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Targeting replication stress in cancer therapy

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Abstract

Replication stress is a major cause of genomic instability and a crucial vulnerability of cancer cells. This vulnerability can be therapeutically targeted by inhibiting kinases that coordinate the DNA damage response with cell cycle control, including ATR, CHK1, WEE1 and MYT1 checkpoint kinases. In addition, inhibiting the DNA damage response releases DNA fragments into the cytoplasm, eliciting an innate immune response. Therefore, several ATR, CHK1, WEE1 and MYT1 inhibitors are undergoing clinical evaluation as monotherapies or in combination with chemotherapy, poly[ADP-ribose]polymerase (PARP) inhibitors, or immune checkpoint inhibitors to capitalize on high replication stress, overcome therapeutic resistance and promote effective antitumour immunity. Here, we review current and emerging approaches for targeting replication stress in cancer, from preclinical and biomarker development to clinical trial evaluation.

One of the most striking characteristics of cancer cells is their ability to sustain chronic proliferation[1]. For these cells, maintaining DNA integrity during DNA replication is a highly complex task that requires the coordination of `DNA replication fork` progression, which in turn requires an adequate supply of deoxyribonucleotides, functional DNA repair mechanisms and intact cell cycle checkpoints[2]. Cancer cells have alterations in many of these processes, which results in obstacles to replication fork progression, slowing down of the replication fork and impaired DNA synthesis. If left unresolved, these obstacles result in detachment of the DNA replication machinery from the DNA fibre, a result known

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Competing interests

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as replication fork collapse[3]. Replication stress is traditionally defined as the slowing or stalling of replication fork progression[2], but this is an evolving concept and other definitions are present in the literature[4].

As replication stress is higher in cancer cells, these cells depend on replication stress response pathways for survival[3]. If the cellular response to replication stress is ineffective, cells may enter mitosis with an excess of DNA error, leading to genomic instability or even cell death owing to `mitotic catastrophe`[2,3,5]. Indeed, replication stress is one of the leading causes of genomic instability in cancer cells[2,3,5], and targeting replication stress has enabled the identification of novel cancer vulnerabilities[6].

Cellular response to replication stress comprises the activation of DNA damage tolerance pathways and the activation of the ataxia telangiectasia mutated (ATM)- and Rad3-related (ATR) pathway. Since the description of ATR in 1996 (REFS.[7,8]), several protein interactions responsible for the cellular response to replication stress have been elucidated[9]. Similarly, new techniques that enable the direct measurement of nucleotide incorporation into the DNA, such as the DNA fibre assay, have unravelled replication fork dynamics under replication stress. Moreover, large-scale genomic studies have highlighted the genomic disorganization of tumour cells and described potential molecular mechanisms of replication stress[9]. In this regard, many types of cancer commonly harbour genomic alterations associated with replication stress (such as amplification of *CCNE1*, which encodes G1/S-specific cyclin E1), including high-grade serous ovarian carcinoma (HGSOC), high-grade serous endometrial carcinoma (HGSEC), non-small-cell lung cancer (NSCLC), triple-negative breast cancer (TNBC), pancreatic cancer and gastric cancer. HGSOC is an excellent tumour model of high replication stress because almost all cases exhibit loss of cell cycle regulation, oncogene activation and/or DNA repair defects[10].

In this Review, we discuss the intrinsic and extrinsic causes of replication stress, outline the biological mechanisms involved in the replication stress response, and review current and emerging approaches for targeting replication stress to induce cancer cell lethality, overcome drug resistance and facilitate effective antitumour immunity.

Response to replication stress

Any obstacle that causes fork slowing or fork stalling can lead to replication stress. In normal cells, the most frequent causes of replication stress include endogenous replicative errors caused by reactive oxygen species (ROS), misincorporation of ribonucleotides, and secondary DNA structures such as hairpins, DNA triplexes and quadruplexes formed in DNA regions with specific base repeats. In addition, replication stress can result from collisions between the replication and transcription machineries and from rehybridization of nascent mRNA to DNA, forming R-loops[2] (FIG. 1).

In cancer cells, unrepaired DNA lesions are among the most frequent causes of replication stress and pose a physical barrier to fork progression. Depletion of nucleotide pools, caused by chemotherapeutic agents such as gemcitabine and 5-fluorouracil, can also limit DNA replication and fork progression. Additionally, activation of oncogenes can lead to depletion

of nucleotide pools and collisions between the replication fork and transcription complexes. Finally, loss of the G1/S checkpoint leads to the accumulation of DNA errors and early entry into S phase[2,5].

ATR kinase occupies a central role in the cellular response to replication stress. Replication protein A (RPA) is the first sensor of this pathway. RPA binds to single-stranded DNA (ssDNA) at the stalled fork and recruits ATR through the partner ATR-interacting protein (ATRIP). ATR is then activated by DNA topoisomerase 2-binding protein 1 (TOPBP1), activating CHK1 through phosphorylation, thus triggering the ATR–CHK1 pathway. ATR–CHK1 pathway activation leads to cell cycle arrest in S–G2, and to activation of the homologous recombination repair (HRR), inter-strand crosslink and nucleotide excision repair (NER) DNA repair pathways. Importantly, ATR–CHK1 pathway also results in replication fork stabilization and suppression of dormant `replication origin firing`, ensuring enough supply of deoxynucleotides (dNTPs)[11,12] (FIG. 2).

CHK1 mediates cell cycle arrest in the S–G2 phase through reduction of cyclin-dependent kinase 2 (CDK2) activation, thus slowing down DNA replication in S phase. CHK1 also limits mitotic entry through phosphorylation of cyclin-dependent kinase 1 (CDK1). CDK1 is negatively regulated via phosphorylation by WEE1 and membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase (PKMYT1, also known as MYT1) kinases and positively regulated by CDC25 phosphatases. CHK1 activates WEE1 and promotes the degradation of CDC25A[11,13].

Replication fork plasticity is a fundamental feature of the replication stress response. To ensure that obstacles in DNA replication do not result in loss of genetic information, cells develop DNA damage repair and tolerance mechanisms to ensure fork progression. Thus, the stalled fork can reverse its direction (fork reversal) (FIG. 3) or replicate over lesions. Fork reversal slows down the replication fork, shifts the position of the replication fork backwards, thus repositioning the DNA damage in a double-stranded DNA segment, and enables an error-free DNA repair, whereas DNA damage tolerance pathways, such as translesion synthesis or `repriming`, lead to replication fork progression through the DNA lesion in an error-prone manner. In the case of translesion synthesis, specialized translesion synthesis polymerases can synthesize the daughter strand through the lesion owing to a more flexible active polymerase site and the lack of exonucleolytic proofreading, whereas in the case of repriming, DNA-directed primase/polymerase protein (PRIMPOL) can restart DNA synthesis immediately after the lesion without removing it, leaving a ssDNA gap behind it[14]. The mechanisms involved in the choice of pathway of fork recovery are not completely understood, but ATR orchestrates many of them, leading to the final result of fork stability[11]. On the one hand, ATR activation causes a global response to fork reversal through RAD51–ZRANB3 activity to slow DNA replication in response to inter-strand crosslinks in *BRCA1*-proficient cells[14]. On the other hand, ATR limits fork reversal promoted by the chromatin remodeller SMARCAL1 and modulates the fork remodelling proteins Bloom syndrome protein (BLM) and Werner syndrome ATP-dependent helicase (WRN), thereby limiting excessive fork cleavage and facilitating fork restart[11]. ATR also has a role in promoting PRIMPOL repriming of stalled forks[14]. These seemingly contradictory effects of ATR in replication fork reversal may be related to different types of DNA damage eliciting ATR

activation in the context of specific genomic backgrounds and constitute an area of active research.

Prevention of the depletion of dNTP supply is another feature of the replication stress response. ATR–CHK1 pathway activation ensures dNTPs supply by suppressing global dormant replication origin firing through phosphorylation of FANCI and the inhibition of cell division cycle 45 (CDC45) recruitment to the minichromosome maintenance 2–7 complex (MCM2–7)[13] (FIG. 3). Replication origins close to the stalled forks are subsequently activated to rescue the fork. In addition, ATR pathway activation leads to upregulation of the cellular dNTP supply by decreasing degradation of dNTPs through proteasome inhibition and increasing dNTP synthesis through upregulation of transcription of ribonucleoside-diphosphate reductase subunit M2 (*RRM2*), which is primarily mediated by WEE1 activity[11,13,15]. Finally, WEE1 activation also contributes to fork stability by inhibiting nucleases such as MUS81, preventing fork degradation[16].

The essential function of the ATR–CHK1–WEE1 pathway in replication stress response is highlighted by the extremely low rates of loss-of-function genomic alterations involving this pathway in various cancer types (<3% for *ATR* and <1% for *CHK1* and *WEE1*)[17]. Of note, mouse models with severe reduction of ATR function are resistant to development of tumours[11].

Replication stress in cancer

Sources of replication stress in cancer cells include loss of G1/S checkpoint (for example, owing to deleterious *TP53* mutations), premature entry into S phase (due to *RB1* loss, *CCNE1* amplification or *FBXW7* loss), oncogenic drive (oncogene-related replication stress such as that due to *KRAS* activating mutations or *MYC* amplification) and DNA repair deficiencies (such as HRR or NER pathway deficiency)[9]. It is important to underscore that different mechanisms may lead to varying levels of replication stress, also depending on the specific genomic context. For example, oncogenic activation or *CCNE1* amplification results in a higher level of replication stress compared with loss of function of p53 or RB1. Moreover, some of these mechanisms, such as *RB1* loss, may lead to genomic instability by means other than replication stress[18].

Oncogene activation from *KRAS*, *CCNE1* or *MYC* can lead to replication stress through the increase in replication origin firing, thus resulting in more topological stress and depletion of nucleotide pools. Oncogene activation also increases transcription, leading to more transcription–replication collisions, and generates ROS through changes in cell metabolism[19,20]. Cyclin E1 regulates the transition from G1 to S phase through binding and activation of CDK2. It also increases replication origin firing while also causing deficiency in pre-replication complex formation. For these reasons, cancers with cyclin E1 overexpression are among those with the highest level of replication stress[19,21].

HGSOC is an excellent tumour model of high replication stress (Supplementary Fig. 1). Almost all HGSOCs bear *TP53* mutations, whereas *CCNE1* amplification occurs in 20% of cases, biallelic *RB1* loss in 10% and *CDKN2A* mRNA downregulation in 32%, all leading

to premature entry into S phase. Of note, low *CDKN2A* mRNA expression is mutually exclusive with *CCNE1* amplification and *RB1* deletion and/or mutation events, reflecting an extreme selection pressure for loss of RB pathway regulation and rapid G1 to S phase entry in HGSOC[10]. *MYC* amplification is present in 40% of cases of HGSOC, *NF1* loss in 10% and *KRAS* amplification and/or mutation in 11%, all leading to oncogene-driven replication stress. Finally, HRR alterations are present in about 50% of cases and NER alterations in 4–8%, leading to accumulation of DNA lesions[10]. Most chemotherapeutic agents used to treat HGSOC, such as gemcitabine, topotecan and platinum salts, are effective, at least in part, because of their ability to further aggravate pre-existing replication stress in these tumours[22] (Supplementary Table 1).

Several other cancers bear different genomic alterations that can lead to a high degree of replication stress. HGSEC shares many molecular features with HGSOC, although it is associated with oncogene-induced replication stress (due to *ERBB2* amplification and *FBXW7* mutations) even more frequently than HGSOC but is less commonly associated with HRR deficiency (Supplementary Fig. 1). In addition, NSCLC, TNBC, pancreatic and gastric cancers commonly exhibit genomic alterations associated with a high degree of replication stress (Supplementary Fig. 1). Human papillomavirus (HPV)-related cancers such as head and neck squamous cell (HNSCC) and cervical cancers also exhibit high levels of replication stress due to loss of RB1 and p53 control of G1/S checkpoint caused by HPV E6 and E7 proteins as well as other mechanisms[23].

