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Dancing with the DNA damage response: next-generation anti-cancer therapeutic strategies

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Abstract: Maintenance of genomic stability is a critical determinant of cell survival and relies on the coordinated action of the DNA damage response (DDR), which orchestrates a network of cellular processes, including DNA replication, DNA repair and cell-cycle progression. In cancer, the critical balance between the loss of genomic stability in malignant cells and the DDR provides exciting therapeutic opportunities. Drugs targeting DDR pathways taking advantage of clinical synthetic lethality have already shown therapeutic benefit – for example, the PARP inhibitor olaparib has shown benefit in *BRCA*-mutant ovarian and breast cancer. Olaparib has also shown benefit in metastatic prostate cancer in DDR-defective patients, expanding the potential biomarker of response beyond BRCA. Other agents and combinations aiming to block the DDR while pushing damaged DNA through the cell cycle, including PARP, ATR, ATM, CHK and DNA-PK inhibitors, are in development. Emerging work is also uncovering how the DDR interacts intimately with the host immune response, including by activating the innate immune response, further suggesting that clinical applications together with immunotherapy may be beneficial. Here, we review recent considerations related to the DDR from a clinical standpoint, providing a framework to address future directions and clinical opportunities.

Keywords: DNA damage response, immunotherapy, PARP inhibitors

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The DNA damage response

Genome instability is described as one of the hallmarks of cancer.[1] Human DNA is continuously exposed to potential sources of damage, both exogenous – such as ultraviolet and ionizing radiations, chemicals and chemotherapeutics – and endogenous – such as reactive oxygen species and faulty DNA replication.[2,3] Cells have evolved a complex DNA damage response (DDR), which is in charge of repairing DNA damage and promoting the maintenance of genome integrity. Defects in DDR are associated with increased mutational load and genome instability and are a well-recognized cause of neoplastic transformation and proliferation. Cells harbouring DDR defects can become reliant on other repair pathways for survival, which makes DDR targeting an attractive therapeutic strategy.[1,2]

Mechanisms of DDR are numerous and partially overlap. Their functioning involves multiple sensors of damage, signalling factors that activate cell-cycle checkpoints and effector proteins of repair. This orchestra is responsible for processing the two main types of DNA lesions: single-strand breaks (SSBs) and double-strand breaks (DSBs) (Figure 1).[4] If DNA is not repaired, replication stress results. Replication stress is slowing of the DNA replication fork.[5] It is a highly dynamic chain of events starting from acutely arrested forks with fully assembled replisomes, uncoupling of the DNA helicase and polymerase, RPA coated ssDNA and ATR activation.[5] If replication stress persists, stalled forks are converted into 'collapsed forks' with dissociation and/or impaired modifications of replisome components.[6,7] Further extension of replication stress

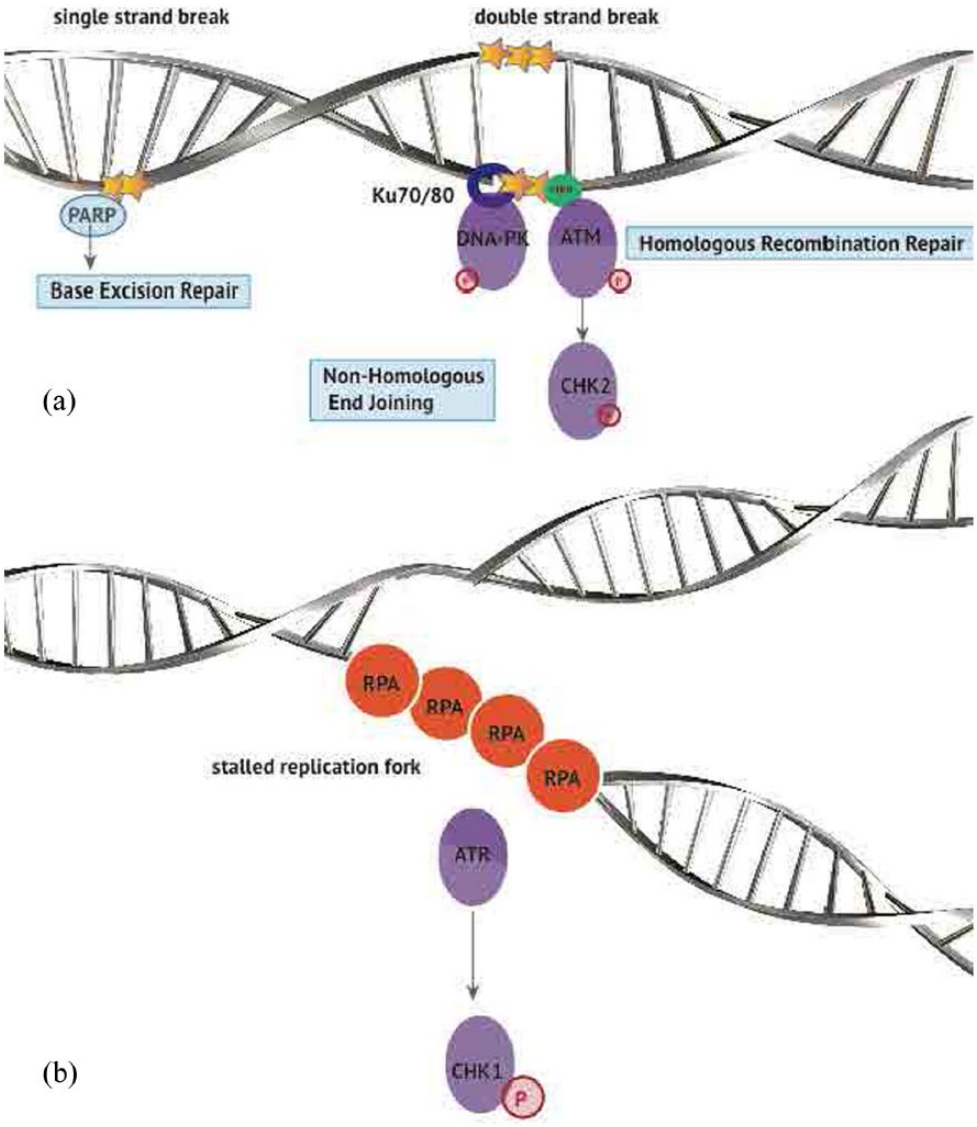

Figure 1. DDR pathways. (a) DNA strand breaks activating DNA repair pathways; (b) stalled replication fork leading to ATR activation.

