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REVIEW

Targeting DNA repair in cancer: current state and novel approaches

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Abstract
DNA damage response, DNA repair and genomic instability have been under study for their role in tumor initiation and progression for many years now. More recently, next-generation sequencing on cancer tissue from various patient cohorts have revealed mutations and epigenetic silencing of various genes encoding proteins with roles in these processes. These findings, together with the unequivocal role of DNA repair in therapeutic response, have fueled efforts toward the clinical exploitation of research findings. The successful example of PARP1/2 inhibitors has also supported these efforts and led to numerous preclinical and clinical trials with a large number of small molecules targeting various components involved in DNA repair singularly or in combination with other therapies. In this review, we focus on recent considerations related to DNA damage response and new DNA repair inhibition agents. We then discuss how immunotherapy can collaborate with these new drugs and how epigenetic drugs can rewire the activity of repair pathways and sensitize cancer cells to DNA repair inhibition therapies.

Keywords Anticancer therapy · DNA repair inhibition · Synthetic lethality

Introduction

The DNA repair machinery has evolved to cope with the endogenous and exogenous insults against the DNA of the cell. Faithful DNA repair is vital for maintaining cell and tissue homeostasis, which is evident by the fact that loss-of-function mutations in DNA repair factors cause genetic disorders and increased susceptibility to cancer. A complex network of sensors, transducers and effectors, coordinates the repair of DNA damage and ensures DNA replication fidelity.

Sensor proteins detect all types of DNA structural alterations, including nicks, gaps, double-strand breaks (DSBs), and replication lesions. Signal transducers are enzymes that control the activity of the cell cycle checkpoints and DNA repair pathways initiating signaling cascades to adjacent nucleoprotein structures. Effectors repair DNA damage and block progression through the cell cycle.

Defects in DNA repair pathways facilitate the accumulation of genomic alterations that contribute to their proliferation and survival of cancer cells. Nonetheless, tumors rely on residual DNA repair capacities to repair the damage induced by replication and genotoxic stress. Mammalian cells employ at least nine distinct pathways to repair a multitude of different genotoxic lesions: mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), translesion synthesis (TLS), homologous recombination (HR), non-homologous end joining (NHEJ), alternative end joining (alt-EJ), the Fanconi anemia (FA) and the O6-methylguanine DNA methyltransferase (MGMT). However, these pathways are neither completely independent of one another nor mutually exclusive processes handling different types of lesions in distinct cell cycle phases. Instead they form a precisely regulated network of multifunctional DNA repair hubs, which are involved in multiple DNA repair pathways. Multiple components of the repair network are deficient in cancer cells due to inactivating mutations or transcriptional silencing, affecting the functionality of different repair hubs and, therefore, the overall capacity for repair in conventional chemotherapeutic treatment.

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Next-generation sequencing has uncovered the frequency of mutations and copy number alterations across different cancer types and demonstrated that alterations of DNA repair mechanisms are common events in carcinogenesis. However, cancer types show different preferences for inactivation of specific DNA repair processes. For instance, mutations in HR genes appear to be enriched in breast and ovarian cancer as well as in bladder cancer, cutaneous skin melanoma and chronic lymphocytic leukemia. On the other hand, certain subgroups of stomach and colorectal adenocarcinoma as well as uterine endometrial carcinoma that harbor alterations in MMR present a hypermutator phenotype with low aneuploidy. Figure 1 presents examples of genomic and transcriptomic data of four tumor types showing distinct profiles in DNA repair components involving HR and MMR [1]. Other cancer types are characterized by enrichment of DNA

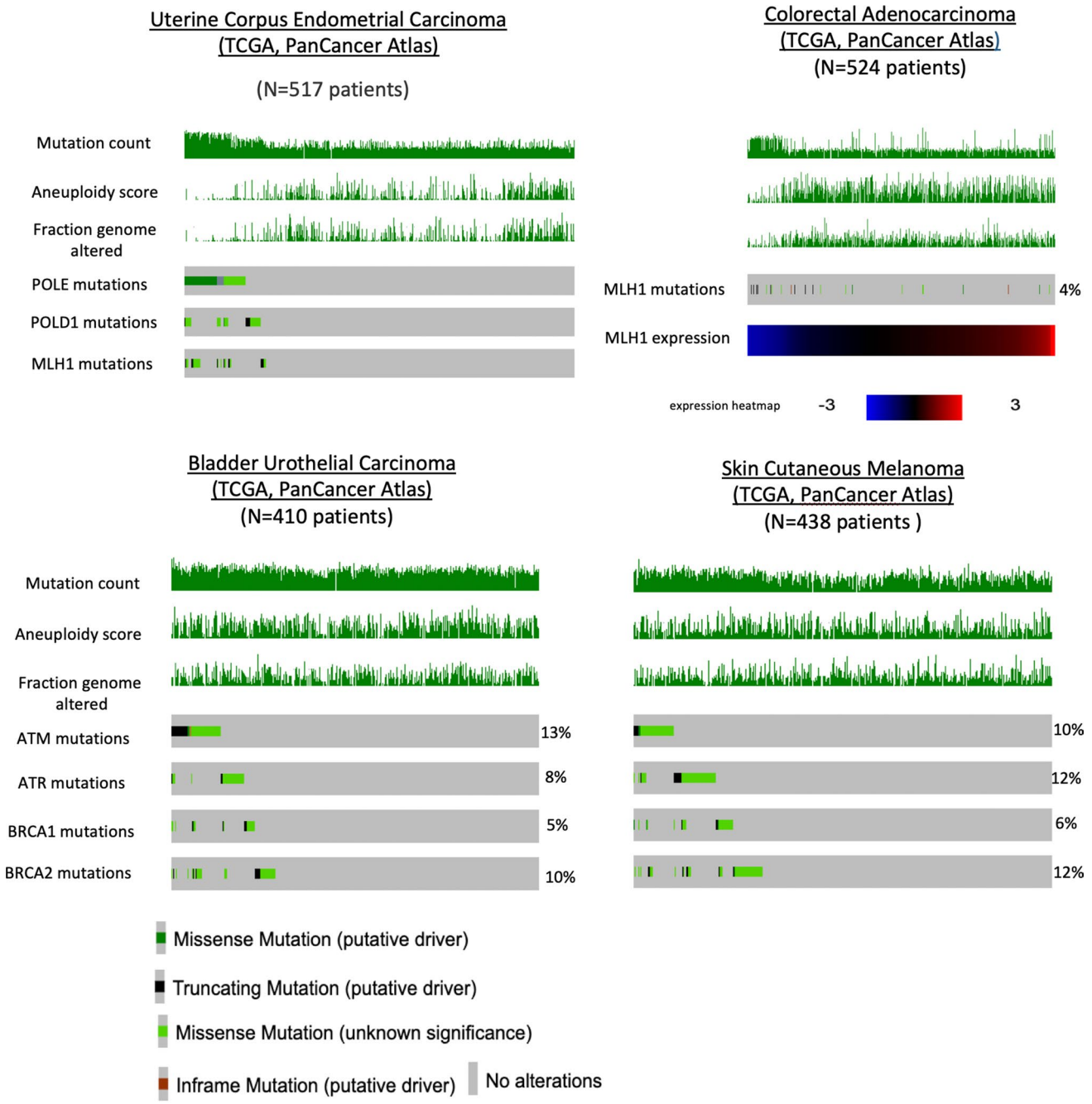

Fig. 1 Genomic and transcriptomic meta-analysis of different solid tumors. TCGA (Pancancer Atlas) mutation, expression and copy number alterations were processed using the cbioportal.org platform

repair deficiencies in more than one pathway. For example, prostate cancer accumulates inactivating mutations within the HR and NER pathways. Similarly, epigenetic deregulation and promoter DNA methylation cause transcriptomic silencing in different components of repair hubs across different cancer types. With increasing understanding of the mutational landscape and epigenetic/transcriptional profile of cancer genomes and the emerging role of DNA repair in tumorigenesis, there has been a shift toward the development of drugs that target specific components of the repair machinery. Cancer cells that harbor repair deficiencies in one DNA repair pathway/component often become hyperdependent upon remaining repair pathways for survival and proliferation. Therefore, knowing the profile of deregulated components of DNA repair networks can help us target specifically the compensatory DNA repair pathways in cancer cells. Next-generation sequencing that permits whole-genome mutational analysis brings forth the dawn of precision medicine, offering the opportunity for personalized treatment strategies based on the deficiencies of patient's DNA repair networks (Fig. 2).

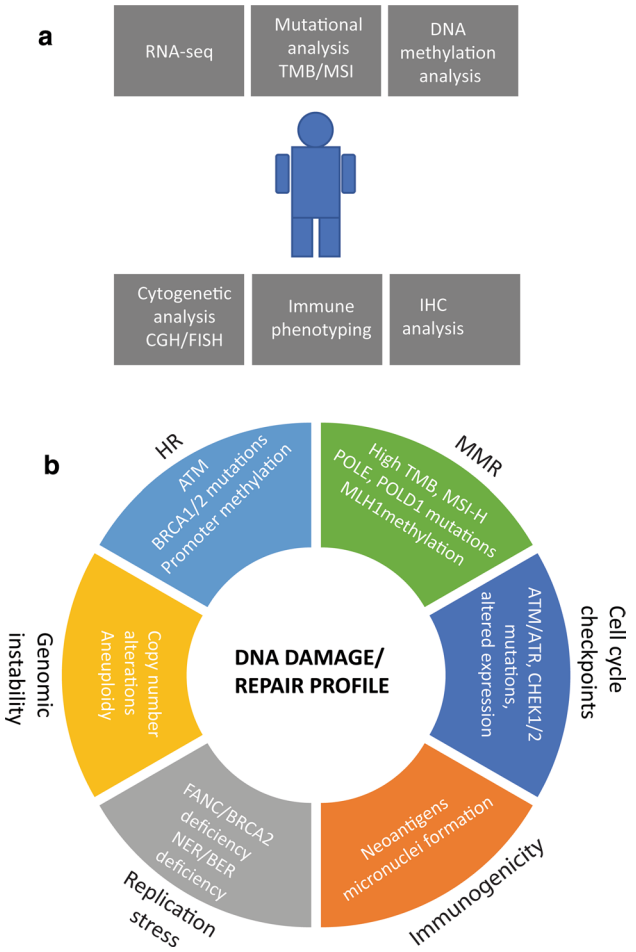

Fig. 2 Diagnostic profiling of defects associated with DNA repair in tumors. **a** Tools used for the molecular and histological characterization of tumor cells. **b** Known genetic alterations associated with compromised genome integrity in cancer cells

DNA damage response (DDR)

DDR involves recognition of double-strand breaks (DSBs) or single-strand breaks (SSBs), followed by initiation of an extended network of signaling cascades to promote DNA repair. These signal cascades can also activate cell cycle checkpoint arrest and apoptosis, and influence the aspects of DNA repair [2, 3]. These two aspects of DDR, DSB repair and checkpoint arrest cooperate to protect genomic integrity and their concomitant loss has a critical impact at the early steps of tumorigenesis. Defective DNA repair increases the mutational load and the genomic instability, whereas malfunctioning cell cycle checkpoints allow cells with DNA damage to proliferate.

