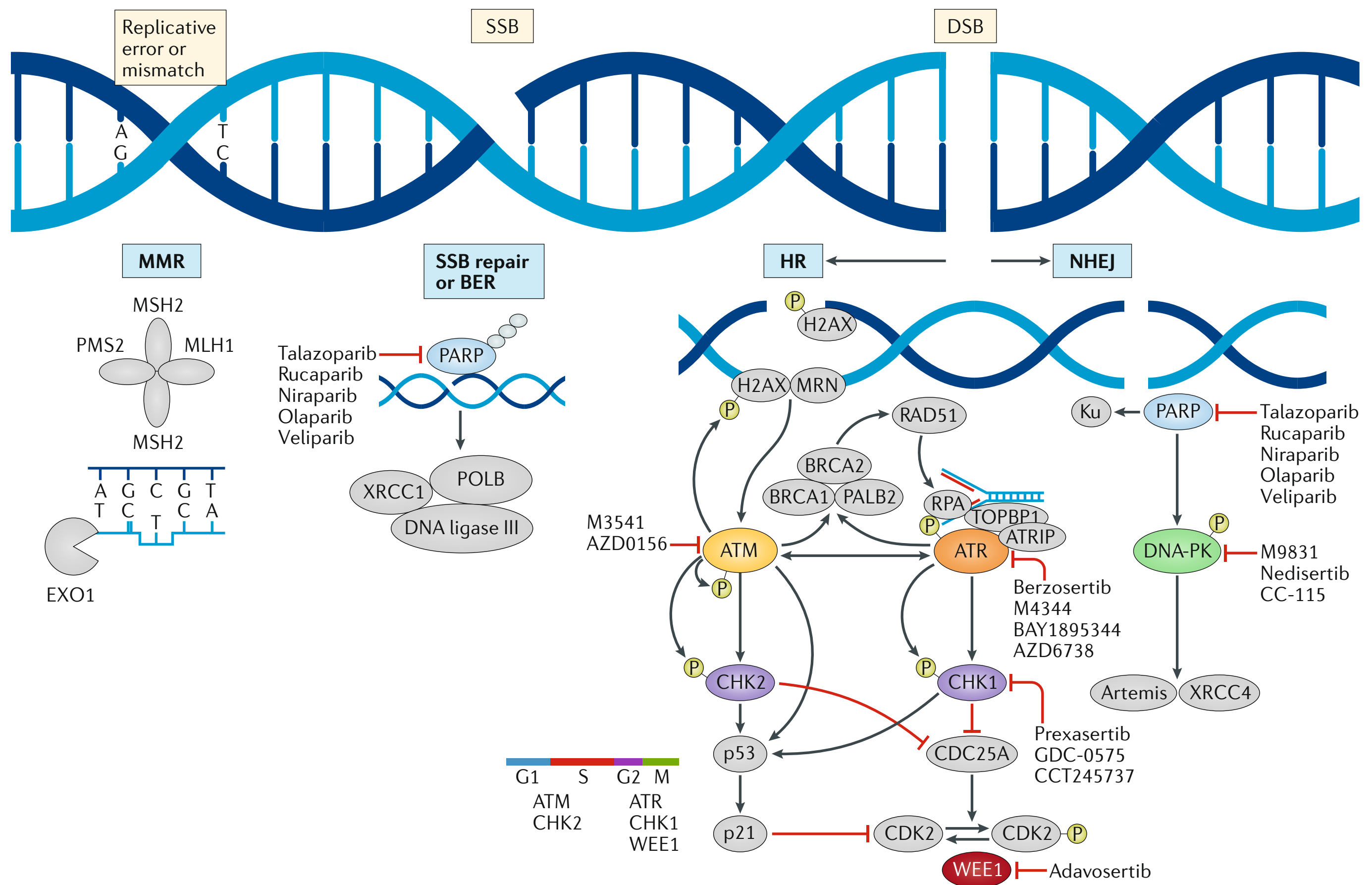

Fig. 1 | **DNA damage response pathways being targeted in the clinic.** Specific types of DNA damage — mismatches due to replication, single-strand DNA breaks (SSBs) or double-strand DNA breaks (DSBs) — result in the activation of specific signalling and repair cascades. DNA damage response (DDR) pathways mitigate replication stress and repair DNA; thus, deficiencies in these pathways result in the accumulation of SSBs and DSBs and increased immunogenicity owing to the generation of neoantigens from mutant proteins. Poly(ADP-ribose) polymerase (PARP) enzymes are key to activating a host of downstream repair mechanisms and are primary proteins involved in SSB repair or base-excision repair (BER). The repair of DSBs occurs predominately through the rapid, error-prone non-homologous end joining (NHEJ) repair pathway in conjunction with the much slower higher-fidelity, error-free homologous recombination (HR) repair pathway. DNA replication is a necessary component of DNA repair and thus cell cycle regulation and replication stress responses are intertwined with DDR pathways. The kinases ATR and ATM have crucial roles in DDR signalling and in maintaining replication fork stability, while also working together via their downstream targets, CHK1 and CHK2, respectively, to regulate cell cycle control checkpoints. The kinase activity of DNA-PK is essential for NHEJ and V(D)J recombination. WEE1 is a distinct nuclear kinase that regulates mitotic entry and nucleotide pools in coordination with DDR. Drugs targeting these key components of the DDR pathways that are undergoing clinical testing are indicated. ATRIP, ATR-interacting protein; EXO1, exonuclease 1; H2AX, histone H2AX; MRN, MRE11, RAD50 and NBS1 complex; POLB, DNA polymerase-β; RPA, replication protein A; TOPBP1, DNA topoisomerase 2-binding protein.

single-agent activity against the advanced-stage cancers evaluated in clinical trials to date[9,18–24], although no head-to-head comparisons have been reported. In addition, the varied clinical characteristics of the patient populations studies in the separate trials make cross-study comparisons difficult[9,18–24]. In clinical studies of PARP inhibitors reported to date, biomarker approaches have focused primarily on single-gene mutations in HR-pathway genes (predominantly *BRCA1/2*); however, the effects of specific mutations on the HR capacity of cells are not well understood, and thus the development of functional biomarkers of HR capacity is an active area of research. Indeed, while *BRCA1/2* mutations decrease HR capacity, cells retain the ability to perform HR, particularly when proteins that push the balance of DNA repair towards the alternative non-homologous end joining (NHEJ) pathway, such as TP53-binding protein 1 (53BP1), are deficient[25,26]. Better defining the DDR pathways involved in removing PARP–DNA complexes in a cell-specific and mutation-specific manner might improve the identification of predictive biomarkers of optimal benefit and inform treatment strategies to overcome or prevent PARP inhibitor resistance.

PARP inhibitors have shown an overall favourable safety profile as monotherapies; common toxicities shared among these drugs include myelosuppression, gastrointestinal symptoms and fatigue[17]. Similar to patients with DDR-deficient cancers treated using DNA-damaging

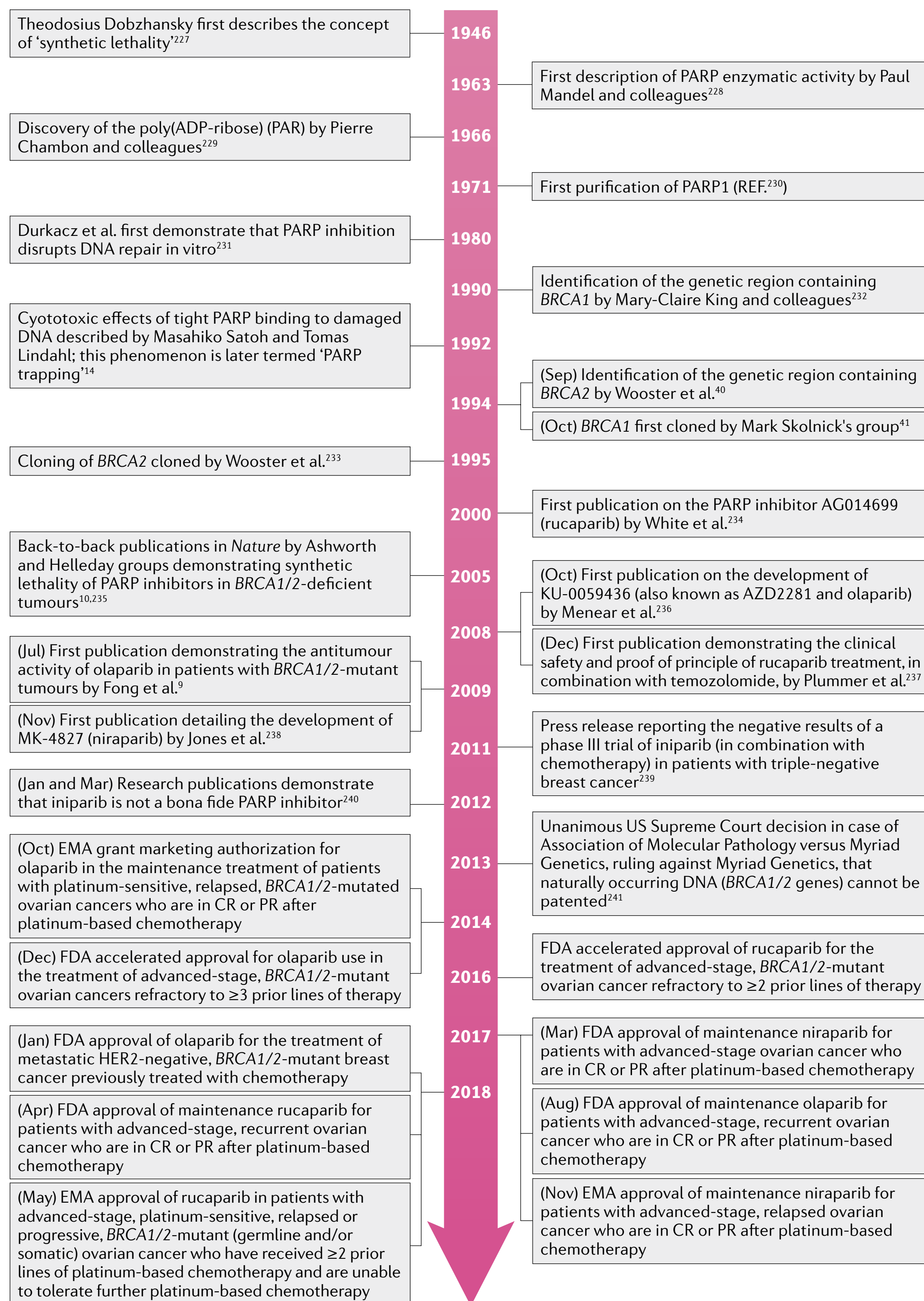

Fig. 2 | **Timeline of key events leading to FDA approvals of PARP inhibitors in cancer medicine.** Landmark discoveries and advances in the development of poly(ADP-ribose) polymerase (PARP) inhibitors are indicated[10,14,40,41,227–235], together with the current approved indications for these agents in the USA and the EU. CR, complete remission; PR, partial remission.

chemotherapies, patients treated with PARP inhibitors have a theoretical risk of secondary malignancies owing to DNA damage and genomic instability generating further mutational events. The risk of secondary malignancies, such as myelodysplastic syndrome and acute myeloid leukaemia, in patients treated with PARP inhibitors has generally been low (<1%)[27,28], although regular complete blood counts should be undertaken during therapy, with prompt referral of patients with persistent cytopenias to haematology specialists.