converts forks to DNA DSBs, causing replication catastrophe and cell death.[8]

SSBs can result from endogenous oxidative damage, defective activity of cellular enzymes or erroneous incorporation of ribonucleotides in DNA.[9] Repair can occur through base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR).[10] BER is involved in the removal of small base adducts. Poly (ADP-ribose) polymerase-1 and 2 (PARP1 and PARP2) are crucial proteins for BER, acting as sensors of SSB and promoting the recruitment and activation of critical downstream SSB repair effectors such as XRCC1.[9] NER is engaged in the repair of lesions that cause a distortion of the DNA helix, generally as a result of UV-induced damage. It promotes the removal of short oligonucleotides, involving ERCC1 as one of the key effector proteins.[11,12] The MMR system is responsible for correcting base–base mispairing and insertion/deletion loops that can occur during DNA replication. These faulty areas of DNA are recognized by the proteins MSH2, MSH3 and MSH6, which recruit MLH1 and PSM2 on the sites of damage, enabling repair.[13] Unrepaired

SSBs can lead to cell death or to the collapse of DNA replication forks, with the formation of DSBs.[9]

DSBs are among the most destructive DNA lesions. Repair occurs through homologous recombination (HR), non-homologous end joining (NHEJ) and single-strand annealing (SSA). HR is an accurate process taking place during the S and G2 phases of the cell cycle, and promoting precise repair of the damaged area of DNA by using the sister chromatid as a template. Several genes with a tumour-suppressor activity are crucial to this process, including *BRCA1, BRCA2, PALB2* and *RAD51*, and their functioning is essential for an error-free repair.[14] Defective HR cells are redirected towards more error-prone DSB repair pathways such as NHEJ, which occurs throughout the whole cell cycle, and SSA. In this circumstance, repair is performed by direct ligation of the two DNA broken strands, which can lead to DNA loss and increased mutagenic potential.[15]

Defects in the components of the DDR network drive a variety of hereditary and sporadic tumours. Loss-of-function mutations in the MMR system are associated with the formation of repeated DNA sequences, a phenotype known as microsatellite instability (MSI). MSI can result from mutations of MMR genes at the germline level which are characteristic of the hereditary non-polyposis colorectal cancer (HNPCC or Lynch syndrome) or from somatic mutations or epigenetic silencing through methylation of MLH1 promoter. Somatic loss of MMR components has been described in 15% of sporadic colorectal cancers as well as in other non-colorectal cancers, particularly endometrial cancer.[16] Aberrations in HR genes correlates with genome instability and cancer susceptibility. Loss of function in the genes *BRCA1* and *BRCA2* is a predisposing factor for the development of hereditary and sporadic cancers: ovarian, breast, pancreatic and prostate cancers.[17,18] BRCA1/2 deficiency can arise from germline or somatic gene mutations or from *BRCA1/2* epigenetic silencing. Methylation of the BRCA1 promoter is described in 11–14% of sporadic breast cancers and 5–31% of ovarian cancers.[19,20] A subset of sporadic tumours has been found to share common features with BRCA-deficient tumours by means of mutation or epigenetic deregulation of genes involved in the HR, including *RAD51, RAD54, DSS1, RPA1, NSB1, ATR, ATM, CHEK1, CHEK2, FANCD2, FANCA* and *FANCC 2.*[21,22] Data from large-scale genome analysis in ovarian cancer from The Cancer Genome Atlas (TCGA) report an incidence of 51% of alterations in HR genes in 316 samples analysed.[23] Sequencing of metastatic castration-resistant prostate cancers has revealed aberrations in key DDR genes in up to 23% of cases.[24]

With the widening use of next-generation sequencing techniques, the plethora of DDR defects being detected is ever increasing, focusing efforts in developing effective therapeutic strategies to target these.

Targeting DDR defects through synthetic lethality

DNA damage has for years represented a backbone of cancer treatment. Platinum compounds such as cisplatin and carboplatin, by inducing interstrand or intrastrand cross-linking, cause double helix disruption leading to DNA damage. Despite their activity in a broad spectrum of cancers, treatment resistance develops through a number of mechanisms which can involve upregulation of DDR components.[25] DDR inhibitors have been developed, and as a first approach were tested in combination with platinum agents. However, the overlapping toxicity profile represented a challenge.[26] Testing as single agents has followed based on the observation of synthetic lethality occurring between two or more DDR components in specific molecular contexts.[27] Two genes are considered synthetic lethal when cell viability is conserved if either of them is inactive, whereas the impairment of both results in cell death.[28] Cancer cells with defects in one DDR pathway often depend on other pathways for their survival, and targeting these pathways of reliance can be exploited to cause selective cancer cell death.

