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Targeting the DDR beyond PARP inhibitors: Novel agents and rational combinations

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Abstract

Purpose of review: Poly(ADP-ribose) polymerase (PARP) inhibitors have transformed treatment paradigms in multiple cancer types defined by homologous recombination deficiency (HRD) and have become the archetypal example of synthetic lethal targeting within the DNA damage response (DDR). Despite this success, primary and acquired resistance to PARP inhibition inevitability threaten the efficacy and durability of response to these drugs. Beyond PARP inhibitors, recent advances in large-scale functional genomic screens have led to the identification of a steadily growing list of genetic dependencies across the DDR landscape. This has led to a wide array of novel synthetic lethal targets and corresponding inhibitors, which hold promise to widen the application of DDR inhibitors beyond HRD and potentially address PARP inhibitor resistance.

Recent findings: In this review, we describe key synthetic lethal interactions that have been identified across the DDR landscape, summarize the early phase clinical development of the most promising DDR inhibitors, and highlight relevant combinations of DDR inhibitors with chemo- and other novel cancer therapies, which are anticipated to make an impact in rationally selected patient populations.

Summary: The DDR landscape holds multiple opportunities for synthetic lethal targeting with multiple novel DDR inhibitors being evaluated on early phase clinical trials. Key challenges remain in optimizing the therapeutic window of ATR and WEE1 inhibitors as monotherapy and in combination approaches.

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Keywords

DNA damage response; targeted therapy; synthetic lethality; ATR; WEE1

Introduction: synthetic lethal interactions across the DDR landscape beyond PARP inhibitors

In the presence of DNA damage, different members of the DNA damage response (DDR) network are activated according to the type of DNA lesion sensed, triggering a downstream cascade of signaling pathways which variably induce cell-cycle arrest, regulate replication fork progression and/or coordinate DNA repair [1, 2]. To this end, the DDR plays a key role in safeguarding the fidelity of DNA replication and in preserving cellular genomic stability [3]. Inherited or acquired deficiencies in the components of the DDR are associated with an increased propensity toward the accumulation of DNA damage and resultant genomic instability, provoking cancer growth and progression [4]. Paradoxically, cancer cells harboring DDR deficiencies develop a dependency on remaining compensatory DDR nodes and pathways, which in turn lends itself as an exploitable vulnerability for synthetic lethal targeting [4, 5].

To date, the success of this strategy is best exemplified by poly(ADP-ribose) polymerase (PARP) inhibitors [6], which have steadily shifted paradigms in the treatment of breast [7–9], ovarian [10–12], prostate [13] and pancreatic [14] cancers harboring deleterious *BRCA1/2* and/or other alterations. Despite this success, primary and acquired resistance to PARP inhibition invariably limit the efficacy and durability of response achieved with these agents [15, 16]. Novel therapies with potential to deepen patient responses, increase durability of response and to widen the application of DDR inhibitors beyond tumors harboring homologous recombination deficiency (HRD) are required. In recent years, the emergence of functional chemogenetic screening efforts aimed at interrogating genetic dependencies through large-scale cancer cell line profiling, have allowed new genetic essentialities conferred by specific genomic alterations to be uncovered. In particular, clustered regularly interspaced short palindromic repeats (CRISPR)-based tools have accelerated the speed of synthetic lethal target discovery across the DDR landscape [17–19]. Growing preclinical and clinical rationale also support DDR targeting as a strategy to overcome PARP inhibitor resistance [20–22], strengthening the interest in this fast-expanding field. Clinically relevant DDR inhibitors in early phase development include small molecule inhibitors targeting ataxia telangiectasia and rad3 related (ATR), wee-like kinase 1 (WEE1), ataxia telangiectasia mutated (ATM), DNA-dependent protein kinase (DNA-PK) and RAD51 (Table 1), with drugs inhibiting membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase (MYT1), polymerase theta (POLQ) and ubiquitin carboxyl-terminal hydrolase 1 (USP1) recently entering phase I trials (Table 2).