DNA-PKs, ATM, ATR: initiation and activation of DDR

Like many intra-cellular signaling cascades, DNA damage signaling is driven by protein phosphorylation. Three kinases, ATM, ATR and DNA-PKs, have a principal role in activating DDR. These large proteins share similarities in domain organization and structure. Their kinase activity is located in the C-terminus and is very similar to that of the phospholipid kinase PI3K, with a specific preference to serine or threonine residue (Ser/Thr) phosphorylation [4–7]. ATM, ATR, and DNA-PKcs must be tightly regulated to prevent aberrant activation. Each kinase requires a specific protein co-factor for stable recruitment to DNA damage sites. DNA-PKc is recruited and activated at DSB ends by a Ku80-Ku70 heterodimer which plays a central role in the non-homologous end-joining (NHEJ) DSB repair pathway ([8, 9]. ATM is activated and recruited to DSBs by the MRE11-RAD50-NBS1 (MRN) complex [10]. ATR is recruited to replication protein A (RPA)-coated single strand DNA (ssDNA) by its stable binding partner ATRIP [11].

Loss of DDR components is an early event in carcinogenesis

In pre-cancerous cells, deregulation of cell cycle control and increased replication stress induce stalling and collapse of DNA replication forks, which in turn leads to DSB formation. This continuous formation of DSBs activates TP53 and DDR components such as ATM and ATR. While this activation represents a barrier to tumor progression, the loss of one or more DDR pathways and TP53 by mutations in early steps of carcinogenesis removes this barrier [12]. This is indeed supported by mutational data across major types

of solid tumors. TP53, ATM and ATR are highly mutated in bladder cancer (50%, 11.2% and 4.1%), lung adenocarcinoma (51.8%, 7.9% and 5.7%) and colorectal adenocarcinoma (58.6%, 5.7% and 2.1%, respectively) [13]. Defects in ATM and ATR genes significantly contribute to cancer initiation and progression via accrual of driver mutations and generation of tumor heterogeneity on a background of genomic instability.

The concept of synthetic lethality

As mentioned above, inactivating mutations in DNA repair components are common and often lead to certain DNA repair deficiencies. Cancer cells thus become hyperdependent on remaining repair pathways for survival and proliferation. Inhibiting the rescue pathway with a specific chemical agent can provide a context for synthetic lethality [14, 15]. Synthetic lethality, a concept first described in the 1920s [16–18], refers to the event where two viable gene mutations lead to cell death when they co-occur. This concept is particularly compelling for cancer therapy, because it allows specific targeting of cancer cells that carry a gene mutation that is not found in normal cells which are therefore spared.

Targeting PARP1

Targeting BRCA1/2-deficient cancers using poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) is a model example of synthetic lethality [19]. Rucaprib (Clovis), Olaparib (AstraZeneca) and Niraparib (Tesaro Inc.) are PARP inhibitors that have been FDA approved for the maintenance treatment of recurrent gBRCAm epithelial ovarian, fallopian tube and primary peritoneal cancer (henceforth referred to as ovarian cancer) which are in a complete or partial response to platinum-based chemotherapy. Importantly, these inhibitors are the first clinically approved drugs to exploit a synthetically lethal interaction in cancer therapy. More recently, Olaparib and a new PARP inhibitor, Talazoparib, were approved for the treatment of gBRCAm recurrent breast cancer (OlympiAD; EMBRACA) and Olaparib was approved as first-line maintenance treatment in ovarian cancer patients that respond to platinum-based chemotherapy (SOLO-1).

Accumulated evidence indicates that PARP1 functions as an important DNA damage sensor protein that recognizes both SSBs and DSBs and catalyzes the formation of linear chains of ADP-ribose residues (PAR chains). The PAR chains form a platform to recruit DNA repair proteins via their PAR-binding domains at the sites of DNA damage [20, 21]. Therefore, it is considered that PARylation can prime the activation of the DNA repair cascades via recruitment of DNA damage response factors to the region near DNA lesions. Upon inhibition, the PARP enzymes which bind to DNA lesions are unable to form signaling scaffolds and dissociate from the DNA [22–24]. These trapped PARP molecules add up to the existing lesions and increase the DNA damage burden of the cell [19, 25].

Homologous recombination (HR)-deficient cells, such as cells with BRCA1/2 mutations, are overly reliant (or "addicted") to other pathways for DNA repair, such as the NHEJ and alt-EJ [26]. The alternative modes of end joining require PARP1 to take place [27]. Therefore, when PARPs are inhibited in HR-deficient cells, there are more DNA lesions, compromised signaling activity and inability to utilize a vital DNA repair pathway. Importantly, PARPi has clinical benefit in non-BRCAm ovarian cancer patients which can be attributed to either unidentified HR deficiency, such as mutations in other HR genes, or to PARP1 actions which are independent of the alt-EJ.

Ongoing clinical trials using PARP1 inhibitors

Ongoing clinical trials examine the efficacy of licensed PARP inhibitors in various solid and hematopoietic malignancies and in combination with DNA damaging agents, and DDR, cell cycle and immune checkpoint inhibitors. The therapeutic strategies, for the most part, fall into one of the two main categories: exploitation of DNA repair defects in tumors using PARPi monotherapy or augmentation of the activity of other agents by combining them with PARPi to treat patients regardless of HR status.

The first category includes trials that use HR deficiency as a stratification biomarker for PARPi therapy through mutations in proteins directly associated with HR such as BRCA1/2 (NCT03344965 and NCT02952534), or other biomarkers suggestive of HR deficiency such as IDH mutations (NCT03561870) [28]. Among the most promising trials are POLO, in which Olaparib treatment shows benefit in BRCAm pancreatic cancer patients who responded to platinum-based chemotherapy, and TRITON2, in which preliminary results show that rucaparib monotherapy benefits BRCAm recurrent prostate cancer patients and is currently at phase III.

The second category of trials examines the efficacy of PARPi as part of a main course of combination treatment (Fig. 3). Traditionally, PARPi drug combinations have focused on DNA damaging agents and this is why the majority of combinations in current trials are with crosslinking agents, topoisomerase inhibitors and irradiation. Additionally, a number of trials aim to determine synergy of PARP inhibitors with other emerging therapeutic agents such as immune checkpoint inhibitors and angiogenesis inhibitors. The potential of immunotherapy to synergize with PARPi was demonstrated in recent literature on genomic instability and immune response [29]. Ongoing trials include ATHENA

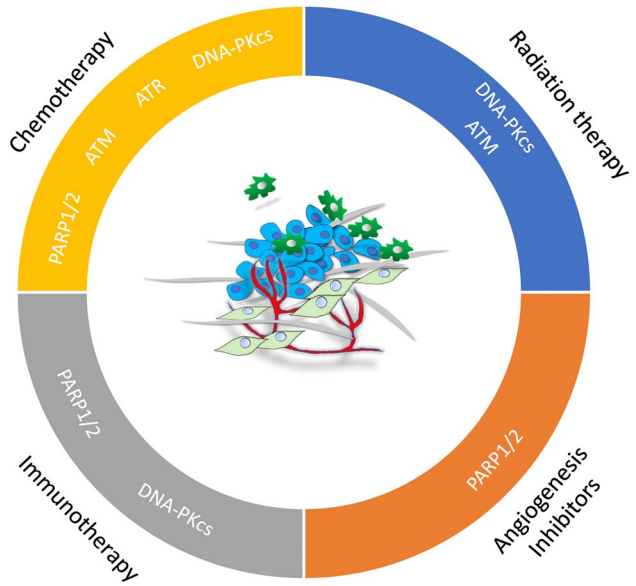

Fig. 3 Strategies for the combinatorial use of DDR inhibitors with known anticancer therapies

and FIRST in ovarian cancer, and KEYLYNK-010 in prostate cancer patients. On the other hand, synergism with angiogenesis inhibitors is based on their negative effect on HR, which in turn sensitizes cells to PARPi [30, 31].

A large number of trials examine novel PARP inhibitors in monotherapy as well as in combination with other agents. Veliparib has currently reached phase III in multiple trials (NCT02470585, NCT02163694 and NCT02264990) in combination with paclitaxel/carboplatin for the treatment of recurrent ovarian, breast and lung cancer patients. Notably, another promising PARP inhibitor, Iniparib, which in combination with gemcitabine/carboplatin reached phase III trials in breast and lung cancer (NCT00938652, NCT01082549), was reported to have significant off-target effects which halted further testing [32].

DDR network and exploitation of synthetically lethal interactions with PARP

Due to extensive crosstalk between pathways involved in DNA damage sensing, repair and cell cycle checkpoint activation pathways, therefore, are ideal candidates for exploitation of synthetically lethal interactions. To this direction, small molecule inhibitors have been designed against ATM, ATR and DNA-PKs and tested in phase I or II clinical trials either as single agents or in combination with chemotherapy or radiotherapy [33, 34].