PARP inhibitors

The paradigm in this field is represented by the development of PARP inhibitors in BRCA1/2-defective cells.[4] PARP1 and PARP2 are key sensors of DNA damage and are crucial in activating the cascade of SSB repair and BER. Their inhibition causes an increase in DSBs, which are normally repaired by HR. In cells harbouring defects in the HR system, such as *BRCA1/2* mutant cells, inhibition of PARP enzymes results in cell-cycle arrest and apoptosis of cancer cells through synthetic lethality.[29,30]

The clinical application of PARP inhibitors is most advanced in ovarian cancer, where the PARP inhibitor olaparib has received regulatory approval in a number of settings. In a phase II study of 57 patients with *BRCA1/2*-mutant ovarian cancer treated with olaparib 400 mg twice per day, Audeh and colleagues have reported an overall response rate of 31%.[31] A phase II trial enrolled 64 patients with high-grade serous ovarian cancers and demonstrated an overall response rate of 41% in the *BRCA1/2*-mutant group.[32] Based on these findings, olaparib received accelerated approval from the FDA in 2014 for fourth-line or later treatment of germline *BRCA1/2*-mutant ovarian cancer. Subsequent trials have investigated the role of olaparib as maintenance therapy after platinum-based chemotherapy with subsequent FDA approval in the platinum-sensitive, non-*BRCA*-mutated setting. In a randomized placebo controlled phase II trial by Ledermann and colleagues, olaparib significantly improved progression-free survival (PFS) in 265 patients with recurrent platinum-sensitive high-grade serous ovarian cancer [median PFS 8.4 months *versus* 4.8 months; hazard ratio 0.35; $p < 0.001$]. A subgroup analysis of the study has reported that the benefit of maintenance olaparib was increased in the BRCA1/2 mutant sub-population (median PFS 11.2 months *versus* 4.3 months; hazard ratio 0.18; $p < 0.000$).[33] This trial has led to the European Medicines Agency (EMA) approval of olaparib in *BRCA1/2*-mutant or platinum-sensitive ovarian cancer (regardless of *BRCA* status) as maintenance after complete or partial response to platinum-based chemotherapy. The SOLO II phase III trial of 295 patients with platinum-sensitive *BRCA1/2*-mutant ovarian cancer, pretreated with at least two lines of chemotherapy, saw a significant PFS benefit of olaparib compared to placebo (19.1 months *versus* 5.5 months; hazard ratio 0.30; $p < 0.0001$), which has led to approval by the FDA for the tablet formulation in this setting.[34]

Olaparib has shown encouraging activity in a phase II trial in 27 patients with *BRCA1/2*-mutant advanced breast cancer, which has reported an overall response rate of 41%.[35] More recently, results from the randomized phase III trial OLYMPIAD compared olaparib *versus* standard chemotherapy in patients with germline *BRCA*-mutant HER2-negative breast cancer treated with two or fewer chemotherapy lines. Among the 302 patients enrolled, olaparib showed a significant benefit in PFS (7.0 months *versus* 4.2 months; HR for disease progression or death, 0.58; $p < 0.001$).[36]

Olaparib has also proven remarkable activity in DDR-defective metastatic castrate-resistant prostate cancer (mCRPC), which represent up to 23% of all prostate cancer cases.[24] Mateo and colleagues conducted a phase II trial (TOPARP-A) of olaparib 400 mg twice daily in unselected mCRPC patients pretreated with docetaxel and abiraterone and/or enzalutamide. Among the 49 patients enrolled, a response by the composite endpoint (comprising RECIST 1.1, PSA or CTC count) was reported in 16 (33%) patients, including PSA decline greater than 50% in 11 patients and 6 radiologic partial responses. Notably, the investigators performed next-generation sequencing on all patients enrolled, which has identified homozygous deletions or deleterious mutations in DNA repair-related genes in 16 out of 49 (33%), including *BRCA1/2, ATM, PALB2, FANCA* and *CHK2*. Among these DDR mutation carriers olaparib showed significantly increased activity, with responses occurring in 14 out of 16 (88%) patients. This trial has granted olaparib breakthrough therapy designation approval in *BRCA1/2*- or *ATM*-mutant mCRPC patients pretreated with one line of taxane chemotherapy and a new-generation antihormonal agent. The study further corroborates the strong rationale in developing PARP inhibition in DDR-defective patients beyond *BRCA* mutations. The second stage of the trial (TOPARP-B) is currently ongoing and prospectively recruiting patients carrying a DDR-defective signature to validate PARP inhibition activity in this subgroup.[37]

Other PARP inhibitors have now reached the late stages of clinical development: rucaparib (AG014699; Clovis), talazoparib (BMN637; Medivation), veliparib (ABT-888; AbbVie) and niraparib (MK4827; Tesaro). Of note, rucaparib has received breakthrough therapy designation in *BRCA1/2*-mutant ovarian cancer progressing on two lines of platinum regimens based on the PFS benefit reported in the phase III trial ARIEL-2;[38] niraparib (MK4827; Tesaro) has been approved by the EMA and FDA as maintenance treatment of recurrent platinum-sensitive ovarian cancer, having showed activity in both *BRCA*-mutant and wildtype patients in the NOVA trial.[39]

Single-agent activity of PARP inhibitors in non-HR-defective tumours has so far been limited; combination strategies of PARP with DNA-damaging cytotoxic agents could enhance sensitivity to PARP inhibition and a number of trials addressing this question are currently under way

Table 1. PARP inhibitor phase II and III trials.