Targeting ATR in the clinic

ATR is an apical signaling kinase and master regulator of single-stranded DNA break (ssDNA) signaling and the replication stress response within the DDR [23]. By activating a

cascade of downstream signaling involving checkpoint kinase 1 (CHK1), cell division cycle 25 (CDC25A/C), WEE1 and others [24, 25], the ATR signaling axis serves to interrupt cell cycle progression, suppress global replication origin firing, and promote replication fork slowing in order allow DNA damage resolution and to prevent cells harboring DNA damage from entering premature mitosis [26, 27].

Several ATR inhibitors are in development (Table 1) and are poised to address important areas of clinical unmet need [28]. Firstly, ATR inhibitors have been proposed as a strategy to target PARP inhibitor-resistant disease [20]. Mitigation of replication stress by replication fork protection has been described as a mechanism of PARP inhibitor resistance and is targetable through ATR inhibition [29]. Amongst reported clinical trials, anecdotal examples of response to ATR inhibitors have been described in patients harboring PARP inhibitor-resistant, *BRCA1/2* mutated cancer [30–32]. For example, early analysis of data from the phase I trial of the orally available ATR inhibitor RP-3500 (Repare Therapeutics) has described clinical responses across a variety of tumors harboring homologous recombination deficiency, but interestingly included several ovarian cancer patients harboring deleterious mutations in *BRCA1* and/or *RAD51C* who were previously resistant to PARP inhibitor treatment [33].

Somewhat analogous to the efficacy of PARP inhibitors in *BRCA1/2* mutated tumors, ATR inhibitors have achieved an early signal of efficacy in ATM-deficient tumors [32]. ATM is a DDR kinase that primarily senses and mediates repair of double stranded DNA breaks (dsDNA), and its loss is hypothesized to increase tumor dependency on the ATR-CHK1 axis [34–36]. Preclinical and clinical studies have supported ATM deficiency as a potential biomarker of sensitivity to ATR inhibition [32, 34, 36, 37]. In a first-in-human phase I trial of the oral ATR inhibitor elimusertib (BAY1895344, Bayer), four of 21 enrolled patients (19%) experienced partial and durable responses, with a median duration of response of 315.5 days [32]. Importantly, all four responders were found to have ATM protein loss and/or deleterious mutations in *ATM*. Expansion cohorts of evaluating *ATM* mutation status as a selective biomarker for ATR inhibition are currently ongoing and will inform the viability of this strategy [32]. Such efficacy will need to be balanced against the potential for mechanism-based toxicity, especially anemia, which was the main dose limiting toxicity on this trial.

Beyond ATM deficiency as a predictive biomarker for ATR inhibitors, Williamson and colleagues have previously identified defects in *ARID1A* as a synthetic lethal partner for ATR inhibition through RNA interference screens [38]. Across a variety of *ARID1A*-mutated models, increased dependency on ATR-mediated cell cycle checkpoints and susceptibility to ATR inhibition was observed [38]. On a prior phase I study of berzosertib (M6620, Merck Serono) monotherapy, complete response was observed in a patient with advanced colorectal cancer harboring *ARID1A* mutation and IHC loss, as well as ATM IHC loss [30]. More recently, interim results from a phase II trial of ceralasertib (AZD6738, AstraZeneca) in *ARID1A*-deficient solid tumors (defined absence of tumor expression of its gene product BAF250a by immunohistochemical (IHC) staining), were reported. Amongst a cohort of 10 participants, the objective response rate (ORR) was 20%, with two complete responses in patients with clear cell endometrial cancer who experienced

durable ongoing response (21.3+ and 16.3+ months) at data cut-off [39]. A further ongoing phase II trial is evaluating ceralasertib monotherapy or in combination with olaparib in patients with relapsed gynecological cancers harboring *ARID1A* deficiency, including a cohort specifically recruiting patients with ovarian or endometrial clear cell cancer (ATARI, NCT04065269). Further data from these studies may help answer key questions that remain in the optimization of *ARID1A* genomic status as a selective biomarker for ATR inhibition, including: the optimal testing modality for *ARID1A*-deficient status (IHC versus deleterious mutation and its zygosity), the optimal tumor type for development of this strategy and further combination therapies that may enhance the efficacy of ATR inhibitors in *ARID1A*-deficient tumors.

