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Review

# Targeting DNA repair as a promising approach in cancer therapy

*Giovanna Damia, Maurizio D'Incalci**

*Department of Oncology, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, 20157 Milan, Italy*

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ABSTRACT

An increased DNA-repair activity in tumour cells has been associated with resistance to treatment to DNA-directed drugs, while defects in DNA repair pathways result in hypersensitivity to these agents. In the past years the unravelling of the molecular basis of these DNA pathways, with a better understanding of the DNA damage caused by different anticancer agents, has provided the rationale for the use of some DNA repair inhibitors to optimise the therapeutic use of DNA-damaging agents currently used in the treatment of tumours. In addition, the possibility to specifically target the differences in DNA repair capacity between normal and tumour cells has recently emerged as an exciting possibility. The present review will mainly cover those approaches that are currently under clinical investigation.



## 1. Introduction

Ionising radiation (IR) and most chemotherapeutic agents currently used in the treatment of cancer, directly or indirectly damage DNA, causing the formation of DNA-breaks (single strand breaks (SSB) or double strand breaks (DBS), DNA-DNA or DNA-protein cross-links) and interfering with important DNA-interacting proteins, e.g. DNA-topoisomerases. The cellular response to these DNA lesions is orchestrated in such a way that the detection of the damage activates a number of signal transduction pathways leading to cell cycle arrest and thus allowing repair, or if the damage is too heavy, induction of apoptosis.[1,2] In the former case the cell fate will be survival, whilst in the latter case, the cell fate will be death. In this scenario the repair activity of the cell is profoundly interplayed with all the other cellular responses to the damage and is an important determinant of cell sensitivity to anticancer agents. Indeed, it has been reported that resistance to DNA-damaging agents can be associated with increased cellular repair activities while defects in DNA repair pathways result in hypersensitivity to these agents.[3–5]

The importance of DNA-repair in cancer treatment is based on the following considerations:

i) most chemotherapeutic agents, including ionising radiations, act causing DNA damage and their effects are influenced by the efficiency of DNA repair pathways;
ii) somatic or inherited mutations in DNA repair genes have been described in tumours determining a selective loss of function that can be exploited to obtain anti-tumour selectivity. In fact, tumour cells will rely much more than normal cells on the remaining functional DNA repair mechanisms and the targeting of these pathways could have a favourable impact;
iii) increased levels of DNA repair proteins have been correlated with resistance to anticancer agents acting as DNA-damaging agents;

* *Corresponding author:* Tel.: +39 2 39014473; fax: +39 2 3546277.
E-mail address: dincalci@marionegri.it (M. D'Incalci).
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iv) the unravelling of the DNA repair pathways and the definition of their molecular partners have made available potentially 'druggable' targets suitable for the identification of new anticancer therapies or for the enhanced anti-tumour activity of DNA-damaging agents.

This review aims at highlighting the mechanisms through which manipulation of DNA repair pathways could be exploited to optimise the therapeutic use of agents currently used in the treatment of tumours mainly focussing on those approaches that are currently under clinical investigation.

## 2. Brief overview of the main DNA repair pathways

As depicted in Fig. 1 cells are endowed with DNA repair machineries able to detect and repair the many different lesions caused by chemical and physical DNA damaging agents. A detailed description of the biochemical basis of the DNA repair pathways is beyond the scope of this article, but it might be helpful for the readers to have a brief outline of the cellular repair pathways. Most of these pathways involve general repair factors such as DNA helicases, replication factor C, proliferating cell nuclear antigen (PCNA), DNA polymerases and ligases and even if each repair pathway recognises specific DNA lesions, more than one of them can cooperate to repair different types of DNA damage.