After DNA damage, sensor proteins such as PARP1, H2AX and sensor complexes including the Ku70/80 and MRN (MRE11/RAD50/NBS1) directly recognize the structure of DSBs and SSBs and recruit ATM, ATR and DNA-PK proteins at the break sites. Like PARP1, the Ku complex, consisting of Ku70 and Ku80 subunits, functions as a DNA sensor protein, binding DSBs after DNA damage. However, PARP1 and Ku complexes mediate DSB repair pathway choice independently and through distinct mechanisms. Binding of the Ku complex to DSBs recruits and activates the DNA-PK catalytic subunit, which facilitates NHEJ [35], whereas the binding of PARP1 promotes alt-EJ [36]. Thus, the antagonism between PARP1 and the Ku complex at DSBs may play an important role in determining repair choices. In addition to PARP1 and Ku proteins, the MRN complex acting as a DNA sensor protein has the ability to bind DNA ends, recruiting and activating ATM [37]. ATM is the main kinase responsible for phosphorylation of H2AX, which is one of the earliest steps for the recruitment of additional DDR factors [38, 39].

ATM-deficient cells exhibit enhanced sensitivity to PARPi

As mentioned above, the rationale for using PARP inhibitors to treat HR-deficient cancers in the clinic is based on the high sensitivity of BRCA1- and BRCA2-defective cells to small molecule PARP inhibitors. As such, ATM inactivation, a known cause of HR deficiency [40], has been also shown to cause sensitivity to PARP inhibition [41]. Recent preclinical studies have focused on the functional interconnection between ATM and PARP1, which also appears to contribute to enhanced PARPi sensitivity [42]. Consistent with these findings, although knockout of PARP-1 and ATM individual does not lead to embryonic lethality, double-null mice die early in embryogenesis [43]. Genome-wide studies have revealed that in patients with metastatic castration-resistant prostate cancer (mCRPC), somatic ATM alterations are detected at a high frequency (5–10%) [44]. In a 49-patient phase II clinical trial, treatment with the PARP inhibitor olaparib of patients whose prostate cancers were no longer responding to standard treatments and who had defects in DNA repair genes (BRCA1/2, ATM, Fanconi's anemia genes, and CHEK2) led to a high response rate [45]. However, preliminary results from another clinical trial (TRITON2) indicate that mCRPC patients harboring ATM and CDK12 mutations seem to benefit less from the PARP1 inhibitor rucaparib compared to patients with BRCA1/2 loss [46].

Pairing ATR and PARP inhibitors

The partial responses to PARP inhibitors, even in BRCA1/2-mutant tumors, indicate the existence of intrinsic or acquired resistance mechanisms [47]. Such resistance mechanisms may include secondary mutations or promoter demethylation in the BRCA1/2 genes which partially restore HR activity

in cancer cells. Other resistance mechanisms include point mutations in PARP1 and drug-efflux pumps affecting drug pharmacokinetics. To overcome such resistance mechanisms as well as to treat non-HR-deficient tumors, the pairing of PARP inhibitors with other DDR pathway inhibitors, such as ATR inhibitors, is ongoing.

Approximately, 50% of high-grade serous ovarian cancer (HGSOC) has defects in genes involved in HR repair [48]. Olaparib monotherapy has modest clinical benefit in recurrent BRCA1/2-mutant HGSOCs, as it results in a 40% response rate, following first-line carboplatin–taxane chemotherapy [49]. Recent studies, however, have shown that PARPi increases the dependence on ATR activity for fork stabilization, and combination of PARPi with ATR blockade is more effective than PARPi alone BRCA-mutant ovarian cancer [50, 51]. Larger prospective studies are clearly needed to confirm or refute these preliminary findings.

Synthetic lethality beyond PARP1

There is a functional crosstalk between ATM, ATR and DNA-PKs in response to DNA damage.

Whereas ATM is primarily activated in response to DSBs, ATR is mainly activated by SSBs, and for this reason older models of DNA repair placed ATM and ATR in separate and distinct repair pathways. The current model proposes that both kinases cooperate for DSB repair during ionizing radiation (IR) or genotoxic stress. Recent studies have demonstrated that ATM and the nuclease activity of Mre11 are both required for the processing of DSBs to generate the RPA-coated ssDNA that is needed for ATR recruitment and Chk1 activation at S and G2 cell cycle phase [52–54].

ATM-mediated phosphorylation of DNA-PKs at Thr2609 is critical for DNA-PKs function in DNA repair and represents another example of functional crosstalk between DNA damage response proteins [55]. It has also been proposed that ATM phosphorylation at Ser1981 can be driven by ATR following replication fork stalling or UV treatment [56]. The survival of tumor cells with impaired ATM or ATR function after DNA damage is compromised and, therefore, the crosstalk between ATM, ATR and DNA-PKs can offer a chance for synthetic lethality when one of these kinases is inactivated (by mutation, deletion or transcriptional repression) in cancer cells.

Targeting the ATM deficiency with ATR inhibitors is an emerging antitumor strategy based on the high frequency of ATM dysregulation in cancer. Somatic mutations in the ATM gene are found in many solid tumors (breast, ovarian, colorectal, and prostate) [57–59]. Regarding hematological malignancies, inactivating mutations of ATM are present in about half the patients with mantle cell lymphoma and T cell prolymphocytic leukemia [60, 61]. There is now significant preclinical evidence supporting that ATM-deficient tumors are sensitive to ATR inhibitors and, therefore, ATM has a synthetically lethal relationship with ATR in chronic lymphocytic leukemia [62], mantle cell lymphoma [63] and mammary cancer cells [64]. Importantly, ATR has also been shown to mediate replication fork stability [65]. This broad role of ATR in many aspects of genome integrity is likely one of the reasons for the success of its inhibitors in the clinic, much like PARPi. The putative clinical significance of inhibiting DNA repair proteins is highlighted by the number of ongoing clinical trials with the use of small molecule inhibitors in monotherapy settings, the majority of which tumors with known DNA repair deficiencies are selected for treatment (Table 1).

Recent studies based on CRISPR/Cas9-mediated knockout and RNAi screens revealed that RecQ DNA helicase WRN is selectively essential in MSI models. Inactivation of WRN in microsatellite instable cells leads to lethality in vitro and in vivo, while it has no effect in microsatellite-stable cells, highlighting a new example of previously undocumented synthetically lethal interaction between MMR and the replication machinery [66, 67]. WRN is a multifunctional enzyme for genome integrity as it is involved in resolving complex DNA structures at replication forks or as a result of HR recombination. Such DNA structures are very common in MMR-deficient cells explaining the dependence of these cells to WRN activity [68].

DDR, genotoxic stress and cell cycle progression

Radiotherapy and chemotherapy with DNA-crosslinking agents that damage DNA are commonly used to treat cancer. Cisplatin, carboplatin, oxaliplatin and other similar platinum-based drugs make up a broad class of DNA-crosslinking agents that target rapidly dividing cancer cells by forming inter-strand crosslinks (ICLs) and disrupting DNA replication. IR used in radiotherapy causes multiple types of DNA damage, including DSBs and SSBs. IR also causes the formation of reactive oxygen species (ROS) which, in turn, promote the production of oxidized nucleotide adducts, such as 8-oxoguanine [69].

DDR defects in cancer can modulate the effectiveness of chemotherapy and radiotherapy

Chemotherapy and radiotherapy rely on the induction of DNA damage which is more cytotoxic for proliferating cells. Unlike normal cells, cancer cells are already burdened by genomic instability, replication stress and, by frequently malfunctioning DNA repair pathways, defects that cause genome-wide DNA damage. Because the apoptosis safety

Table 1 DDR inhibitors in monotherapy clinical trials

Agents	Target	Selected clinical trials	Disease	Selection factors	therapeutic regimen	Phase
FDA approved						
Olaparib	PARP1/2	NCT02184195 (POLO)	Metastatic pancreatic cancer	Platinum sensitivity; BRCAm[a]	Single agent; maintenance	III
		NCT03786796 (ORCHID)	Metastatic renal cell carcinoma	HRD[b]	Single agent	II
		NCT03561870 (OLA-GLI); PARADIGM-2	High-grade glioma	IDHm[c]	Combination with low-dose chemotherapy	II
Rucaparib	PARPi	NCT02975934 (TRITON3)	Metastatic castration-resistant prostate cancer (mCRPC)	HRD	Single agent	III
		NCT02505048 (RUBY)	Metastatic breast cancer	HRD	Single agent	III
		NCT03140670	Pancreatic adenocarcinoma	Platinum sensitivity; HRD	Single agent	II
Niraparib	PARP1/2	NCT02655016	Advanced ovarian cancer	Platinum sensitivity	Single agent; maintenance; 1st line	III
		NCT03709316	Advanced ovarian cancer	Platinum sensitivity	Single agent; maintenance; 1st line	III
		NCT03601923	Advanced pancreatic cancer	Platinum sensitivity; HRD	Single agent	II
		NCT03925350	Metastatic melanoma	HRD	Single agent	II
Talazoparib	PARP1/2	NCT01945775 (EMBRACA)	Advanced breast cancer	Platinum sensitivity; BRCAm	Single agent	III
		NCT02034916 (ABRAZO)	Advanced breast cancer	Platinum sensitivity; BRCAm	Single agent	II
Under evaluation						
Veliparib	PARP1/2	NCT02163694	Her2-negative advanced breast cancer	BRCAm	Combination with chemotherapy; 1st-2nd line	III
AZD6738	ATR	NCT03878095	Solid tumors	IDHm	Combination with olaparib	II
SRA737	CHK1	NCT02797977	Solid tumors	DNA repair deficiency	Combination with low-dose chemotherapy	I/II
LY2606368	CHK1/2	NCT02203513	High-grade serous ovarian cancer (HGSOC), fallopian or primary peritoneal cancer or triple-negative breast cancer (TNBC)	BRCAm	Single agent	II
		NCT02873975	Solid tumors	HRD or replication stress	Single agent	II

[a]BRCA mutated

[b]Homologous recombination deficient

[c]IDH mutated

check is often inactivated, cancer cells need to repair any DNA damage to continue to proliferate. DNA damaging by chemotherapy/radiotherapy exploits this principle and this is why they have been the cornerstone of first-line therapeutic schemes for many unresectable or metastatic malignancies for decades now. Although dysregulation of the DNA damage response is associated with cancer initiation and progression, it can also result in hypersensitivity or resistance of tumors to genotoxic cancer therapy.