Targeting replication stress

Cytotoxic agents and replication stress

Most chemotherapeutic agents that are used to treat cancer patients induce replication stress through various mechanisms (FIG. 4). Platinum salts and alkylating agents generate intra-strand and inter-strand crosslinks. Intra-strand crosslinks are bypassed by translesion synthesis polymerases or repaired via NER, whereas inter-strand crosslinks cause fork stalling and require a synchronized response of several pathways, including NER, base excision repair (BER), HRR and Fanconi anaemia (FA) pathways[24] (BOX 1). Topoisomerase 1 inhibitors, such as topotecan and irinotecan generate topoisomerase 1–DNA cleavage complexes that block replication by forming a barrier to fork progression[9]. Nucleoside analogues, such as gemcitabine, promote replication stress through two different mechanisms. First, they are directly incorporated into DNA during replication, leading to fork stalling. Secondly, they reduce intracellular nucleotide pools through inhibition of the enzymes responsible for dNTP synthesis (gemcitabine inhibits ribonucleotide reductase, whereas 5-fluorouracil inhibits thymidylate synthetase)[25].

Among targeted agents, poly[ADP-ribose]polymerase (PARP) inhibitors (PARPi) are potent inducers of replication stress via multiple mechanisms including inhibition of BER, which leads to impairment of single-strand break repair, PARP entrapment on the DNA, causing replication forks to stall, and inhibition of PARP activities in fork protection and restart[26,27]. PI3K–mTOR pathway inhibitors also induce replication stress by promoting new origin firing, leading to decelerated fork speed; they also attenuate DNA HRR activity by decreasing trans-nuclear localization of RAD51 and downregulating BRCA2 and RAD51

expression[28]. AXL is a receptor tyrosine kinase that promotes cell proliferation and survival via the RAS–MEK–ERK and the PI3K–AKT–mTOR pathways and drives epithelial to mesenchymal transition. Inhibition of AXL promotes DNA damage and replication stress, leading to activation and dependence on the ATR–CHK1 pathway[29,30]. AXL promotes resistance to chemotherapy and to many targeted therapies such as EGFR, ALK and BRAF inhibitors in lung cancer and HNSCC[31,32]. Bromodomain and extra-terminal domain (BET) inhibitors, which have been used to target *MYC*-driven cancers, also induce replication stress. Besides its role as transcription factor, BRD4, one of the BET family proteins, plays a part in the DNA damage response. BRD4 interacts with the pre-replication factor CDC6, regulating the activation of CHK1–WEE1 signalling and preventing the formation of R-loops. BET inhibitors (BETi) also promote double-strand breaks through inhibition of the non-homologous end-joining repair (NHEJ) pathway[33–37].

More recently, the implication of DNA damage tolerance pathways such as translesion synthesis and repriming by PRIMPOL as alternative responses to replication stress has led to the development of inhibitors of these pathways[14,38]. Furthermore, the improved characterization of specific post-translational modifications of replication stress response factors through ubiquitylation and deubiquitylation has renewed the interest in drugs that target these processes, such as inhibitors of the deubiquitylating enzymes USP13 and USP1, which take part in ATR activation[39] and translesion synthesis[39] regulation, respectively, and inhibitors of the ubiquitylating enzyme TRAF4, which is involved in proper CHK1 activation[40].

Given their ability to induce replication stress, all aforementioned agents represent ideal partners for synergistic combinatorial strategies with agents that target the replication stress response such as ATR inhibitors (ATRi), CHK1 inhibitors (CHK1i) and WEE1 inhibitors (WEE1i).

Replication stress and drug resistance.

Although tumours with HRR deficiency are highly sensitive to platinum salts and PARPi, acquired resistance commonly occurs via distinct mechanisms. First, tumours may restore HRR function through secondary somatic reversion mutations or re-expression of HRR-related genes that had been silenced through promoter hypermethylation. Second, HRR-deficient tumour cells may restore double-strand break end resection and HRR through loss of checkpoint proteins that inhibit end resection and promote NHEJ such as 53BP1, REV7 (also known as MAD2L2) or dynein light chain 1 (DYNLL1), or by upregulation of proteins that promote end resection and activate HRR such as TRIP13 and ubiquitin carboxyl-terminal hydrolase 15 (USP15)[41–43]. Third, HRR-deficient tumour cells can stabilize their replication forks through loss of PAX-interacting protein 1 (PTIP), which recruits end resection proteins, loss of SMARCAL1 or loss of Schlafen family member 11 (SLFN11)[41,42]. Fourth, mutations or post-translational modifications of *PARP1* itself can lead to PARPi resistance[41,42]. Fifth, loss of expression of poly(ADP-ribose) (PAR) glycohydrolase (PARG) can lead to upregulation of PAR despite PARP1 inhibition[44]. Finally, the overexpression of the PARPi efflux pump MDR1 (REFS.[41,42,44]) can cause PARPi resistance (FIG. 5). Restoration of HRR function has a central role in

PARPi resistance, whereas stabilization of the replication fork is important for platinum resistance[45].

Preclinical studies have demonstrated that ATRi, CHK1i and WEE1i may overcome PARPi resistance and platinum resistance, addressing different resistance mechanisms[46–55]. ATRi, CHK1i and WEE1i can promote HRR deficiency in cases in which HRR function is restored. In the cases in which stable replication forks drive PARPi resistance, ATRi, CHK1i and WEE1i can cause replication stress and replication fork instability as measured by the `DNA fibre assay`[46,48–50]. Interestingly, ATRi, CHK1i and WEE1i may also overcome primary PARPi resistance in *BRCA1/2* wild-type tumour models, such as *CCNE1*-amplified cancers[46,51]. Finally, cells that become PARPi resistant through PARG downregulation exhibit high replication stress and dependence on the ATR–CHK1–WEE1 pathway for survival. Accordingly, PARG downregulation confers sensitivity to CHK1 and WEE1 inhibition[56,57].

Loss of SLFN11 expression is a unique mechanism of PARPi and platinum resistance. SLFN11 is an executioner of replication stress, as it binds to RPA-coated ssDNA and permanently blocks replication independently of ATR, leading to cell death[58]. SLFN11 expression changes are dynamic during tumour evolution and loss of SLFN11 expression, mainly through methylation and silencing, has been associated with resistance to DNA-damaging agents such as PARPi, cisplatin, gemcitabine, topotecan, etoposide and doxorubicin[58]. Tumours with low expression of SLFN11 depend on the ATR–CHK1–WEE1 axis to tolerate replication stress and inhibition of this pathway resensitizes tumour cells, in vitro and in vivo, to both platinum and PARPi[59,60]. Taken together, ATR–CHK1–WEE1 inhibitors may overcome multiple mechanisms of de novo and acquired PARPi and platinum resistance.

Replication stress and immunotherapy

Combination of DNA-damaging agents with immunotherapy has recently yielded promising results. Indeed, PARPi can elicit an innate immune response by releasing DNA particles into the cytoplasm, activating the cGAS–stimulator of interferon genes protein (STING) pathway, and upregulating the type I interferon-γ response and, subsequently, the nuclear factor-κB (NF-κB) pathway[61,62]. Besides this pro-inflammatory signal, cGAS–STING can also upregulate programmed cell death 1 ligand 1 (PDL1) expression. Hence, there is a strong rationale for combining monoclonal antibodies against PD1 or PDL1 with PARPi[61,62]. However, although DNA damage checkpoint inhibitors may contribute to cGAS–STING activation through the induction of double-strand breaks[63], some studies indicate that the ATR–CHK1 pathway also has a fundamental role in the PDL1 upregulation caused by DNA damage[64,65]. Indeed, at least one preclinical study demonstrated that ATR inhibition leads to PDL1 downregulation[66]. In that setting, although PDL1 downregulation could sensitize tumour cells to T cell-mediated killing, there may not be a synergism between DNA damage checkpoint inhibitors and PD1/PDL1 blockade. Moreover, at least one study using different cell lines demonstrated that inhibition of ATR, CHK1 or WEE1 increases cytoplasmic double-stranded DNA and activates the STING pathway but does not result in activation of a type I interferon response[67].

However, other studies have demonstrated synergistic antitumour activity for combinations of DNA damage checkpoint inhibitors with PD1/PDL1 blockade[68–71]. In models of NSCLC, the addition of a CHK1i to PD1 inhibition was synergistic owing to activation of the cGAS–STING pathway and upregulation of PDL1 expression[69]. Remarkably, two other studies using syngeneic mouse and organoid models of HGSOC with *CCNE1* amplification and *KRAS* mutation demonstrated a significant benefit from the combination of immunotherapy agents (anti-CTLA4 or anti-PDL1) with replication stress-inducing agents (the CHK1i prexasertib or gemcitabine), compared with the immunotherapy doublet or combinations of immunotherapy and other chemotherapies[72,73].

In one study that addressed the various effects of PARPi and ATRi in activating the immune response and regulating PDL1 expression, both PARPi and ATRi activated the cGAS–STING pathway; however, ATR inhibition led to PDL1 protein degradation by activating the CDK1–speckle-type POZ protein (SPOP) pathway. Conversely, PARPi led to CDK1 inactivation and did not activate the CDK1–SPOP pathway, therefore having no effect on PDL1 degradation. By downregulating PDL1, ATR inhibition derepressed an IFNβ-mediated pro-apoptotic pathway, the cGAS–STING–IFNα/β receptor (IFNAR1) pathway, resulting in autocrine cytotoxic signalling. Whether these results are tumour specific or restricted to ATRi, compared with CHK1i or WEE1i, remains to be clarified[74] (FIG. 6).

A different strategy is to combine ATR, CHK1 and WEE1 inhibition with radioimmunotherapy. Although radiotherapy stimulates an immune response against tumour cells, it also upregulates PDL1 expression and increases tumour infiltration of regulatory T (T_{reg}) cells through the activation of DNA damage repair pathways[75]. In a hepatocellular carcinoma model, the addition of ATRi promoted $CD8^+$ T cell infiltration and activity, reduced infiltration of T_{reg} cells, and increased memory T cell infiltration[76]. Thus, triple therapy with radiotherapy, immune checkpoint inhibitors and ATRi prolonged survival and delayed recurrence in tumour-bearing mice.

Taken together, these studies highlight the active role of replication stress and DNA damage response in immunomodulation and suggest that there may be synergism between DNA damage checkpoint and immune checkpoint blockade, although this synergism seems to be context specific.

ATRi, CHK1i, WEE1i and MYT1i

ATR–CHK1–WEE1 inhibitors are potent and selective agents when used as single agents. Their primary mechanism of action is to increase origin firing and to inhibit the S phase and G2/M cell cycle checkpoints, which leads to enhanced replication stress and premature entry to mitosis[77–83]. It also leads to impairment of HRR during the S and G2 phases and induction of double-strand breaks[83–91].

Early studies hypothesized that cells that bear *TP53* mutations would be more sensitive to ATR–CHK1–WEE1 inhibition. ATRi, CHK1i and WEE1i have shown selective efficacy in p53-deficient cells[92–95], but this effectiveness is irrespective of p53 status[90,91,96–98]. The development of these drugs then followed two paths, the first searching for predictive

biomarkers to identify tumour cells that are particularly sensitive to monotherapy and the second testing various drug combinations. Several compounds that target ATR, CHK1 and WEE1 are currently in preclinical and clinical development (TABLE 1 and Supplementary Tables 2, 3 and 4).

ATRi monotherapy.—ATRi have shown promising efficacy and are currently being tested in randomized, late-phase clinical trials. At least five compounds have reached clinical trial evaluation: berzosertib (M6620/VE822), ceralasertib (AZD6738), elimusertib (BAY1895344), M1774 and RP-3500.

A phase I trial evaluated ceralasertib, an oral ATRi, as monotherapy in advanced-stage solid tumours that were refractory to conventional treatment. Ceralasertib was well tolerated at 160 mg delivered twice daily on a 2-week-on, 2-week-off schedule and showed an overall response rate (ORR) of 7%[99]. Furthermore, preliminary clinical data from a phase II study indicated promising antitumour activity of ceralasertib given as a single agent (at a dose of 160 mg twice daily for days 1–14 of a 28-day cycle) in patients with ARID1A-deficient solid tumours, including two of ten patients (both with endometrial cancer) with durable and ongoing complete responses[100].