Table 1 | **Registration clinical trials of PARP inhibitors**

Study	Phase	Disease setting	Treatments	Most common grade ≥3 AEs	Efficacy	Reason for notability
Study 19; Ledermann et al. (2012 and 2014)[33,34]	II (randomized)	Recurrent ovarian cancer with a PR or CR to most recent line of platinum-based chemotherapy (after ≥2 lines chemotherapy)	Olaparib vs placebo	Fatigue 7% vs 3%; anaemia 5% vs 1%; and diarrhoea 2% vs 2%	• Median PFS 8.4 mo vs 4.8 mo (HR 0.35, 95% CI 0.25–0.49; *P* < 0.001) • *BRCA1/2*-mutant: median PFS 11.2 mo vs 4.3 mo (HR 0.18, 95% CI 0.10–0.31; *P* < 0.0001) • *BRCA1/2*-wild-type: median PFS 7.4 mo vs 5.5 mo (HR 0.54, 95% CI 0.34–0.85; *P* = 0.0075)	• Led to the first approval of a PARP inhibitor, by the EMA in 2014, as a maintenance treatment of patients with relapsed, *BRCA1/2*-mutated ovarian cancers who are in CR or PR after platinum-based chemotherapy • In conjunction with preliminary results from SOLO2, led to 2017 FDA approval of olaparib maintenance therapy in women with advanced-stage ovarian cancer in CR or PR after platinum-based chemotherapy (irrespective of *BRCA1/2* status)
Kaufman et al. (2015)[11]	II	Advanced-stage and/or recurrent solid tumours with germline *BRCA1/2* mutations	Olaparib	Anaemia 17%, fatigue 6% and abdominal pain 6%	• Overall: ORR 78/298 (26.2%) • Ovarian cancers: ORR 60/193 (31.1%) • Breast cancers: ORR 8/62 (12.9%) • Pancreatic cancers: ORR 5/23 (21.7%) • Prostate cancers: ORR 4/8 (50.0%)	Led to 2014 FDA approval of olaparib for refractory *BRCA1/2*-mutant ovarian cancer after ≥3 prior lines of chemotherapy
ARIEL2 part 1; Swisher et al. (2017)[18]	II (non-randomized)	*BRCA1/2*-mutant, *BRCA1/2*-wild-type and LOH-high, or BRCA1/2-wild-type and LOH-low recurrent ovarian cancer[a]	Rucaparib	Anaemia 22%, serum ALT and/or AST elevations 12% and fatigue 9%	• *BRCA1/2*-mutant: ORR 32/40 (80%); median PFS 12.8 mo • *BRCA1/2*-wild-type, LOH-high: ORR 24/82 (29%); median PFS 5.7 mo • *BRCA1/2*-wild-type, LOH-low: ORR 7/70 (10%); median PFS 5.2 mo	Preliminary results in conjunction with data from Study 10 (REF.[39]) led to the 2016 FDA and 2017 EMA approvals[b] of rucaparib in for *BRCA1/2*-mutant ovarian cancer refractory to ≥2 prior lines of treatment
Study 10; Kristeleit et al. (2016)[39]	I/II	Phase I: advanced-stage ovarian cancer Phase II: germline *BRCA1/2*-mutant ovarian cancer	• Phase I: escalating rucaparib doses • Phase II: rucaparib 600 mg BID	Not disclosed separately from ARIEL2	Phase II: ORR 25/42 (60%)	In conjunction with preliminary results of ARIEL2 (REF.[18]), led to the 2016 FDA and 2017 EMA approvals[b] of rucaparib for *BRCA1/2*-mutant ovarian cancer refractory to ≥2 prior lines of treatment
NOVA; Mirza et al. (2016)[37]	III	Platinum-sensitive, recurrent ovarian cancer stratified into two subgroups: germline *BRCA1/2* mutant and *BRCA1/2* wild type	Niraparib vs placebo	Thrombocytopenia 34% vs 1%; anaemia 25% vs 0%; neutropenia 20% vs 2%	• *BRCA1/2*-mutant: median PFS 21.0 mo vs 5.5 mo (HR 0.27, 95% CI 0.17–0.41; *P* < 0.001) • *BRCA1/2*-wild-type: median PFS 9.3 mo vs 3.9 mo (HR 0.45, 95% CI 0.34–0.61; *P* < 0.001); 12.9 mo vs 3.8 mo in those with HRD (HR 0.38, 95% CI 0.24–0.59; *P* < 0.001)	Led to 2017 FDA and EMA approvals of niraparib maintenance therapy for patients with advanced-stage ovarian cancer who are in CR or PR after platinum-based chemotherapy
SOLO2/ ENGOT-Ov21; Pujade-Lauraine et al. (2017)[36]	III	Recurrent *BRCA1/2*-mutant ovarian cancer with PR or CR to most recent line of platinum-based chemotherapy (after ≥2 lines of chemotherapy)	Olaparib vs placebo	Anaemia 19% vs 2%; fatigue 4% vs 2%; and neutropenia 5% vs 4%	Median PFS 19.1 mo vs 5.5 mo (HR 0.30, 95% CI 0.22–0.41; *P* < 0.0001)	In conjunction with results from Study 19, led to 2017 FDA approval of olaparib maintenance therapy for patients with advanced-stage ovarian cancer in CR or PR after platinum-based chemotherapy (irrespective of *BRCA1/2* status)

Table 1 (cont.) | **Registration clinical trials of PARP inhibitors**

Study	Phase	Disease setting	Treatments	Most common grade ≥3 AEs	Efficacy	Reason for notability
ARIEL3; Coleman et al. (2017)[38]	III	Recurrent ovarian cancer with PR or CR to most recent line of platinum-based chemotherapy (after ≥2 lines chemotherapy)	Rucaparib vs placebo	Anaemia 19% vs 1%; and increased serum ALT and/or AST levels 10% vs 0%	• Median PFS 10.8 mo vs 5.4 mo (HR 0.36, 95% CI 0.30–0.45; $P<0.0001$) • *BRCA1/2*-mutant: 16.6 mo vs 5.4 mo (HR 0.23, 95% CI 0.16–0.34; $P<0.0001$). • HRD: 13.6 mo vs 5.4 mo (HR 0.32, 95% CI 0.24–0.42; $P<0.0001$)	Led to 2018 FDA approval of rucaparib maintenance therapy for patients with advanced-stage ovarian cancer in CR or PR after platinum-based chemotherapy (irrespective of *BRCA1/2* status)
OlympiAD; Robson et al. (2017)[31]	III	Metastatic germline *BRCA1/2*-mutant, HER2-negative breast cancer after ≤2 prior lines of chemotherapy	Olaparib vs physician's choice of single-agent chemotherapy (capecitabine, eribulin or vinorelbine)	Anaemia 16% vs 4%; neutropenia 9% vs 26%; and decreased white cell count 3% vs 10%	ORR 100 /167 (59.9%) vs 19/66 (28.8%); median PFS 7.0 mo vs 4.2 mo (HR 0.58, 95% CI 0.43–0.80; $P<0.001$)	Led to 2018 FDA approval of olaparib for metastatic, HER2-negative breast cancers with *BRCA1/2* mutations

AEs, adverse events; ALT, alanine aminotransferase; AST, alanine aminotransferase; BID, twice daily; CR, complete remission; HRD, homologous recombination deficient; LOH, loss of heterozygosity; ORR, objective response rate; mo, months; PARP, poly(ADP-ribose) polymerase; PFS, progression-free survival; PR, partial remission. [a]Threshold for LOH-high versus LOH-low was at a prespecified cut-off of 14%. [b]EMA approval stipulates that patients must have platinum-sensitive disease but no longer be able to tolerate platinum-based chemotherapy.

FDA approvals in ovarian cancer

The development of PARP inhibitors for the treatment of ovarian cancer has provided clinical proof of concept and paved the way for the development of selective DDR inhibitors in cancer medicine, with three agents — olaparib, rucaparib and niraparib — currently approved in different therapeutic settings (TABLE 1). FDA approvals have typically preceded EMA approvals for similar indications (FIG. 2).

In clinical trials assessing olaparib in patients with ovarian cancer, the BRACAnalysis CDx PCR-based sequencing platform has been used to identify patients with deleterious germline mutations in the protein coding region or the intron–exon boundaries of *BRCA1* and *BRCA2*, and is FDA-approved for this purpose[11,29]. In clinical studies of rucaparib, investigators used the FoundationFocus CDx platform to perform next-generation sequencing (NGS) for the detection of both somatic and germline *BRCA1/2* aberrations[18,30]; consequently, this test was FDA-approved as a companion diagnostic to select patients with advanced-stage, *BRCA1/2*-mutated ovarian cancers who have previously received ≥2 lines of chemotherapy to receive rucaparib[30]. Current National Comprehensive Cancer Network (NCCN) guidelines recommend that deleterious somatic and/or germline *BRCA1/2* mutations can be identified using any FDA-approved or other validated test undertaken in a CLIA-approved facility when selecting patients with advanced-stage ovarian cancer to receive olaparib or rucaparib[18,29,31,32].

Approvals in the platinum-sensitive, maintenance setting. In clinical studies to date, PARP inhibitors have provided substantial benefit with tolerable toxicities when used as maintenance therapy following a response to platinum-based chemotherapy (TABLE 1). Indeed, olaparib first gained regulatory approval in 2014 from the EMA for the maintenance treatment of patients with platinum-sensitive, relapsed, germline or somatic *BRCA1/2*-mutant ovarian cancers who are in complete or partial remission after platinum-based chemotherapy (FIG. 2). This approval was based on data from the randomized phase II Study 19 trial (NCT00753545), which demonstrated improved progression-free survival (PFS) in olaparib capsule-treated versus placebo-treated patients[33]. A preplanned retrospective analysis of these data demonstrated a PFS benefit[34], albeit modest, even for patients lacking *BRCA1/2* mutations (TABLE 1), suggesting a need for expanded biomarkers to identify patients with *BRCA1/2*-wild-type disease who might benefit from PARP inhibitor maintenance therapy. Of note, however, treatment with olaparib capsules was not associated with an improvement in overall survival (OS) in either the *BRCA1/2*-wild-type or *BRCA1/2*-mutant groups of this study[35]. The follow-on, placebo-controlled, phase III SOLO2/ENGOT-Ov21 trial involving women with *BRCA1/2*-mutant ovarian cancers[33,36] confirmed that olaparib (tablet formulation) improves PFS in this population, with a tolerable adverse effect profile and no detrimental effects on quality of life (TABLE 1). In 2017, the FDA approved olaparib for the maintenance treatment of ovarian cancer, irrespective of *BRCA1/2*-mutation status, on the basis of data from these trials (FIG. 2).

In the phase III NOVA study, 553 patients with ovarian cancer who had an objective response to platinum-based chemotherapy were randomly assigned (2:1) to receive either maintenance niraparib or placebo[37]. The NGS-based myChoice HRD companion diagnostic was used to identify not only *BRCA1/2* variants, but also HRD tumours that share molecular hallmarks of *BRCA1/2*-mutated tumours — that is, loss of heterozygosity (LOH), large-scale translocations (LSTs) and telomeric allelic imbalance (TAI)[37]. Exploratory analyses revealed that median PFS durations were significantly longer in patients who received niraparib;

while the PFS benefit was highest in patients with germline *BRCA1/2* mutations and intermediate in patients with *BRCA1/2*-wild-type HRD tumours, a small but statistically significant PFS benefit was demonstrated in patients without detectable HRD[37] (TABLE 1). These findings supported the 2017 FDA and EMA approvals of niraparib for this indication, independent of *BRCA1/2* status (FIG. 2).

Similarly, positive data have been reported with maintenance rucaparib in the phase III ARIEL3 trial for patients with high-grade ovarian cancer who had responded to platinum-based chemotherapy in the second-line or third-line settings[38]. Testing for LOH, somatic *BRCA1/2* mutations and other prescribed HR-gene aberrations was conducted using the T5 NGS assay, while germline *BRCA1/2* testing was performed using the BRCAnalysis CDx test. Patients who received rucaparib had significantly longer PFS than those who received placebo (TABLE 1), again regardless of *BRCA1/2*-mutation status or the presence of HRD (as defined by high levels of LOH)[38], leading to the FDA-approval of rucaparib in this maintenance setting regardless of biomarker status in April 2018 (FIG. 2).

Approvals in the relapsed-disease setting. PARP inhibitor monotherapy also has demonstrated clinical benefit in selected patients with ovarian cancer that has progressed on prior chemotherapy. For example, on the basis of data from a single-arm phase II trial by Kaufman et al.[11] in patients with various advanced-stage, *BRCA1/2*-mutant cancers (TABLE 1), the FDA in December 2014 granted accelerated approval to olaparib monotherapy for patients with deleterious or suspected deleterious germline *BRCA1/2*-mutant, advanced-stage, ovarian cancer after ≥3 lines of chemotherapy[29] (FIG. 2). In December 2016, the FDA also granted accelerated approval to rucaparib for the treatment of patients with advanced-stage ovarian cancers harbouring deleterious germline and/or somatic *BRCA1/2* mutations who have received ≥2 prior lines of chemotherapy[30]. This approval was based on promising efficacy data from the single-arm, phase II ARIEL2 and Study 10 trials[18,39] (TABLE 1). Talazoparib and veliparib are currently in late phase trials in patients with newly diagnosed ovarian cancer (NCT02470585) and in other advanced-stage cancers, mainly in combination with chemotherapies for the latter agent (Supplementary Table 1).

Beyond ovarian cancer

BRCA1 and *BRCA2* are the two most studied genes in the HR repair pathway. Germline variants of these genes were initially discovered in patients with hereditary breast or ovarian cancer[40–42]; however, somatic and germline *BRCA1/2* mutations, as well as aberrations affecting other HR genes including *ATM*, *ATR, BARD1, BRIP1, CHK1, CHK2, PALB2, RAD51* and *FANC*, are increasingly being detected in patients with other tumour types through NGS of paired tumour and non-malignant DNA samples[43–47]. Antitumour responses have already been reported in a number of phase I/II trials of olaparib monotherapy in patients with various advanced-stage *BRCA1/2*-mutant cancers, including breast, prostate and pancreatic cancers (Supplementary Table 2).