For instance, ovarian cancer patients with hereditary mutations in BRCA1 or BRCA2 genes that impair HR activity are more sensitive to platinum agents [70]. Notably, the appearance of compensatory mutations in BRCA1 and BRCA2 that restore HR functionality in initially cisplatin sensitive tumors is able to develop cisplatin resistance [71]. In bladder cancer, patients with impaired NER pathway due to somatic ERCC2 mutations or low ERCC1 expression are more sensitive to platinum agents [72]. Furthermore, genomic alterations in ATM and FANCC genes predict response and clinical benefit after cisplatin-based chemotherapy for muscle invasive bladder cancer [73]. NSLC patients with ERCC1-negative tumors appear to benefit

from postoperative cisplatin-based chemotherapy, whereas patients with ERCC1-positive tumors do not [74]. Similarly, patients with ERCC1-negative locally advanced esophageal cancers benefit from preoperative chemoradiotherapy [75]. On the contrary, high ERCC1 expression has been positively correlated with cisplatin resistance in several human neoplasms including bladder [76], colorectal [77], gastric [78], head and neck [79–81] and ovarian cancers [82]. MMR deficiency due to mutations or downregulation of MSH2, MSH6 and MLH1 proteins has also been correlated with acquired cisplatin resistance. Functional MMR proteins recognize cisplatin adducts on DNA [83] and initiate proapoptotic signals [84] or generate gaps and strand breaks that lead to cell death [85]. Therefore, loss of MMR rescues cancer cells from apoptosis or cell death upon cisplatin treatment. Consistent with these observations, methylation-dependent silencing of MLH1 has been shown to predict poor survival in ovarian cancer patients [86]. In addition, patients with stages II and III MMR-deficient colon cancer do not benefit from fluorouracil-based adjuvant therapy [87].

Interestingly, defects in MLH1 and MSH6 are associated with increased level of translesion synthesis, which efficiently permits DNA synthesis in the presence of cisplatin adducts [88]. Replicative bypass of adducts by translesion synthesis is mediated by the coordinated activity of a specific group of DNA polymerases including POLH, POLK, and POLZ [89]. The activity of POLH and POLZ has a pivotal role on the replicative bypass of GpG adducts and is associated with cisplatin resistance [89, 90]. Defects in POLH and REV3L subunit of POLZ have been linked to increased sensitivity to cisplatin in multiple tumor cell lines [91], whereas POLH expression levels predict the survival of NSLC patients treated with platinum-based chemotherapy [92] and of metastatic gastric adenocarcinoma patients treated with oxaliplatin-based chemotherapy [93].

Despite the fact that platinum-containing chemotherapy and radiotherapy have been used in clinic for the treatment of a wide variety of solid tumors for many decades, intrinsic or acquired resistance during treatment cycles and high toxicity in patients are major obstacles that severely limit the clinical benefit.

Targeting of DDR components modulates cell cycle checkpoint activity

In the context of their role in genome integrity, ATM, ATR and DNA-PKs facilitate communication between damage recognition proteins and the cell cycle checkpoints (G1/S, intra-S and G2/M) to temporarily arrest the cell cycle and increase the opportunity for DNA repair before moving to the next stage of cell cycle. In cases of extensive DNA damage, cells permanently exit the cell cycle (senescence) or undergo programmed cell death (apoptosis) [94].

The G1/S checkpoint is believed to be controlled primarily by ATM rather than ATR. By contrast, both ATM and ATR contribute to the establishment and maintenance of the intra-S and G2/M checkpoints. The G1/S checkpoint allows the repair of DNA damage prior to the start of DNA replication, whereas the intra-S phase checkpoint can delay replication origin firing, providing time for DNA repair [95]. The G2/M checkpoint represents the last major barrier that prevents DNA damage from being transferred into mitosis which would lead to mitotic catastrophe and cell death [96].

Whereas ATM signals DSBs through the Chk2 checkpoint kinase, activated ATR in SSBs or in replication-linked DSBs signals through the Chk1 checkpoint kinase. In response to DSBs, the MRN complex recruits ATM to DSB sites. Once activated, the ATM/Chk2 pathway phosphorylates Cdc25A and p53 simultaneously within minutes promoting p53 stabilization, p21 upregulation, S-phase cell cycle arrest and activation of the p53-associated G1/S-phase checkpoint [97]. DSBs in the G2 can directly activate ATM, and indirectly, via ATM-dependent strand resection, ATR [54]. The G2/M checkpoint is initiated by the ATM/ATR-driven phosphorylation of CHK1 and CHK2 checkpoint kinases. In turn, these kinases mediate by phosphorylation (Ser216) the inhibition of Cdc25C. This prevents dephosphorylation of CDK1–cyclin B, which is required for progression into mitosis [98].

In response to SSBs at sites of DNA damage or stressed replication forks, RPA-coated ssDNA activates ATR and its binding partner ATRIP [11, 99, 100]. The kinase CHK1 is the key downstream regulator of the ATR response and is phosphorylated by ATR on Ser317 and Ser345 [65]. Activated CHK1 promotes by phosphorylation the proteasomal degradation of CDC25A and CDC25C which leads to a decrease in the CDK activity in S and G2/M cell cycle phase, thereby causing activation of intra-S and G2/M-phase checkpoints [101].

Chemical inhibition of ATM ATR, CHK1, CHK2 and Wee1 is a promising and attractive avenue for anticancer therapy because inhibition of these proteins can lead to cell cycle checkpoint abrogation (S and G2) in cancer cells. Checkpoint abrogation under genotoxic stress is associated with cell death due to excessive unrepaired DNA damage that is accumulated by rapid and unregulated cell cycle progression. In this direction, potent inhibitors of ATR, ATM, CHK1, CHK2, and WEE1 are under clinical evaluation. This family of compounds has been poorly tolerated in early monotherapy clinical trials [102, 103].

Combinatorial targeting of DDR and the G2/M checkpoint

Unrepaired DNA damage can be resolved before entering mitosis through activation of the G2 cell-cycle checkpoint.

Abrogation of the G2 checkpoint allows cells with unrepaired DNA damage to enter into premature mitosis resulting in mitotic catastrophe [104–106]. A strong rationale exists for combined therapy with ATR and WEE1 inhibitors. Given that ATR inhibitors promote origin firing, replication stress and DSB generation [107–111], the parallel WEE1 inhibition can abrogate the G2 arrest in these cells allowing chromatin with unrepaired DNA damage to enter into mitosis and undergo mitotic catastrophe [112, 113].

Similarly to ATRi, PARPi also induces replication stress and DNA damage [114]. Combined WEE1 and PARP inhibition has demonstrated antitumor activity in a number of preclinical models [115, 116]. However, overlapping WEE1i and PARPi toxicity profiles hinders the development of combinations, which has been largely confirmed in early clinical trials [117]. Interestingly, the sequential administration of PARP and WEE1 inhibitors seems to maintain efficacy while ameliorating toxicity [118].

DNA repair deficiencies and immunotherapy

Advances in immunotherapy have changed the treatment landscape in many cancers. Immune checkpoint inhibitors such as anti-CTLA4 and anti-PD1/PD-L1 antibodies have demonstrated successful clinical effect in a wide range of cancers. The anti-PD-1 monoclonal antibodies, nivolumab, atezolizumab and pembrolizumab, have been shown compared to standard systemic chemotherapy, to improve overall survival in head and neck cancer, advanced melanoma, non-small cell lung cancer, and urothelial cancer [119–122]. The development of predictive biomarkers is needed to optimize patient benefit, minimize risk of toxicities and guide combination strategies. Significant therapeutic responses have recently been observed in patients presenting tumors with high mutational burden that produce substantial levels of neoantigens [123–125]. Previous studies have shown that tumor mutation burden (TMB) is associated with increased T cell cytolytic activity supporting the notion that neoantigens can drive cytotoxic T cell responses [126].

Tumor mutation burden correlates with predicted neoantigens and immune infiltration

DNA repair safeguards genomic stability and the functional loss of components involved in this process can lead to high mutational burden due to the accumulation of DNA damage. Therefore, DNA damage defects are associated with acquired somatic mutations resulting in generation of neoantigens. Neoantigens are able to trigger the activation of cytotoxic T-cells [127] and make cancer cells more immunogenic. Since the cellular response to DNA damage determines the mutational load of cancerous cells, it has become clear that DNA damage and DNA repair activity have a major impact on the interaction between the tumor and the immune system and, furthermore, that the DNA damage and repair landscape have important therapeutic implications in the context of immunotherapy. A schematic representation of the crosstalk between DNA repair and immunological response is shown in Fig. 4.

Previous studies have reported that specific defects in DNA repair proteins could be potential predictive biomarkers of clinical response to immune checkpoint inhibitors in a wide spectrum of tumors. The most robust current evidence supporting the association between DNA repair deficiency, the TMB and the efficiency of immune checkpoint blockade (ICB) comes from tumors with loss of MMR function. Tumors with MMR deficiency (dMMR) have high response rates to ICB, and the FDA recently approved pembroluzimab for treatment of microsatellite instability-high (MSI-H) colorectal tumors [128]. In microsatellite-unstable endometrial cancer, due to mutations in the exonuclease ('proofreading') domain of polymerase DNA polymerase ε (POLE), there is increased mutation burden and, as a result, a higher number of cytotoxic T tumor-infiltrating lymphocytes (TILs), compared with microsatellite-stable tumors [129]. Clinical and immunological response to immune checkpoint inhibition with pembrolizumab has been also demonstrated in hypermutated glioblastoma with POLE mutations [130].

Several reports have also linked somatic mutations (BRCA1/2, RAD51, ATM, ATR, PTEN) leading to HR repair deficiency with higher neoantigen levels and ICB response [131–134]. Similarly, in urothelial cancer, tumors harboring alterations in DNA damage response genes ATM, POLE, FANCA, ERCC2, and MSH6 were correlated with high TMB and improved clinical outcomes to ICB [135].