Another phase I trial evaluated another oral ATRi, elimusertib, in 21 patients with advanced-stage solid tumours. The ORR was 19% (four of 21 patients), and all four responding tumours harboured loss or alterations in *ATM*. Grade 3 anaemia, a class effect of ATRi, occurred in 81.8% of patients. Notably, one long-term responder patient carried a *BRCA1* pathogenic mutation and was resistant to previous treatment with PARPi[101]. Phase I evaluation of berzosertib, an intravenous ATRi administered as monotherapy in 17 patients, showed a complete response in one patient with colorectal cancer bearing *ATM* loss and an *ARID1A* mutation[102]. Finally, RP-3500 was tested in a phase I/II clinical trial in patients with solid tumours selected by genomic alterations in DNA damage repair genes. Interestingly, the ORR was 25% (5/20) among patients with ovarian cancer, 17 of whom presented with platinum-resistant disease and 18 of whom had prior PARPi treatment[103].

CHK1i monotherapy.—Several CHK1i have reached early-phase clinical trials, including prexasertib, rabusertib, MK-877, SRA737, GDC-0575, PF-00477736 and AZD7762. In many cases, such as with rabusertib, MK-877, GDC-0575, PF-0047773 and AZD7762, toxicity or low efficacy led to termination of further drug development[104–111].

Two phase I trials tested prexasertib, an intravenous agent, as monotherapy. The first trial included 45 patients with advanced-stage solid tumours; one patient with HNSCC and one patient with squamous cell carcinoma of the anus had a partial response[112]. This result led to an expansion cohort, including patients with squamous cell carcinoma of various origins; 15% of patients with anal cancer and 5% of patients with HNSCC had partial responses. Genomic alterations in *BRCA1*, *BRCA2*, *ATR*, *MRE11A*, *PIK3CA* and the E3 ubiquitin ligase genes *FBXW7* and *PARK2* were present in patients with clinical benefit. HPV positivity correlated with higher clinical benefit for patients with HNSCC[113]. Prexasertib was also tested in patients with ovarian cancer in a single-arm phase II trial that included high-grade serous or endometrioid histologies, regardless of platinum sensitivity. Among 24

patients without mutations in *BRCA1* or *BRCA2*, eight patients had partial responses (ORR 33%). Interestingly, four of the eight responses occurred in patients with tumours bearing *CCNE1* amplification[114]. Among 18 patients with a mutation in *BRCA1* or *BRCA2*, only two responses were observed (ORR 11%), suggesting that *BRCA1* and *BRCA2* wild-type tumours may be enriched for other replication stress mechanisms[115]. Unlike ovarian cancer, prexasertib treatment resulted in lower response rates in other tumour types.

In these monotherapy studies, a high incidence of myelosuppression was observed, including grade ≥3 neutropenia in 70–90% of patients, febrile neutropenia in 6–12% of patients and grade ≥3 thrombocytopenia in 20% of patients[112–115]. This degree of haematological toxicity with CHK1i seems higher than that of other DNA damage checkpoint inhibitors, possibly reflecting concurrent inhibition of CHK2 or the presence of independent crosstalk mechanisms in haematopoietic cells. Other toxicities associated with earlier CHK1i such as hyperglycaemia and cardiotoxicity are generally considered off-target effects. In this regard, prexasertib is considered a more specific CHK1i[116].

SRA737 is an orally bioavailable CHK1i that was first tested as monotherapy in a phase I/II trial with an expansion cohort of HGSOC enriched for *CCNE1* amplification. Intriguingly, despite a disease control rate of 54%, there were no partial or complete responses, and there was no association of *CCNE1* with clinical benefit[117]. LY2880070 is an oral CHK1i tested in an initial phase I trial as monotherapy with a maximal tolerated dose of 200 mg delivered twice daily[118].

WEE1i monotherapy.—The WEE1i adavosertib was initially tested in two phase I trials, with partial responses observed in two of eight patients with mutations in *BRCA1* or *BRCA2* (REF.[119]), four of ten patients with HGSOC and two of three patients with endometrial cancer, both of whom had *TP53* mutations[120]. Frequent adverse events included gastrointestinal effects (nausea, vomiting, diarrhoea), fatigue and haematological toxicity. Similarly, in a phase I trial with another WEE1i, ZN-c3, the most frequent adverse events were nausea, vomiting, diarrhoea and fatigue. In that study, two of 16 patients had a partial response[121]. ZN-c3 is also being tested in combination with standard-of-care chemotherapy in platinum-resistant HGSOC. Preliminary results showed an ORR of 30.2% among 43 patients[122].

In a phase II single-arm study of adavosertib in HGSEC, 34 patients who had received at least one prior chemotherapy line were treated with adavosertib as a single treatment 300 mg orally, delivered once daily on days 1–5 and 8–12 of a 21-day cycle. In that study, adavosertib monotherapy demonstrated very promising and durable evidence of activity in women with HGSEC, with an ORR of 29.4%, median progression-free survival (PFS) of 6.1 months and median duration of response of 9 months[123]. Of note, one patient with two-copy loss of *FBXW7*, which is expected to lead to upregulation of both MYC and cyclin E, experienced a significant and prolonged response of more than a year. In a phase I study, ZN-c3 treatment resulted in one complete response and two partial responses among 11 patients with metastatic HGSEC, further suggesting the sensitivity of HGSEC to WEE1i[124].

In another phase II trial in patients with *CCNE1* amplification (defined as ≥7 copy numbers) using the same dosing schedule, ORR was 27%, with five objective responses among 13 patients with ovarian cancer (ORR 38.5%)[125].

MYT1i monotherapy.—MYT1 (FIG. 2) is another target for inhibition of the cellular response to replication stress. MYT1 targets both Tyr14 and Tyr15 residues of CDK1, and MYT1 inhibition causes unscheduled activation of CDK1, leading to early mitosis. MYT1 is more CDK1-selective than WEE1, which regulates both CDK1 and CDK2. Unlike WEE1, which is localized primarily in the nucleus, MYT1 is associated with the endoplasmic reticulum and Golgi apparatus and can promote CDK1 cytosolic segregation[126]. Preclinical studies have demonstrated that the MYT1i RP-6306 has higher selectivity for cyclin E1-overexpressing cells both in vitro and in vivo and that synergizes with gemcitabine[127].

Taken together, ATRi, CHK1i or WEEi have only modest activity as monotherapy in biomarker-unselected patient populations. Certain genomic alterations such as *CCNE1* amplification, *ATM* loss and mutations in *BRCA1* or *BRCA2* as well as specific tumour subtypes such as HGSOC and HGSEC, which are characterized by high prevalence of alterations associated with a high degree of replication stress, may predict response to these agents, but more studies are needed to confirm these observations. All these agents are characterized by dose-limiting haematological toxicity, with anaemia more prominent with ATRi and neutropenia more prominent with CHK1 and WEE1i, although individual differences do exist.

Combining ATRi, CHK1i, WEE1i and MYT1i

Cytotoxic and targeted agents that induce replication stress are attractive partners for combinatorial strategies with ATRi, CHK1i and WEE1i, and many combinations are currently under development (FIG. 4).

Gemcitabine combinations.—Gemcitabine induces a high level of replication stress and has been effectively combined with ATRi, CHK1i and WEE1i in preclinical studies[128–139]. Interestingly, CHK1i are less effective than ATRi and WEE1i in sensitizing tumour cells to gemcitabine[129,132].

A phase I trial tested adavosertib alone or in combination with carboplatin, cisplatin or gemcitabine in advanced solid tumours that were refractory to standard treatments. Among 81 patients treated with the gemcitabine combination, four (4.9%) achieved a partial response[140]. In pancreatic cancer, adavosertib was combined with gemcitabine and radiotherapy. Carbohydrate antigen 19–9 (CA19.9) levels were downregulated in the 26 patients for whom measurements of this biomarker were available[141].

CHK1i SRA737 and LY2880070 were also tested in combination with gemcitabine. In a phase I study in 81 patients with tumours from various primary sites (28 patients with HGSOC), ORR was 11.7%, including four of 16 patients (25%) who had FA pathway/BRCA mutations and who showed an objective response. Grade 3, or higher, diarrhoea occurred in 20% of patients, and nausea and vomiting occurred in 50% of patients[142]. Another phase I trial tested LY2880070 plus low-dose gemcitabine in patients with

advanced-stage solid tumours; one partial response was observed among 12 patients with ovarian cancer[143].

Two randomized phase II trials compared gemcitabine monotherapy with gemcitabine plus ATRi or WEE1i. The first trial compared berzosertib plus gemcitabine with gemcitabine alone in 70 patients with HGSOC and platinum-resistant relapse stratified by platinum-free interval (PFI) (PFI <3 months against PFI 3–6 months). The median PFS was 22.9 weeks in the gemcitabine plus berzosertib group versus 14.7 weeks in the gemcitabine-alone group (HR 0.57). Interestingly, benefit was observed primarily in the PFI <3 months subgroup, in which median PFS was 27.7 weeks with gemcitabine plus berzosertib compared with 9.0 weeks with gemcitabine alone[144].

The other randomized phase II study compared gemcitabine plus adavosertib with gemcitabine alone in 124 patients with platinum-resistant ovarian cancer. Median PFS was 4.6 months versus 3.0 months, ORR was 23% compared with 6% and median overall survival was 11.4 versus 7.2 months, all in favour of the adavosertib plus gemcitabine combination. Patients with tumours that had *CCNE1* amplification showed a higher response rate with the combination regimen, whereas *SLFN11* expression was not associated with clinical benefit[123].

The combination of adavosertib with various chemotherapies resulted in significant bone marrow toxicity. Grade 3 or higher thrombocytopenia was observed in 31–48% of patients, grade 3 or higher neutropenia in 37–46% of patients, and dose reductions were necessary in 38–80% of patients[123,145–147] (TABLE 1).

Platinum combinations.—As discussed above, platinum compounds induce replication stress and dependency on ATR–CHK1–WEE1 through formation of intra- and inter-strand crosslinks leading to slowing/stalling of the replication fork. Synergism of ATRi, WEE1i or CHK1i with platinum is further mediated by the additional role of these kinases in the activation of the FA–inter-strand crosslink DNA repair pathway that is responsible for repair of the DNA inter-strand crosslinks induced by platinum[55,133,148–151].

Platinum agents were among the first agents to be tested in phase I trials in combination with ATRi and WEE1i. In a phase I trial, among 120 patients with various solid tumours treated with carboplatin or cisplatin in combination with adavosertib, 13 (10.8%) achieved a partial response[140]. Combination of adavosertib with docetaxel and cisplatin in patients with HNSCC was well tolerated, and five of ten patients had a partial response[152]. The ATRi berzosertib in combination with carboplatin[102] produced a complete response in one patient with platinum-refractory and PARPi-resistant HGSOC[102]. Finally, in a phase I trial of ceralasertib plus carboplatin, two of 36 patients with absent or low expression of ATM or SLFN11 had a partial response[153].

Two phase II studies evaluated carboplatin plus adavosertib in platinum-resistant ovarian cancer. The first trial included patients with *TP53*-mutated ovarian cancer refractory or resistant (PFI <3 months) to first-line platinum and showed an ORR in 43% of patients significantly higher than expected in that setting[145]. The other phase II trial was a non-

randomized study testing four different combinations of adavosertib with gemcitabine, paclitaxel, carboplatin or liposomal doxorubicin in six different schedules. Of 94 patients who entered the study, 31.9% had an ORR. Among the various combinations, carboplatin and adavosertib resulted in an ORR of 66.7% (eight of 12) patients. Among 13 patients whose tumours exhibited *CCNE1* amplification, six patients showed partial responses, for an ORR of 46.1% of patients. The small number of patients in each group limits any definitive conclusions about the best drug to combine with adavosertib and these data require confirmation in larger cohorts of patients[147]. Besides ovarian cancer, a phase II trial of cisplatin plus adavosertib in 34 patients with TNBC demonstrated an ORR in 26% of patients, with median PFS of 4.9 months[123].