Deficiencies in homologous recombination DNA repair (HRR) are also potential biomarkers for ATR inhibition [26, 40]. Several ongoing clinical trials are actively recruiting patients harboring alterations in HRR genes. In a phase I trial of RP-3500 monotherapy, 17 biomarkers which were previously identified on a CRISPR technology-based genome-wide screening platform were investigated as selective biomarkers for ATR inhibitor sensitivity. An early analysis of antitumor activity from this trial demonstrated promising clinical activity across a spectrum of genetic alterations, with responses observed in *BRCA1*-mutated breast cancer, head and neck squamous cell carcinoma, ovarian cancer and *BRCA2*-mutated melanoma patients [33]. Further correlatives including the allele-specific copy number, zygosity and germline versus somatic status of these genomic alterations are important data that are still pending from this and other studies, which may further refine these biomarkers. Other intriguing potential biomarkers with preclinical rationale for ATR inhibition are summarized in Table 3.

Targeting WEE1 in the clinic

WEE1 regulates cyclin dependent kinase (CDK) 1 and 2 which modulate cell cycle progression through the S and G2/M checkpoints. WEE1 inhibitors thus abrogate G2/M cell cycle arrest, promoting premature mitotic entry and mitotic catastrophe [41, 42]. At present, several WEE1 inhibitors are in developmental pipelines (Table 1). Adavosertib (AZD1775, AstraZeneca), the first-in-class WEE1 inhibitor, has shown preliminary efficacy in uterine serous carcinoma (USC), a rare and aggressive cancer characterized by ubiquitous *TP53* mutations and frequent *CCNE1* amplification [43]. This rationale formed the basis of a single arm phase II clinical trial of adavosertib monotherapy in recurrent USC patients, which reported an ORR of 29.4%, median progression-free survival (PFS) of 6.1 months and duration of response of 9.0 months [44]. In another phase II trial of newly diagnosed *TP53* and *KRAS*-mutated advanced colorectal cancer, patients who achieved stable disease or partial response after 16 weeks of first-line chemotherapy were randomized to adavosertib versus active monitoring. The trial met its primary endpoint, demonstrating improvements in PFS for adavosertib over active monitoring (median 3.61 versus 1.87 months, hazard ratio (HR)= 0.35, 95% confidence interval (CI) 0.18–0.68) [45].

CCNE1 amplification has emerged as a putative biomarker for WEE1 inhibitor activity [46]. *CCNE1* encodes cyclin E which interacts with CDK2 to phosphorylate retinoblastoma (Rb) shortly prior to S-phase entry, activating E2 factor (E2F) transcription factors and

cell cycle-related genes [47]. Amplification of *CCNE1* thus promotes dysregulated and early S-phase entry, leading to the accumulation of genomic instability [48, 49]. While the CDK2-Cyclin E node itself has remained undruggable due to challenges in achieving target selectivity over other CDK and cyclin E isoforms, abrogation of the G2/M checkpoint in the context of *CCNE1* amplification provides a therapeutic opportunity by provoking mitotic catastrophe [50]. In a phase I dose-escalation trial of adavosertib monotherapy in advanced cancers, correlative biomarker analyses by RNA sequencing and whole-exome sequencing of paired tumor biopsies revealed *CCNE1* overexpression and/or copy number alteration to be a potential biomarker of treatment response [51]. In a phase II single-arm trial evaluated adavosertib monotherapy in *CCNE1*-amplified advanced refractory cancer patients, the ORR was 25.9% (95% CI 15.1–47.5%), median PFS was four months and one-year overall survival was 55% [52]. Other potential biomarkers that may predict for response with WEE1 inhibition are described in Table 3.

Development of rational PARP and ATR inhibitor combinations

PARP inhibitors trap PARP1 and 2 on DNA strands [53], generating DNA adducts and stalled replication forks which require ATR for resolution. Therefore, combined PARP and ATR inhibition holds potential to further increase replication stress, DNA damage, and provoke cell death. Preclinical data have also described a marked increase in sensitivity of PARP inhibitor-resistant cells to combined PARP and ATR inhibition in ovarian cancer patient-derived xenograft (PDX) models [21]. Yet, the overlapping toxicities of combined ATR and PARP inhibition remain a concern. In a dose-finding phase I trial of ceralasertib plus olaparib, thrombocytopenia and neutropenia were dose limiting toxicities (DLTs), which restricted the continuous dosing of ceralasertib beyond the first 7 days when given with the full monotherapy dose of olaparib [54]. Despite this, in a small phase II trial of this combination in platinum-sensitive but PARP inhibitor-resistant ovarian cancer, an ORR of 46% and PFS of 7.5 months (95% CI, 4.7- not reached) were reported. The ORR was 69%, 23% and 8% amongst patients with germline *BRCA* mutations, somatic *BRCA* mutations and HRD positivity, respectively [55].