In human cancers, a deficiency in a DNA repair pathway can result in dependence on a compensatory DNA repair pathway [136]. HR-deficient tumors are hyperdependent on polymerase θ (POLQ)-mediated repair. POLQ appears to channel DNA repair by antagonizing HR and promoting PARP1-dependent alt-EJ repair [137]. POLQ and alternative end-joining activity have been described to be intrinsically mutagenic [138, 139], explaining the high neoantigen levels of HR-deficient tumors.

BRCA1/2-mutated ovarian tumors have been associated with higher neoantigen loads, higher T cell infiltration and improved overall survival compared to HR-proficient tumors [134]. Patients with BRCA2 mutated melanoma were found to have better response to anti-PD-1 treatment [132]. Furthermore, microsatellite-unstable tumors harboring mutations in MMR genes are characterized by TMB and enhanced response to immune therapy [124, 140].

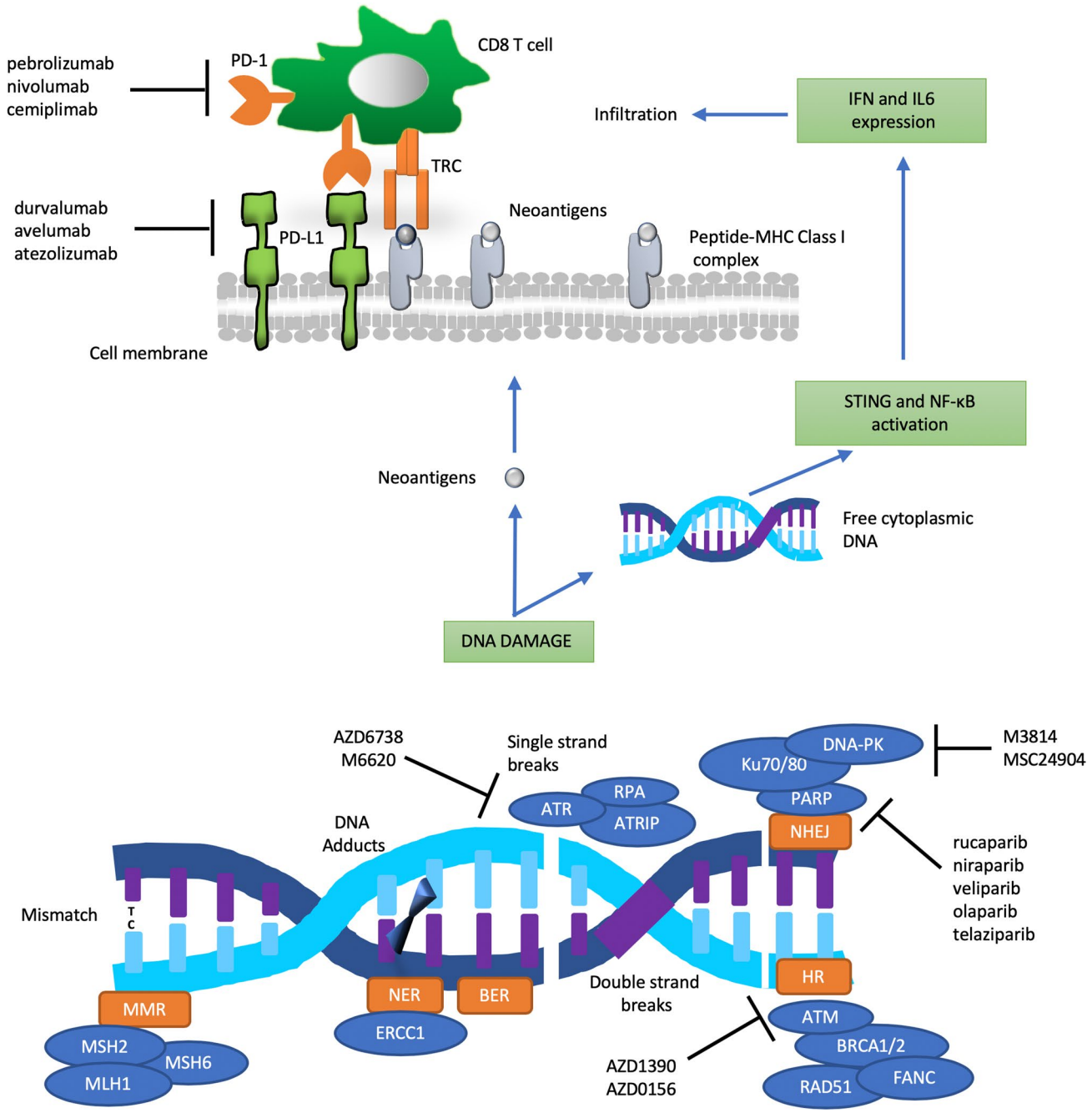

Fig. 4 Therapeutic targeting of the DNA repair–immune response interconnection in cancer. Approved and under evaluation anticancer agents targeting distinct components of the DNA repair machinery as well as modulators of the immunological response

DNA repair defects that generate intratumoral heterogeneity may confer to immune escape

An important aspect of defective DNA repair and cell cycle checkpoints is persistence of unrepaired DSBs during M phase, which lead to mis-segregation of chromosomes and chromosomal instability (CIN) [141–143]. CIN is characterized by an increased rate of gains and losses of fragments or whole chromosomes, leading to aneuploidy, and is a principal driver of tumor heterogeneity [144, 145]. Heterogeneous tumors contain multiple subclones and under selection pressure, such as chemotherapy or immunotherapy, subclones with either intrinsic or acquired resistance can survive the pressure and potentially drive disease progression.

Although ICB induces significant responses in many patients, response rates vary significantly both within and

across tumor types. The use of next-generation sequencing and single-cell sequencing from different sites within the same tumor (multi-region analysis) has revealed a high degree of genetic heterogeneity within the same tumor (intratumoral heterogeneity) [146–149]. Comparative analysis of tumor subclones between the primary tumor and distant metastases from the same patient has also revealed differences, arising either from evolution of subclones that were either present at the primary tumor or through emergence of subclones that represent new branching points during the evolutionary process and the Darwinian selection. DNA repair defects and epigenetic alterations in tumor progression have a crucial role in sub-clonal evolution.

Due to intratumoral heterogeneity, PD-L1 expression levels may vary among different tumor subpopulations and, therefore, clones with low PD-L1 expression can drive immune escape. In clinic, a substantial number of patients with PD-L1 positivity (at least 40–50%) do not achieve objective response to anti-PD-1/PD-L1 therapies [150].

Oncogenic mutations may also drive immunotherapy resistance in specific subclones. Activation of the canonical WNT-β-catenin signaling pathway in melanoma cells has been shown to correlate with suppression of the chemokine CCL4, reduced T cell infiltration and resistance to anti-PD-L1 and anti-CTLA-4 mAb-based therapy [151]. Oncogenic activation of the PI3-kinase pathway due to PTEN loss in melanoma cancer cells can also promote resistance to immune therapy [152]. Similarly, activation of the epidermal growth factor receptor (EGFR) pathway has been associated with immunosuppression in lung adenocarcinoma [153].

There is growing evidence that intratumoral neoantigen heterogeneity is associated with low response to ICB therapy. Recent studies in non-small cell lung cancer (NSCLC) have demonstrated that tumors with high levels of clonal neoantigens have improved responses to ICB and that the loss of clonal neoantigens due to elimination of tumor subclones or through deletion of chromosomal regions can lead to ICB resistance [154, 155]. In addition, genomic instability can lead to evolution of immunotherapy resistant subclones that harbor somatic copy number loss of neoantigens that are critical to immune response [156]. An analysis of more than 5000 TCGA tumors across 12 tumor types demonstrated that high levels of somatic copy number alterations (SCNAs) are associated with reduced expression of cytotoxic immune cell markers [157].

DNA repair targeting agents and immunotherapy

Previous reports have shown that DNA damage that arises from genotoxic stress can activate the STING pathway, an innate immune pathway activated by cytoplasmic DNA [158]. Cyclic guanosine monophosphate (GMP)-AMP synthase (cGAS) is a DNA sensor that triggers innate immune responses through production of the second messenger cyclic GMP-AMP (cGAMP), which binds and activates the adaptor protein STING. Activated STING triggers phosphorylation and nuclear translocation of IRF3 which in turn activates transcription of inflammatory genes [159, 160]. cGAS is considered to detect DNA damage derived by the formation of micronuclei during mitotic progression and leakage of DNA into the cytosol [161]. Activation of the cGAS/STING pathway leads to expression of interferons and chemokines and has been shown to attract and activate immune cells in the tumor microenvironment [162, 163].

Recently, Dunphy et al. showed that detection of genotoxic stress-induced DNA damage by ATM and PARP1 can activate the DNA sensor adaptor STING independently of the cytosolic DNA receptor cGAS and cGAMP production through a non-canonical pathway. According to this alternative pathway, the non-canonical STING signaling complex that includes the tumor suppressor p53 and the E3 ubiquitin ligase TRAF6 promotes the activation of an NF-kB-dependent transcriptional program [164].

There is pre-clinical evidence that DDR inhibitors, such as PARP inhibitors, activate the STING pathway and the type I IFN signaling in BRCA1 or ERCC1-deficient cancer cells, stimulating augmented cytotoxic-T cell infiltration [165, 166]. In addition, it has been proposed that PARP inhibition promotes adaptive overexpression of cell surface PD-L1 in these cells [167, 168]. Based on the biological evidence for the immunomodulatory function of DDR inhibitors, various ongoing clinical trials investigate the efficiency of combinatorial treatment with PARP inhibitors and immune checkpoint inhibitors targeting the PD-1 and/or PD-L1 (NCT03834519, NCT03851614, NCT03602859, NCT03308942).

In vitro and in vivo mouse experiments in pancreatic cancer showed that combination of ATM inhibition with radiation enhanced antigen presentation and type I interferon signaling. Furthermore, ATM silencing increased PD-L1 expression and increased the sensitivity of pancreatic tumors to PD-L1 blocking antibodies in association with increased tumoral CD8+ T cells and established immune memory. Interestingly, low ATM expression was inversely correlated with PD-L1 expression in clinical samples of patients with pancreatic tumors in the same study [169]. Further work is needed to uncover the mechanisms by which PARP inhibitors or other DNA repair-directed agents (such as ATM, ATR, or DNA-PKcs inhibitors) modulate the immune response and sensitivity to IC blockade.