Of note, two randomized phase II studies compared ATRi or WEE1i plus chemotherapy versus chemotherapy alone. The first trial randomly assigned patients with *TP53*-mutated ovarian cancer to receive carboplatin and paclitaxel together with adavosertib or placebo. The study met its primary end point of an `enhanced response evaluation criteria in solid tumours` (RECIST) PFS criterion (ePFS), showing an ePFS of 7.9 months versus 7.3 months (HR 0.63, 95% CI 0.38–1.06, $P = 0.080$)[146]. The other study was conducted in patients with urothelial carcinoma who were randomly assigned to receive cisplatin and gemcitabine or cisplatin, gemcitabine and berzosertib. There was no difference in ORR or PFS, and patients in the experimental arm had higher rates of grade 3 or 4 thrombocytopenia (59% versus 39%), neutropenia (37% versus 27%), dis continuation of treatment (24% versus 15%), and lower cumulative cisplatin dose, likely explaining why the addition of berzosertib did not provide benefit[125].

Topotecan and taxane combinations.—Combinations with topoisomerase I inhibitors such as topotecan were also synergistic in preclinical studies[133,148]. A phase I trial evaluated berzosertib plus topotecan[154]; toxicity was mild, with no dose-limiting toxic events in the monotherapy group and only one patient in the combination treatment group reporting toxicity. Three of five patients with platinum-refractory small-cell lung cancer (SCLC) achieved partial response or prolonged stable disease for 10 months, more than 6 months and more than 7 months, suggesting that this may be a promising regimen for this challenging tumour type[154].

Combinatorial strategies with taxanes are supported by various mechanisms. The most obvious of these mechanisms is to further enhance the activity of taxanes by bypassing the G2/M checkpoint and forcing cells with DNA damage and replication stress into mitosis. However, an additional mechanism may be relevant to the emerging role of ATR in controlling chromosome instability during mitosis[155]. Specifically, during mitosis, Aurora A promotes the localization of ATR to centromeres by enabling its binding to centromere protein F. Centromere protein F recruits ATR to RPA-coated centromeric R-loops, where ATR stimulates Aurora B and prevents formation of lagging chromosomes and ensures accurate chromosomal segregation during mitosis. Accordingly, ATRi, CHK1i and WEE1i may synergize with anti-mitotic agents such as taxanes[5,155]. Indeed, a phase I study tested ceralasertib plus paclitaxel. The response rate was 33% in patients with melanoma post-immunotherapy. Interestingly, although not clearly associated with response, patients with melanoma exhibited frequent somatic *NF1* or *NRAS* activating mutations[156].

In conclusion, combinations of ATRi and WEE1i with chemotherapy have shown promising preliminary results. Gemcitabine and platinum have been the agents most successfully used in these combinations so far, especially in platinum-resistant HGSOC. Conversely, chemotherapy combinations with CHK1i were limited by toxicity and demonstrated only modest activity.

PARPi combinations.—Several preclinical studies demonstrated synergism of PARPi with ATRi, CHK1i and WEE1i in ovarian cancer models[148,157–160], models of TNBC[51,161,162] and other primary tumours[163–165]. The synergism was present in both tumours with wild-type and those with mutated *BRCA1* and *BRCA2*, although the effect was more evident in wild-type tumours[159,166]. As discussed above, ATR–CHK1–WEE1 inhibition may overcome key mechanisms of PARPi resistance including restoration of HRR deficiency, replication fork stabilization, SLFN11 inactivation and loss of PARG expression. Mechanistically, preclinical studies have demonstrated decreased levels of pATR and pCHK1, decreased RAD51 recruitment and increased pH2AX accumulation[51,63,148,157,158,161,166].

Concomitant or sequential administration of PARPi with WEE1i in ovarian cancer xenografts had similar effects in cells with high degree of replication stress, but the sequential regimen led to a smaller effect in cells with a low degree of replication stress and was less toxic. Simultaneous administration led to higher levels of replication stress and more DNA damage than sequential administration of the drugs, which may explain the different cell sensitivity according to basal levels of replication stress[158]. This suggests that sequential administration may be an equally effective, less toxic dosing strategy for PARPi combinations with cell cycle checkpoint inhibitors.

A proof-of-mechanism phase I trial tested prexasertib in combination with the PARPi olaparib in an expansion cohort of patients with HGSOC who had disease progression after PARPi treatment. Four of 18 patients with PARPi-resistant disease had partial responses[143]. In a phase I study of ceralasertib plus olaparib, the recommended phase II dose was 160 mg delivered once daily from day 1 to day 7 plus olaparib 300 mg delivered twice daily on days 1–28. Thrombocytopenia and neutropenia were the dose-limiting toxic effects, and one complete response and five partial responses were seen among 45 patients[167].

PARPi in combination with the WEE1i adavosertib and the ATRi ceralasertib was evaluated in two phase II studies in patients with PARPi-resistant ovarian cancer. The CAPRI trial assessed ceralasertib plus olaparib in patients with platinum-sensitive ovarian carcinoma who had progressed through PARPi therapy after at least 6 months of treatment. Among 13 patients, six patients had a partial response for an ORR of 46%[168]. The olaparib plus ceralasertib combination resulted in mostly haematological toxicity with grade 3 or 4 thrombocytopenia in 23.1% of patients, and anaemia and neutropenia in 8% of patients, each. In the EFFORT study, patients who showed disease progression while taking PARPi were randomly assigned to treatment with adavosertib alone or in combination with olaparib. The ORR was 23% and 29% of patients, and median PFS was 5.5 months and 6.8 months, respectively. Among patients with wild-type *BRCA1* and *BRCA2*, ORR was 31% in the single treatment arm and 39% in the combination treatment arm[169]. The combination

treatment resulted in grade 3 or 4 thrombocytopenia and anaemia in 10% of patients, each, and in grade 3 or 4 diarrhoea and fatigue in 12% of patients, each[168,169]. The results of CAPRI and EFFORT studies are clearly promising, with objective responses higher than expected for PARPi monotherapy in these settings.

Immunotherapy combinations.—The combination of the CHK1i prexasertib with the anti-PDL1 antibody LY3300054 in a phase I clinical trial was safe, without unexpected toxicity. Three of six patients with *CCNE1*-amplified HGSOC had partial responses and one patient had stable disease for more than 12 months[170]. A phase I study tested the ATRi ceralasertib plus the anti-PDL1 antibody durvalumab in 21 patients, leading to one complete response and two partial responses in patients with NSCLC and HNSCC[167].

A phase II trial tested ceralasertib plus durvalumab in patients with melanoma who had progressed after treatment with anti-PD1 or anti-PDL1 therapy immediately before enrolment in the trial. The ORR was 30% and the median PFS was 7.1 months among 30 patients. Grade 3 or 4 toxicity was mainly haematological, with anaemia in 33.3% and thrombocytopenia in 16.7% of patients[171].

Combinations with other targeted drugs.—Other targeted drugs are synergistic in combination with ATRi, CHK1i or WEE1i. PIK3CA inhibitors and mTOR inhibitors have shown synergism with CHK1i and WEE1i, leading to increased DNA damage, chromosomal breaks and mitotic catastrophe in preclinical models of HGSOC, NSCLC, TNBC and glioblastoma[172–177]. A phase I trial tested prexasertib plus the PI3K/mTOR inhibitor samotolosib. The addition of samotolosib resulted in a higher rate of haematological toxicity and nausea; ORR was 13.3% among patients with *PIK3CA*-mutated tumours and 25% among patients with TNBC in the expansion cohorts[143].

Besides PI3K pathway inhibitors, AXL inhibitors plus ATRi were synergistic in melanoma and NSLC cells, notably in cells with low expression of SLFN11, a marker of platinum and PARPi resistance[29,30]. BETi synergized with ATRi, CHK1i or WEE1i in various preclinical models of lung cancer, ovarian cancer, melanoma and lymphoma. In these studies, inhibition of BRD4 led to increased replication stress and activation of pCHK1 (REFS.[34–37]). Translesion synthesis inhibitors may also be attractive partners for ATRi, CHK1i and WEE1i, given the role of translesion synthesis as an alternative to the ATR–CHK1–WEE1 pathway in overcoming replication stress[178]. Other drugs tested in combination with ATRi, CHK1i or WEE1i in preclinical studies have been Aurora kinase inhibitors[179], histone deacetylase inhibitors[180–182] and BCL2 inhibitors[183], but more data are needed[179].

Dual blockage of ATR–CHK1–WEE1 pathway.—ATR, CHK1 and WEE1 function at different points of the replication stress response and have distinct roles. Therefore, their inhibition leads to varied cellular toxic effects and elicits different compensatory mechanisms. Because WEE1 is the downstream effector of the pathway, WEE1i are more potent than ATRi or CHK1i, inducing cell cycle arrest and death in the S phase of the same cell cycle owing to replication catastrophe[77,131,184]. Moreover, WEE1 upregulation by various mechanisms has been implicated as an adaptive mechanism of resistance to ATRi and CHK1i[185,186]. Therefore, combinations of WEE1i with ATRi and CHK1i are

mechanistically relevant, and preclinical models have already demonstrated the synergistic potential of such combinations[131,187–192].

Emerging replication stress biomarkers

Functional assays combined with genomic analyses may be useful to identify tumours with high levels of replication stress, but these assays are far from being incorporated into routine clinical practice. In preclinical models, the DNA fibre assay can directly measure the speed and symmetry of replication fork progression. Hill et al.[45] described the generation of short-term patient-derived organoids from patients with ovarian cancer that are suitable for evaluation by functional assays such as the DNA fibre assay. In these organoid models, replication fork instability was associated with response to ATRi and CHK1i and replication stress-inducing chemotherapies.

Biomarkers of response to ATRi, CHK1i or WEE1i may be grouped according to the following principles: first, genomic alterations that are potential causes of replication stress; second, biomarkers of the presence of an active cellular response to replication stress; third, biomarkers that present a synthetic lethal relationship with the ATR–CHK1–WEE1 pathway; and fourth, biomarkers of treatment resistance to ATRi, CHK1i and WEE1i (Supplementary Tables 5 and 6). Biomarkers in the first and second groups can also be used as surrogate markers of replication stress.

Among genomic alterations, tumour cells with amplification of *CCNE1* overexpress biomarkers of replication stress such as pRPA32, pKAP1, pATR, pCHK1 and pCDK1 and are more sensitive to ATRi, CHK1i or WEE1i in preclinical models[47,48]. Moreover, patients with *CCNE1*-amplified tumours had higher response rates to WEE1i and CHK1i in early-phase clinical trials[114,119,123,125,145].

MYC-amplified tumours show high levels of replication stress. MYC induces CDK4 and cyclin D expression and inhibits transcription of the CDK inhibitors CDKN2B (also known as p15) and CDKN1A (also known as p21). These alterations lead to increased replication origin firing and increased cell cycle progression from G1 to S phase[19,193]. In this regard, *MYC*-amplified multiple myeloma and mantle-cell lymphoma models are sensitive to ATR and CHK1 inhibition[77,194,195]. *FBXW7* and *PPP2R1A* mutations, which are both prevalent (each in 22% of patients) and mutually exclusive with *CCNE1* amplification in HGSEC (Supplementary Fig. 1) may also be biomarkers of response to WEE1i and MYT1i and may explain the excellent response of adavosertib in this setting[196].

DNA repair defects also promote replication stress through the accumulation of DNA errors and the disturbance of replication fork dynamics. In the first phase I study of the WEE1i adavosertib, two patients carrying mutations in *BRCA1* and *BRCA2* had partial responses[119] consistent with preclinical studies demonstrating an association between loss of FA and HRR pathway components with response to WEE1 inhibition[89].

NF1 is a negative regulator of the RAS pathway, and deletions in *NF1* lead to activation of the RAS pathway, resulting in replication stress[19,193,197]. In addition, *RB1* loss and *CDKN2A* mRNA downregulation lead to early S phase entry. These alterations, together

with *KRAS*, *CCNE1* and *MYC* amplification, were incorporated as candidate biomarkers of replication stress, which was associated with response to gemcitabine compared with combined gemcitabine and berzosertib in the randomized phase II study in HGSOC discussed previously[198]. This study suggested that the degree of replication stress in a tumour is probably determined by more than one genomic alteration.

The phosphorylated forms of ATR, CHK1 and RPA32 are markers of ATR pathway activation and their expression is widely used as a marker of replication stress in preclinical studies using protein detection methods such as western blot[45,48,196]. KAP1 and H2AX are primary substrates of the ATM kinase. Because replication stress generates secondary double-strand breaks and subsequently activates ATM, the expression of pKAP1 and pH2AX is also used as a marker of cellular response to replication stress[45,48,196]. The clinical utility of these biomarkers depends on the development of reproducible immunohistochemistry methods to enable the evaluation of archival tumour samples and is currently under investigation[101]. Gene expression profiling is another alternative to identify a cellular state of high replication stress; a recent study identified a six-gene signature of differentially expressed genes in cells harbouring genomic alterations related to replication stress[199].