PARP inhibition for breast cancer. Data from the phase III OlympiAD trial demonstrated a doubling of the objective response rate (ORR), a significant PFS benefit and a more favourable safety profile for olaparib versus single-agent chemotherapy (not including platinum-based agents) in patients with germline *BRCA1/2*-mutant, HER2-negative, metastatic breast cancer[31] (TABLE 1), leading to FDA approval of olaparib in this patient population (FIG. 2). The efficacy of olaparib in a post-chemotherapy maintenance setting or concurrently with chemotherapy in patients with *BRCA1/2*-mutant breast cancer is currently being tested in the phase III OlympiA (NCT02032823) and PARTNER (NCT03150576) trials, respectively (Supplementary Table 1).

Encouraging data from the phase III EMBRACA trial investigating single-agent talazoparib for the treatment of advanced-stage, *BRCA1/2*-mutant, HER2-negative breast cancers indicate a PFS benefit of talazoparib (median 8.6 months versus 5.6 months with chemotherapy; HR 0.54; $P < 0.0001$), which extended to patients with hormone receptor-positive disease (HR 0.47; 95% CI 0.32–0.71) and those with central nervous system metastases (HR 0.32; 95% CI 0.15–0.88)[24]. Importantly, the talazoparib-treated patients in this study also had improvements in quality of life and delayed clinically meaningful deterioration compared to patients treated with standard chemotherapies[48]. A randomized trial of investigator's choice chemotherapy versus niraparib (NCT01905592) is ongoing.

Substantial efforts are also being applied to early stage clinical testing of PARP inhibitors in the neoadjuvant setting. Veliparib plus carboplatin for triple-negative breast cancer (TNBC) was the first combination to graduate from the I-SPY2 trial, a multicentre, adaptive, platform trial designed to screen multiple experimental compounds on a standard chemotherapy backbone for the treatment of patients with breast cancer[49]. Data from the phase III BrighTNess trial[50], however, did not show a benefit of the addition of veliparib to carboplatin and paclitaxel chemotherapy in terms of pathological complete response (pCR) rate (53% versus 58%; $P = 0.36$), with carboplatin plus paclitaxel alone providing clinical advantage over single-agent paclitaxel. This lack of benefit could be multifactorial, but, as discussed previously, veliparib is the least potent PARP inhibitor and thus might not be the optimal choice of agent to use in synthetic lethality approaches. By contrast, encouraging data have been reported for the use of single-agent talazoparib in the neoadjuvant setting in patients with *BRCA1/2*-mutant, HER2-negative breast cancer, with 9 of 17 patients (53%) achieving a pCR[51,52]. Thus, earlier targeting of *BRCA1/2*-mutant tumours with potent PARP inhibitors, when the accumulation of driver mutations is perhaps more limited, might prove to be particularly effective; however, long-term data are not yet available to discern whether early PARP inhibition has survival benefits.

PARP inhibition for prostate cancer. Considerable interest surrounds the use of PARP inhibitors in selected patients with metastatic castration-resistant prostate cancer (mCRPC), following positive results from the single-arm phase II TOPARP-A trial of olaparib[53] (Supplementary Table 2): the ORR was substantially higher in patients with DDR gene mutations (including *BRCA1/2* or *ATM* mutations) than in the unselected population (88% versus 33%)[53]. Interestingly, serial analyses of circulating cell-free DNA (cfDNA) revealed that declines in cfDNA concentrations and mutant allele frequencies correlated with better outcomes after olaparib treatment[54]. The ongoing phase III PROfound (NCT02987543) and TRITON3 (NCT02975934) trials are currently testing olaparib and rucaparib, respectively, versus investigators' choice therapy for mCRPC with HR gene mutations (Supplementary Table 1).

PARP inhibition for gastrointestinal cancers. In a subgroup analysis of the phase II trial by Kaufman et al.[11], 5 of 23 patients with *BRCA1/2*-mutant pancreatic cancers (22%) had an objective response and an additional 11 (47%) had stable disease lasting ≥8 weeks (Supplementary Table 2). A phase I/II study of olaparib combined with gemcitabine in patients with advanced-stage pancreatic cancer revealed an ORR of 27% (versus 14% with gemcitabine alone); however, grade ≥3 toxicities were common[55] (Supplementary Table 2). In the ongoing phase III POLO trial (NCT02184195; Supplementary Table 1), a switch maintenance approach is being used whereby patients with *BRCA1/2*-mutated pancreatic cancers who have been on treatment with first-line platinum-based therapy for ≥16 weeks without progression are being randomly assigned to receive either olaparib or placebo.

BRCA1/2 mutations are rare in gastric cancers; however, these tumours often have loss of ATM expression, providing a biological rationale for PARP inhibitor therapy[56,57]. A randomized phase II study has been conducted to compare olaparib plus paclitaxel with placebo plus paclitaxel[58]. Unsurprisingly, adverse events, including grade ≥3 neutropenia, were more frequently observed with the olaparib combination[58]. Interestingly, although no difference in PFS was detected, the patients receiving combination therapy with olaparib had longer OS durations (median 13.1 months versus 8.3 months; $P = 0.005$)[58]. In addition, a subset of patients with low baseline levels of tumour ATM expression had an even greater OS benefit (median not reached versus 8.2 months; $P = 0.002$) in a prespecified secondary analysis[56], again, without a PFS benefit. Nevertheless, the subsequent placebo-controlled, phase III GOLD trial of the same regimen in Asian patients with gastric cancers did not meet its primary end point of improved OS in the olaparib-treated group, in neither the overall population nor an ATM-low subpopulation[59] (Supplementary Table 2). The lack of benefit of olaparib in this setting despite the promising phase II results is likely multifactorial, as discussed elsewhere[60]. For example, additional factors outside of ATM loss might predict PARP inhibitor sensitivity or resistance in patients with gastric cancer, even in the ATM-low population, highlighting that single-gene or single-protein biomarker approaches might be inadequate[60,61].

Other cancer types and chemotherapy combinations. Large-scale paired tumour and germline sequencing studies have revealed a substantial number of incidental deleterious variants in DDR-related genes, including *BRCA1/2* and *ATM*, across a variety of cancer types, such as prostate cancer, hepatobiliary cancers, sarcomas and bladder cancer[43,62,63]. Interestingly, prior studies have also revealed a high germline-to-somatic ratio for *BRCA1/2* mutations[64]; therefore, any patient found to have a somatic *BRCA1/2* variant in their tumour should be considered for germline testing. Whether context dependency across tumour types will affect antitumour responses remains unclear. In the absence of this knowledge, patients with cancers harbouring pathogenic HR gene mutations should be considered for clinical trials involving PARP inhibitors and other DDR inhibitors, agnostic of tumour type; however, the definition of which genes qualify as 'HR genes' is actively evolving[65].

In contrast to switch maintenance therapy with chemotherapy followed by PARP inhibition, which has proved successful, simultaneously combining DDR inhibitors, including PARP inhibitors, with chemotherapy has been problematic owing to substantial toxicity. Attempts to combine the first DDR inhibitor, O^6-benzylguanine, with alkylating agents were terminated owing to high rates of toxicities. For example, a trial of temozolomide plus O^6-benzylguanine for patients with gliomas revealed limited responses, with >45% of patients having grade 4 haematological toxicities[66]. Multiple subsequent studies of PARP inhibitors, particularly those with a high capacity for PARP trapping, given concurrently with chemotherapeutic agents, have revealed improved response rates across tumour types, compared with chemotherapy alone, but increased toxicity — predominantly myelosuppression — requiring dose reductions or treatment delays in a substantial proportion of patients[55,67,68].

Biomarkers beyond BRCA1/2 mutations

Identifying novel predictive biomarkers of benefit from PARP inhibitors is important: although germline or somatic *BRCA1/2* mutations can be used to enrich for responders, a substantial number of patients who lack these mutations benefit from PARP inhibitor monotherapy[37,38]. Furthermore, patients with tumours harbouring *BRCA1/2* mutations frequently do not respond to PARP inhibitors (TABLE 1). Multiple studies have been undertaken to investigate whether specific molecular features of *BRCA1/2*-mutated tumours can serve as biomarkers for selection of patients with *BRCA1/2*-wild-type disease to receive PARP inhibitors, thereby expanding the potential benefit of these targeted therapies. The term 'BRCAness' was originally coined to describe a molecular phenocopy of *BRCA1/2*-mutated tumours, which can arise through a range of genomic, epigenetic or post-translational alterations[69]. What BRCAness truly reflects, however, is a HRD phenotype beyond the narrow scope of defects in the BRCA pathway. As such, we propose that the term 'BRCAness' should be broadened

to ‘HRDness’ to recognize these non-BRCA-related, yet ‘HRD-like’ mechanisms of PARP inhibitor sensitivity (Supplementary Figure 1). Notably, a subset of tumours might also demonstrate ‘PARPness’ — that is, responsiveness to PARP inhibitors in the absence of HRD, potentially owing to PARP trapping or related to abrogation of the activity of PARP in processes other than base-excision repair (BER), such as alternative-NHEJ (alt-NHEJ) or replication-fork protection[70,71].

BRCA-like tumours are characterized by frequent genomic structural rearrangements and LSTs resulting in high levels of genomic instability via global LOH and TAI[72,73]. Within the NOVA[37], and ARIEL2 and ARIEL3 trials[18,38], different assays designed to quantify HRD via genomic-based analysis of LOH and global genomic alterations were studied as potential companion diagnostics for selecting patients with *BRCA1/2*-wild-type tumours who are more likely to benefit from treatment with PARP inhibitors[74].

The ARIEL2 investigators capped the number of patients with known hereditary *BRCA1/2* mutations enrolled in order to test the ability of the T5 NGS LOH assay to predict PARP inhibitor sensitivity in patients with *BRCA1/2*-wild-type disease[18,75]. Importantly, the results of this study, and subsequently ARIEL3 (REF.[38]), showed that maintenance rucaparib improved PFS even in women with *BRCA1/2*-wild-type, LOH-low ovarian cancer. Similarly, the myChoice HRD assay used in the NOVA trial enabled assessment of BRCAness on the basis of global genomic scarring, including a high degree of LOH, LSTs and TAI; however, PARP inhibition with niraparib improved PFS regardless of biomarker status, albeit with different efficacy in the different patient subsets[37]. The results of these trials demonstrate that patients with deleterious *BRCA1/2* variants achieve the greatest benefit from PARP inhibition, which is biologically in line with preclinical evidence of synthetic lethality, followed by those with molecular genomic features of BRCAness, including high LOH, as defined using companion diagnostic assays[37,38] (TABLE 1). However, the observation of clinical benefit from PARP inhibitors in the absence of HRDness defined using the companion diagnostic indicates that genomic scarring assays are either not inclusive enough in defining molecular signatures of HRD tumours or fail to capture mechanisms of PARP inhibitor sensitivity outside of HRDness, or both. Indeed, the inclusion of patients with repeated responses to platinum-based chemotherapy in these trials might have enriched for tumours with HRD, even if this HRD was undetectable in some patients using the companion diagnostic assays, thus skewing the results[76,77].

In addition to the aforementioned genomic scarring assays, multiple other mutational signatures of HRDness have been developed through whole-exome or whole-genome sequencing of *BRCA1/2*-mutant tumours and retrospectively tested in a range of different solid tumour settings. In a seminal study[78], all somatic point mutations and larger-scale genomic alterations across 7,042 cancers were catalogued, with subsequently identified mutational signatures serving as avatars of specific aberrant pathways and biological processes. For example, ‘Signature 3’ was strongly associated with *BRCA1/2*-inactivating mutations across a variety of tumour types[78]; however, this signature lacks a discreet cut-off to determine *BRCA1/2*-deficient versus *BRCA1/2*-proficient tumours. Building on these data, the HRDetect test was subsequently designed and trained by using known *BRCA1/2*-mutated tumours to generate a unique somatic mutational profile that enabled the identification of BRCA pathway-deficient tumours with 98.7% sensitivity[79].

Researchers have also sought to pair data from somatic and germline sequencing of DDR genes with whole-exome or whole-genome mutational signatures[80]. Whereas Signature 3 and HRDetect have high sensitivity for the detection of *BRCA1/2*-mutant and so-called BRCA-like tumours, they have failed to enable the identification of tumours with known functional mutations in other HR pathway genes, including *ATM, CHEK* and *ATR*, as being HRD[79,80]. These findings again suggest that some tumours have HRDness through mechanisms unrelated to BRCA pathway function specifically; thus, deleterious variants, epigenetic modifications or post-translational changes affecting other DDR gene products that lead to HRDness might have unique molecular features or signatures that do not entirely align with a BRCA-like or BRCAness signature. Multiple studies assessing PARP inhibitors have reported antitumour responses in patient populations enriched for these non-*BRCA* HRD mutations or even changes in protein expression levels (for example, loss of ATM)[53,57,58,81,82]. Knowledge of genes with direct or indirect roles in DDR, cell cycle regulation and chromatin remodelling is constantly expanding, thereby increasing the discovery of aberrations that can lead to HRDness. For example, *ARID1A*, which encodes a component of the SWI/SNF chromatin remodelling complex, is one of the most frequently mutated genes in cancer and has been shown to facilitate DSB processing, sustain DDR signalling and regulate the cell cycle via CDC25C[83]. Unsurprisingly, therefore, ARID1A deficiency has been shown to render tumours sensitive to a variety of DDR inhibitors, including PARP inhibitors, in preclinical models[84]. Aditionally, BAP1, another key SWI/SNF complex unit, has been shown to regulate HR and cellular recovery via its phosphorylation sites and catalytic activity; BAP1 deficiency predicts for PARP inhibitor sensitivity[85,86]. CDK12 is a transcriptional regulator with roles in maintaining genomic stability by regulating the expression of other DDR genes; loss of CDK12 function leads to HRD and sensitizes ovarian cancer cells to PARP inhibition[87]. Approximately 5% of mCRPCs harbour deleterious aberrations in *CDK12*, which have been shown to predict increased T cell infiltration, thus highlighting a new potential biomarker based on genomic instability for selecting patients with prostate cancer to receive immunotherapy[88].