Epigenetic factors and chromatin remodelers as components of the DNA repair machiner: the chromatin context

Recent advances in epigenetics have shed light onto the dynamic changes of chromatin modifications after DNA damage, supporting a model where specific chromatin-remodeling factors are essential components of the repair network [170]. The study of how these epigenetic and chromatin-remodeling factors modulate the chromatin dynamics and cooperate with DNA repair proteins in response to genotoxic stress is important for the identification of new synthetically lethal interactions. Therefore, pharmacological targeting of these epigenetic factors could be a new approach to enhance the efficacy of DDR inhibitors in producing DSB cytotoxicity and mitigate drug resistance.

Chromatin-remodeling and epigenetic factors mediate dynamic alterations of chromatin structure that facilitate DSB repair

Regulating the accessibility of damaged DNA to repair complexes requires a high degree of coordination between DSB repair machineries and chromatin-modifying enzymes. One of the best characterized changes in chromatin organization is the rapid formation of open chromatin structures at DSBs. The response to DSBs requires localized chromatin expansion to facilitate the assembly of repair complexes. A large number of studies, based on biochemical approaches, highlight that the chromatin expansion at the sites of damage is characterized by density loss of core and linker histones. This chromatin decompaction is thought to facilitate access of the repair machinery to damaged DNA. In this direction, recent work in human cells showed that the CHD2 remodeling activity promotes euchromatin expansion at IR-induced DSBs and the recruitment of NHEJ factors. Interestingly, recruitment of CDH2 at the sites of damage is directed by PARP1 catalytic activity [171].

Several groups have also demonstrated that the rapid formation of open chromatin is driven by an increased acetylation of histone H4 (H4K16ac) on nucleosomes, a modification that extends for many kilobases away from the break, following the spreading pattern of γH2AX [172–175]. The N-terminal tail of histone H4 interacts with the acidic patch on the surface of H2A-H2B dimers of adjacent nucleosomes. Disruption of this interaction by acetylation of histone H4 at DSBs promotes chromatin unpacking and formation of open, relaxed chromatin structures detected at DSBs [176]. Acetylation of H4K16 at DSBs is carried out by the histone acetyltransferase TIP60 and its inactivation is sufficient to block chromatin decompaction during DNA damage [175].

The chromatin state has also been shown to influence DNA repair pathway choice. After DNA damage and ATM activation, γ-H2AX recruits a number of repair factors to DNA damage sites. Among these are the E3 ubiquitin ligases RNF8 and RNF168 which generate ubiquitin marks on histones near the breaks. RNF8 promotes K63-linked polyubiquitylation of H1 linker histones [177, 178]. This polyubiquitylation is a recruitment signal for RNF168, which in turn ubiquitylates H2A-type histones at K13/K15 [177, 179, 180]. TP53BP1, a DSB pathway choice protein that actively promotes NHEJ and inhibits BRCA1-mediated HR, is strongly dependent on RNF8/RNF168-mediated chromatin ubiquitylation and binds to monoubiquitylated H2A-K15 [179].

The role of H3K36me3 histone marks in DNA mismatch repair

MMR maintains genome stability primarily by correcting single nucleotide misincorporations, and small insertion/deletion loops (IDLs) created by the DNA polymerase, improving replication fidelity [181]. In human cells, these mismatches are recognized by hMSH2–hMSH6 (hMutSα) and hMSH2–hMSH3 (hMutSβ) protein complexes. The MSH2–MSH6 complex primarily recognizes single base pair mismatches and 1–2 base IDLs while MSH2–MSH3 recognizes larger IDLs. Both MSH6 and MSH3 harbor a conserved N-terminal PIP (PCNA-interacting protein) motif and interact with the proliferating cell nuclear antigen (PCNA) in replication forks recognizing errors. Defective expression of components of these complexes leads to a mutator phenotype and MSI. Recently, it was demonstrated that H3K36me3 marks, generated by the histone methyltransferase SETD2, facilitate the binding of hMutSα complex to the chromatin [182]. Consistent with this, cells depleted of SETD2 fail to recruit hMutSα to chromatin and display a mutator phenotype characterized by MSI [182]. Lines of evidence have also highlighted a role for H3K36 dimethylation (H3K36me2) on the choice for NHEJ repair pathway [183].

Epigenetic context and nuclear architecture components impact heterochromatin DSB repair

In eukaryotic cells, euchromatin (EC) that contains the majority of the transcribed genes is loosely packed compared to heterochromatin (HC). Centromeres, pericentromeric regions, telomeres and repetitive elements comprise the constitutive heterochromatin, while developmentally silenced genes constitute the facultative heterochromatin. Constitutive heterochromatin contains histones that are hypoacetylated and hypermethylated at histone H3 lysine 9 (H3K9me2/3) and lysine 27 (H3K27me3). These histone

marks are associated with anchoring to nuclear lamina [184]. The writing and maintenance of these histone modifications require a large number of epigenetic factors including the histone methyltransferases SETDB1, SUV39 (H3K9 methylation) and histone deacetylases 1 and 2 (HDAC1/2) [185]. The H3K9me2/3 histone marks are specifically recognized and bound by the heterohromatin protein 1 (HP1) which recruits other proteins such as the KAP-1 and the SMARCAD1. The KAP1 protein recruits epigenetic factors that maintain the chromatin compaction (SETDB1, HDAC1, HDAC2), while the SMARAD1 facilitates the deacetylation of newly assembled histones during replication to maintain gene silencing [186]. In response to DNA damage, the chromatin compaction in heterochromatin and the dense array of proteins that bind its domains present a barrier for DSB repair factors to access the sites of damage. Furthermore, the presence of repetitive DNA elements within heterochromatin can initiate aberrant homologous recombination events during repair, such as sister chromatid exchanges or interchromosomal recombination, leading to deletions, duplications, translocations, and formation of dicentric or acentric chromosomes. Therefore, heterochromatin DSB repair requires a more stringent control of HR to prevent inappropriate recombination events. Upon formation of heterochromatic DSBs, ATM phosphorylates the HP1-interacting protein Kap1 (KRAB-associated protein-1), thus reducing the strength of Kap1 interaction with damaged heterochromatin, and promoting the release of the chromatin modifier CHD3.1 (Chromodomain Helicase DNA binding protein 3). The release of CHD3.1 drives chromatin relaxation which provides access to repair complexes [187, 188].

Recent studies in Drosophila and mouse cells have shown a relocation of heterochromatin DSBs to anchoring points at the nuclear periphery. This relocation ensures safe and precise HR while preventing aberrant recombination, by isolating the DSBs and their templates away from ectopic sequences before strand invasion [189]. Notably, SUMO and the SUMO E3 ligases dPIAS and Nse2 are required to relocalize DSBs to the nuclear periphery [190, 191].

The role of chromatin remodelers in BER and NER

In eukaryotes, BER is the major pathway for the repair of alkylatively and oxidatively generated lesions such as 8-oxoguanine (8-oxoG). BER is initiated by a glycosylase, which cleaves the glycosidic bond that attaches the lesion to the sugar-phosphate backbone and generates an abasic site. Eleven glycosylases have been identified in humans and are categorized based on their structural architecture [192]. The apurinic/apyrimidinic sites are bound by the endonuclease APE1, which cleaves the DNA backbone on the 5′ side of the abasic deoxyribose phosphate, creating a nick in the DNA [193]. The synthesis step of BER employs either repair polymerase Pol β which adds a single nucleotide, or one of the processive polymerases Pol δ or Pol ε, adding up to 13 nucleotides to the 3′ hydroxyl group of the nucleotide 5′ of the nick [194]. The remaining deoxyribose phosphate is removed by the dRPase activity of Pol β, whereas the 5′ stretch of nucleotides when added by Pol δ or Pol ε is cleaved by the flap endonuclease FEN-1 [195]. The final step of BER is ligation of the nicked strand by DNA ligase IIIα in a complex with its partner protein XRCC1.

For execution of this multi-step process, it is necessary that DNA is accessible to all components of BER. Each step of BER requires access to DNA for its enzymatic activity. A number of studies in cells have demonstrated an inverse correlation between the level of chromatin compaction and BER activity [196]. Several in vitro studies also indicate that chromatin remodeler activity is sufficient to dramatically increase the BER efficiency. In vitro experiments showed that purified members of SWI/SNF subfamily and purified ISW1 and ISW2 chromatin remodelers significantly facilitate the glycosylase, APE-1 and polymerase synthesis step during BER [197–199]. It is considered that chromatin remodelers provide accessibility to BER repair proteins by either remodeling, or combined remodeling and sliding mechanisms.

NER is the major pathway for repair of bulky DNA lesions caused by UV, environmental mutagens and cancer chemotherapeutic drugs. Two distinct DNA damage recognition cascades can activate NER, depending on the location of DNA damage. Genome NER (GG-NER) is activated by helix distortions associated with DNA lesions anywhere in the genome. The main damage sensor in GG-NER is the XPC–RAD23B–CETN2 protein complex. Transcription-coupled NER (TC-NER) is activated by stalled RNA Pol II during transcript elongation by a lesion in the template strand [200].

Similarly to BER repair pathway, numerous in vitro NER assays have shown that the nucleosome structure can be a barrier to efficient NER function and purified SWI/SNF complexes increase accessibility of damaged DNA and stimulate NER repair were found to be within in vitro reconstituted mononucleosomes [201, 202]. However, it is not clear yet whether SWI/SNF complexes are involved in early NER steps facilitating XPF recruitment and lesion detection, or are recruited by XPF and promote the binding of late NER factors XPG and PCNA. The latter is supported by experiments showing that knockdown of chromatin remodelers BRG1, BRM and ARID1A/B has no effect on XPC recruitment but can impair the recruitment of late NER factors ERCC1 and XPA [203, 204].

Given the broad role of SWI/SNIF complexes in NER and BER repair pathways and the high incidence of mutations in family members across different cancer types, the exploitation of SWI/SNF deficiency-induced

susceptibilities is crucial for the development efficient and precise therapies for SWI/SNF-mutated cancers.