Besides their utility as candidate predictive biomarkers of response to ATRi, CHK1i or WEE1i, changes in expression and phosphorylation of proteins related to the cellular response to replication stress may also be used as pharmacodynamic biomarkers of target engagement. Although all DNA damage inhibitors lead to increased expression of pH2AX and fork instability as determined by the DNA fibre assay, the effects of different ATRi, CHK1i or WEE1i on pATR and pCHK1 depend on the point of the pathway that is blocked. Supplementary Table 6 presents various pharmacodynamic biomarkers for ATRi, CHK1i or WEE1i[200].

Inhibition of ATR in cells with *ATM* loss is synthetically lethal in preclinical models of various primary tumours[197,201–204]. ATM and ATR are the primary sensors of the DNA damage response. Cells with *ATM* loss rely on ATR response to orchestrate DNA repair and cell cycle checkpoints. Indeed, in early-phase clinical studies of ATRi monotherapy, patients with ATM-deficient tumours responded to treatment[101].

Another synthetic lethal interaction to highlight is that between WEE1 inhibition and deficiency of histone methyltransferase SETD2 (REF.[15]). RRM2 is the target of this synthetic lethal interaction. Specifically, RRM2 is regulated by two pathways: first, trimethylation of the histone 3 protein (H3K36me3) by SETD2 upregulates transcription of *RRM2*; and second, WEE1 increases RRM2 protein levels by inhibiting CDK1 and preventing RRM2 degradation. When SETD2 is lost (*SETD2* mutations occur in about 15% of endometrial cancers) or the demethylase gene *KDM4A* is amplified (as is in ~6% of ovarian cancers), then there is a decrease in histone trimethylation, decrease in RRM2 levels and decrease in dNTP pools. In that setting, WEE1 inhibition, which also leads to aberrant origin firing, induces even further, critical, reduction in RRM2, critical dNTP depletion and subsequent lethality[15].

Mutations in *ARID1A* and *SMARCA4* (encoding transcription activator BRG1) are also synthetic lethal with ATR inhibition. ARID1A and BRG1 are subunits of the BAF complex, one of the SWI/SNF complexes. These are multisubunit protein complexes that interact with histones and transcription factors and modulate DNA accessibility for cellular processes such as transcription, DNA replication and DNA repair[205]. Mutations in the genes encoding SWI/SNF complex components are present in 20% of all cancer types, mutations in *ARID1A* are present in up to 50% of ovarian clear cell carcinomas and 30% of ovarian endometrioid carcinomas, and mutations in *SMARCA4* are almost universally present in the rare small-cell hypercalcaemic ovarian tumours[205]. Loss of *ARID1A* leads to a reduced localization of topoisomerase 2A (TOP2A) to DNA, resulting in a defect in chromosome `decatenation`. This is relevant to the role of ATR in regulating the G2 phase decatenation checkpoint, which delays entry into mitosis until chromosomes have been decatenated (disentangled) by TOP2A[206]. ATR mediates the delay of entry to mitosis by inhibition of Polo-like kinase 1, which leads to inhibition of cyclin B1. Interestingly, the SWI/SNF or BAF complexes have a very important function in this process by recruiting TOP2 to the chromatin[207]. ARID1A interacts with TOP2A, and BRG1 is required for TOP2A binding to chromatin. So, when there is loss of ARID1A function or loss of *SMARCA4*, this results in a deficiency in decatenation and dependence on the ATR decatenation checkpoint. As a result, *ARID1A*-deficient cells have a reduced rate of S phase progression and an increased dependency on the G2/M checkpoint[208]. Moreover, loss of ARID1A increases the abundance of R-loops and transcription–replication transcripts, thus increasing replication stress[197]. Therefore, in the setting of *ARID1A* deficiency, ATR inhibition results in increased S phase DNA damage and premature mitotic entry, leading to chromosomal instability and apoptosis[208]. Consistent with these preclinical data, preliminary clinical data from a phase II study presented at the European Society of Medical Oncology (ESMO) annual meeting in 2021 indicated promising antitumour activity of the ATRi ceralasertib given as a single agent in patients with *ARID1A*-deficient solid tumours, including two patients (both with endometrial cancer) with durable and ongoing complete responses[100].

Finally, certain studies have suggested that the alternative lengthening of telomeres (ALT) pathway used by some cancer cells to overcome replicative senescence is synthetically lethal with ATR inhibition. ALT depends on stabilizing the RPA coating of telomeres during the G2 phase to start the HRR pathway to elongate telomeres. RPA activates the HRR pathway through the ATR pathway. As a consequence, cancer cells that rely on ALT are particularly sensitive to ATR inhibition[209]. Although ALT is uncommon in HGSOC, it has been found in 4% of clear cell ovarian carcinomas and 1% of ovarian endometrioid cancers[210]. However, other studies did not find an association of ATR sensitivity with ALT, and this synthetic lethal relationship remains to be better defined[211,212].

ATM and *ARID1A* mutations can be detected by gene sequencing, but as the functional impact of *ATM* mutations is occasionally difficult to determine, ATM protein levels have been evaluated by immunohistochemistry as a complementary method in clinical trials[101]. Although loss of H3K36me3 may be caused by mutations in *SETD2* or amplification of *KDM4A*, both detectable by genomic evaluation, immunohistochemistry may interrogate the expression of H3K36me3 or its direct target, RRM2 (REF.[15]). Clinical studies are currently

using these methods to confirm the preclinical findings of the synthetic lethal relationship between them and ATR, CHK1 and WEE1 inhibition.

More recent studies have focused on potential biomarkers of resistance to ATRi, CHK1i or WEE1i. Most of these biomarkers are downstream factors of the ATR pathway. Change in expression of these proteins may develop as compensatory mechanisms during treatment or may be present as a constitutive survival mechanism of the cell and result in the bypass of the upstream inhibition of the pathway by ATRi, CHK1i and WEE1i. FAM122A is a negative regulator of the G2/M checkpoint and forms a complex with the phosphatase PP2A. When CHK1 phosphorylates FAM122A, PP2A is disinhibited and dephosphorylates WEE1, preventing its degradation and activating the G2/M checkpoint by WEE1 (FIG. 2). Cells with low expression of FAM122A exhibit constitutional activation of the G2/M checkpoint independently of ATR–CHK1 activity, becoming more tolerant to replication stress and resistant to treatment with CHK1i. Interestingly, the addition of WEE1i to CHK1i overcomes this resistance mechanism[185,186].

As described above, MYT1 is a kinase related to WEE1, with redundant roles in the phosphorylation and inhibition of CDK1. Cells treated with WEE1i upregulate MYT1 as a compensatory mechanism, leading to reactivation of the G2/M checkpoint. Moreover, patients with breast cancer with high levels of *MYT1* mRNA have worse prognosis, underscoring that *MYT1* mRNA expression could be both a prognostic biomarker and a predictive biomarker of WEE1i response[213]. Another downstream complex in the ATR–CHK1–WEE1 pathway is the MMB–FOXM1 complex, the presence of which is required to activate premature mitosis triggered by CDK1 activity induced by CHK1 inhibition, resulting in replication catastrophe[214]. Other potential resistance biomarkers for CHK1i and WEE1i, such as p21 and YAP, are also under investigation[213,215].

Conclusions

Thus far, clinical evaluation of ATRi, CHK1i and WEE1i in early-phase clinical trials has demonstrated significant and durable monotherapy activity only in a subset of cancer patients. Monotherapy activity has been promising in very specific settings, such as WEE1i in HGSEC and *CCNE1*-amplified HGSOC, and ATRi in tumours with ATM loss and *ARID1A* mutations. Combinatorial strategies have shown considerable promise, with gemcitabine combinations with WEE1i and ATRi showing superior activity to gemcitabine alone in randomized phase II trials. The combination of gemcitabine and berzosertib is planned to undergo randomized phase III clinical trial evaluation in platinum-resistant ovarian cancer, and adavosertib monotherapy is being evaluated with a registrational intent in HGSEC in the ADAGIO trial. Significant interest exists for combination of ATRi, CHK1i or WEE1i with PARPi to overcome or prevent PARPi resistance as well as combination with radiotherapy and immune checkpoint inhibitors; a summary of ongoing clinical trials evaluating ATRi, CHK1i and WEE1i is provided in Supplementary Table 7. In addition, several new compounds are under preclinical development and/or undergoing first-in-human clinical trials, such as the ATRi M1774, RP-350 and ART0380, and the PKMYT1 inhibitor RP-6306.

Nonetheless, a major challenge for the clinical development of these agents remains toxicity, especially bone marrow toxicity. To address this, alternative dosing schedules and sequencing of administration of drug combinations are being explored and this is an active area of clinical investigation. For instance, the STAR trial is designed to test sequential therapy of WEE1i plus PARPi in an attempt to limit the toxicity of this promising combination[158].

Another challenge is that there is currently no single, clinically validated biomarker of replication stress or response to ATRi, CHK1i and WEE1i. Also, there is no accurate way to measure replication stress in cancer cells, nor is it known what threshold of baseline replication stress is necessary to confer response to these agents. *CCNE1* amplification represents a biomarker of particular interest, given its high prevalence among some types of cancer such as HGSOC and HGSEC, its homogeneity within tumour samples, its mutual exclusivity with *BRCA* mutations and its association with platinum-resistant or refractory disease. *CCNE1* amplification confers enhanced replication stress and possibly response to cell cycle checkpoint inhibitors, based on emerging, early-phase, clinical data. Other genomic alterations such as *FBXW7* and *PPP2R1A* mutations, which are prevalent and mutually exclusive in HGSEC, may also represent genomic biomarkers of response to these agents, particularly WEE1i and MYT1i. Preclinical studies and early clinical data suggest that mutation status of *BRCA1* and *BRCA2* may be associated with response to ATRi, CHK1i and WEE1i, including in the setting of PARPi resistance. This is consistent with preclinical studies showing that ATR–CHK1–WEE1 inhibition can overcome multiple mechanisms of PARPi resistance in tumours that are deficient in *BRCA* and HRR. These studies suggest that BRCA-mutated, PARPi-resistant tumours may be a particularly attractive group to consider for cell cycle checkpoint inhibition (preferably combinatorial strategies, as monotherapy seems to have low activity based on early clinical data). Furthermore, the synthetic lethality between ATM deficiency and ATR inhibition, which has been demonstrated preclinically, seems to be supported by early-phase clinical data (mostly in non-ovarian cancers), although more data are needed. Taken together, the development of biomarkers of response to ATR–CHK1–WEE1 inhibition is an evolving field with a rapidly growing body of preclinical data. Clinical data are immature as they include only small, early-phase trials with results that can only be considered hypothesis-generating at this juncture.

In conclusion, targeting replication stress has emerged as a very promising anticancer strategy over the past decade. Outstanding challenges include identification of predictive biomarkers to optimize selection of patients and addressing toxicity, which may be overcome by using alternative dosing and administration schedules based on preclinical, pharmacokinetic and pharmacodynamic data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

DNA replication fork
A structure that is formed during DNA replication by the unwinding of the DNA double helix, so that the fork has a DNA double helix downstream, towards which the fork progresses, and two single-stranded DNA strands upstream. The replisome, a multiprotein complex present at the DNA fork, is responsible for DNA unwinding and synthesizes new DNA strands.

Mitotic catastrophe
A mechanism of cell death that is the consequence of the appearance of numerous DNA breaks during DNA replication owing to obstructions of the replication fork progression that the cell is not able to overcome.

Replication origin firing
Replication origins are the genomic regions in which DNA replication starts in a two-step process. First, origin licensing occurs during G1 phase when the pre-replication complex assembles to the DNA and identifies the origin sites. Then, origin firing happens in the G1–s transition and s phase by the formation and activation of the replisome, starting DNA replication at the origin site.

Repriming
A DNA damage tolerance pathway through which the replication fork bypasses DNA damage sites. The enzyme primase–polymerase (PRIMPOL) inserts a new primer immediately after the damage site and allows polymerases to restart DNA synthesis.