Combining PARP and WEE1 inhibitors

The combination of olaparib and adavosertib has previously demonstrated synergy in several tumor models including PARP inhibitor-resistant ovarian cancer xenografts [56–60]. In a phase II trial of PARP inhibitor-resistant ovarian cancer patients, the cohort treated with adavosertib plus olaparib demonstrated an ORR of 29%, clinical benefit rate (CBR) of 89% and median PFS of 6.8 months (90% CI 4.3–8.3). Amongst patients treated with adavosertib monotherapy, the ORR was 23% and CBR was 63%. However, grade 3–4 toxicities occurred in 76% of patients treated with adavosertib plus olaparib, including prominent myelosuppression, nausea and fatigue, which led to dose interruptions in 88%, dose reductions in 71% and treatment discontinuation in 10% of patients [61].

Thus, for combinations of PARP inhibitors with ATR or WEE1 inhibitors, establishing a sufficiently wide therapeutic window which maintains tolerability while preserving treatment efficacy, remains a major challenge to their successful development. Sequential

dosing of DDR inhibitors may be a rational strategy to improve upon patient tolerability while maintaining concomitant combination efficacy. This has been feasible in ovarian cancer PDX models, where sequential dosing of PARP inhibitor monotherapy followed by adavosertib monotherapy on a weekly schedule led to markedly improved tolerability compared with concurrent dosing of both drugs but maintained efficacy that mirrored concurrent therapy in tumor cells harboring a high basal level of replication stress [60]. A phase I trial is currently investigating sequential olaparib-adavosertib scheduling in PARP inhibitor-resistant patients harboring defects in known DDR genes (STAR, NCT04197713). As newer and more selective WEE1 inhibitors progress in developmental pipelines, including potential best-in-class candidates (Table 1), it is also hoped that these may improve upon the therapeutic window of this class and advance the development of combination approaches.

Combining ATR or WEE1 inhibitors with chemotherapy

Different cytotoxic chemotherapies including platinum salts [62, 63], gemcitabine [64–68], topoisomerase inhibitors [69] and taxanes [70] have been evaluated in combination with ATR or WEE1 inhibitors in preclinical and clinical settings [71]. Thus far, ATR/WEE1 combinations with non-platinum chemotherapy classes appear to be better tolerated, some with promising efficacy, including combinations of gemcitabine with adavosertib or berzosertib [65–67], paclitaxel plus ceralasertib [70] and topotecan plus berzosertib [69].

Combining DDR inhibitors with antibody-drug conjugates

Combining DDR inhibitors with antibody-drug conjugates (ADCs) is a feasible strategy due to fewer overlapping toxicities between these classes. Pyrrolobenzodiazepine dimers (PBD) are alkylators that form intra-strand cross-links within the DNA minor groove, leading to dsDNA breaks, and are a potent ADC payload [72]. A preclinical study has described promising anti-tumor activity in *BRCA* deficient tumor xenografts when treated with MEDI0641 [5T4 oncofetal antigen (5T4)-PBD, AstraZeneca], compared with *BRCA*-wildtype models. MEDI0641 was also successfully combined with olaparib *in vivo*, without associated myelotoxicity, suggesting an acceptable therapeutic window [73]. In triple negative breast cancer (TNBC) models, combinations of the trophoblast cell surface antigen 2 (Trop-2) targeting ADC, sacituzumab govitecan (IMMU-132, Gilead Sciences), with olaparib, rucaparib or talazoparib led to synergistic growth inhibition, increased dsDNA breaks and accumulation of cells in S-phase, regardless of *BRCA1/2* status [74]. In *BRCA1/2*-wildtype mouse models, the combination of sacituzumab govitecan plus olaparib led to significant tumor regression compared with each monotherapy and was well tolerated with no significant myelosuppression observed [74]. An ongoing trial is investigating the novel PARP1-selective inhibitor, AZD5305 (AstraZeneca), in combination with the human epidermal growth factor receptor 2 (HER2)-targeting ADC, trastuzumab deruxtecan (T-Dxd, AstraZeneca), or the Trop2-targeting ADC, datopotamab deruxtecan (Dato-Dxd, AstraZeneca), in advanced cancer patients harboring selected HRR gene alterations (PETRA, NCT04644068).