### 2.1. *Enzyme $O^6$-alkylguanine-DNA-alkyltransferase (AGT/MGMT)*

The enzyme $O^6$-alkylguanine-DNA-alkyltransferase (AGT/MGMT) transfers the methyl/alkyl adducts from the $O^6$ position of guanine to the cysteine residue within its active site, by a direct repair process. This protein is unique in its ability to remove the methyl/alkyl group from the $O^6$ position of guanine independently from any other proteins and without causing DNA strand break. In addition, it repairs with a stechiometric and auto-inactivating reaction rendering this repair system saturable. Many alkylating agents, including temozolomide, streptozotocin, procarbazine, dacarbazine and nitrosoureas, cause $O^6$ adducts that are repaired by MGMT.[6–9]

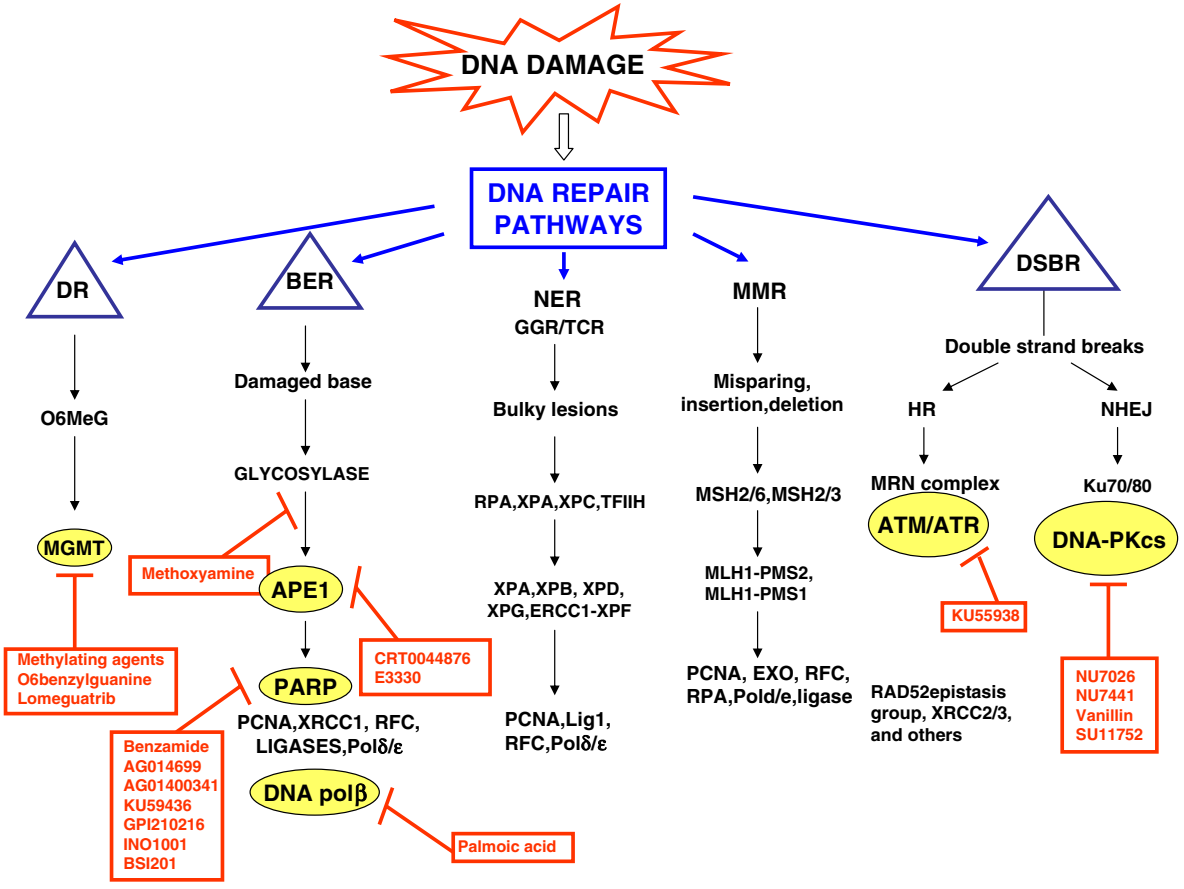


**Fig. 1 – Schematic representation of the main DNA repair pathways and the inhibitors under investigation. DR: direct repair; MGMT: $0^6$-alkylguanine-DNA-alkyltransferase; BER: base excision repair; APE1: apurinicendonuclease; PARP: poly(ADP-ribose)polymerase; NER: nucleotide excision repair; GGR: global genome repair; TCR: transcription coupled repair; MMR: mismatch repair; DSBR: double strand break repair; HR: homologous recombination; NHEJ: nonhomologous end joining. Triangled pathways are the ones for which an inhibiting strategy has been pursued. The yellow highlighted proteins are the targets to which inhibitors have been selected.**

### 2.2. Base excision repair (BER)

The base excision repair (BER) pathway recognises and removes damaged bases such as oxidised-reduced, alkylated and deaminated bases, caused by environmental mutagens and by anticancer agents, such as alkylating agents and ionising radiation.[10] In this process the damaged base is removed by a DNA glycosylase with the formation of a potentially cytotoxic apurinic or apirimidinic (AP) site, that is processed by an AP endonuclease (APE1), with the generation of a strand break. Replacement of the damaged base and re-ligation of the DNA involve binding of poly(ADP-ribose)polymerase (PARP) and recruitment of DNA polymerase $\beta$ and ligase III. The broad substrate specificity of BER relies on the existence of diverse damage-specific glycosylases that remove the damaged bases.