Histone methyltransferases KMT2C/D and stalled fork stability

KMT2C and KMT2D belong to the family of mammalian mixed-lineage leukemia (MLL) genes that encode histone methyltransferases, responsible for monomethylation at H3K4 at a subset of active gene promoters and enhancers [205]. The distribution of H3K4me1 marks across cis-regulatory elements is characteristic of accessible enhancer regions and, therefore, KMT2C/D activity in enhancers is positively correlated with transcriptional activation of neighbor genes. Recent studies that focus on enhancer regulation also indicate that KMT2C/KMT2D can promote transcription independently of their methylation activity [206, 207]. Cancer genome sequencing studies have identified the histone methyltransferases KMT2C and KMT2D among the most frequently mutated genes across different types of solid tumors [13]. Besides the inactivation of these genes by loss of function mutations, their expression is significantly downregulated across different cancer types [208].

Recent studies have also uncovered an important role of KMT2C and KMT2D proteins in genome stability and in precise control of DNA repair. Chaudhuri and collogues showed that recruitment of MRE11 nuclease to stalled replication forks in HR-deficient cells is mediated by KMT2C/KMT2D [209]. Stalled replication forks are featured by exposed DNA ends in the form of ssDNA or dsDNA, which makes them susceptible to various cellular nucleases that generate HR substrates during DSB repair, including MRE11, which promote 3′–5′ short-range resection during initial steps of homologous recombination repair. The MRE11 nuclease activity at the sites of replication stress is tightly control to prevent excessive fork degradation by BRCA1/2 and RAD51. More specifically, during replication stress, BRCA1 and BRCA2 proteins relocate to stalled replication forks and promote the formation of stable RAD51 nucleoprotein filaments, thereby suppressing deleterious fork degradation mediated by the MRE11 nuclease [210, 211]. In BRCA1/2-deficient cells, stalled replication forks are unprotected from MRE11 activity and, therefore, these cells are characterized by increased genomic instability and chemosensitivity [212–214]. However, KMT2C/D inactivation in BRCA1/2-deficient tumors decreases MRE11 recruitment to stalled forks, which in turn restores fork stability, and renders BRCA1/2-deficient cells resistant to cisplatin and PARP inhibitors [209], indicating a general resistance mechanism to genotoxic stress.

Dysregulated epigenome as a driver for DNA methylation and transcriptional silencing of DNA repair genes

Comprehensive analyses of human cancer genomes over the past decade have revealed that epigenetic factors and chromatin-remodeling complexes are highly mutated in cancer. Since epigenetic factors regulate precisely gene transcription, mutations on these factors in cancer cell offer an adaptive plasticity to the transcriptome. Dysregulated epigenome in cancer is often associated with altered chromatin context in enhancers and promoters of DNA repair genes, which favors DNA methylation and transcriptional silencing. DNA methylation represents the epigenetic biomarker with the highest translational potential due to its stable nature.

Methylation of lysine 27 on histone 3 (H3K27me), a modification associated with gene repression, is a focal point of epigenetic deregulation in cancer [215]. The genetic basis of H3K27me deregulation may include either components of the H3K27 methyltransferase complex PRC2 (Polycomb repressive complex 2) and associated proteins or the H3K27 demethylase UTX [216]. Several studies have demonstrated that H3K27me3-marked genes are targets for aberrant DNA methylation in cancer cells [217, 218].

MSI has been reported in 15% of sporadic colorectal cancer cases which represent a distinct molecular subtype characterized by hypermutator phenotype and global CpG methylation [219]. Most tumors in this subtype have lost expression of the MLH1 DNA mismatch repair gene due to acquired DNA hypermethylation of the MLH1 promoter region [220]. As a result, tumors of this subtype are deficient for DNA mismatch repair (MMR) and exhibit a hypermutable phenotype.

BRCA1 and BRCA2 proteins play a basic role in the regulation and promotion of HR. Germline mutations in these genes are the most important causes of hereditary breast and ovarian cancer [221]. In addition to germline mutations, somatic mutations and epigenetic silencing of these genes occur in a variety of cancers in the general population. BRCA1 methylation is observed in approximately 11–14% of breast cancers and 5–31% of ovarian cancers [222]. BRCA1 methylation has also been detected in 18.6% and 12.1% of non-small cell lung (NSCLC) and bladder cancer (BLCA), respectively [223, 224]. BRCA2 methylation has been reported in NSCLC [225].

ATM is also epigenetically silenced in primary head and neck and breast cancers by aberrant methylation in promoter region [226, 227].

Individuals with Fanconi anemia (FA) are predisposed to develop ovarian cancer than those without FA; this is largely contributed to promoter methylation of the FANCF gene and subsequent disruption of the FA–BRCA pathway [228, 229]. Epigenetic inactivation of FANCF has also been proposed

as a mechanism of sensitization to platinum chemotherapy [230].

In bladder cancer (BLCA), the excision repair cross-complementing group 1 (ERCC1) gene, encoding a key enzyme of the nucleotide excision repair (NER) pathway, and the RBBP8/CtiP gene that has an important role in HR repair exhibit a tumor-specific promoter methylation in up to 17% and 45% of BLCA patients, respectively [231]. It, therefore, is evident that epigenetic status is sufficient to cause DNA repair defects and should, therefore, be considered in patient selection for targeted therapies.

Transcriptional silencing of HR DNA repair genes due to epigenetic deregulation as predictive biomarker for response to PARP1 inhibition

Methylation of DNA repair genes seems to represent a good predictive biomarker for response to PARP inhibitors. For instance, in high-grade serous ovarian carcinoma (HGSOC), patient-derived xenografts (PDXs) with homozygous methylation of BRCA1 alleles have been found to respond to PARP1 inhibitor rucaparib. Moreover, clinical data from HGSOC patients who participated to ARIEL2 Part 1 clinical trial indicate a correlation between high levels of BRCA1 methylation in homozygous status and response to PARP inhibitors [232]. Experiments with breast cancer cell lines have also demonstrated that the status of BRCA1 methylation is correlated with sensitivity to PARP1 inhibition. In these experiments, BRCA1 silencing as well as PARPi sensitivity was abolished by the demethylating agent 5-azacytidine [233].

Recently, the DNA methyltransferase inhibitors (DNMTi) and PARP inhibitors (PARPi) have been reported to act synergistically inducing cell death in acute myeloid leukemia (AML), breast and ovarian cancers [234, 235]. The most widely used DNMTis are the cytosine analogs 5-azacytidine (Aza) and 5-aza-2′-deoxycytidine (Decitabine). These analogs are incorporated into DNA during replication leading to the formation of DNMT-DNA adducts that inhibit the catalytic activity of DNMT1 leading to global DNA demethylation [236, 237]. Besides epigenetic effects, the DNMTis are also able to increase the PARP-1 trapping at the DNA damage sites, enhancing the DSBs cytotoxic effects induced by PARP inhibitors [238].

There is accumulated evidence that deregulation of epigenetic factors that promote open chromatin conformation in promoters of HR genes can confer to their transcriptional silencing in a subset of cancers. Previous studies have demonstrated the involvement of the histone methyltransferase EZH2 in inducing epigenetic silencing of RAD51 [239, 240]. Moreover, EZH2 expression was shown to be associated with the activation of RAF1–MEK signaling and expansion of breast cancer stem cells [240].

Recently our group showed that KMT2C binds the promoter region of BRCA1/2 and other HR repair genes controlling their transcription in bladder cancer cell lines. KMT2C silencing in these cell lines leads to BRCA1/2 hypo expression, HR deficiency and increased sensitivity to PARP inhibitor olaparib. Meta-analysis of TCGA RNA-seq data showed that KMT2C downregulation is associated with BRCA1/2 downregulation in bladder, head and neck, lung and colon cancer [208].

HDAC and BET inhibitors can suppress the expression of HR genes and synergize with PARPi

HDAC activity has been linked to histone deacetylation that is often associated with chromatin condensation and gene repression. They are also part of complexes involved in transcription silencing [241, 242] and it has been proposed that in cancer, HDACs activity mediates the transcriptional repression of tumor suppressor genes [243, 244]. Pharmacological inhibition of these enzymes by HDAC inhibitors causes global changes to the chromatin acetylation landscape, re-shaping the boundaries between transcriptionally active and quiescent chromatin. This results in transcriptome changes including re-expression of silent genes and repression of highly transcribed genes [245]. Interestingly, recent studies have shown that the expression of repair factors that are critical components of HR repair pathway is sensitive to HDAC inhibition. More specifically, the HDAC inhibitor vorinostat has been shown to suppress the transcription of DNA damage repair proteins, such as RAD50 and MRE11, and induce DNA DSBs in human prostate cancer cells (LNCaP), and human lung adenocarcinoma cells (A549) [225]. The ability of HDACs inhibitors to suppress HR repair leads to sensitization of cancer cells to PARP inhibition [246, 247] and to the DNA damaging agents doxorubicin and cisplatin [248, 249]. Combination of chemo/radiotherapy with HDACi in phase I and phase II clinical trials has been well tolerated and showed encouraging efficacy [250–252], while investigation of HDACi in combination with PARPi is also underway in a phase I trial (NCT03742245).

The bromodomain and extraterminal domain (BET) family comprises four members (BRD2, BRD3, BRD4, and BRDT) and is the best characterized class of acetylation readers. These proteins bind hyper-acetylated chromatin regions as active promoters or enhancers and serve as scaffolds for the recruitment of transcription factors that promote the transcription of target genes [253, 254]. BRD4 is the best characterized member of this family and has been heavily implicated in transcriptional regulation and tumorigenesis [255]. BRD4 localizes on both gene promoters and enhancers and has been shown to

accumulate specifically on regulatory regions termed "super-enhancers" [256]. BET inhibitors have been shown also to impair the HR pathway by directly downregulating BRCA1 and RAD51 expression. This finding has provided a rationale to use BET inhibitors to sensitize cancer cells to PARPi, showing preclinical efficacy in breast and ovarian cancer models [257–259], and is now being further investigated in the clinic (NCT03901469). Figure 5 outlines basic aspects of epigenetic regulation of DNA repair and possible therapeutic implications, while Table 2 summarizes the clinical trials with the use of DNA repair inhibitor in combination with targeted and conventional anticancer therapies.