DNA fibre assay
An in vitro image technique to visualize single DNA molecules and DNA replication forks. Replicating DNA is labelled with two thymidine analogues such as 5-iodo-2′-deoxyuridine (IdU) and 5-chloro-2′-deoxyuridine (CldU), cells are lysed and DNA fibre stretched on glass coverslips. Fibres are then visualized under a microscope by immunofluorescence, allowing the study of replication fork dynamics.

Enhanced response evaluation criteria in solid tumours
Response evaluation criteria in solid tumours (RECIsT) sets the criteria to define tumour response or progression to treatments. Enhanced RECIsT are modified RECIsT criteria that assess changes in the longest diameter of the lesion, allowing earlier detection of response or progression.

Decatenation

The disentanglement of chromosomes during mitosis to allow proper cell division. Sister chromatids entangle as a consequence of DNA replication, but non-replicative entanglements may also occur during interphase.

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Box 1 |

Summary of DNA repair pathways

Base excision repair (BER):

The BER pathway is responsible for the repair of single-nucleotide lesions that usually do not lead to distortion in the double helix of DNA.

Nucleotide excision repair (NER):

NER is responsible for the repair of bulky adducts that cause helix distortions, and intra- and inter-strand DNA crosslinks.

Homologous recombination repair (HRR):

HRR is a high-fidelity repair pathway that has an essential role in double-strand break repair, replication fork stabilization and recovery of stalled or collapsed replication forks during DNA replication. It is active during S and G2 cell cycle phases and uses the sister chromatid as a template to rebuilt lost sequences in double-strand breaks.

Non-homologous end-joining (NHEJ):

NHEJ is a simpler, faster and more versatile response to double-strand breaks. It detects double-strand breaks, protects them from nuclease activity that would otherwise lead to other repair pathways, binds the double-strand break ends together and processes them to seal the break.

Fanconi anaemia (FA) pathway:

The FA pathway repairs inter-strand crosslinks in the genome. FA proteins work as sensors of inter-strand crosslinks, recruit the FA core complex proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL), which activate a second FA complex (FANCI–FANCD2). Once activated, the FA complex cleaves the DNA damage site and generates two sister chromatids, one with a double-strand break that is repaired by HRR and one with an inter-strand crosslink that will be repaired by translesion DNA synthesis.

Translesion synthesis (TLS):

TLS is a DNA damage tolerance pathway. It rescues stalled forks, allowing fork progression using special error-prone polymerases that are able to synthesize DNA across a DNA damaged template.

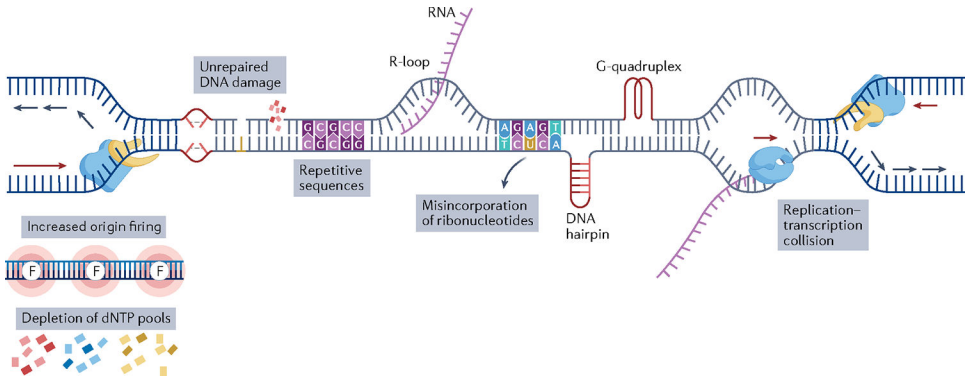

Fig. 1 |. Illustration of causes of replication stress.

A replication fork consists of an enzyme with helicase activity (shown in yellow) and an enzyme with polymerase activity (shown in blue). Replicative stress results from endogenous or exogenous obstacles to DNA replication. The red arrows indicate the direction of the continuous DNA synthesis on the leading strand and the blue arrows indicate the direction of the non-continuous DNA synthesis on the lagging strand. Oncogenic activation and some chemotherapeutic agents such as gemcitabine cause depletion of deoxynucleotides (dNTPs), which impairs the progression of ongoing DNA replication. Increased origin firing (shown by pink circles labelled with the letter F) may be caused by oncogenic activation or loss of tumour suppressor genes. Different types of DNA damage caused by endogenous and exogenous agents lead to replication stress if left unrepaired. Repetitive DNA sequence, rehybridization of the nascent RNA to DNA, forming R-loops, misincorporation of ribonucleotides and secondary DNA structures such as hairpins and quadruplexes pose a challenge to DNA replication and are endogenous causes of replication stress even in healthy cells. Finally, collisions between replication and transcription machinery result in replication stress. RNA polymerase is shown as the blue protein and the newly synthesized RNA molecule is shown in purple.

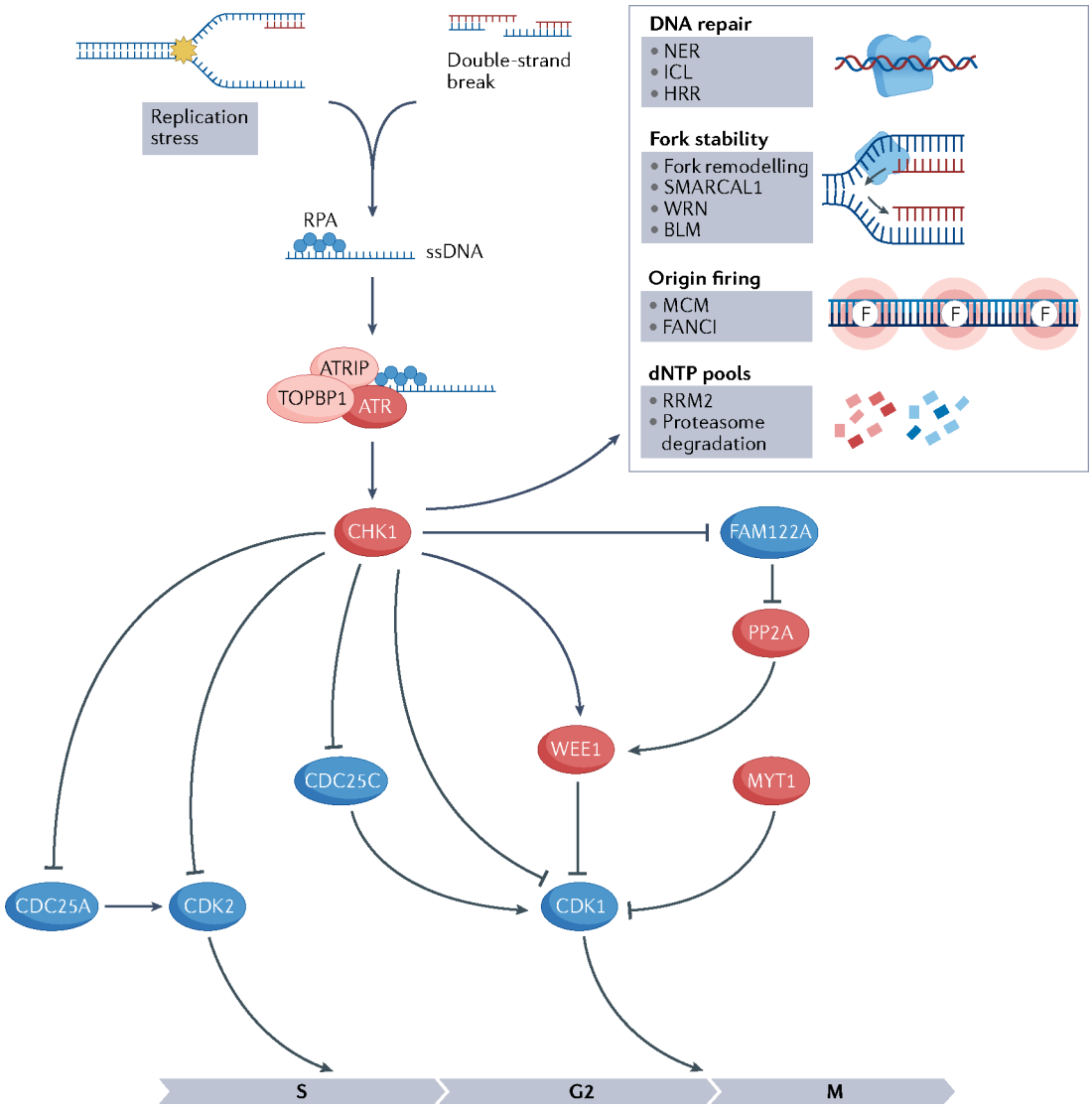

Fig. 2 |. Schematics of the ATR pathway.

Activation of ataxia telangiectasia mutated (ATM) and Rad3-related (ATR) pathway because of replication stress or double-strand breaks results in cell cycle arrest, activation of DNA repair pathways, fork stabilization, inhibition of origin firing, decrease in deoxynucleotide (dNTP) degradation and increase in dNTP synthesis. Barriers to replication fork progression (shown by the yellow star) lead to the uncoupling of helicases and polymerases, generating single-stranded DNA (ssDNA) at the stalled fork. Replication protein A (RPA) binds to ssDNA at the stalled fork and recruits ATR through the partner ATR-interacting protein (ATRIP). ATR is then activated by DNA topoisomerase 2-binding protein 1 (TOPBP1), activates CHK1 through phosphorylation and triggers the ATR–CHK1 pathway. CHK1 mediates cell cycle arrest in the S–G2 phase through phosphorylation and reduction of cyclin-dependent kinase 2 (CDK2) activation. CDK1 is negatively regulated via phosphorylation by WEE1 and MYT1 kinases and positively regulated by CDC25 phosphatases. CHK1 phosphorylates and inactivates CDC25C, activates WEE1 and promotes the degradation of CDC25A[11,13]. FAM122A is a negative regulator of the G2/M

checkpoint and forms a complex with the phosphatase PP2A. When CHK1 phosphorylates FAM122A, PP2A is disinhibited and dephosphorylates WEE1, preventing its degradation and activating the G2/M checkpoint by WEE1. Proteins whose activity leads to cell cycle arrest are shown in red and proteins whose activity leads to cell cycle progression are shown in blue. Red DNA strands indicate newly synthesized DNA. Pointed arrows indicate action on that protein (phosphorylation) and blunted arrows indicate inhibition. BLM, Bloom syndrome protein; HRR, homologous recombination repair pathway; ICL, inter-strand crosslink pathway; MCM, minichromosome maintenance; NER, nucleotide excision repair pathway; RRM2, ribonucleoside-diphosphate reductase subunit M2; WRN, Werner syndrome ATP-dependent helicase.