Combinations of ADCs with other DDR inhibitors are also being explored. In multiple myeloma models, the novel B-cell maturation antigen ADC MEDI2228 (AstraZeneca) which delivers a PBD payload, was demonstrated to activate the ATR signaling axis prior to cell apoptosis. When combined with DDR inhibitors targeting ATR or WEE1, significant synergy occurred leading to increased cell death in previously refractory myeloma cells [75]. A further study has described downregulation of schlafen family member 11 (SLFN11) as a mechanism of PBD ADC resistance [76]. The combination of ceralasertib with PBD-based ADCs was able to reverse PBD ADC resistance in previously-resistant breast cancer cells [76], providing further rationale for future combinations of these drug classes.

Combining ATR or WEE1 inhibitors with immunotherapy

Inextricable links between innate immunity with the DDR pathway [77] have driven interest in the combined targeting of DDR and programmed death (ligand) 1 (PD(L)1) checkpoints. We have previously summarized the rationale and preclinical data surrounding the co-targeting of ATR and PD(L)1 [78]. Further early phase clinical trial data of ATR-PD(L)1 combination therapy has recently been reported. In a phase II trial of anti-PD1 refractory-melanoma patients, ORR and disease control rate to the durvalumab plus ceralasertib combination was 31% and 63.3%, respectively, with a median PFS of 7.1 months (95% CI, 3.6–10.6 months). Notably, grade three or higher treatment-emergent adverse events occurred in 53.3% of patients, of which myelosuppression was most common (anemia (33.3%), thrombocytopenia (16.7%), neutropenia (16.7%)), which improved following drug interruptions and intermittent transfusions as required [79]. Ongoing trials are combining ATR, WEE1 and other DDR inhibitors with immune checkpoint blockade (Table 4). Translational analyses from these studies will be instrumental to guide our understanding of factors predisposing to treatment response. A big advantage of such a combination is the general lack of overlapping drug-related toxicities, potentially enabling full monotherapy doses of both drugs to be administered concurrently.

The New Kids on the Block: MYT1, POLQ and USP1

Similar to WEE1, MYT1 is a cytoplasmic membrane-associated serine/threonine protein kinase which negatively regulates CDK1. Loss of MYT1 leads to dysregulation of the G2/M checkpoint, allowing premature mitotic entry, ultimately triggering apoptosis or mitotic catastrophe [80]. Despite their seemingly redundant functions in regulating CDK1, several key differences exist between MYT1 and WEE1, including the dual activity of MYT1 for phosphorylating CDK1 at Thr14 and Tyr15 [81] and its ability to sequester CDK1 in the cytoplasm owing to its extranuclear localization [82]. Loss of MYT1 function has been proposed to be synthetic lethal with *CCNE1* amplification [83, 84] or FBXW7 loss [84]. FBXW7 targets cyclin E for ubiquitin-dependent proteolysis, and its loss is anticipated to increase cyclin E dosage in cells [85, 86]. This hypothesis has formed the basis for a phase I trial of the orally available and selective MYT1 inhibitor, RP6306 (Repare Therapeutics), in patients harboring these genomic aberrations (MYTHIC, NCT04855656).

PolQ is a widely conserved DNA polymerase and central mediator of the error-prone microhomology-mediated end joining (MMEJ) pathway [87]. PolQ also interacts with