Reference	Phase	Patient numbers	Population	Drug	Results
Audeh and colleagues[31]	II	57	*BRCA1/2*-mutant, recurrent, ovarian cancer	Olaparib 400 mg twice daily (cohort 1) and 100 mg twice daily (cohort 2)	ORR 31% (95% CI 20–51) in cohort 1
Gelmon and colleagues[32]	II	64	Advanced high-grade serous and/or undifferentiated ovarian carcinoma or triple-negative breast cancer	Olaparib 400 mg twice daily	ORR 41% (95% CI 22–64) of 17 patients with *BRCA1/2* mutations
Ledermann and colleagues[33]	II	265	Platinum-sensitive, relapsed, high-grade serous ovarian cancer who had received two or more platinum-based regimens and had had a partial or complete response to their most recent platinum-based regimen	Olaparib 400 mg twice daily, or placebo	Median PFS 8.4 months *versus* 4.8 months; hazard ratio 0.35; $p < 0.001$ Benefit increased in the *BRCA1/2*-mutant sub-population (median PFS 11.2 months *versus* 4.3 months; hazard ratio 0.18; $p < 0.000$).
Pujade-Lauraine and colleagues[34]	III	295	Platinum-sensitive *BRCA1/2*-mutant ovarian cancer, pretreated with at least two lines of chemotherapy	Olaparib 300 mg twice daily, or placebo	PFS benefit compared to placebo (19.1 months *versus* 5.5 months; hazard ratio 0.30; $p < 0.0001$)
Tutt and colleagues[35]	II	27	Metastatic breast cancer with germline *BRCA*-mutant breast cancer	Olaparib 400 mg twice daily (cohort 1) and 100 mg twice daily (cohort 2)	ORR was 11 (41%) of 27 patients (95% CI 25–59) in cohort 1
Robson and colleagues[36]	III	302	Metastatic breast cancer with germline *BRCA* mutation and HER2-negative who had received no more than two previous chemotherapy regimens for metastatic disease	Olaparib 300 mg twice daily *versus* standard chemotherapy	Median PFS significantly longer in the olaparib than the standard-therapy (7.0 months *versus* 4.2 months; HR for disease progression or death, 0.58; 95% CI 0.43 to 0.80; $p < 0.001$).
Mateo and colleagues[37]	II	49	mCRPC patients pretreated with docetaxel and abiraterone and/or enzalutamide	Olaparib 400 mg twice daily	ORR 33% Of 16 patients with DDR gene deficit ORR 88%
Mirza and colleagues[39]	II	206	Recurrent, platinum-sensitive, high-grade ovarian carcinoma	Rucaparib 600 mg twice daily	PFS 12.8 months (95% CI 9.0–14.7) in the *BRCA*-mutant subgroup
del Rivero and Kohn[40]	III	553	Platinum-sensitive, recurrent ovarian cancer	Niraparib 300 mg or placebo once daily	Niraparib group had a longer median PFS than placebo group, including 21.0 *versus* 5.5 months in the g*BRCA* cohort (hazard ratio 0.27; 95% CI 0.17 to 0.41, $p < 0.001$).

(Table 1).[40] Furthermore, acquired resistance to PARP inhibition is common. As observed with platinum agents, modifications in DDR pathways can occur through activation of NER, MMR and/or HR pathways, allowing for increased repair and promoting treatment resistance.[41] Similarly, increasing evidence is revealing a number of mechanisms underlying resistance to PARP inhibition.[42] Several mechanism of resistance have been proposed, among which the restoration of *BRCA* function through secondary frameshift mutations is the most well established.[43] Restoration of HR function by somatic mutations confers olaparib resistance.[44,45] Combination of PARP inhibitors with other DDR agents, potentially exploiting DDR synthetic lethalities, or with chemotherapeutic agents are currently explored approaches in trying to overcome PARP inhibitor resistance.[42,46] In the era of new DDR agents, treatment resistance will have to be taken into account.

ATM inhibitors

ATM is a key protein in HR repair of DSB *via* HR. ATM acts as a signalling protein with hundreds of downstream substrates, including CHK2, a cell-cycle checkpoint activator. In preclinical studies, ATM inhibitors have sensitized cells to ionizing radiation and DSB-inducing agents; early-phase clinical testing of ATM inhibitors is currently ongoing.[47] ATM has a synthetic lethal relationship with PARP1 and preclinical models exhibit enhanced sensitivity to PARP inhibition of ATM-deficient cells.[48] Synthetic lethality exists also between ATM and ATR and between both ATM and ATR with XRCC, a relevant component of SSB repair through BER.[49]

ATR inhibitors

ATR is an essential DDR kinase activated in response to replication stress and stalled replication forks. Through activation of multiple downstream effectors of which CHK1 and Wee1 are the most well characterized, ATR signalling promotes cell-cycle control and DNA repair through HR. Cancer cells, which harbour high levels of replication stress, are more likely to rely on the ATR pathway for survival.[50] Among agents in clinical testing, VX-970 (Vertex Pharmaceutical; now M6620, Merck), an intravenous ATR inhibitor, has shown target modulation and meaningful tumour control in a phase I trial as a single agent and in combination with carboplatin, including tumour responses in a patient with an ATM-deficient colorectal cancer and a patient with a *BRCA1*-mutant, platinum- and PARP-inhibitor-resistant high-grade serous ovarian cancer.[51,52] Early-phase combination trials of VX-970 with gemcitabine and cisplatin exhibited encouraging activity results.[53,54] Another oral ATR inhibitor, AZD6738 (Astrazeneca), has shown promising preclinical activity and is currently being tested in phase I trials as monotherapy or in combination with olaparib, radiotherapy regimens, carboplatin and immunotherapy agents (Table 2).[4,55] ATR inhibition has been found to be synthetic lethal, with a number of DDR components in preclinical models including ERCC1,[56] XCRCC1,[49] CHK1[57] and ATM,[27] which could serve as a background for new clinical trials.