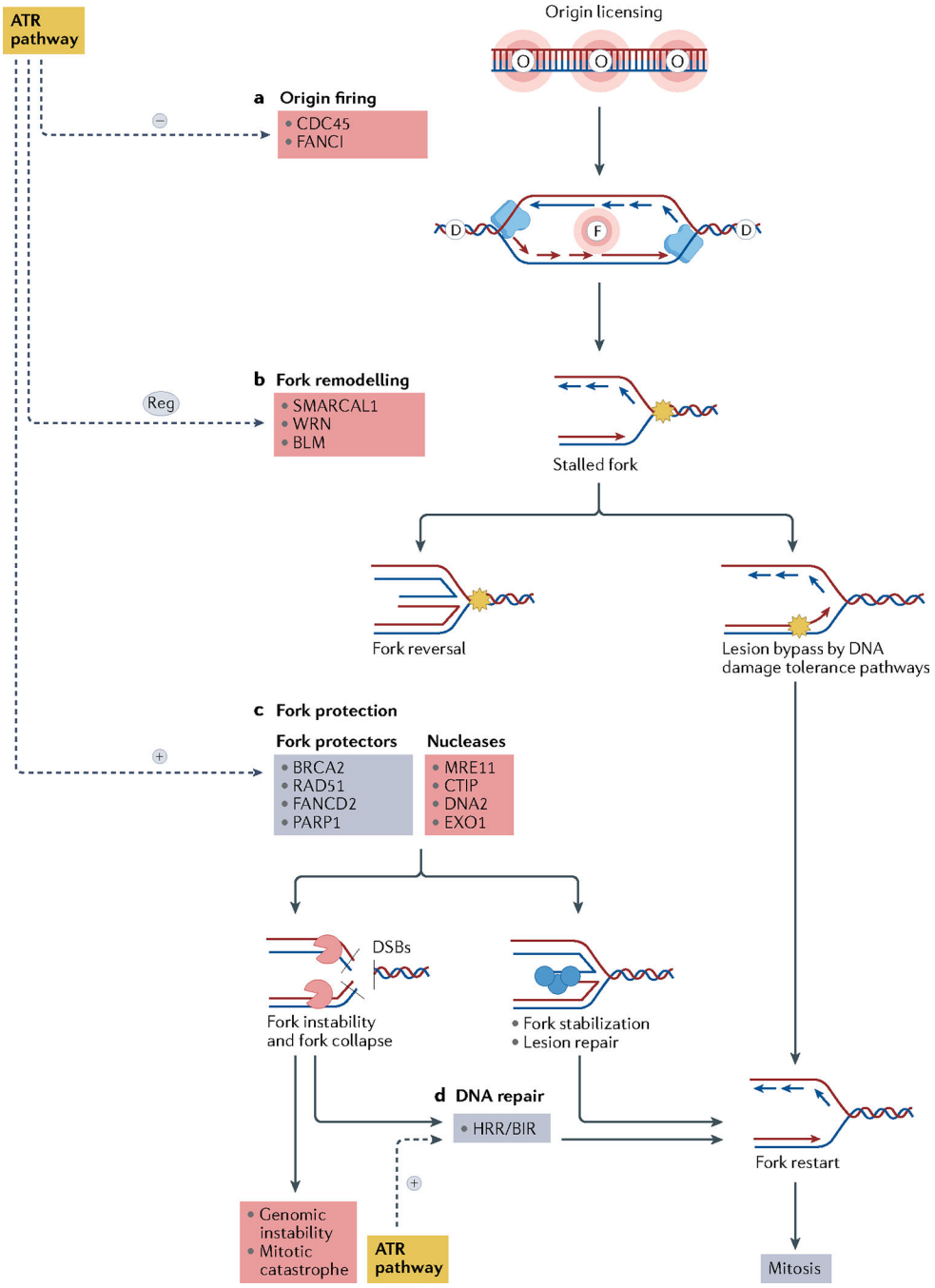

Fig. 3 |. Fork dynamics regulation by the ATR pathway.

a | The ataxia telangiectasia mutated (ATM) and Rad3-related (ATR) pathway inhibits replication fork origin firing through inhibition of the minichromosome maintenance 2–7 complex (MCM2–7) and Fanconi anaemia group I protein (FANCI). Replication origin firing is indicated by the letter F, and dormant replication origins — licensed but not activated — are indicated by the letter D. Replisome (blue), is a protein complex with helicase and polymerase activities that progresses bidirectionally, starting from the origin firing and forming the replication forks. **b** | Stalled forks owing to an obstacle to fork

progression (shown by the yellow star) may be rescued directly by DNA damage tolerance pathways or may be reversed by fork remodelling proteins. Fork reversal may result in fork protection or fork degradation depending on the balance of activity of various remodelling factors and on the DNA repair proficiency of the cell. **c** | The ATR pathway modulates fork reversal and activates fork protectors, promoting fork stabilization. Reversed forks are substrates for nucleases that cleave the DNA to form double-strand breaks (DSBs). Fork protectors such as BRCA2, RAD51 and poly[ADP-ribose]polymerase 1 (PARP1) prevent fork degradation by nucleases, allowing lesion repair and fork restart. **d** | Collapsed replication forks may be repaired by the homologous recombination repair (HRR) and the break-induced replication (BIR) pathways, allowing fork restart and normal mitosis. If DNA repair is not effective, fork collapse may lead to genomic instability and mitotic catastrophe. Mechanisms that lead to fork stabilization and restart and normal mitosis are shown in blue, mechanisms that lead to fork instability, fork collapse and genomic instability are shown in red. BLM, Bloom syndrome protein; CDC45, cell division cycle 45; WRN, Werner syndrome ATP-dependent helicase.

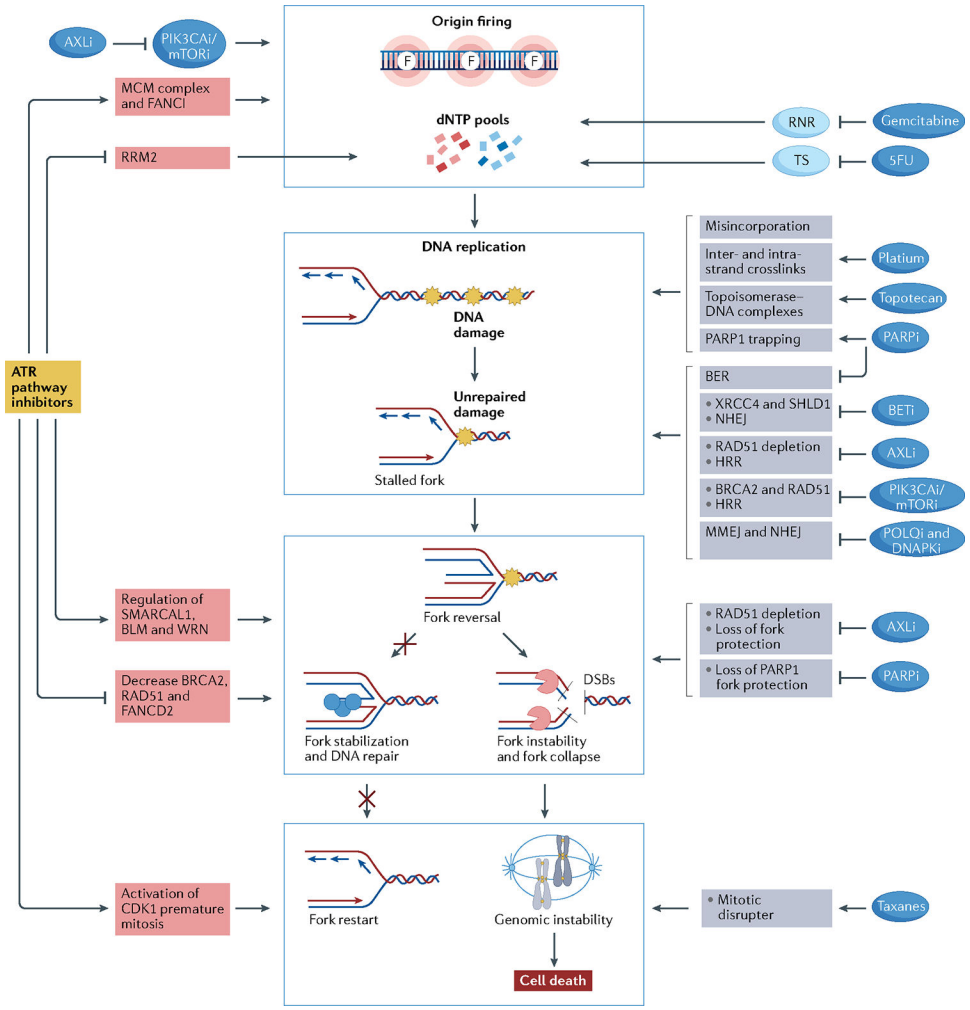

Fig. 4 |. Drug combinations with ATR–CHK1–WEE1 inhibitors.

Rationale for drug combinations that promote replication stress, potentiating the effect of ATR–CHK1–WEE1 inhibitors. Red boxes indicate mechanisms of action related to ataxia telangiectasia mutated (ATM) and Rad3-related (ATR) pathway inhibitors; grey boxes indicate mechanisms of action of the other drugs. Central blue squares group the main mechanisms through which drugs may activate replication stress. Drugs may activate replication stress by decreasing deoxynucleotide (dNTP) pools directly or through increasing origin firing (indicated by the letter F, top blue box), increasing DNA damage (second blue box), promoting fork instability (third blue box) and disrupting mitosis (bottom blue box). ATR inhibitors (ATRi), CHK1 inhibitors (CHK1i) and WEE1 inhibitors (WEE1i) decrease dNTP pools by activating origin firing through loss of Fanconi anaemia group I protein (FANCI) phosphorylation and minichromosome maintenance 2–7 complex (MCM2–7) inhibition and by decreasing ribonucleoside-diphosphate reductase subunit M2 (RRM2) expression. PIK3CA/mTOR inhibitors synergize with ATRi, CHK1i or WEE1i by promoting origin firing through increasing cell division cycle 45 (CDC45) expression, which leads to activation of the MCM2–7 complex. Moreover, AXL inhibitors (AXLi) decrease dNTP pools by inhibiting the PI3K–mTOR pathway. Nucleoside analogues reduce

intracellular dNTP pools by inhibiting the enzymes responsible for dNTP synthesis. Gemcitabine inhibits ribonucleotide reductase (RNR), and 5-fluorouracil (5FU) inhibits thymidylate synthetase (TS). Drugs may increase DNA damage by directly damaging the DNA or by inhibiting DNA repair mechanisms. Gemcitabine and 5FU lead to DNA damage by their misincorporation into DNA. Platinum salts cause inter- and intra-strand crosslinks, and topotecan generates topoisomerase–DNA complexes, which become obstacles to fork progression. Poly[ADP-ribose]polymerase (PARP) inhibitors (PARPi) create obstacles to fork progression through PARP1 trapping and inhibition of the base excision repair (BER) pathway. Bromodomain and extra-terminal domain (BET) inhibitors (BETi) lead to non-homologous end-joining (NHEJ) inhibition through downregulation of XRCC4 and SHLD1. AXL inhibition results in inhibition of homologous recombination repair (HRR) through RAD51 depletion. PIK3CA/mTOR inhibition leads to HRR inhibition through downregulation of BRCA2 and RAD51. POLQ inhibitors and DNAPK inhibitors directly inhibit microhomology-mediated end-joining (MMEJ) and NHEJ pathways, respectively. ATRi, CHK1i and WEE1i cause fork instability by regulating fork remodellers such as SMARCAL1, Bloom syndrome protein (BLM) and Werner syndrome ATP-dependent helicase (WRN) and by decreasing the activity of fork protectors such as BRCA2, RAD51 and FANCD2. AXLi and PARPi also lead to fork instability by depletion of RAD51 and inhibition of PARP1 fork protection, respectively. Finally, ATRi, CHK1i and WEE1i lead cells to premature mitosis and may synergize with mitotic disrupter agents such as taxanes.