### 2.3. Double strand breaks (DSBs)

Double strand breaks (DSBs) are generally considered the most lethal of all DNA lesions.[11–13] They can be induced by IR, free radicals and many anticancer agents or when the replicative fork encounters a single strand break (SSB) and are dealt with both by an error-free repair pathway (homologous recombination repair-HR and single strand annealing-SSA) and by an error-prone pathway (non homologous end joining repair-NHEJ). The HR repairs the DSBs through the alignment of homologous sequence on the sister chromatid and is mediated by the *RAD52* epistasis genes and is the predominant repair pathway during G2 phase of the cell cycle. SSA is similar to HR and is dependent upon homologous sequences flanking the site of break. The NHEJ is the main route for the repair of DSBs in G0/G1 phases of the cell cycle and it involves the alignment of the broken ends through Ku70/Ku80 eterodimers and the recruitment and activation of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). DNA-PKcs processes the DNA ends before XRCC4 and DNA ligase IV along with other factors to facilitate the final ligation step. During this process, however, DNA residues are lost at the side of the DSBs so that NHEJ can be mutagenic.

### 2.4. Nucleotide excision repair (NER)

The nucleotide excision repair (NER) is certainly the most versatile DNA repair mechanism recognising and dealing with bulky, helix-distorting lesions, such as the ones induced by UV (cyclobutane dimers and 6–4 photoproducts).[14] This is a multi-step process with many (more than twenty) different proteins involved in sequential steps that deal with lesion recognition, damage removal and DNA synthesis. Two major pathways can be recognised in NER: the transcription coupled repair (TCR) and the global genome repair (GGR); the former is a highly specific and efficient system that detects and removes the DNA damage that blocks the progression of RNA polymerase II, while the latter is a slow process that inspects the entire genome. Cell derived from Xeroderma Pigmentosum or Cockaine Syndrome patients are deficient in NER and are exquisitively sensitive to cisplatin and alkylating agents, whereas they are resistant to Trabectedin,[15–17] a marine natural product that binds in the minor groove of DNA. In fact for Trabectedin evidence exists that the activation of NER following exposure with the drug causes cytotoxicity through a mechanism still to be elucidated.[18]