DNA-PK inhibitors

DNA-PK (DNA-dependent serine/threonine protein kinase catalytic subunit) acts as a sensor of DNA damage and is crucial for repair through NHEJ. High levels of DNA-PK are correlated to poor prognosis and resistance to chemotherapy and radiotherapy in several tumour models.[59,60] The inhibition of DNA-PK through small molecules has proven particularly active when combined with agents inducing DSBs, such as radiotherapy and topoisomerase inhibitors. Clinical studies involving DNA-PK inhibitors as a single agent or in combination with chemotherapy or radiotherapy are currently ongoing in patients with solid and haematological neoplasms (Table 2).[61,62]

CHK1 inhibitors

CHK1 is the most important phosphorylation target of ATR and mediates DNA repair and checkpoint activation.[50] CHK1 inhibitors are currently undergoing clinical testing both in combination with cytotoxics and as single agents. Among them, LY2606368 (Eli Lilly) as monotherapy has shown promising signs of anti-tumour activity and safe toxicity profile in a phase I trial,[63] and combination trials with cytotoxics are ongoing. Other CHK1 inhibitors currently in early-phase trials are GDC-0575 and SRA737, which are being tested as single agents and in combination with enhancers of replication stress (Table 2). Preclinical studies have reported a synergistic effect of combination of CHK1 inhibitors with PARP inhibitors and a trial investigating this strategy is now recruiting (Table 2).[64,65]

Table 2. Selected ongoing DDR inhibitor trials.[58]

ClinicalTrials.gov identifier	Title	Phase	Drug target
NCT01844986	Olaparib Maintenance Monotherapy in Patients with BRCA Mutated Ovarian Cancer Following First Line Platinum Based Chemotherapy. (SOLO-1)	III	PARP inhibitor
NCT02282020	Olaparib Treatment in Relapsed Germline Breast Cancer Susceptibility Gene (BRCA) Mutated Ovarian Cancer Patients Who Have Progressed at Least 6 Months After Last Platinum Treatment and Have Received at Least 2 Prior Platinum Treatments (SOLO3)	III	PARP inhibitor
NCT02446704	Study of Olaparib and Temozolomide in Patients With Recurrent Small Cell Lung Cancer Following Failure of Prior Chemotherapy	I	PARP inhibitor
NCT02789332	Assessing the Efficacy of Paclitaxel and Olaparib in Comparison to Paclitaxel/Carboplatin Followed by Epirubicin/Cyclophosphamide as Neoadjuvant Chemotherapy in Patients with HER2-negative Early Breast Cancer and Homologous Recombination Deficiency (GeparOla)	II	PARP inhibitor Chemotherapy
NCT02264678	Ascending Doses of AZD6738 in Combination With Chemotherapy and/or Novel Anti Cancer Agents Drug: administration of AZD6738 in combination with carboplatin Drug: administration of AZD6738 Drug: administration of AZD6738 in combination with olaparib Drug: administration of AZD6738 in combination with MEDI4736	I/II	PARP inhibitor Chemotherapy
NCT00535353	AZD2281 and Irinotecan in Treating Patients with Locally Advanced or Metastatic Colorectal Cancer	I	PARP inhibitor Chemotherapy
NCT00678132	AZD2281 and Cisplatin Plus Gemcitabine to Treat Solid Tumor Cancers	I	PARP inhibitor Chemotherapy
NCT00515866	Study to Assess the Safety & Tolerability of a PARP Inhibitor in Combination with Gemcitabine in Pancreatic Cancer	I	PARP inhibitor Chemotherapy
NCT01460888	Radiotherapy & Olaparib in COmbination for Carcinoma of the Oesophagus (ROCOCO)	I	PARP inhibitor Radiotherapy
NCT02308072	Phase I Study of Olaparib Combined with Cisplatin-based Chemoradiotherapy to Treat Locally Advanced Head and Neck Cancer (ORCA-2)	I	PARP inhibitor Chemoradiotherapy
NCT02797964	A Phase 1 Trial of SRA737 in Subjects with Advanced Cancer	I	CHK1 inhibitor
NCT02797977	A Phase 1 Trial of SRA737 in Combination with Gemcitabine Plus Cisplatin or Gemcitabine Alone in Subjects with Advanced Cancer	I	CHK1 inhibitor Chemotherapy

(Continued)

Table 2. (Continued)

ClinicalTrials.gov identifier	Title	Phase	Drug target
NCT03057145	Combination Study of Prexasertib and Olaparib in Patients with Advanced Solid Tumors	I	CHK1 inhibitor PARP inhibitor
NCT02516813	Phase 1 Trial of MSC2490484A, an Inhibitor of a DNA-dependent Protein Kinase, in Combination with Radiotherapy	I	DNA-PK inhibitor Radiotherapy
NCT03308942	Phase 2, Multi-Arm Study of Niraparib Administered Alone and in Combination with PD-1 Inhibitor in Patients with Non-Small Cell Lung Cancer	II	PARP Inhibitor PD-1 Inhibitor
NCT02660034	The Safety, Pharmacokinetics and Antitumor Activity of BGB-A317 in Combination with BGB-290 in Subjects with Advanced Solid Tumors	I	PARP Inhibitor PD-1 Inhibitor
NCT02264678	Ascending Doses of AZD6738 in Combination with Chemotherapy and/or Novel Anti Cancer Agents	I	ATR Inhibitor Chemotherapy PDL-1 Inhibitor

ATR and CHK1 activity is closely interrelated and there is an established synthetic lethal relationship between the two, which provides a rational for combination therapies of ATR and CHK1 inhibitors.[57] CHK1 inhibition has been found to be synthetic lethal with the Wee1 kinase in preclinical models (see below).[66,67]

Wee1 inhibitors

Wee1 is a key controller of cell-cycle checkpoint, particularly at the G2 phase; when activated by DNA damage it prevents progression of the cell to mitosis, allowing time for repair.[68] The Wee1 inhibitor AZD1775 (MK1775; Astrazeneca) exhibited single-agent activity as well as in combination with cytotoxic agents (gemcitabine, carboplatin, cisplatin) in early-phase trials and a favourable safety profile. Interestingly, two partial responses were observed in *BRCA1*-mutant patients, suggesting a role for Wee1 inhibition in HR-defective cancers.[69,70] AZD1775 has undergone testing in a phase II trial in combination with carboplatin in patients with p53 mutant platinum-resistant ovarian cancers and has shown an overall response rate of 43%.[71] A phase II trial of AZD1775 in 121 ovarian cancer patients with platinum-sensitive disease randomized patients to AZD1775 in combination with paclitaxel-carboplatin or paclitaxel-carboplatin alone. A small PFS benefit was seen with a PFS of 34.14 *versus* 31.85 weeks (hazard ratio 0.63; $p = 0.080$).[72]