Ultimately, the utility of NGS-based biomarkers of sensitivity to PARP inhibitors is limited because they typically provide only a historical, static record of genomic alterations present in the tumour at the time of biopsy sampling or tumour resection and lack the capacity to inform on dynamic, active signalling and, in particular,

adaptive processes that occur in the tumour during PARP inhibition. Monitoring cfDNA before and serially during therapy and at the time of disease progression can provide greater insights into tumour genomic evolution as it relates to therapeutic response[54]. As mentioned previously, decreases in total cfDNA concentration and the allele frequencies of HR gene variants have been independently associated with responsiveness to olaparib in patients with mCRPC, while NGS of cfDNA at the time of progression unveiled somatic reversion mutations in HR genes, thus marrying restoration of DDR gene function to drug resistance[54].

Biomarkers of sensitivity to DDR inhibitors that rely on the identification of mutations in cancer-related genes, such as *BRCA1/2*, are inherently limited by the fact that most variants discovered in these genes are still of undetermined functional significance, and thus their potential for predicting synthetic lethality is unclear. However, the expansion of mutational library screens and collaborative pooling of data from specialist functional laboratories have great potential to differentiate true functional loss from benign exonic point mutations within specific clinical contexts[89]. For example, the identification of differential gene-expression profiles in known HRD versus HR-proficient cell lines has led to the development and preclinical validation of multiple transcriptomic HRD scores[90–93]. These RNA profiling-based scores remain in development, but hold great promise as dynamic biomarkers of HR repair function and PARP inhibitor sensitivity[90–93]. Nevertheless, caution should be exercised in the use of transcriptional signatures that are derived from comparisons of drug-sensitive versus drug-resistant tumour models because results from drug-sensitivity assays in preclinical models have been shown to be highly variable, with low levels of inter-assay concordance[94].

A variety of other causes of HRD and/or replication stress have been shown in preclinical models and small cohorts of patients to predict sensitivity to certain DDR inhibitors. For example, a response to platinum-based chemotherapy can be a biomarker in itself, providing insight into the underlying defects in HR and cell cycle control. In a retrospective molecular analysis[76], patients with advanced-stage ovarian cancer who responded to multiple lines of platinum-based chemotherapy and, to a lesser extent, long-term responders and/or survivors had tumours enriched for HR defects. Moreover, the co-occurence of HRD and loss of retinoblastoma-associated protein (RB) expression was associated with prolonged survival[76]. Interestingly, patients with long-term responses to platinum-based chemotherapy and protracted OS also had highly replicative tumours with high Ki67 levels and a high degree of tumour immune infiltration, suggesting increased replication stress and S phase-specific DNA damage[76,77].

Other causes of HRDness in cancer cells include the generation of oncometabolites that result from a general shift towards glycolytic metabolism (the Warburg effect), which can result in HRD. For example, hypoxic tumour states and mutations in Krebs cycle genes, such as *IDH1* or *IDH2*, can result in the production of oncometabolites — D-2-hydroxyglutarate in the case of IDH variants — that lead to the downregulation of HR proteins and increased sensitivity to PARP inhibitors in preclinical models[95].

In addition, cancers that lack mutations in HR repair genes and do not typically display HRDness, such as small-cell lung cancer (SCLC), have demonstrated sensitivity to platinum-based agents and PARP inhibitors[71,96]. This increased susceptibility might be a consequence of high levels of replication stress owing to loss of *RB1* and *TP53* expression combined with MYC overactivation and, therefore, a reliance on the HR and replication stress response pathways for cell viability. High levels of PARP1 expression in the tumour cells, resulting in lethal levels of PARP trapping, is another potential explanation. Thus, even tumours lacking HRDness can be PARP inhibitor sensitive, with these tumours showing features we have termed 'PARPness' (Supplementary Figure 1). Potential biomarkers of PARPness include high tumoural levels of PARP1, E-cadherin and/or Schlafen 11 (SLFN11), which predicted sensitivity to platinum-based agents and PARP inhibitors in preclinical models and select clinical trials[96–98]. High levels of replication stress combined with replication fork instability, as in the context of replication protein A (RPA) exhaustion, can also contribute to PARP and platinum sensitivity[99]. *IDH1* mutations have been shown to confer PARP inhibitor sensitivity via reduced production of NAD^+ that is required for PARP1-mediated DNA repair[100], in addition to oncometabolite production[95]. Histological phenotypes, including neuroendocrine differentiation (for example, in SCLC and aggressive-variant prostate cancer), also seem to predict sensitivity to platinum-based agents and PARP inhibitors; studies investigating the underlying mechanisms of responsiveness are ongoing[98,101,102]. Finally, robust multigene expression signatures based on sensitivity and resistance profiles of cancer cell lines of different lineages have been shown to accurately predict PARP inhibitor sensitivity in preclinical models, as well as benefit from cisplatin chemotherapy in retrospective clinical studies[103]. These RNA-based expression scores of PARP inhibitor sensitivity reflect tumour PARPness, rather than inherent *BRCA1/2* or HR defects[103], although the additional mechanisms by which sensitivity to PARP inhibition arise remain under active investigation. To establish the biological rationale for the wider use of DDR inhibitors to treat a broader range of tumours, it is important to move beyond a *BRCA1/2*-centric view of DDR biomarkers and to consider tumours that demonstrate HRDness and PARPness phenotypes (Supplementary Figure 1).

PARP inhibitor resistance mechanisms

Long-term data from clinical trials of different PARP inhibitors have demonstrated durable responses, mostly in patients with *BRCA1/2*-mutant cancers, although the majority of patients inevitably develop resistance to platinum-based and/or PARP inhibitor therapy[104]. Indeed, with the regulatory approval of PARP inhibitors in multiple indications, as well as off-label use of PARP inhibitors, an emerging population of patients with disease that has progressed after PARP inhibition requires new therapeutic options to overcome resistance.

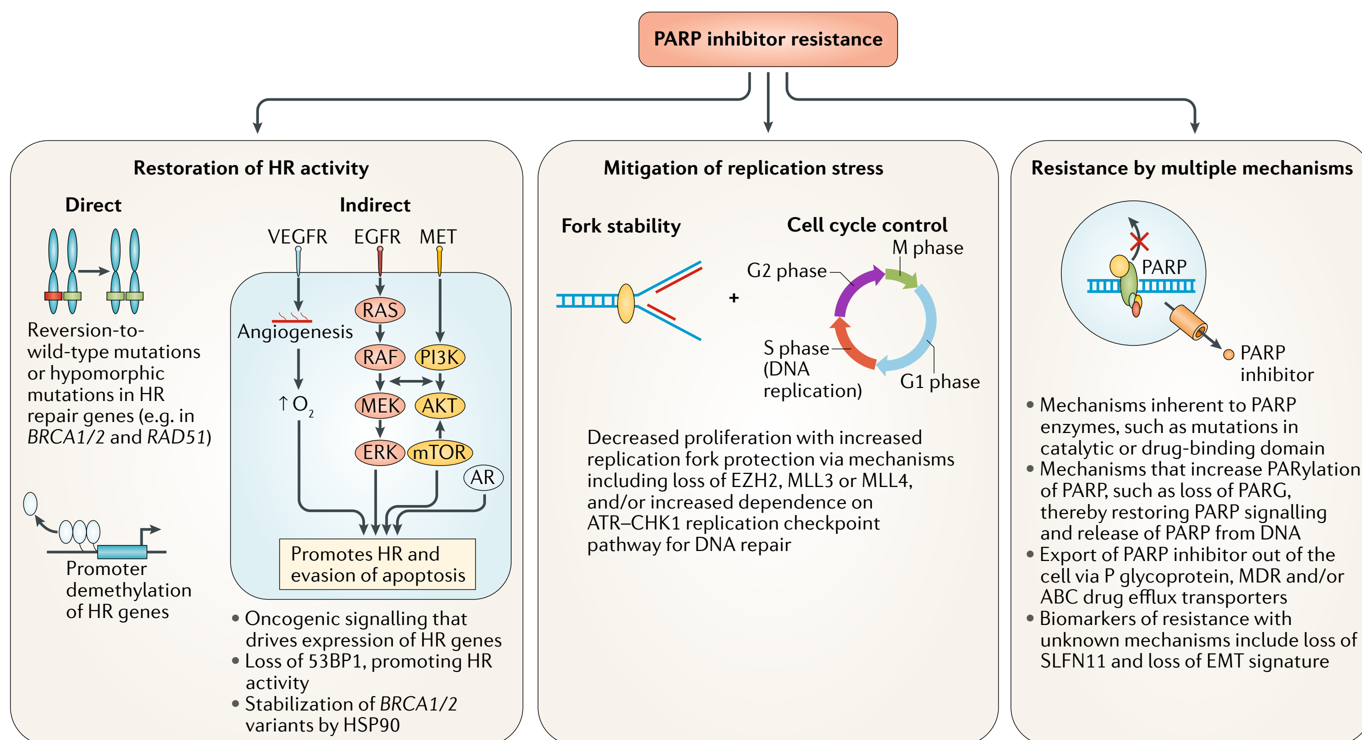

Fig. 3 | **Mechanisms of resistance to PARP inhibitors.** Resistance of cancers to poly(ADP-ribose) polymerase (PARP) inhibitors can be inherent or acquired. The potential mechanisms of resistance are varied and can be multifactorial but centre around three main categories: restoration of homologous recombination (HR) repair activity through direct (genomic, epigenetic or post-translational changes in the HR machinery itself) or indirect mechanisms (signalling that increases the activity and/or expression of the HR machinery); replication stress mitigation, whereby the cancer cell slows the cell cycle and stabilizes replication forks; and mechanisms not currently assigned to a single DNA repair pathway-related process but still alter the response to PARP inhibition, such as mutations in PARP itself, genomic events that alter protein poly ADP-ribosylation (PARylation) and/or PARP trapping, upregulation of drug efflux pumps and loss of biomarkers of sensitivity to PARP inhibition, such as expression of Schlaffen 11 (SLFN11) and/or a epithelial-to-mesenchymal (EMT) signature. 53BP1, TP53-binding protein 1, AR, androgen receptor; HSP90, heat-shock protein 90; MDR, multidrug resistance protein; MLL3, histone-lysine *N*-methyltransferase 2C; MLL4, histone-lysine *N*-methyltransferase 2B; PARG, poly(ADP-ribose) glycohydrolase.

In clinical and preclinical studies, resistance to PARP inhibitors seems to occur by three general mechanisms: acquisition of aberrations that increase HR repair capacity; activation of signalling pathways that decrease cell cycle progression and replication stress; and miscellaneous alterations that cannot currently be assigned to a single DDR pathway-related mechanism (FIG. 3).

Mechanisms of acquired resistance to PARP inhibition in patients with *BRCA1/2*-mutant tumours have been determined through genomic analyses of sequential tumour biopsy and cfDNA samples. Across multiple cancer types, secondary mutations have been discovered that cause a reversion to the wild-type sequence and/or function of the DDR gene or protein, including *BRCA1/2*, *RAD51C*, *RAD51D* and *PALB2*, thereby restoring HR repair capacity and negating synthetic lethality[54,105,106] (FIG. 3). These types of mutations have also been implicated in resistance to platinum-based chemotherapy[107]. However, these reversion mutations are identified in only a small subset (approximately 20–25%) of patients with PARP inhibitor resistance[108]. Moving forward, larger clinical data sets incorporating liquid or tumour biopsy sampling before and during treatment and at the time of disease progression are needed to better understand the prevalence and influence of these reversion mutations in the context of PARP inhibition and to better define changes in the function of DDR pathways during therapy[108].

Observations from preclinical models have revealed other mechanisms of resistance to PARP inhibitors that rely on the restoration of HR repair function. For example, HSP90 can stabilize and prevent the degradation of a subset of BRCA1 variants, resulting in retention of some HR repair function[109] (FIG. 3). In addition, changes in the promoter regions of DDR genes, in the form of gene fusions or loss of promoter hypermethylation, can restore the activity of HR genes, such as *BRCA1* (REF.[110]). Loss of 53BP1 expression has been shown to influence responsiveness and resistance to PARP inhibitors, probably by shifting the balance of DNA repair from NHEJ to HR[25,26,111]. Mitigation of replication stress via replication fork stabilization, by a variety of potential pathways, often combined with slowing of cell cycle progression, might also have an important role in PARP inhibitor resistance, distinct from restoration of HR repair[112,113] (FIG. 3).