### 2.5. Mismatch repair (MR)

The mismatch repair removes biosynthetic errors from newly synthesised DNA and in particular it efficiently recognises base-base mismatches and insertion/deletion loops improving the fidelity of DNA replication by several orders of magnitude. Lack of MR is responsible for a mutator phenotype and predisposes to cancer. In addition it has been clearly demonstrated how the cellular MR status influences the cytotoxic activities of different anticancer agents such as (mainly) cisplatin, methylating agents and some antimetabolites.[19]

### 2.6. The Fanconi anaemia/BRCA (FA/BRCA) pathway

The Fanconi anaemia/BRCA (FA/BRCA) pathway is involved in the repair of interstrand DNA cross-links possibly through the coordination of NHEJ, HR and postreplication/translesion DNA synthesis pathways.[20] Clinically this pathway is important as many anticancer agents cause DNA crosslinks, e.g. cisplatin, mytomicin, cyclophosphamide, melphalan, nitrosoureas.

## 3. Targeting DNA repair pathways

Since the function of DNA repair systems is to protect living organisms from the mutagenic and toxic lesions caused by DNA-damaging agents, it is understandable that any strategy aimed at inhibiting DNA repair enzymes is inherently associated with a high degree of risk to produce damage to normal tissues. For this reason DNA-repair inhibitor strategies have been directed to enzymes/pathways for which there are reasons to believe that some degree of selectivity for some human neoplasms can be achieved. In addition it should be considered that many of the proteins involved in some complex DNA repair pathways, e.g. NER or MR, do have a role in other important cellular pathways and/or important physiological backup functions and inhibition of their activities might have deleterious side effects for normal cells. This explains why the available inhibitors of DNA repair that are under clinical investigation are so far directed to pathways such as MGMT, BER and PARP, involving few steps with key proteins and for which some degree of selectivity seems to exist.[21,22]

### 3.1. Targeting MGMT

The repair protein $O^6$-alkylguanine-DNA alkyltransferase, also known as $O^6$-methylguanine-DNA methyltransferase (MGMT), acts by transferring the alkyl groups present on the $O^6$ position of guanine to a cysteine residue in the active site of the protein.[7–9,23] Therefore, the repair process of $O^6$-alkylguanine occurs without generating DNA breakage.

This repair mechanism is evolutionarily well conserved from bacteria to mammalian cells, as repair methylation of $O^6$-guanine can occur as an endogenous metabolic reaction and is highly mutagenic. MGMT is not a typical enzyme as

the transfer of the alkyl group to a cysteine residue inactivates the protein (i.e. suicide enzyme), that is then ubiquinated and digested by the proteosome.

It has been known for more than 20 years that MGMT is involved in the mechanism of resistance to several anticancer drugs that act as methylating agents, like temozolomide, dacarbazine and procarbazine, or as chloroethylating agents such as chloroethylnitrosoureas like BCNU, CCNU or fotemustine.[23–26]

Plenty of preclinical data support the notion that there is an inverse correlation between the amount of MGMT and the sensitivity to methylating and chloroethylating agents of cancer cell lines and xenografts. It has been suggested that the reason why most murine tumours are extremely sensitive to nitrosoureas is that these tumours have a very low expression of MGMT. These considerations have provided the rationale for studies conducted on clinical samples aimed at establishing a link between the tumour sensitivity to alkylating agents and MGMT expression. The most convincing data have certainly been obtained in primary CNS tumours. Higher overall survival was reported in patients with osteocytoma and glioblastoma treated with CCNU combined with radiotherapy when the tumour concentration of MGMT was low.[27] Other studies indicate that when the levels of MGMT in osteocytomas and glioblastomas were low, the response rate to temozolomide was 60% compared to 9% in the cases in which tumour concentrations of MGMT were high.[28]