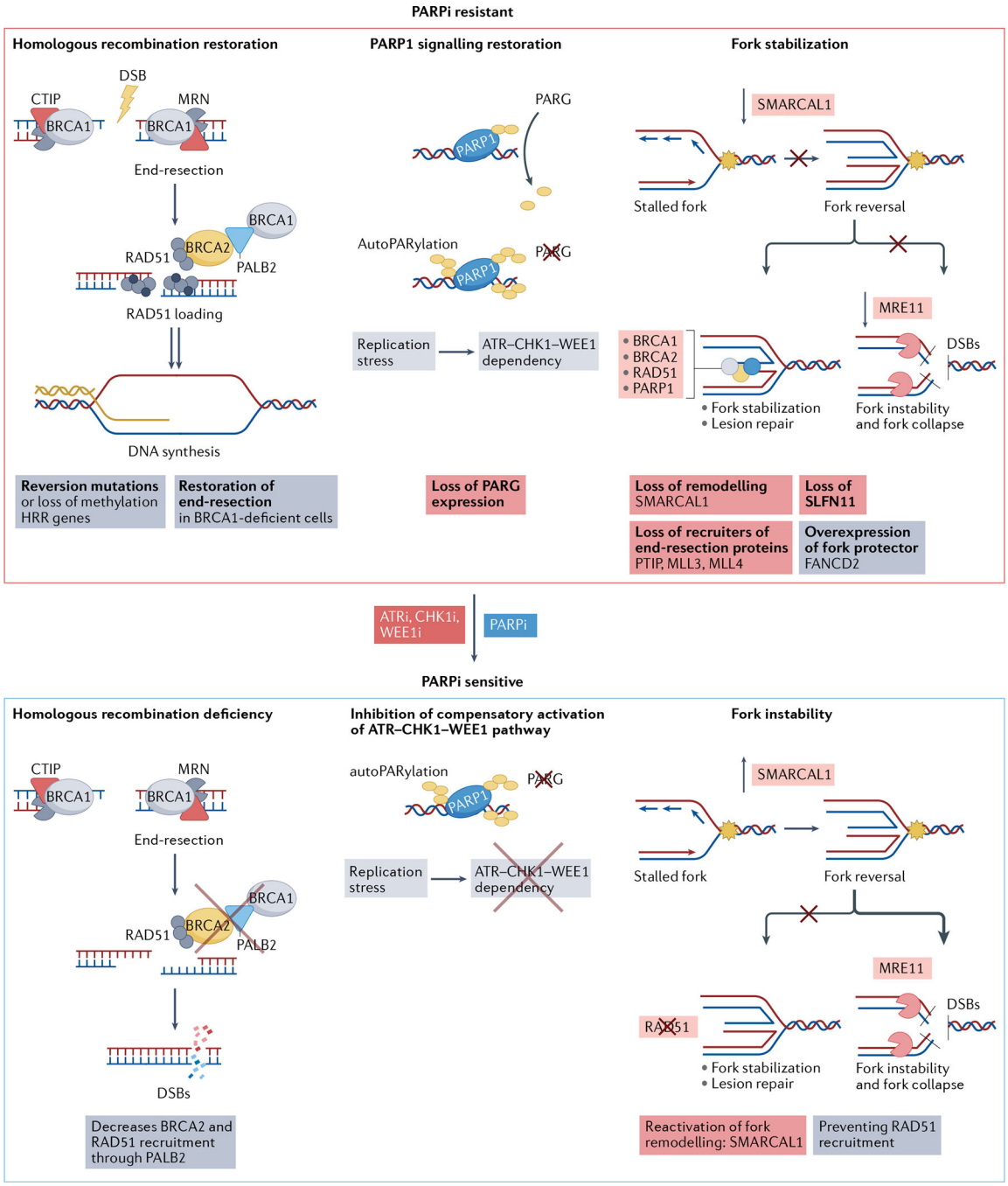

Fig. 5 |. Mechanisms of ATR–CHK1–WEE1 inhibitors to overcome PARP inhibitor resistance.

The top panel shows mechanisms of poly[ADP-ribose]polymerase (PARP) inhibitor (PARPi) resistance. The main steps of the homologous recombination repair (HRR) pathway are shown at the left: DNA end resection on double-strand breaks (DSBs), RAD51 loading, homologous strand invasion and DNA synthesis. HRR deficiency makes cells sensitive to PARPi. Reversion mutations in *BRCA1* and *BRCA2*, and RAD51 paralogues can restore HRR. CtIP and MRN are nucleases responsible for initial DNA end resection. MRN is a nuclease complex formed by MRE11-RAD50-NBS1. Loss of proteins that inhibit DNA end

resection, such as 53BP1 and shieldin complex proteins, or upregulation of proteins that promote end resection, such as TRIP13 and USP15, can restore end resection in BRCA1-deficient cells, promoting PARPi resistance. The central panel shows how poly(ADP-ribose) glycohydrolase (PARG) counteracts PARP1-mediated PARylation (shown in yellow). Accordingly, loss of expression of PARG can lead to upregulation of PAR despite PARP1 inhibition, increasing replication stress and activating the ATR–CHK1–WEE1 pathway. The top right panel shows a stalled fork undergoing fork reversal. Fork protectors (shown by the grey, yellow and blue circles) such as BRCA1, BRCA2, RAD51 and PARP1 stabilize the fork, allowing DNA repair and fork restart. In the absence of fork protectors, nucleases (shown in red) such as MRE11 can degrade the reversed forks. Accordingly, loss of fork remodelling factors such as SMARCAL1, loss of recruiters of end resection such as PTIP, MLL3 and MLL4, and overexpression of fork protectors such as Fanconi anaemia group D2 protein (FANCD2), can lead to fork stabilization and PARPi resistance. The bottom panels show how ataxia telangiectasia mutated (ATM) and Rad3-related inhibitors (ATRi), CHK1 inhibitors (CHK1i) and WEE1 inhibitors (WEE1i) can overcome PARPi resistance. ATRi, CHK1i and WEE1i can cause HRR deficiency by decreasing BRCA2 and RAD51 recruitment (bottom left panel). They can suppress activation of the ATR pathway, which may be overactivated by PARG loss (bottom centre panel), and promote fork instability by reactivating SMARCAL1 and preventing RAD51 recruitment to the reversed fork (bottom right panel). SLFN11, Schlafen family member 11.

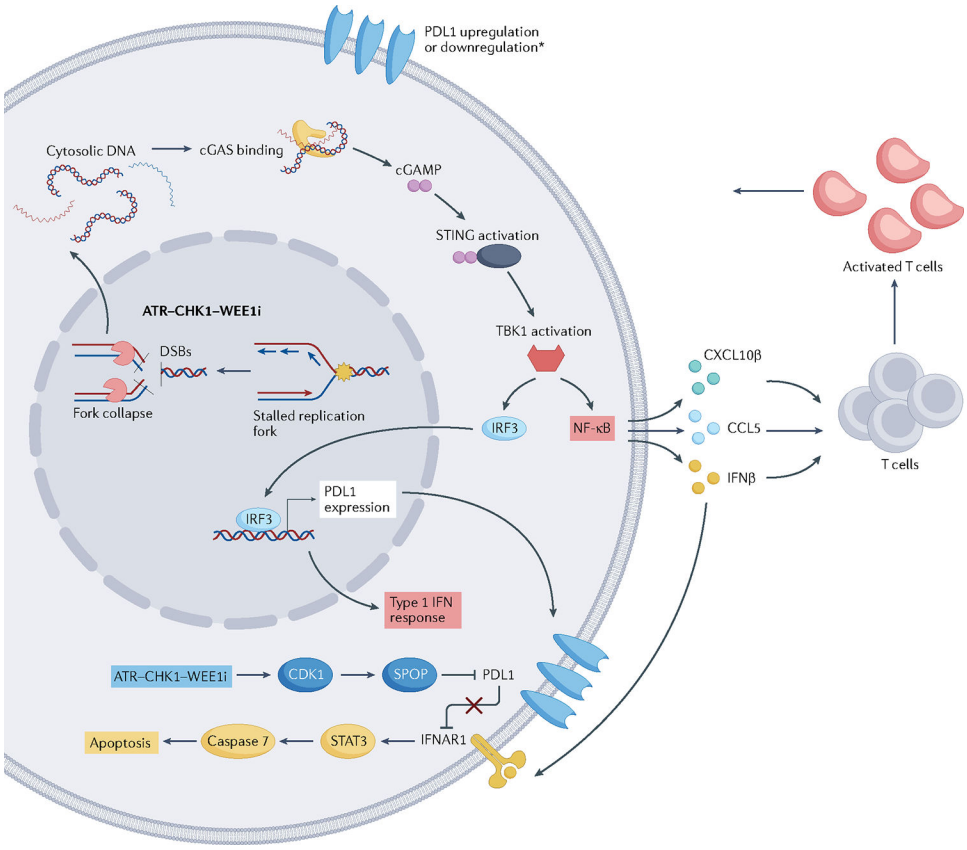

Fig. 6 |. Mechanism of immune response sensitization.

Replication stress can elicit an innate immune response by releasing DNA particles into the cytoplasm, activating the cGAS–stimulator of interferon genes (STING) pathway, and upregulating the type I interferon-γ (IFNγ) response and, subsequently, the nuclear factor-κB (NF-κB) pathway. Besides this pro-inflammatory signal, cGAS–STING can also upregulate PDL1 expression. In addition, the ATR–CHK1 pathway can also lead to PDL1 protein degradation by activating the CDK1–speckle-type POZ protein (SPOP) pathway. Downregulation of PDL1 can inhibit an IFNβ-mediated pro-apoptotic pathway, resulting in autocrine cytotoxic signalling. IFNAR1, interferon-α/β receptor 1. *The resulting effect of ATR-CHK1-WEE1 inhibition on PD-L1 is disputable with studies showing both upregulation or downregulation upon blockage of the ATR-CHK1-WEE1 pathway.

Table 1 |

Selected ongoing clinical trials with ATR, CHK1 and WEE1 inhibitors according to strategy

Design	Target	Agents	Indication	Status	Study Identifier
Biomarker driven monotherapy trials					
Phase II	CHK1	Prexasertib	Advanced solid tumours with replication stress or homologous recombination deficiency	Active, not recruiting	NCT02873975
Phase II	WEE1	Adavosertib	Advanced refractory solid tumours	Suspended	NCT03253679
Combination with chemotherapy					
Phase I	ATR	Gemcitabine, carboplatin and berzosertib	Platinum-sensitive ovarian cancer	Recruiting	NCT02627443
Phase I	ATR	Gemcitabine and BAY1895344	Incurable solid tumours; expansion cohort platinum-resistant ovarian cancer	Not yet recruiting	NCT04616534
Phase I	WEE1	Gemcitabine, nab-paclitaxel and adavosertib	Unresectable pancreatic adenocarcinoma	Active, not recruiting	NCT02194829
PARPi synergism					
Phase II	ATR	Ceralasertib and olaparib	Recurrent HGSOC irrespective of platinum sensitivity	Recruiting	NCT03462342 (CAPRI trial)
Phase II	ATR	Ceralasertib, olaparib	Recurrent gynaecological cancers, including CCC and rare gynaecological cancer subtypes with frequent *ARID1A* loss	Recruiting	NCT04065269 (ATARI trial)
Phase II	ATR; WEE1	Olaparib alone; olaparib and capivasertib; olaparib and ceralasertib; olaparib and adavosertib	Advanced solid tumours with positive predictive biomarkers	Not yet recruiting	NCT02576444 (OLPACO trial)
Randomized phase II	ATR	Ceralasertib, olaparib; placebo, olaparib	HGSOC relapsed after at least 6 months maintenance with PARPi	Recruiting	NCT04239014 (DUETTE trial)
Randomized phase II	ATR; WEE1	Olaparib; ceralasertib and olaparib; adavosertib and olaparib	Metastatic triple-negative breast cancer	Active, not recruiting	NCT03330847 (VIOLETTE trial)
Phase I	ATR	BAY1895344 and niraparib	Advanced solid tumours and ovarian cancer; two expansion ovarian cancer cohorts: PARPi naive, PARPi resistant	Recruiting	NCT04267939
Phase I	WEE1	Olaparib, adavosertib	Advanced refractory solid tumour with mutations in DDR genes	Recruiting	NCT04197713 (STAR trial)
Phase I/II	ATR	Ceralasertib; ceralasertib and carboplatin; ceralasertib and olaparib; ceralasertib and durvalumab	Head and neck squamous cell carcinoma, non-small-cell lung carcinoma, gastric, breast and ovarian cancer	Recruiting	NCT02264678
Overcoming PARPi resistance					
Phase I/II	ATR	Olaparib and ceralasertib	Platinum-sensitive ovarian cancer after PARPi progression	Recruiting	NCT02264678
Phase I	ATR	Niraparib once daily plus M4344 100–200 mg daily	Patients with ovarian cancer with disease progression while on PARPi	Not yet recruiting	NCT04149145

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Design	Target	Agents	Indication	Status	Study Identifier
Phase I	CHK1	Olaparib and prexasertib	Advanced solid tumours with prior PARPi treatment	Active, not recruiting	NCT03057145
Phase II	WEE1	Adavosertib; adavosertib and olaparib	Patients with ovarian cancer with disease progression while on PARPi	Recruiting	NCT03579316
Immunotherapy combinations					
Phase I/II	ATR	Berzosertib and avelumab	Advanced solid tumours	Recruiting	NCT04266912
Phase I	ATR	BAY1895344 and pembrolizumab	Advanced solid tumours	Recruiting	NCT04095273

ATR, ataxia telangiectasia mutated (ATM)- and Rad3-related; CCC, clear cell cancer; DDR, DNA damage response; HGSOC, high-grade serous ovarian cancer; PARPi, poly[ADP-ribose]polymerase 1 inhibitor.