Multiple biomarkers of PARP inhibitor resistance do not coalesce on a single DDR pathway-related mechanism (FIG. 3). For example, SLFN11 expression predicts PARP inhibitor sensitivity[96–98] and unsurprisingly, therefore, SLFN11 inactivation can confer PARP inhibitor resistance[114]. SLFN11 responds to replication stress by binding chromatin at stressed replication foci and stalling replication, making cancer cells with overexpression of SLFN11 susceptible to synthetic lethal therapies; thus, loss of SLFN11 expression, which is often achieved through promoter hypermethylation in a variety of cancers, leads to resistance to such treatments, probably through increased reliance on the ATR–CHK1 axis[97,114–116]. Overexpression of the multidrug efflux transporter P glycoprotein has also been implicated in resistance to both PARP inhibitors and chemotherapy[117]. Inherent resistance to PARP inhibition might also be attributable to deficient PAR glycohydrolase (PARG) activity, which increases PARP1 auto-PARylation, thereby restoring PARP signalling, releasing PARP1 from DNA and ultimately decreasing PARP inhibitor-induced DNA damage[118]. Additionally, resistance can arise through mutations or loss of PARP1 that decrease PARP inhibitor binding and/or PARP trapping[119].

PARP inhibitor resistance is probably multifactorial and research on strategies to overcome these various forms of acquired resistance is ongoing[114,120,121]. Interestingly, certain markers of resistance to PARP inhibitors, such as loss of SLFN11 expression, might predict for sensitivity to other DDR inhibitors. Finally, genomic, transcriptomic and proteomic profiling of sensitive and resistant cell lines has revealed that the tissue-specific activation of certain oncogenic pathways, such as RAS, PI3K or androgen receptor (AR) signalling, can promote HR repair activity and PARP inhibitor resistance in certain cancer contexts, providing the rationale for targeted combination strategies[90,122–125] (FIG. 3).

Moving beyond PARP in targeting the DDR

Inhibitors of ATR and ATM

ATR inhibition. Unlike PARP inhibitors, the use of other antitumour agents targeting key components of the DDR is currently limited to early phase clinical trials (TABLE 2; Supplementary Table 3). ATR and ATM are prime targets of DDR inhibitors, given their central regulatory function in activating the response to both SSBs and DSBs[126]. Both of these proteins work through distinct but overlapping pathways to halt the cell cycle and initiate DDR pathways (FIG. 1). Four ATR inhibitors are currently undergoing clinical trial testing: M6620 (VX-970 or berzosertib), M4344 (VX-803), AZD6738 and BAY1895344 (Supplementary Table 3).

M6620 is the first-in-class ATR inhibitor and has been tested as monotherapy and in combination with different chemotherapies, including topotecan, carboplatin, gemcitabine and cisplatin[127–130]. M6620 monotherapy was well tolerated, with no dose-limiting toxicities (DLTs) observed; a durable complete response (CR) lasting >19 months was observed in a patient with advanced-stage colorectal cancer and 100% ATM loss on immunohistochemistry[127] (TABLE 2). Preliminary data from phase I trials have also now been reported for the chemotherapy combinations; while signals of antitumour activity were observed, unlike monotherapy, the chemotherapy combinations were associated with higher rates of bone marrow toxicities, requiring frequent dose delays and reductions[127–130]. Unsurprisingly, the MTDs of M6620 in combination with chemotherapy were lower than the recommended phase II dose (RP2D) of M6620 monotherapy[127]. Together with the aforementioned studies combining PARP inhibition with chemotherapy[55,67,68], these findings emphasize that DDR inhibitor–chemotherapy combinations have problematic toxicities, particularly bone marrow suppression, without any clear clinical benefits over the use of either treatment alone (although direct comparisons are lacking).

The safety and efficacy of AZD6738 monotherapy in patients with advanced-stage solid tumours have been investigated in the phase I PATRIOT study; two partial responses (PRs) were observed, although one was unconfirmed[131]. Owing to bone marrow suppression observed beyond cycle 1 with continuous dosing, the investigators are exploring whether different dosing schedules improve long-term tolerability[131]. Patients with Ki67-high and/or HRD tumours will be enrolled in future expansion cohorts[131]. In a parallel phase of the study, AZD6738 treatment is being combined with palliative radiotherapy[131]. Early data on AZD6738 in combination with carboplatin, olaparib or the anti-programmed cell death 1 ligand 1 (PD-L1) antibody durvalumab have also been reported[132] (TABLE 2); drug-related toxicities were substantially more common with the AZD6738–carboplatin combination, including grade ≥3 thrombocytopenia, neutropenia and anaemia requiring dose delays and modifications[132]. With the AZD6738–carboplatin combination, three PRs (one unconfirmed) were reported, two of which involved patients with ATM-aberrant tumours (rectal and clear cell ovarian cancers)[132]. In comparison with the carboplatin regimen, the AZD6738–olaparib combination seemed to be well tolerated, although myelosuppression remained the predominant reported toxicity[132]. PRs were observed in two patients with *BRCA1*-mutant TNBC and in a patient with *BRCA2*-mutant oestrogen receptor-positive breast cancer[132]. Testing of AZD6738–durvalumab revealed that this combination was well tolerated, with no grade ≥3 or DLTs reported; PRs were reported in a patient with HNSCC and in another with non-small-cell lung cancer (NSCLC)[132]. While objective responses were observed with each combination, it remains unclear whether these are truly biologically active combinations because the antitumour activity could be attributable to any of the individual agents. However, the non-overlapping toxicities of DDR inhibitors and immune-checkpoint inhibitors makes combinations of these agents attractive. The oral ATR inhibitors BAY1895344 and M4344 are currently in phase I testing in combination with different chemotherapies and/or as single agents in patients with advanced-stage solid tumours (NCT03188965 and NCT02278250, respectively; Supplementary Table 3).

Table 2 | **Trials of DDR inhibitors other than PARP inhibitors**

Study	Phase	Disease setting	Treatments	Most common grade ≥3 AEs	ORR	Reason for notability
Yap et al. (2015)[128]	I	Advanced-stage and/or recurrent solid tumours	Escalating doses of M6620 (VX-970 or berzosertib; ATR inhibitor) alone or in combination with carboplatin	• M6620 alone: none • M6620 plus carboplatin: neutropenia 38%, hypersensitivity 8% and thrombocytopenia 8%	• M6620 alone: 1/11 (9%) • M6620 plus carboplatin: 1/15 (7%)	First-in-human study of an ATR inhibitor alone and with chemotherapy
Do et al. (2015)[172]	I	Advanced-stage and/or recurrent solid tumours	Escalating doses of adavosertib (AZD1775; WEE1 inhibitor)	Leukopenia 8%, lymphopenia 8% and neutropenia 8%	2/21 (10%); both responders had *BRCA1/2* mutations	First-in-human trial of a WEE1 inhibitor
Leijen et al. (2016)[171]	I	Advanced-stage and/or recurrent solid tumours	Escalating doses of adavosertib alone or combined with gemcitabine, cisplatin or carboplatin	Overall: thrombocytopenia 23%, neutropenia 22%, anaemia 12%, leukopenia 10% and diarrhoea 7%	• Adavosertib (multiple doses[a]) plus gemcitabine: 3/67 (4%) • Adavosertib (multiple doses[b]) plus cisplatin: 7/45 (16%) • Adavosertib (multiple doses[c]) plus carboplatin: 2/46 (4%)	First-in-human trial combining WEE1 inhibitor with chemotherapy
Hong et al. (2016)[161]	I	Advanced-stage and/or recurrent solid tumours and CLL	Escalating doses of prexasertib (LY2606368; CHK1 inhibitor)	Neutropenia 89%, leukopenia 71% and thrombocytopenia 53%	2/45 (4%)	First-in-human trial of a CHK1 inhibitor
Munster et al. (2016)[148]	I	Advanced-stage and/ or recurrent solid and haematological cancers	Escalating doses of CC-115 (dual mTOR and DNA-PK inhibitor)	Not reported	2/44 (4%)	First-in-human trial of a DNA-PK inhibitor
Yap et al. (2017)[132]	I	Advanced-stage and/or recurrent solid tumours	Escalating doses AZD6738 (ATR inhibitor) plus carboplatin, olaparib (PARP inhibitor) or durvalumab (anti-PD-L1 antibody)	• Carboplatin plus AZD6738: anaemia 19%, neutropenia 19% and thrombocytopenia 19% • Olaparib plus AZD6738: anaemia 7%, low white blood cell count 7% and neutropenia 7% • Durvalumab plus AZD6738: anaemia 8%, lung infection 8% and hyponatraemia 8%	• AZD6738 plus carboplatin: 3/37 (8%) • AZD6738 plus olaparib: 3/28 (11%) • AZD6738 plus durvalumab: 2/12 (17%)	First-in-human study of ATR inhibitor in combination with a PARP inhibitor or a immune-checkpoint inhibitor

AEs, adverse events; BID, twice daily; CLL, chronic lymphocytic leukaemia; DDR, DNA damage response; ORR, objective response rate; PARP, poly(ADP-ribose) polymerase; PD-L1, programmed cell death 1 ligand 1. [a]Various dosages once a day for days 1 and 2 of 3 consecutive weeks out of every 4-week cycle; maximum tolerated dose (MTD) 175 mg. [b]Various dosages twice daily for 2.5 days (5 doses) per 21-day cycle; MTD was 200 mg.[c]Various dosages twice daily for 2.5 days (5 doses) per 21-day cycle; MTD was 225 mg.

Predictive biomarkers of ATR inhibitor sensitivity. Preclinical screens for predictive biomarkers have revealed that HR defects, including deleterious *ATM* and *BRCA1/2* mutations, confer sensitivity to ATR inhibition[133–135]. This observation suggests that many biomarkers of HRDness might predict benefit from ATR inhibitors, as well as PARP inhibitors (Supplementary Figure 1). Indeed, anecdotal responses of patients with ATM-aberrant tumours to regimens containing ATR or PARP inhibitors have been reported in early phase clinical trials[53,56,58,132], although the optimal method of assessing ATM status — assays for deleterious mutations or loss of protein expression — remains unclear[126]. Of note, the failure of ATM loss (defined as nuclear staining of ATM in <25% of tumour cells) to predict benefit from olaparib in the aforementioned phase III GOLD trial[59] might in part reflect limitations of the immunohistochemistry assay and scoring system used. Moving

forward, it might be prudent to define only tumours with 100% loss of ATM expression as ATM deficient. Notwithstanding, ATM levels should be better quantified to help determine whether a more precise threshold would enable more accurate prediction of ATR inhibitor sensitivity. In addition, p53 deficiency, as an indication of compromised ATM signalling and DNA damage checkpoints, has been shown in preclinical studies to confer cancer cells with sensitivity to ATR inhibition; this association has not been recapitulated in early phase clinical studies, but warrants further investigation[126,136]. As with PARP inhibitors, mutations in *ARID1A* have also been shown to predict for ATR inhibitor responsiveness, which is related to the reliance of ARID1A-deficient cells on ATR checkpoint activity to prevent premature mitotic entry and subsequent apoptosis[137].

Alternative lengthening of telomeres (ALT), a telomerase-independent mechanism of maintaining telomere length and overcoming replicative senescence, is a biological process not directly related to HRD that has been investigated as a predictor of ATR inhibitor sensitivity. ALT is detected across a variety of tumour types and arises predominantly owing to deleterious mutations in the *ATRX* gene and less commonly owing to mutations in *DAXX*[138,139]. ATR inhibition in ALT-positive cancer cells has been shown to disrupt ALT, leading to chromosome fragmentation and ultimately apoptosis[140]. Fluorescence in situ hybridization (FISH)-based assays that enable the identification and quantification of ALT in tumour specimens have been developed[138]. However, preclinical data on ALT positivity as a predictor of ATR inhibitor sensitivity remain conflicting[139–141], and clinical investigations of the predictive value of ALT biomarkers are warranted.

HER2 signalling has been shown in preclinical models to promote activation of the ATM–ATR signalling cascades in response to DNA damage, and selected HER2-positive breast cancer cell lines display sensitivity to ATR inhibition with AZD6738 (REFS[142,143]). However, the mechanism of this sensitivity is not well understood and clinical trials of ATR inhibitors are warranted in this setting.

ATM inhibition. ATM is another logical therapeutic target for DDR inhibitors, given its close association with ATR and its crucial function as a central regulator of the DDR, especially in DSB repair[144]. Two ATM inhibitors are currently being tested in phase I trials: M3541, combined with fractionated palliative radiotherapy, in patients with solid tumours (NCT03225105); and AZD0156, as a monotherapy and in combination with olaparib or 5-fluorouracil, folinic acid and irinotecan, in patients with advanced-stage solid cancers (NCT02588105).