Biomarkers of DDR inhibitors

As demonstrated by the clinical development of PARP inhibitors to date, the clinical utility of DDR inhibitors relies on establishing biomarkers of response to allow appropriate patient selection. Many putative biomarkers reflect aberrations in DDR pathways or genomic signatures that result from DNA damage.[73]

Clinical biomarkers

Sensitivity to platinum-based chemotherapy is often taken as a surrogate biomarker of 'BRCAness' and thus sensitivity to PARP inhibitors. The FDA has approved platinum sensitivity as a biomarker for olaparib in the maintenance setting. However, this clinical biomarker is limited; some patients who respond to platinum do not respond to PARP inhibitors (for example, due to *NER* mutations) and some whom are resistant to platinum respond to PARP inhibition (for example, due to loss of *TP53BP1* or *REV7*).[32,74–76]

Genomic biomarkers

As discussed, olaparib's EMA (though not FDA) approval for treatment of ovarian cancer mandates germline *BRCA1/2*-mutant ovarian cancer and *BRCA1/2* or *ATM* mutation in mCRPC. Attempts are being made to predict the larger patient population who can benefit from DDR inhibitors. Single-agent activity of olaparib has been reported in patients with sporadic cancers, which is likely explained by the presence of non-*BRCA1/2* HR defects conferring susceptibility to PARP inhibition.[32,33] Nevertheless, reliable biomarkers of response to PARP inhibition are yet to be defined and the increasing availability of genomic analysis can be expected to add meaningful information for patient selection.[21,77]

Several oncogenic features, such as alterations of replication timing and progression, lead to replication stress and are thus proposed as potential biomarkers for DDR inhibitor response, including ATR and ATM inhibitors.[78] *RAS* mutant cancers have been shown to have dependence on the DDR and *KRAS* mutations have been shown to induce hypersensitivity to ATR in cell lines,[79] as have *CCNE1, CCND2* and *MYC*.[80] In ovarian cancer, lipid phosphatase inositol polyphosphate 4-phosphatase type II (INPP4B) loss (found in 40% of patients) causes a DNA repair deficit.[81] Other cell-cycle regulators including *CDC25A* also increase replication stress.[82]

Aberrations in DDR/cell-cycle checkpoint genes cause replication stress in preclinical data. These include aberrations in including *FA, Rb*,[80] *ERCC1*, ribonucleotide reductase,[83] *XRCC1*[49] and *ATM*,[84] and can thus be proposed as potential biomarkers for DDR inhibition.

The SWI/SNF chromatin-remodelling complex is composed of multiple components including ARID1A, ARID1B, SMARCA4 and SMARCB1, and modulates DNA replication, transcription and repair.[85,86] Defects in ARID1A sensitize tumour cells to ATR inhibition *via* topoisomerase 2A and cell-cycle defects.[87] Other epigenetic modulators including loss of the chromatin-remodelling protein ATRX and H3K36me3-deficiency have been shown to render cells hypersensitive to CHK1 and ATR inhibition.[88,89]

Significant heterogeneity characterizes DNA repair defects; prevalence varies across different tumour types. MSI occurs more frequently in colorectal cancer, whereas HR defects are more frequently detected in breast and ovarian cancers.[90] While genome sequencing has broadened the detection of DDR defects in other tumour types, precise estimates across different cancers are yet to be defined. In addition, the degree to which each DNA repair deficiency constitutes a catastrophic event, therefore rendering cells more susceptible to DDR inhibition, is still a point of uncertainty. Heterogeneity also exists in the impact of different DNA repair defects on patient outcome – for example, MSI colorectal cancers are characterized by better prognosis compared to genomically stable CRC.[91] These aspects constitute a limitation in the development of DDR agents. Despite encouraging results from early-phase trials, development is still challenged by lack of predictive biomarkers. A better understanding of each deficiency in the context of specific tumour types and the identification of validated biomarkers for patient selection will be critical for the development of these compounds.

Genomic scars

In DDR-deficient cells, DNA damages accumulates. This 'genomic scar' has different features depending on the pathway affected. For example, in HR deficiency there are large genomic deletions and loss of heterozygosity (LOH).[92–94] *BRCA1/2* mutations manifest as tandem duplications, and microhomology-mediated deletions.[95] Mismatch repair manifests as microsatellity instability.[95] The use of genomic scars as a predictive marker is not established. In the ARIEL3 trials of rucaparib maintenance following platinum chemotherapy in ovarian cancer, LOH was assessed. In those patients with *BRCA* wildtype tumours and LOH, 30% of patients in the rucaparib group achieved a benefit of over 1 year compared to 5% in the placebo group. LOH was, however, clearly not completely predictive of benefit.[96] In the NOVA trial of niraparib maintenance following platinum chemotherapy in ovarian cancer, HR deficiency was assessed by the myChoice HRD test which measures LOH, large-scale transitions and telomeric allelic imbalances. The HRD score did not predict niraparib benefit.[38]

Functional assays

Functional assays are being developed, that aim to give a real-time read-out of DNA repair. These include the RAD51 focus formation assay that has been shown to correlate with HRR defects[97,98] and the gamma-H2AX foci that correlates with