PARP1 in DDR pathways, as PARP1 facilitates the recruitment of PolQ to dsDNA breaks [88]. Several studies have reported on synthetic lethal interactions between HRD and PolQ targeting [88, 89], due to the increased dependency of HRD cells on compensatory low-fidelity MMEJ for DDR [89]. Knockdown of PolQ in ovarian cancer cells harboring HRD led to increase cell death compared with cells proficient in HRR [89]. In another study, depletion of PolQ in mouse embryonic fibroblast cells lacking *BRCA1/2* was associated with a 4-fold increase in genomic instability reflected by chromatid and chromosome breaks, ultimately compromising cell survival [88]. ART558 (Artios Pharma) is a low molecular weight, potent and selective allosteric inhibitor of PolQ which effectively inhibits PolQ mediated MMEJ and was not only able to effect selective synthetic lethality in *BRCA1/2*-mutated models, sparing wild-type cells, but also displayed some therapeutic activity in *BRCA2*-mutated pancreatic cancer cells harboring reversion mutations that restored the native open-reading frame of BRCA2 [90]. Interestingly, siRNA targeting components of the 'Shieldin' complex, namely REV7 or SHLD2, greatly enhanced ART558 sensitivity in *BRCA1*−/− cells. Defects in the 53BP1/Shieldin DNA repair complex have been described as a source of PARP inhibitor resistance in *BRCA*-mutated cancers, suggesting that PolQ targeting holds potential to overcome some forms of PARP inhibitor resistance [90]. On another preclinical study, high-throughput screens for compounds that reduced PolQ ATPase activity led to the discovery of the antibiotic novobiocin as a potent and selective PolQ inhibitor that selectively reduced survival of *BRCA1/2* deficient cells compared to their isogenic wildtype counterparts [91]. Novobiocin was also able to potentiate the effect of PARP inhibitors in HRD ovarian cancer models, *in vitro* and *in vivo*, and led to synergistic growth inhibition in combination with olaparib in a PDX model of acquired PARP inhibitor resistance, established from a patient's tumor harboring germline *BRCA1* mutation and biallelic *TP53BP1* mutations [91]. Early phase trials of PolQ inhibitors are ongoing (Table 2) and clinical data from these studies are eagerly anticipated.

USP1 is a key enzyme that regulates the Fanconi anemia (FA) complex and translesion synthesis (TLS) through its de-ubiquitination of key FA and TLS substrates [92]. USP1 has also been reported to contribute to dsDNA break repair via HR through suppression of non-homologous end joining (NHEJ) [93]. Through a CRISPR screening platform, USP1 emerged as a potential cancer-selective target, leading to further interest in its inhibition [94]. KSQ-4279 (KSQ Therapeutics) is a potent, first-in-class USP1 inhibitor which has displayed selective inhibitory activity cells characterized by the presence of HRD, including mutations in *BRCA1/*2. In *BRCA1/2*-mutated breast cancer cells, KSQ-4279 induced S-phase cell cycle arrest, marked accumulation of gamma-H2A histone family member X (ϒH2AX) and increased apoptosis [94]. As monotherapy and in combination with olaparib, KSQ-4279 showed dose-dependent, robust and durable anti-tumor regression in multiple TNBC and ovarian cancer PDXs with varied genomic status including *BRCA1*-mutated and -wildtype models. Synergistic effects were also observed when KSQ-4279 was combined with olaparib, *in vitro* and *in vivo*, with no evidence of dose-limiting hematologic toxicities *in vivo*. Furthermore, CRISPR screens using KSQ-4279, cisplatin and olaparib revealed a distinct and non-overlapping resistance profile of KSQ-4279 to olaparib, supporting the combination of these agents [94]. A phase I trial of KSQ-4279 as monotherapy is currently

enrolling, with separate cohorts of combination therapy with PARP inhibitors or platinum-based chemotherapy planned (NCT05240898).

Conclusion

Numerous interactions for synthetic lethal targeting have been identified across the DDR landscape, beyond PARP inhibitors. Importantly, these herald opportunities to tackle PARP inhibitor resistance and to expand synthetic lethal DDR targeting beyond HRD. As we have learnt from our initial clinical experience with DDR inhibitors, important challenges remain in terms of the optimization of the therapeutic window of these agents, as well as the refining of patient selection through the identification of robust predictive biomarkers.

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Key points

- The DDR landscape hold multiple opportunities for synthetic lethal targeting.
- Key DDR inhibitors in early phase clinical trials and their target patient populations are summarized here.
- Optimizing the therapeutic window of ATR and WEE1 inhibitors has been a key challenge in the development of combination approaches with these agents.