Inhibitors of DNA-PK

DNA-PK, a member of the PI3K–mTOR enzyme family, is a critical enzyme involved in the NHEJ pathway of DNA repair[145] (FIG. 1). The specific targeting of NHEJ makes this class of drug especially attractive for combination with radiation because NHEJ is the predominant mechanism for repair of traditional (non-heavy ion) radiation therapy[146]. Three DNA-PK inhibitors are currently being investigated in phase I/II trials (Supplementary Table 3): M9831 (VX-984), nedisertib (M3814; MSC2490484A) and CC-115 (FIG. 1). CC-115 is a small-molecule inhibitor of both DNA-PK and mTOR that was developed through optimization of a novel series of triazole-containing mTOR inhibitors[147]. CC-115 monotherapy has been evaluated in a phase I study (NCT01353625) with an initial 44 patients treated across 10 dose-escalation cohorts[148]. Preliminary antitumour activity was reported, although whether these responses are attributable to activity against DNA-PK or mTOR is unclear — especially considering that CC-115 led to hyperglycaemia, which is consistent with mTOR inhibition, and that associated pharmacodynamic studies provided evidence of mTOR complex 1 (mTORC1) and mTORC2 inhibition[148]. Planned phase Ib/II trials include combination studies of CC-115 with androgen-deprivation therapy (ADT) in patients with CRPC (NCT02833883) or with radiation in those with glioblastoma (NCT02977780).

The DNA-PK inhibitor nedisertib has been tested in combination with palliative radiation therapy in a phase I trial involving patients with tumours or metastases in the head and neck or thoracic regions; 2 of 7 patients had grade 3 mucositis, and 2 patients had local disease control lasting >300 days[149]. Multiple trials of nedisertib alone or with definitive chemotherapy and/or radiotherapy are underway (Supplementary Table 3). M9831 is being assessed with and without pegylated doxorubicin in an ongoing phase I study initially in patients with advanced-stage solid tumours, followed by an expansion cohort comprising patients with metastatic endometrial cancer that has progressed after platinum-based chemotherapy (NCT02644278). Biomarkers of responsiveness to DNA-PK inhibition remain in preclinical development, but HRDness could theorectically predict sensitivity to DNA-PK inhibitors given the increased reliance of HRD cells on NHEJ.

Inhibitors of CHK1/2

The cell cycle checkpoint kinases CHK1 and CHK2 act in coordination with DDR pathways and are immediate targets of ATR and ATM, respectively (FIG. 1). Inhibitors of CHK1 and/or CHK2 have a long development history with numerous compounds being discontinued before phase III testing, in most cases owing to toxicity[150], such as UCN-01 (7-hydroxystaurosporine)[151,152]. Many of these inhibitors, including UCN-01 are nonspecific, inhibiting both CHK1 and CHK2, as well as other targets[150]. AZD7762, an inhibitor of CHK1 with equal potency against CHK2, has been tested alone or in combination with gemcitabine or irinotecan in patients with advanced-stage solid tumours[153–155]. In addition to low efficacy, severe cardiac toxicities, including myocardial infarction, ventricular dysfunction and troponin elevations, were observed[153–155]. Interestingly, one patient with *RAD50*-mutant, *ATM*-deficient SCLC achieved a CR with the combination of irinotecan and AZD7762, suggesting that biomarkers of sensitivity to CHK1 inhibition will likely overlap with those of ATR inhibitors[156]. The relatively selective CHK1 inhibitor rabusertib (LY2603618), has also been assessed in combination

with different chemotherapies, but these trials revealed limited antitumour activity and a high incidence of serious thromboembolic events[157,158]. Another selective CHK1 inhibitor, MK-8776 (SCH 900776) has undergone phase I testing as a monotherapy and in combination with gemcitabine and cytarabine for solid tumours and acute myeloid leukaemia, respectively; a high frequency of QTc prolongation with both MK-8776 monotherapy and combination regimens was a concern[159,160]. Neither AZD7726, rabusertib, nor MK-8776 are being tested in active clinical trials.

Currently, clinical evaluation of three selective CHK1 inhibitors is ongoing: prexasertib (LY2606368), GDC-575 (ARRY-575; RG7741) and CCT245737 (SRA737). A high frequency of grade 4 neutropenia (~73%) was observed in patients treated on the phase I and phase Ib trials with single-agent prexasertib, a second-generation CHK1-selective inhibitor, which was generally manageable and transient (lasting <5 days)[161,162]. Preliminary data from a phase II trial in 22 patients with advanced-stage, high-grade serous ovarian cancers demonstrated 5 PRs in patients without detectable *BRCA1/2* mutations; again, grade 3–4 prexasertib-related neutropenia was common[163]. This drug is undergoing further testing in a number of phase I–II trials across various treatment and disease settings (Supplementary Table 3). GDC-575 (NCT01564251) and CCT245737 (NCT02797964 and NCT02797977) are in phase I testing as single agents or in combination with gemcitabine-based chemotherapy.

Inhibitors of WEE1

WEE1 is a protein kinase that inhibits cyclin-dependent kinase 1 and 2 (CDK1/2), thereby activating the G2/M cell cycle checkpoint, causing cell cycle arrest and providing time for DNA damage repair (FIG. 1). Thus, inhibition of WEE1 prevents G2 checkpoint initiation, leading to unscheduled mitotic entry, increased replication stress via uncontrolled firing of replication origins, subsequent nucleotide starvation and loss of genomic integrity[164,165]. Given these effects, a strong biological rationale supports the targeting of p53-deficient cells with WEE1 inhibitors, given the key role of p53 in the regulation of the G1 checkpoint and, therefore, the increased reliance on the G2 checkpoint in p53-deficient cells[164,165]. Indeed, preclinical studies with adavosertib (AZD1775; MK-1775) have shown that this WEE1 inhibitor abrogates the G2 checkpoint and sensitizes p53-deficient cells to DNA-damaging chemotherapies and radiation owing to mitotic lethality[164–166]. As such, current developmental strategies have focused on using WEE1 inhibitors in combination with other DNA-damaging treatments (including PARP inhibitors, chemotherapy or radiation therapy) in patients with tumours harbouring *TP53* mutations. In addition, preclinical data also suggest increased sensitivity to WEE1 inhibition through mechanisms outside of cell cycle checkpoint defects, such as DDR aberrations and nucleotide resource starvation, with single-agent activity observed even in *TP53*-wild-type cancer cells[167–170].

Adavosertib is the first-in-class WEE1 inhibitor and the only WEE1 inhibitor currently in clinical development (TABLE 2; Supplementary Table 3). Establishing an optimal dose and schedule of adavosertib that achieves an acceptable therapeutic index at active drug doses seems to be a major challenge. The phase I study of adavosertib either as monotherapy or in combination with DNA-damaging chemotherapy in patients with advanced-stage solid tumours resulted in a somewhat unconventional dose and schedule[171] (TABLE 2). The rationale for the MTDs of adavosertib in combination with chemotherapeutic agents was based on pharmacokinetic data indicating that, when given in combination with chemotherapy, serum adavosertib concentrations after the fifth dose exceeded the threshold established in preclinical models to be safe[171]. Nevertheless, grade 3 adverse events were common (55% in all evaluable patients), including haematological and gastrointestinal toxicities[171] (TABLE 2). Promisingly, in another phase I study[172], adavosertib monotherapy led to 2 PRs in 25 evaluable patients, both in patients with refractory *BRCA1*-mutant solid tumours. Retrospective tissue analysis showed 5 patients had tumours with *TP53* mutations, but none had a response (despite the strong preclinical rationale for WEE1 inhibition in this context)[172]. While the sample set was limited, pharmacodynamic analyses of paired skin biopsy samples showed a reduction in the levels of phosphorylated CDK1/2, with phosphorylated histone H2AX staining providing evidence of DSBs[172].

Adavosertib has been tested in combination with gemcitabine, cisplatin or carboplatin in the aforementioned phase I study[171]. When adavosertib was administered in multiple doses with chemotherapy, 47–67% of patients had grade ≥3 adverse events, primarily haematological toxicities (frequencies of 29–57%). *TP53* mutations were only weakly associated with an antitumour response (across treatments), with an ORR of 21% in 19 evaluable patients with *TP53*-mutant tumours versus 12% in 33 patients with *TP53*-wild-type disease[171]. In a phase II study of adavosertib plus carboplatin in patients with *TP53*-mutant refractory ovarian cancer, the ORR was 43%, with one patient having a prolonged CR (lasting >30 months); however, toxicities were again frequent, particularly fatigue, nausea and thrombocytopenia[173]. In a subsequent phase II study, 121 patients with platinum-sensitive, *TP53*-mutant ovarian cancers were randomly assigned to receive carboplatin plus paclitaxel with or without adavosertib, with preliminary data at 57% maturity revealing improved PFS with the addition of adavosertib (median 42.9 weeks versus 34.9 weeks; HR 0.55, 95% CI 0.32–0.95; $P = 0.03$)[174]. Clinical studies to evaluate adavosertib combined with cisplatin and docetaxel as neoadjuvant therapy before resection (NCT02508246) or with definitive chemoradiation and concurrent cisplatin[175] (NCT02585973) in patients with locoregionally advanced HNSCC, as well as in other settings, are ongoing (Supplementary Table 3).

In addition to p53 deficiency[164,165], HR repair defects owing to mutations in *BRCA1/2* and/or the *FANC* genes have been shown to predict a response to WEE1 inhibitors[176], similar to PARP inhibitors. Other predictive biomarkers being explored for WEE1 inhibition include high levels of EZH2 and mitotic cyclins[165]. Moreover, deficiencies in trimethylation of histone

H3K36 (H3K36me3), which are observed in a range of cancers and specifically in *SETD2*-mutant cancers[168], have been shown to predict for sensitivity to WEE1 inhibition in preclinical models via a mechanism of nucleotide resource depletion and cancer cell starvation[167,168]. Mechanistically, H3K36me3 deficiency leads to reduced expression of the ribunucleotide-diphosphate reductase subunit M2 (RRM2) and depletion of deoxynucleoside triphosphate pools resulting in apoptosis; in this context, WEE1 inhibition induces synthetic lethality by promoting degradation of RRM2 via dysregulated CDK1/2 activity[167]. H3K36me3 deficiency and *SETD2* mutations might thus serve as potential biomarkers of response to WEE1 inhibitors across different cancer types, and a clinical trial of single-agent adavosertib for patients with *SETD2*-deficient cancers is underway (NCT03284385).

DDR inhibitor combinations

The lack of regular and prolonged responses to DDR inhibitors, even in biomarker-selected populations, points to inherent or acquired mechanisms of resistance to single-agent therapy. In general, tumour sensitivity and resistance to DDR inhibitors will largely be dependent on the remaining proficiency of the underlying SSB and DSB response and repair, cell cycle regulation and chromatin remodelling pathways, as well as the active oncogenic pathways, which can also influence DDR and the availability and/or utilization of cellular resources. Understanding the system-wide biology of these sensitivity and resistance patterns can directly inform combination treatment strategies in order to overcome or prevent resistance and to expand the potential patient populations that might benefit from DDR inhibitors (FIG. 4).

Combinations with DNA-damaging agents

Given the ability of radiation to cause anatomically targeted DNA damage, combination of DDR inhibitors with radiotherapy has been the subject of considerable ongoing clinical research. However, early data, mostly with PARP inhibitors, have not demonstrated compelling and consistent evidence of synergy, although nor have they shown unexpected toxic effects[177–179]. This lack of synergy might be attributable to the fact that DSB repair in response to conventional radiation damage occurs predominately via the NHEJ pathway, rather than by the BER pathway that is primarily mediated by PARP (FIG. 1). Moreover, most DDR inhibitors, including PARP and ATR inhibitors, exploit aberrations in HR repair pathway components, rather than defects in the NHEJ pathway; DNA-PK inhibitors are a notable exception and are promising combination partners for radiotherapy. Furthermore, preclinical studies have revealed that, in comparison with conventional photon-based radiation, more densely ionizing DNA damage induced by irradiation with heavier ions (carbon and iron) or high energy protons results in greater engagement of HR repair and might, therefore, be more attractive for combination with DDR inhibitors[146,180].

As discussed, multiple studies of concurrent treatment with DDR inhibitors and chemotherapy have been performed, with the majority of these combinations resulting in additive toxicities with only modest clinical benefit; in most cases, studies comparing the use of DDR inhibitor alone versus in combination with chemotherapy have not been performed[55,67,68]. By contrast, approaches using sequential administration of chemotherapy followed by DDR inhibitor have proven to be more tolerable and clinically successful, as discussed previously[34,36,37,104].

DDR inhibitor–DDR inhibitor combinations

Preclinical studies combining agents targeting key components of the DDR have been shown to overcome acquired resistance to single-agent DDR inhibitors and to induce synthetic lethality. Multiple drug combinations have been evaluated in preclinical studies with the aim of overcoming acquired resistance to DDR inhibitors, predominantly PARP inhibitors (for which the mechanisms of resistance are best understood). For example, PARP inhibitor resistance due to SLFN11 inactivation can be overcome using ATR inhibition, owing to the reliance of SLFN11-deficient cells on the ATR pathway for survival under PARP inhibitor treatment[114]. Similarly, in BRCA-deficient cancer cells with resistance to PARP inhibition, ATR inhibition further disrupts HR repair leading to replication fork collapse, supporting the development of PARP–ATR inhibitor combinations[120] (FIG. 4).