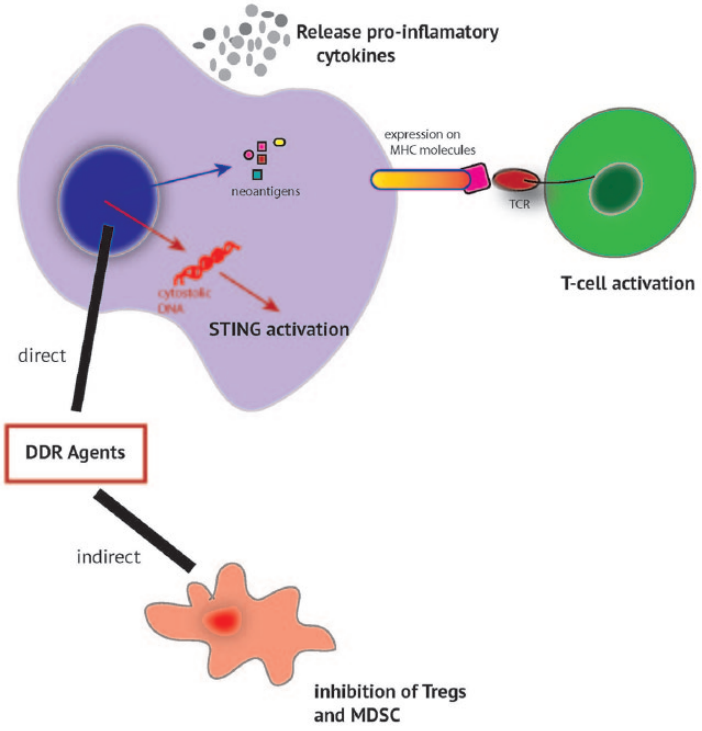

Figure 2. DDR agents and interaction with the immune environment.

DNA DSBs.[99,100] Assays for replication stress include the DNA fibre assay. This technique utilizes labelled nucleotides that become incorporated into DNA at the activated replication forks. Cells are lysed and DNA fibres stained using fluoroprobes and visualized using fluorescent microscopy. Information or origin firing, elongating forks and replication fork stalling can thus be obtained.[101,102] Clinical validation is awaited.

DDR inhibitors and the immune system

In recent years the key role that immune modulation has in oncogenesis has been recognized and the classic "hallmarks of cancer" have been updated to include "evasion of the immune system" as a key factor in tumourigenesis.[1] Emerging work is also revealing a tight coordination between the DDR and the immune defence systems (Figure 2).

Mutational burden and the immune system

DNA damage and repair influences responses to immunotherapy.[103] The burden of somatic mutations varies greatly between tumours, with melanoma, lung and bladder having the highest mutational load.[95] Mutations that cause a change in protein expression result in mutant proteins being bound by MHC class I and presented to T-cells, resulting in T-cell stimulation. These proteins are known as neoantigens and can arise from any changes that alter the open reading frame (ORF) sequences in the genome, such as missense mutations, fusion transcripts, frameshifts and stop losses.[104] Thus, neoantigen expression is closely correlated with mutational load.[105–107]

It has been found that tumour mutational load correlates with response and survival in CTLA-4 antagonists in metastatic melanoma.[105,106] This has also been demonstrated in non-small cell lung cancer, where, in two independent cohorts, higher nonsynonymous mutation burden in tumours was associated with improved objective response, durable clinical benefit and PFS.[107] Tumours with a mutational landscape in which C>A transversions are common, typical of tobacco exposure, are more likely to benefit from immune checkpoint inhibition.[108] Measures of mutational load have classically been burdens of single nucleotide variants (SNVs). It has also been demonstrated that a number of small insertions and deletions (indels) cause frameshifts correlating

with immunogenicity in a pan-cancer panel with correlation with immune checkpoint inhibitor responses seen in melanoma.[109]

Neoantigen expression has been shown to correlate with response to immune checkpoint inhibitor and survival.[105–107] Loss of neoantigens is also implicated in immune checkpoint inhibitor resistance.[110] The clonality of neoantigens is thought to play a role in response; loss of clonal expression of neoantigens is associated with immune checkpoint inhibitor resistance.[110,111]

DDR, mutational burden and response to immune checkpoint inhibitors

Deficits in DDR increase mutational burden; indeed, on large-scale genomic screens, defects in components of the DDR (including *BRCA1/2* and *ATM*) result in unique mutational signatures in tumours.[95,112] It can also be expected that the mutational burden will result in neoantigen burden and so influence responses to immunotherapy. Defects in DDR result in distinct immunological characteristics – for example, in breast cancer *BRCA1/2*-mutant tumours having higher levels of tumour-infiltrating lymphocytes and PD-L1.[113,114]

The most well-established example of DDR deficit and its influence on response to immunotherapy comes from MMR-deficient tumours. It has been demonstrated the MMR-deficient colorectal cancers have an activated immune microenvironment and upregulation of immune checkpoints such as PD-L1 and CTLA-4.[115] A phase II trial of the PD-1 inhibitor pembrolizumab demonstrated an objective response rate of 40% for MMR-deficient non-colorectal cancer patients and 71% for MMR-deficient colorectal cancer patients, contrasting to 0% for MMR-proficient patients. This clearly correlated with mutational load, with 1782 somatic mutations in MMR-deficient tumours compared to 73 somatic mutations in MMR-proficient tumours.[116] MMR-deficient tumours had a higher somatic mutational burden and neoantigen load. The study has since expanded to 12 MMR-deficient tumour types with objective radiographic responses seen in 53% of patients, and complete responses in 21% of patients. Responses were durable, with median PFS and overall survival not yet reached.[117] Following this trial, the FDA has approved pembrolizumab for treatment of MMR-deficient tumours.