Table 1:

Inhibitors of ATR, WEE1, ATM, DNA-PK and RAD51 currently in development

Drug name	Company	Phase	Route
ATR inhibitors			
VX-970/M6620/Berzosertib	EMD Serono	II	IV
AZD6738/Ceralasertib	AstraZeneca	II	PO
BAY1895344/Elimusertib	Bayer	I/II	PO
RP-3500	Repare Therapeutics	I	PO
ART0380	Artios Pharma	I	PO
M1774	EMD Serono	I	PO
ATRN 119	Atrin Pharmacueticals	Preclinical/I	PO
WEE1 inhibitors			
AZD1775/MK-1775/Adavosertib	AstraZeneca	II	PO
Debio0123	Debiopharm	I	PO
Zn-c3	Zentalis Pharmaceuticals	I/II	PO
DNA-PK inhibitors			
M3814/Nedisertib/Peposertib	EMD Serono	I/II	PO
AZD7648	AstraZeneca	I/II	PO
CC-115	Celegene Corporation	I	PO
ATM inhibitors			
AZD0156	AstraZeneca	I	PO
AZD1390	AstraZeneca	I	PO
M4076	EMD Serono	I	PO
RAD51 inhibitors			
CYT-0851	Cyteir Therapeutics	I/II	PO

IV: intravenous; PO: per oral; NCT: ClinicalTrials.gov identifier

Table 2:

Inhibitors of MYT1, PolQ and USP1 currently in development and ongoing clinical trials

Drug name	Company	Route	Phase	Ongoing clinical trials
MYT1 inhibitors				
RP-6306	Repare Therapeutics	PO	I	NCT05147350 NCT05147272 NCT04855656
PolQ inhibitors				
ART4215	Artios Pharma	PO	I	NCT04991480
USP1 inhibitors				
KSQ-4279	KSQ® Therapeutics	PO	I	NCT05240898

IV: intravenous; PO: per oral; NCT: ClinicalTrials.gov identifier

Table 3:

Potential selective biomarkers for ATR and WEE1 inhibition

Biomarker	Function	References
ATR inhibition		
ATM deficiency	• ATM deficiency increases dependency on ATR/CHK1 and leads to replication catastrophe in the presence of ATR inhibition.	[34–37]
ARID1A deficiency	• Loss of ARID1A leads to BAF complex dysfunction and defects in TOP2A, leading to reduced S-phase rate and replication stress. • ARID1A-loss also leads to dysfunctional modulation of R-loop associated replication stress.	[38, 39]
APOBEC3A and B amplification	• APOBEC3A and APOBEC3B induce replication stress by introducing abasic sites at replication forks. • ATR inhibition in APOBEC3A expressing cells led to a surge of abasic sites at replication forks, increased ssDNA accumulation and replication catastrophe.	[98]
Homologous recombination repair protein deficiency e.g. deleterious mutations in *BRCA1/2, PALB2, RAD51C/D, BARD1, BRIP1, CHEK2*	• HRD, modelled through RAD51 inactivation, led to aberrant replication dynamics, increased ATR reliance and preferential killing of HRD cells in response to ATR inhibition.	[41]
RNASEH2 deficiency	• RNASEH2 removes aberrantly incorporated ribonucleotides during replication and resolves R loops. • CRISPR/Cas9 whole-genome screens in cells treated with an ATR inhibitor uncovered RNASEH2 as a gene whose depletion affects ATR inhibitor sensitivity. • RNASEH2-deficient cells developed increased DNA damage, apoptosis or senescence upon ATR inhibition.	[99]
WEE1 inhibition		
CCNE1 amplification	• *CCNE1* encodes cyclin E which interacts with CDK2 to phosphorylate Rb shortly prior to S-phase entry. • Amplification of *CCNE1* promotes dysregulated, early S-phase entry, and accumulation of genoccnemic instability. • WEE1 inhibition in the context of *CCNE1* amplification provokes mitotic catastrophe.	[47, 48, 51]
H3K36me3-deficiency	• H3K36me3 physiologically facilitates the expression of RRM2 by recruiting transcription factors to initiation sites. • WEE1 inhibition in cells deficient in H3K36me3 leads to reduced RRM2 recruitment, depletion of dNTP, cell cycle arrest in S-phase and apoptosis.	[100]
TSC2 deficiency	• TSC2 depletion led to hyper-mTORC1 activity and accelerated G2/M checkpoint recovery. • WEE1 inhibition in TSC2-null cells led to mitotic catastrophe.	[101]