Preclinical studies combining adavosertib with olaparib revealed that WEE1 inhibition enhances PARP inhibitor-mediated radiosensitization of pancreatic cancer cells, apparently by reducing HR repair capacity and abrogating the G2 cell cycle checkpoint[181]. Similarly, depletion of DNA topoisomerase 2-binding protein 1 (TOPBP1) has been shown to induce sensitivity to PARP inhibition by reducing HR efficiency. The bromodomain and extraterminal (BET) protein BRD4 promotes global gene transcription by RNA polymerase II; BET inhibition has also been shown to suppress the transcription of key DDR genes, including *CTIP*, *BRCA1*, *RAD51*, *TOPBP1* and *WEE1*, thereby increasing the sensitivity of HR-proficient cell lines to PARP inhibition[182–184]. Furthermore, BET inhibition has been shown to synergize with PARP inhibition in multiple mouse models of HR-proficient tumours[182–184].

Early phase clinical trials of PARP inhibitors combined with ATR or WEE1 inhibitors are currently underway (NCT02723864, NCT02576444 and NCT02511795; Supplementary Table 3). Given the vital roles of CHK1, DNA-PK and ATM, as well as ATR and WEE1, at the interface between DDR and cell cycle checkpoint signalling, combination of one or more of these agents with PARP inhibition to induce replication fork collapse and/or mitotic lethality is biologically feasible, even in HR-proficient cells[185]. In addition, HRD cancers have an increased reliance on alternative repair pathways, including microhomology-mediated end joining (MMEJ) for DSB repair. Thus, a biological rationale exists for combining PARP inhibitors with inhibitors of DNA polymerase-θ (POLQ), a key protein mediating MMEJ repair, to achieve synthetic lethality[186]. However, these agents all have overlapping toxicity profiles, most notably bone marrow suppression, which will pose a challenge to such combination strategies; both dosing

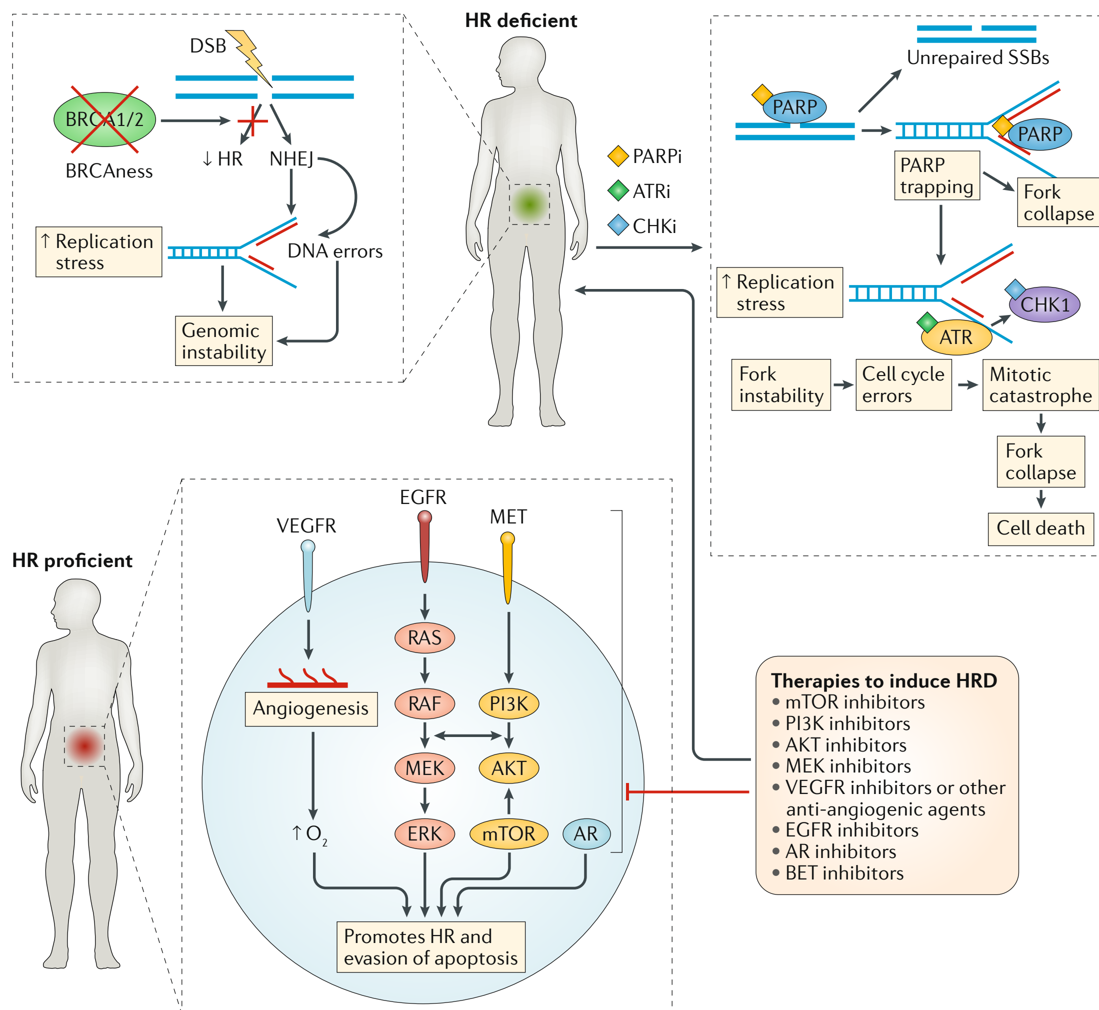

Fig. 4 | **Biomarker-driven combination strategies to augment PARP inhibitor responses.** Cancers that are inherently homologous recombination deficient (HRD) or display 'BRCAness'/'HRDness' are susceptible to poly(ADP-ribose) polymerase (PARP) inhibition (PARPi). Acquired PARP inhibitor resistance arises owing to the phenotypic rescue of homologous recombination (HR) or by mitigation of replication stress, and could potentially be overcome through combination of PARP inhibitors with ATR inhibition (ATRi) and/or inhibition of cell cycle checkpoint kinases (CHKi), such as CHK1. Selected oncogenic drivers and metabolic pathways specific to certain tumour types can drive HR activity to enable cancer cell survival and PARP inhibitor resistance. Therefore, HR-proficient cancer cells can be induced to become HRD through the concept of chemical HRDness by targeting these pro-survival pathways with different molecularly targeted agents, thereby engendering PARP inhibitor sensitivity. Given the rapidly growing number of rational combinations, functional biomarkers of HR, replication stress and PARP trapping are now urgently needed to provide guidance as to which combination should be used for which tumour type and at what time point. AR, androgen receptor; BET, bromodomain and extraterminal motif; DSB, double-strand DNA break; NHEJ, non-homologous end joining; SSBs, single-strand DNA breaks.

and scheduling of the agents will be crucial to optimizing their application in the clinic.

Inducing HRDness with targeted agents

Preclinical studies have shown that actionable oncoproteins can directly or indirectly regulate DDR and cell cycle checkpoint pathways by driving HR gene expression. For example, prior studies have demonstrated that the RAS, PI3K and AR signalling pathways can all promote HR repair; thus, similar to inhibition of BET proteins[182–184], targeting of these oncoproteins together with DDR pathway components has the potential to pharmacologically induce an HRDness phenotype and lead to synthetic lethality in otherwise DDR inhibitor-resistant cells[123–125,187], which we have termed 'chemical HRDness' (FIG. 4). Preclinical experiments providing evidence of such a chemical HRDness concept have paved the way for a diverse array of combination strategies using a variety of molecularly targeted agents in combination with inhibitors of PARP and potentially other DDR proteins (Supplementary Figure 2), which hold great potential for the treatment of a wide range of tumour types[122–125,187–190]. In addition, DDR pathways overlap considerably with chromatin remodelling mediated by histone modifications, providing the rationale for combining DDR inhibitors with epigenetic agents, beyond those targeting BRD4 (REF.[191]). For example, inhibition of PARP attenuates the PARP1-mediated

downregulation of EZH2 induced by alkylating DNA damage, and the addition of an EZH2 inhibitor further sensitizes *BRCA1/2*-mutant cells to PARP inhibition in preclinical models[192]. Histone deacetylase inhibitors have also been reported to synergize with PARP inhibitors in preclinical studies, a finding associated with downregulation of HR repair genes, suggesting induction of HRDness[191,193]. Furthermore, DNA methyltransferase (DNMT) inhibitors can improve PARP inhibitor efficacy in *BRCA1/2*-wild-type cells and leukaemia models by increasing tight binding of PARP1 to chromatin at sites of DNA damage[194].

In the preclinical setting, genetically induced PARP knockout models have been shown to inhibit in vivo angiogenesis; although, paradoxically, PARP inhibition has been shown to selectively target hypoxic tumour cells and increase tumour blood vessel perfusion as a potential mechanism of engendering chemosensitivity and radioresponsiveness[195–197]. Despite the lack of a complete understanding of the effects of PARP inhibition on hypoxia and angiogenesis, promising preclinical findings have led to multiple clinical trials assessing the combination of anti-angiogenic agents with PARP inhibitors. The synergy of such combinations might partially be explained by hypoxia induced by anti-angiogenic therapy leading to HRD through the downregulation of key repair proteins, thereby enhancing sensitivity to PARP inhibitors[95,198]. In the clinical setting, a phase I trial to assess olaparib combined with the anti-VEGFA antibody bevacizumab showed that the regimen was safe and tolerated, with no DLTs[199]. In a proof-of-concept trial[200], the small-molecule VEGFR inhibitor cediranib was also combined with olaparib in patients with recurrent ovarian cancer ($n = 20$) or metastatic TNBC ($n = 8$), resulting in an ORR of 44% in 18 evaluable patients with ovarian cancer; no objective responses were observed in 7 evaluable patients with TNBC. Notably, 75% of the patients had grade ≥3 toxicities, predominantly bone marrow suppression, hypotension and fatigue[200]. Preliminary data from the ongoing phase II study of this same combination (NCT01116648) in 90 patients with recurrent platinum-sensitive ovarian cancer demonstrated a PFS benefit compared with single-agent olaparib across the entire study population (HR 0.50, 95% CI 0.30–0.83, $P = 0.007$), with a trend towards improved OS, although not statistically significant[201]. Grade 3–4 toxicities, including fatigue, diarrhoea and hypertension, were more common with the combination[188,201]. Interestingly, the PFS benefit of the combination was not seen in subgroup analysis of patients with known germline *BRCA1/2* mutations[201]. These findings are perhaps unsurprising considering that this combination is likely to benefit patients with *BRCA1/2*-wild-type disease to a greater extent than those with pre-existing HRD due to germline *BRCA1/2* mutations, who will already gain benefit from olaparib monotherapy. These data potentially justify the increased risk of toxicities with the combination therapy in the former group. Nevetheless, biomarker-driven trials are needed to determine the optimal subgroup of patients to target. A phase III trial of the olaparib–cediranib combination in patients with *BRCA1/2*-mutant ovarian cancers is underway (Supplementary Table 1).

Findings from preclinical models have provided a biological rationale and robust evidence of antitumour activity that support combined inhibition of PARP and the PI3K–AKT–mTOR pathway[122,187,189] (FIG. 4). Genetic or pharmacological inhibition of PI3K in *BRCA1/2*-wild-type TNBC cells was shown to induce HRDness via suppression of *BRCA1/2* expression, increased DNA damage and compensatory PARP activation, and subsequently sensitized tumours to PARP inhibition[187]. Downregulation of *BRCA1/2* expression upon PI3K inhibition is hypothesized to be mediated via a compensatory increase in MEK–ERK signalling resulting in binding of the transcriptional repressor ETS1 to the *BRCA1/2* promoter regions[187]. By contrast, marked synergy between PARP and MEK inhibitors via reduced HR gene expression, HR repair capacity and cell cycle checkpoint activity and increased PARP1 expression and PARP inhibitor-mediated DNA damage has been reported in other preclinical studies, at least in *RAS*-mutant or *RAF*-mutant tumours[124]. Not surprisingly, in vivo synergy between PI3K and PARP inhibitors has been shown in mouse models of *BRCA1*-mutant breast cancer, *PIK3CA*-mutant ovarian cancer and *PTEN*-mutant endometrial cancers, among others[189,202,203]. Transcriptomic profiling and pathway analysis has led to the identification of mTOR inhibitors as top candidates for inducing HRDness, even in *BRCA1/2*-wild-type breast cancer cell lines and xenografts[90,123]. Indeed, the combination of the mTOR inhibitor everolimus and talazoparib had greater efficacy than either agent alone in preclinical models[123]. Interestingly, mTOR inhibition did not alter the expression of key HR genes, such as *BRCA1/2* or *RAD51*, but instead induced HRD via the suppression of SUV39H1, a histone methyltransferase involved in DSB repair[123].