Concluding remarks

For many years, the study of DNA repair was fueled by the early findings indicating a tumor suppressor role for the BRCA1/2 proteins, which were commonly mutated in gynecological primarily cancers. With the identification of mutations of other DDR and DNA repair proteins in cancer, the field expanded. Nevertheless, the primary focus of these studies was the role of DDR and DNA repair proteins in early steps of carcinogenesis and particularly the resulting genomic instability. Genomic instability leads to mutagenic events which are rightfully considered to drive carcinogenesis as well as contribute to tumor heterogeneity, a feature that we know now is crucial for therapeutic resistance. We now know that the response of cells to DNA damage is a complex mechanism involving multiple

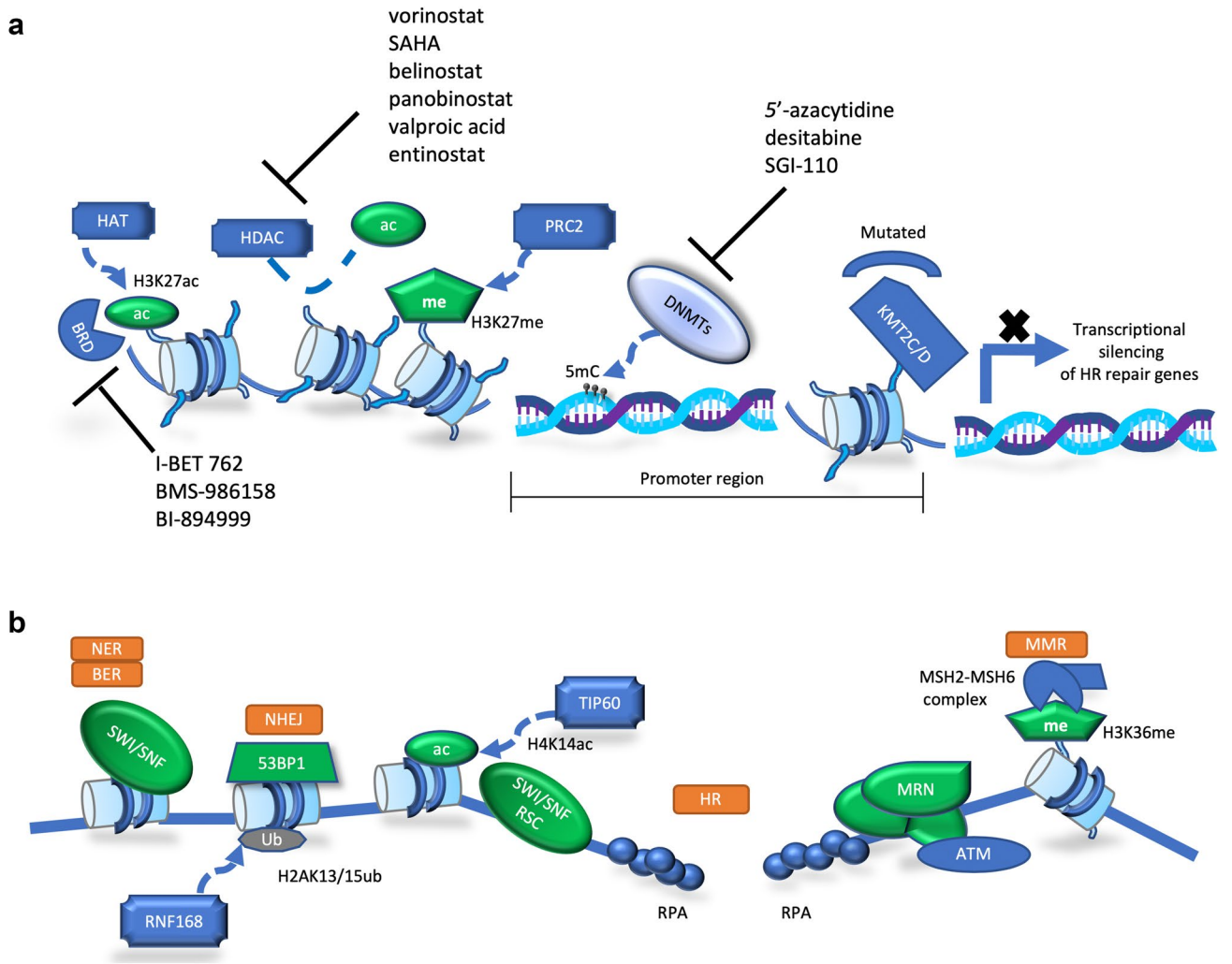

Fig. 5 Therapeutic implications of the epigenetics of DNA repair. **a** Epigenetic regulation of HR repair components by histone modifiers, histone binding proteins and DNA modifying enzymes. **b** Direct involvement of epigenetic regulators in different DNA repair processes

Table 2 Combinatorial schemes of DDR inhibitors in clinical trials

Agents	TARGET	Selected clinical trials	Disease	Therapeutic regimen	PHASE
FDA approved					
Olaparib	PARP1/2	NCT03150576 (PARTNER)	Triple-negative breast cancer	Combination with chemotherapy; neoadjuvant	II/III
		NCT02446600, NCT02502266	Platinum-sensitive high-grade ovarian cancer	Single agent and combination with angiogenesis inhibition	III
		NCT03834519 (KEYLYNK-010)	Metastatic castration-resistant prostate cancer (mCRPC)	Combination with immunotherapy	III
		NCT03851614 (DAPPER)	Advanced colorectal or pancreatic cancer or leiomyosarcoma	Combination with immunotherapy	II
		NCT03742245	Breast cancer	Combination with HDAC inhibition	I
Niraparib	PARP1/2	NCT03602859 (FIRST)	High-grade non-mucinous ovarian cancer	Single agent and combination with immunotherapy; switch maintenance	III
		NCT03308942	Advanced non-small cell lung cancer	Single agent and combination with immunotherapy	II
		NCT03574779 (OPAL)	High-grade ovarian cancer	Combination with immunotherapy and angiogenesis inhibition	II
Rucaparib	PARP1/2	NCT03476798	Cervical or endometrial carcinoma	Combination with angiogenesis inhibitor	II
Talazoparib	PARP1/2	NCT03642132 (JAVELIN OVARIAN PARP100)	High-grade serous ovarian cancer	Maintenance after 1st line chemotherapy; single agent or combination with immunotherapy	III
		NCT03672773	Small cell lung cancer	Combination with low-dose chemotherapy	II
		NCT03901469	Triple negative breast cancer	Combination with BET inhibition	II
		NCT02878785	AML or R/R AML	Combination with DNA demethylating agent	I/II
Under evaluation					
Veliparib	PARP1/2	NCT02264990	Advanced non-small cell lung cancer	Combination with chemotherapy; 1st line	III
		NCT02470585	High-grade serous ovarian cancer	Combination with chemotherapy; 1st line and maintenance	III
AZD6738	ATR	NCT03740893 (PHOENIX)	Triple-negative breast cancer	Single agent; neoadjuvant and adjuvant	II
		NCT03682289	Any solid tumor except ovarian cancer	Single agent or combination with olaparib	II
		NCT03462342 (CAPRI)	High-grade serous ovarian cancer	Combination with olaparib	II
M6620	ATR	NCT03896503	Small cell lung cancer	Combination with chemotherapy	II
		NCT02595892	High-grade serous ovarian cancer	Combination with chemotherapy	II
		NCT03517969	Metastatic castration-resistant prostate cancer (mCRPC)	Combination with chemotherapy	II
		NCT03641313	Gastric or GEJ adenocarcinoma	Combination with chemotherapy	II
		NCT03787680 (TRAP)	Metastatic castration-resistant prostate cancer (mCRPC)	Combination with olaparib	II
AZD1390	ATM	NCT03423628	Brain tumor—GBM or Solid tumor metastasis	Combination with radiation	I
AZD0156	ATM	NCT02588105 (AToM)	Solid tumors	Single agent or combination with olaparib or chemotherapy or other	I

Table 2 (continued)

Agents	TARGET	Selected clinical trials	Disease	Therapeutic regimen	PHASE
M3814	DNA-PKcs	NCT03770689	Rectal	Combination with chemotherapy	I/II
		NCT03724890	Advanced solid tumors	Combination with radiation and/or immunotherapy	I
MSC2490484A	DNA-PKcs	NCT02516813	Advanced solid tumors	Combination with chemotherapy and/or radiation	I
LY3300054	CHK1	NCT03495323	Advanced solid tumors	Combination with immunotherapy	I

networks of proteins and interconnected functions which are responsible for damage detection, cell cycle regulation and DNA repair. In this process, other chromatin modifiers and epigenetic regulators seem to play particularly important roles. Next-generation sequencing revealed that DNA repair proteins are frequently mutated in sporadic cancers from various anatomic sites. Moreover, for the first time we realized that epigenetic regulators are frequent targets of mutation events in cancer. Beyond mutations, there is accumulating evidence that gene downregulation as a result of epigenetic silencing is another mode of loss-of-function events involving loci encoding proteins that function in the context of DNA repair and also epigenetic regulation.

Physicians have been exploiting therapeutically the defects ensuing from mutations in proteins involved in DNA repair. Challenging a system with DNA repair defects with additional damage induced by genotoxic agents has been particularly successful in certain cancers with DNA repair deficiencies. Yet, the complexity of the cellular machinery in charge of DNA repair and the large number of proteins involved in this process allow cells to develop resistance to therapy. At the time, however, this complexity allows the identification of additional targets which can be targeted therapeutically in cancers carrying silenced alleles or mutated proteins with roles in DNA repair. Cell or organismal death as a result of combinatorial mutations was first described in Drosophila 100 years ago. This phenomenon, later coined as synthetic lethality, has been fueling research in the field of cancer for over a decade now. As novel inhibitors are developed and more synthetically lethal interactions are being described, promising avenues for anticancer therapy are being opened. Recent advances in our understanding of how DNA damage modulates the immune response and how repair is regulated by the epigenome have spurred interest in investigating novel drug combinations in treatment of resistant disease. Molecular characterization of each and every single tumor will provide the ground for a more personalized therapeutic exploitation of novel therapeutic options, hopefully leading to the highly praised Precision Medicine.

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