APOBEC3A/B: Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A/B; ssDNA: single-stranded DNA breaks; HRD: homologous recombination deficiency; RNASEH2: Ribonuclease H2 subunit A; H3K36me3: histone H3 trimethylation at lysine 36; RRM2; ribonucleotide reductase regulatory subunit M2; TSC2; TSC complex subunit 2; mTORC1: mammalian target of rapamycin complex 1

Table 4:

Ongoing clinical trials combining DDR inhibitors with anti-PD(L)1 immune checkpoint blockade

Drug	Phase	Study population	Treatment	Efficacy	Safety	Reference
ATR inhibitors						
Berzosertib (M6620; VX-970)	I	Advanced solid tumors harboring markers of DDR deficiency; N=36 Expansion cohort in subjects with *ATM*-mutation	• Avelumab + Berzosertib	Recruitment ongoing	N/A	NCT04266912
	II	Recurrent platinum-sensitive ovarian cancer, that is resistant to PARPi; N=90	• Part A: Carboplatin + Avelumab + Berzosertib • Part B: Carboplatin + paclitaxel/ gemcitabine/PLD +/− bevacizumab and bevacizumab maintenance; vs carboplatin + avelumab + berzosertib and avelumab maintenance	Recruitment ongoing	N/A	NCT03704467
Ceralasertib (AZD6738)	II	Advanced NSCLC who with progression on prior immunotherapy; N=410	• Novel durvalumab combinations including ceralasertib + durvalumab	• ORR: 8.3% • Median PFS: 7.43 months (95% CI, 3.45–9.46) • Median OS:15.80 months (95% CI, 11.01-NR) • No correlation between *ATM* or PD-L1 status and treatment response was observed	N/A	NCT03334617
	II	Advanced melanoma with progression on prior immunotherapy; N=30	• Ceralasertib + durvalumab	• ORR: 31.0% • DCR: 63.3%	• TEAEs (any grade): 96.7% • Grade ≥3 TEAEs most commonly: anemia (33.3%),	NCT03780608

Drug	Phase	Study population	Treatment	Efficacy	Safety	Reference
				• Responses occurred across cutaneous, acral and mucosal melanoma patients • Median duration of response: 8.8 months (range, 3.8–11.7 months) • Median PFS: 7.1 months (95% CI, 3.6–10.6 months) • Median OS: 14.2 months (95% CI, 9.3–19.1 months) • No correlation between *ATM* or PD-L1 status and treatment response was observed	thrombocytopenia (16.7%), neutropenia (16.7%)	
	II	Advanced biliary tract cancers with progression on prior immunotherapy; N=26	• Ceralasertib + durvalumab	Recruitment ongoing	N/A	NCT04298008
	II	Advanced NSCLC with progression on prior immunotherapy; N=120	• Ceralasertib + durvalumab vs docetaxel	Recruitment ongoing	N/A	NCT03833440
Elimusertib (BAY1895344)	Ib/II	Advanced solid tumors harboring markers of DDR deficiency; N=110	• Elimusertib + pembrolizumab	Recruitment ongoing	N/A	NCT04095273
	I	Recurrent HNSCC; N=38	• Elimusertib + pembrolizumab + SBRT	Recruitment ongoing	N/A	NCT04576091
WEE1 inhibitors						

Drug	Phase	Study population	Treatment	Efficacy	Safety	Reference
Adavosertib	I	Advanced solid tumors; N=54	• Adavosertib + durvalumab in varying doses	• Overall DCR: 36%	• Grade≥3 TEAEs most commonly: fatigue (15%), diarrhoea (11%), nausea (9%)	NCT02617277
ZN-c3	I/II	Advanced solid tumors; N=360	• ZN-c3 + pembrolizumab	Recruitment ongoing	N/A	NCT04158336

N: number of participants expected; D: day of cycle; BD: twice-daily dosing; ORR: objective response rate; PFS: progression-free survival; OS: overall survival; NR: not reached; DCR: disease-control rate; NSCLC: non-small cell lung cancer; HNSCC: head and neck squamous cell carcinoma; SBRT: stereotactic body radiotherapy; TEAE: treatment-emergent adverse events