In the clinical setting, a phase I trial of the pan-class I PI3K inhibitor buparlisib (BKM120) in combination with olaparib (NCT01623349) was undertaken in 70 patients with advanced-stage, high-grade serous ovarian or breast cancers[204,205]. The ORRs were 29% in those with ovarian cancer, irrespective of platinum sensitivity, and 28% in those with breast cancer[204,205] (Supplementary Table 2). Nausea and fatigue were the most common toxicities, whereas DLTs included transaminitis and depression[204,205]. In view of multiple buparlisib dose reductions needed owing to drug-related toxicities, olaparib was subsequently combined with the PI3Kα-specific inhibitor alpelisib (BYL719)[205,206]. The MTD was lower than the monotherapy RP2D; DLTs included hyperglycaemia, rash and fever with decreased neutrophil counts. The most common toxicities were those expected with PI3K inhibition, including gastrointestinal symptoms, hyperglycaemia and fatigue. Impressively, among 28 patients with advanced-stage ovarian cancer, 26 (93%) of whom had platinum-resistant disease, the ORR was 36%, with a median duration of response of 167 days (range 16–398 days). The ORR was similar between patients with or without germline *BRCA1/2* mutations in both the buparlisib and alpelisib cohorts[204–206].

A phase I trial of AKT inhibitor capivasertib (AZD5363) in combination with olaparib in patients with advanced-stage solid tumours (NCT02338622) revealed that the combination was well tolerated; antitumour activity was observed independent of *BRCA1/2* status or previous exposure to platinum-based chemotherapy, PARP inhibitors or PI3K pathway inhibitors[207].

Supported by the aforementioned preclinical findings[124], the combination of the MEK inhibitor selumetinib and olaparib is currently being assessed in a phase I trial involving patients with endometrial, ovarian or other solid tumours with RAS pathway alterations, as well as those with difficult to treat PARP inhibitor-resistant ovarian cancers (NCT03162627). This combination could potentially be of great importance, given the difficulties associated with targeting *RAS*-mutant tumours (and considering that *RAS* pathway alterations are among the most common drivers of tumorigenesis). Furthermore, the approval of PARP inhibitors for multiple indications and the almost inevitable emergence of resistance is rapidly leading to a population of patients for whom resensitization to PARP inhibitors is an urgent need. In PARP inhibitor-resistant preclinical models, the combination of PARP and MEK or other MAPK inhibitors is highly active[124,208].

Induction of oncogenic *MYC* in myeloma cell lines induces replication and oxidative stresses, which cause DSBs requiring the activation of HR repair pathways[209]. Similarly, amplification of *MYC* correlates with increased RAD51 expression in both germline *BRCA1/2*-mutant and sporadic TNBCs, and women with tumours overexpressing both *MYC* and *RAD51* have an unfavourable prognosis[190]. Treatment with the pan-CDK inhibitor dinaciclib (SCH-727965) downregulates MYC expression in TNBC cells, although studies have shown that MYC loss alone is insufficiently lethal, thus providing the rationale for combination therapy[190]. The combination of dinaciclib with niraparib results in increased DNA damage and downregulated HR activity, resulting in suppression of epithelial-to-mesenchymal transition, and importantly resensitizes PARP inhibitor-resistant TNBC cells to niraparib[190]. This synthetic lethal treatment strategy is active across ovarian, prostate, pancreatic, colon and lung cancer cell lines[190]. The precise mechanism of dinaciclib-induced MYC downregulation remains unclear, although several CDKs are implicated in regulating DNA repair[190]. A phase I study of dinaciclib in combination with veliparib (NCT01434316) has been undertaken in patients with HR-proficient advanced-stage solid tumours. Overall, the combination was poorly tolerated, probably owing to the toxicities associated with dinaciclib monotherapy, and limited antitumour activity was observed[210]. Combinations involving more-selective CDK inhibitors and more-potent PARP inhibitors might be a better approach to testing this hypothesis in patients.

Emerging data also support a synthetic lethal relationship between AR signalling and the PARP and ATR–CHK1 axes in prostate cancers. AR signalling has a key role in regulating HR repair, whereas AR inhibition activates the PARP pathway in mouse models of this disease[125,211,212]. Moreover, ADT has been shown to functionally impair HR repair before the development of castration resistance in prostate cancer[180], providing the rationale for the use of PARP inhibitors in combination with ADT upfront in patients with advanced-stage or high-risk prostate cancers. Preclinical models have revealed that the combined or sequential targeting of the AR with PARP and/or ATR inhibition leads to synthetic lethality, regardless of DDR gene mutation status[125,211,212]. Correspondingly, data from a phase II trial of abiraterone combined with olaparib in patients with mCRPC showed improved radiographic PFS compared with that observed with abiraterone alone, including in patients without DDR gene mutations[213]. Another randomized phase II trial is currently underway to compare the AR antagonist enzalutamide, olaparib and combination of both agents specifically in patients with mCRPC harbouring DNA repair defects (NCT03012321).

Thus, multiple combination or sequential treatment strategies involving DDR inhibitors are now being tested. In order to optimize their development, efforts should be focused on using adaptive trial designs with dynamic, functional biomarkers of DDR and replication stress in order to pursue strategies to maintain the cancer cells in a state of perpetual DDR deficiency and minimize issues of acquired resistance.

Combinations with immunotherapies

Multiple lines of evidence have provided a biological rationale, and demonstrated synergistic benefit, for combining immune-checkpoint inhibitors (ICIs) with DDR inhibitors, while defects in DDR pathways might potentially serve as predictive biomarkers of responsiveness to ICIs. DDR proteins maintain the integrity of the genome and, therefore, the use of DDR inhibitors might increase the tumour mutational burden (TMB), which in turn could lead to neoantigen production and enhanced anticancer T cell activity. Studies have supported the association of a high TMB and dynamic neoantigen renewal with neoepitope-specific T cell responses against mismatch repair (MMR)-deficient tumours treated with ICIs[214–216]. Thus, the high ORRs (40–71%) are observed in patients with MMR-deficient tumours treated with anti-programmed cell death protein 1 (PD-1) antibodies are unsurprising, given the mutator phenotype engendered by defects in this DNA repair pathway[214,215]. These impressive clinical findings led to the histology-agnostic FDA approval of the anti-PD-1 antibody pembrolizumab for patients with MMR-deficient cancers. However, direct evidence that targeting of DSB repair proteins with DDR inhibitors causes an increased TMB is only beginning to emerge[217]. The mechanisms underlying the potential benefit of immunotherapy in patients with HRD tumours and/or the rationale for combining ICIs with DDR inhibitors are multifactorial.

First, S phase-specific DSBs in cells with DDR gene aberrations result in the accumulation of cytosolic DNA, which activates stimulator of interferon genes (STING); STING subsequently mediates the release of type I interferons, which activate T cells and facilitate the innate immune recognition of immunogenic tumours, but is associated with upregulation

of immunosuppressive PD-L1 expression[5,180,218]. Preclinical studies have also revealed that PARP inhibition upregulates PD-L1 expression via inactivation of GSK3β and consequently attenuates antitumour immunity[219]. Moreover, BRCA2 or Ku70/80 depletion has been shown to enhance CHK1-dependent and interferon signalling-dependent upregulation of PD-L1 on cancer cell lines following PARP inhibition and/or irradiation[220].

Second, a high burden of indel mutations in tumours predicts for higher levels of neoantigens and better responses to ICIs in preclinical models[221]. Given that HRD typically results in a higher number of indels, neoantigen generation might contribute to the improved immunotherapy responses of these tumours. In HRD tumours, DSBs might also contribute to genomic instability and copy-number aberrations, which seem to be associated with responsiveness to both PARP inhibitors and ICIs[222]. Thus, the neoantigen burden and profile of a tumour is probably partially dependent on the specific defect in the DDR, resulting in substantial heterogeneity.

Together, these findings suggest that HRD and/or DDR inhibitors might create immunological vulnerabilities in tumours, while simultaneously activating immunosuppressive pathways. Thus, targeting HRD or even HR-proficient tumours with anti-PD-1 or anti-PD-L1 antibodies in combination with PARP inhibitors or other DDR inhibitors is attractive, particularly given that these agents have distinct, mostly non-overlapping toxicities. Indeed, combinations of ICIs and DDR inhibitors are currently undergoing clinical testing.

In a small cohort of patients with advanced-stage gynaecological or breast cancers ($n = 12$), Lee and colleagues[223] established the combination RP2D at full monotherapy doses of both olaparib and durvalumab, with no DLTs observed (Supplementary Table 2). Overall, two PRs (lasting ≥15 months and ≥11 months) and eight stable disease responses with a median duration of 8 months (range 4 months to 14.5 months) were observed, equating to a disease control rate (DCR) of 83%[223]. Preliminary data from the phase II MEDIOLA trial of the same drug combination indicated a similarly impressive DCR of 80% for patients with advanced-stage germline *BRCA1/2*-mutant breast cancers; the ORR (all PRs) was 52%[224].

Early data from a phase II trial of the olaparib–durvalumab combination in patients with mCRPC (NCT02484404) demonstrated an acceptable toxicity profile and serum prostate-specific antigen responses in 12 of 17 patients (71%), with a median PFS of 16.1 months for all patients who received the combination[225]. Importantly, biochemical responses were observed independently of known mutations in DNA repair genes[225]. Interestingly, T cell activation was shown to be associated with improved PFS[225]. At this early stage of clinical development, although apparently well tolerated, the extent to which anti-PD-1 or anti-PD-L1 antibodies will enhance the effects of PARP inhibition in patients with germline *BRCA1/2*-mutant cancers remains unclear. Other active areas of research include the determination of other tumours or molecular subtypes of cancers in which this combination will ultimately have biological and clinical activity.

Outside of PARP inhibitors, the ATR inhibitor AZD6738 is currently being tested in combination with durvalumab in a phase Ib trial involving patients with advanced-stage NSCLC or HNSCC (NCT02264678; Supplementary Table 3); patients will receive AZD6738 daily during a run-in period before each dose of durvalumab in 28-day cycles. Initial data from this trial indicate that the combination is well tolerated, with no DLTs observed, with preliminary signals of activity[132] (TABLE 2). In the phase Ib, randomized, multi-drug, biomarker-directed BISCAY study in patients with metastatic muscle-invasive bladder cancer (NCT02546661), patients with any HR repair defect detected will receive durvalumab with or without olaparib; those with detectable *CDKN2A* or *RB1* deficiency and/or amplifications of *CCNE1*, *MYC*, *MYCL* or *MYCN* will receive durvalumab with or without the WEE1 inhibitor adavosertib, owing to the increased replication stress and dysfunction in cell cycle response associated with these genomic aberrations[226]. Adavosertib is also being combined with durvalumab in a phase I trial to test different treatment schedules in patients with advanced-stage solid tumours (NCT02617277).

Conclusions

Great strides have been made in successfully targeting DNA repair in cancer medicine, with multiple selective and potent DDR inhibitors emerging in the clinic. The regulatory approvals of different PARP inhibitors in the management of patients with *BRCA1/2*-mutant ovarian and breast cancers and in the maintenance setting for those with platinum-sensitive ovarian cancer, regardless of mutation status, are major breakthroughs. Indeed, ovarian cancer has served as the poster child for the use of PARP inhibitors, although clear signals of acceptable safety and antitumour activity have been observed across multiple other cancer types and molecular subtypes, including in patients who are wild type for canonical DDR genes. The oncology community's clinical research focus for the development of PARP and other DDR inhibitors should now shift to transforming more nonresponders into responders and turning more responders into 'super-responders' with a markedly increased depth and duration of response.

To produce a large step change and improvements in patient outcomes, clear priority should now be given to the development of more inclusive and functionally based predictive biomarker assays. Moreover, an improved understanding of resistance mechanisms to different DDR inhibitors and their interactions with one another is needed to facilitate the development of novel combination strategies. While combinations designed to induce tumour HRDness are a rational approach and have demonstrated clinical potential, the appropriate dosing and scheduling of each agent to minimize adverse events while maximizing benefit will also be key to optimizing outcomes. The clear and mounting evidence that genomic instability strongly influences the tumour immune microenvironment is paving the way for biomarker development as well as treatment strategies to

target this hallmark of cancer with DDR inhibitors in conjunction with immunotherapy.

Preclinical and clinical data discussed herein emphasize the complex and far-reaching nature of DDR pathways. The ability to maximize the benefit of DDR inhibitors requires a better system-wide understanding of the dynamic mechanisms that tissue-specific cancer cells use to mitigate genomic instability and how this instability influences innate and acquired immunity. In the clinic, treatment strategies using DDR inhibitors should be optimized through the use of adaptive, longitudinal trial designs with integrated functional biomarkers to truly personalize antitumour DDR therapies and increase patient benefit.

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