In endometrial cancer with *POLE* mutations causing MSI there is a higher number of CD3+ and CD8+ tumour-infiltrating lymphocytes and increased PD-1 expression on tumour-infiltrating lymphocytes compared to microsatellite stable tumours.[118] In trials of pembrolizumab in non-small cell lung cancer, patients with prolonged responses were more likely to have mutations in DDR genes such as *POLE, POLD1* and *MSH2*.[107] In melanoma patients treated with the PD-1 inhibitors pembrolizumab or nivolumab, a high proportion of responders (6/21) had a *BRCA2* mutation compared to 1/17 non-responders.[119] Breast cancer patients with *BRCA1/2*-mutated tumours have a greater number of clonal mutations compared to wildtype.[120]

DDR, immune cytokines and STING

DNA damage results in an increase in levels of inflammatory cytokines, including TNF-α and IL-6 *via* ATM and ATR.[121,122] PARP inhibition synergizes with CTLA-4 blockade in a mouse ovarian model *via* IFN-gamma secretion.[123] The stimulator of interferon (STING) pathway plays a key role in innate immunity. Agonists of the STING pathway have been identified as enhancing anti-cancer immunity, with inhibitors of the pathway (β-catenin/wnt) inhibiting anti-cancer immunity.[124] STING pathway activating drugs are in development.[125,126] DDR is intimately linked to the innate immune system.[127] There is evidence that cytosolic DNA sensors directly activate the STING pathway,[128,129] which activate type I interferons which are known to augment cytotoxic T-cell priming,[130] and promote immunogenic cell death. DDR pathways may also directly activate the STING pathway.[131,132]

DDR and downregulation of MDSC and TREGs

Regulatory T-cells (TREGS) have an immunomodulatory role. DNA damage resulting from chemotherapy such as cyclophosphamide, temozolamide, gemcitabine and 5-FU has been shown to reduced TREG levels.[133–136] Whether DDR inhibitors can indirectly cause a similar effect is, as yet, unknown.

DDR inhibitors in combination with immunotherapy

Given the interplay of DDR pathways and the immune system, synergy of DDR inhibitors and immunotherapy can be proposed. DDR inhibitors

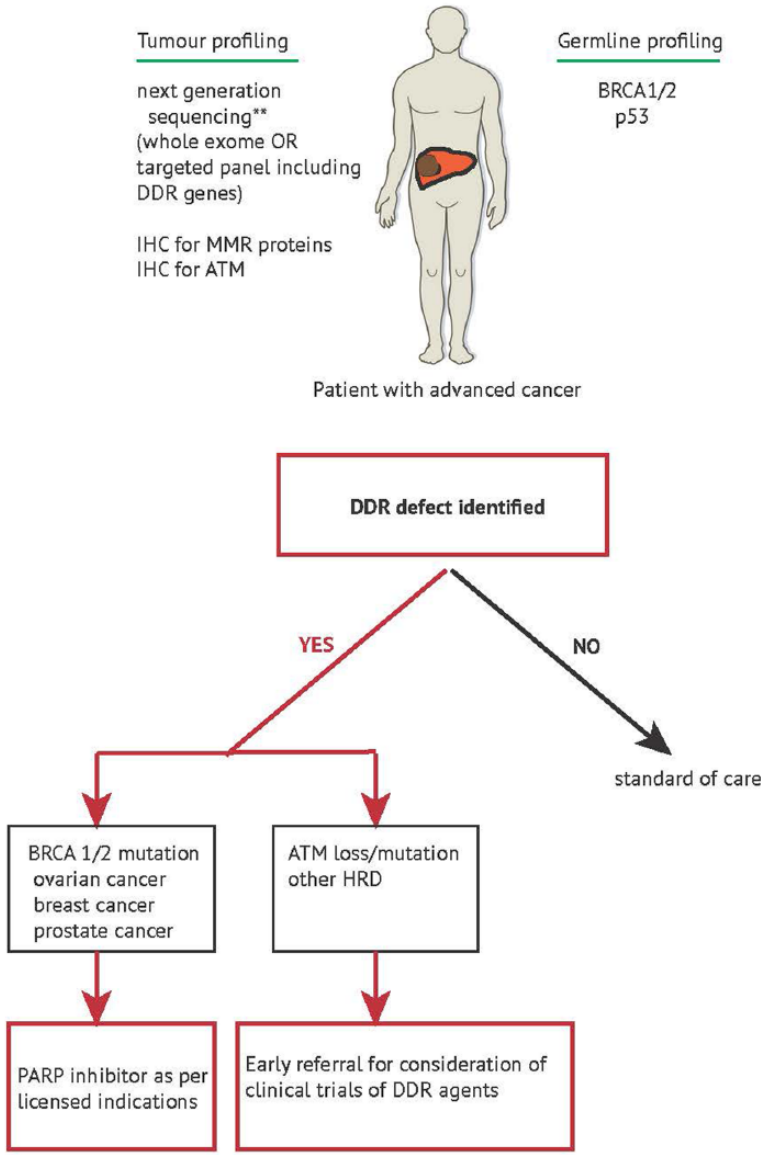

Figure 3. The DDR treatment paradigm.

could increase the mutational burden, making tumours more immunogenic. However, it is, as yet, unknown whether treatment with a DDR inhibitor results in a similar change in mutational load, neoantigen profile or STING pathway activation as occurs with DDR genomic deficits. Some early preclinical data are supportive. BMN 673, a PARP inhibitor, increases CD8+ T-cells and increased IFN-gamma and TNF-α in *BRCA1*-deficient murine ovarian cancer,[137] and PARP inhibition has also been shown to upregulate PD-L1.[138] Clinical trials of combination immunotherapy and DDR inhibitors are ongoing (Table 2).[4]

Conclusions and future challenges

We are at an exciting time in the clinical development of DDR inhibitors, where the dynamic interplay between DDR, therapeutic inhibition and the immune response offers a window of opportunity for the augmentation of anti-tumour effects.

For the patient-facing clinician, the take-home message is that a subset of cancers can be molecularly stratified for treatment with DDR inhibitor agents. While the frequency of DNA repair defects in each specific subtype of cancer remains

to be determined, access to molecular profiling is becoming more widespread and affordable. The identification of patients with DDR defects should prompt early referrals for inclusion into clinical trials of rational combinations of DDR inhibitors which will hopefully lead to improved patient outcomes and survival (Figure 3). Envisaging the future, adaptive combinatorial treatments with DDR inhibitors tailored to match evolving tumour profiles and their resulting vulnerabilities is likely to become a reality.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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