More recently the efficacy of both CCNU and temozolomide in glioblastoma patients was found to be correlated to the methylation status of the promoter of the MGMT gene. The most extensive studies were performed by Hegi et al.[29] who found that MGMT promoter was methylated in 45% of 206 glioblastoma cases investigated. The survival benefit observed in patients treated with temozolomide and radiotherapy was significantly greater for patients whose tumours contained a methylated MGMT promoter. Esteller et al.[30] found that methylation of MGMT is a useful indicator of responsiveness of gliomas to alkylating agents such as nitrosoureas. Levin et al.[31] found that in oligodendrogliomas the response to temozolomide treatment was associated with 1p deletion and low protein expression. The importance of MGMT for the response to alkylating agents has been highlighted not only in studies performed in adult glioblastomas but also in paediatric CNS tumours treated with methylating agents.[32,33] Interesting to note that MGMT promoter methylation was found to be a useful marker for predicting survival of patients with diffuse large B cell lymphomas too,[34] independent from other relevant molecular alterations such as 10q loss or p53 expression.[35]

MGMT also appears to play a role in the modulation of response to poisons of DNA-topoisomerase I,[36] an effect presumably related to the fact that $O^6$methylguanine containing DNA appears to favour the enzyme entrapment compared to the same sequence containing not alkylated guanines.

### 3.1.1. *Depletion of MGMT*

The well documented evidence that a major mechanism of resistance to methylating or chloroethylating agents is related to an increased expression of MGMT, led to the development of methods to deplete MGMT as a strategy to overcome drug resistance. Two different approaches have been pursued: an indirect inhibition of MGMT by the use of methylating agents and a direct inhibition by using analogues of guanine.

The first approach involves the methylation of $O^6$ guanine by methylating agents, then subsequent removal of the methyl group by MGMT that is inactivated by the repair reaction itself, as previously explained.[7,8,25] The extent of the depletion of MGMT is related to the number of methylguanine present in DNA and thus ultimately to the methylating agent dose, whereas the duration of the depletion will depend on the cellular rate of biosynthesis of new MGMT molecules. In several cell lines the turn-over time of MGMT has been estimated to be around 4 h, thus suggesting that giving a first dose of a methylating agent, e.g. temozolomide, followed after 4 h by a second dose of the same methylating agent or of a chloroethylating agent, e.g. BCNU or fotemustine could result in a potentiation of the effect. This approach with multiple doses of drugs given with selected intervals on the MGMT depletion concept have been shown to be feasible,[37–39] but large studies to assess the antitumour efficacy compared to standard protocols are still to be reported. The most likely reason for the lack of these studies is related to the fact that in small pilot studies the bone marrow toxicity appeared to be higher than expected with sequential combinations.[8,37,40] A further reported drawback was related to lung toxicity (manifested as interstitial pneumonitis) that was shown in patients receiving sequential treatment with dacarbazine and nitrosoureas.[41,42]

A weak aspect of most published studies is that most information was obtained by determining the enzyme level in lymphocytes,[8,37] that does not necessarily reflect what happens in the tumour. A further limitation is the finding that MGMT levels assessed by immuno-histochemistry appear to be variable in different areas of the same tumour, e.g. melanomas. Thus, if a significant MGMT depletion is achieved, this does not mean that all tumour cells have a similar depletion of the enzyme. A variety of different proportions of cells with low or high levels of MGMT may co-exist; the cells that express high concentrations of MGMT presumably being resistant.

The second approach involves the use of direct MGMT inhibitors. Most available data on the two inhibitors that have been developed in the clinic, $O^6$-benzylguanine ($O^6$-BG) and $O^6$-(4-bromothenyl) guanine (lomeguatrib) are herein shortly reviewed. $O^6$-BG is a non-toxic inhibitor of MGMT that works by transferring the benzoyl group to the cysteine residue in the active site of the protein. The preclinical activity of $O^6$-BG was demonstrated both in cultured cells and in xenografts, where it was shown to restore the sensitivity to nitrosoureas.[43] Phase I clinical trials indicated that $O^6$-BG can effectively suppress MGMT activity, but the doses required were different for different tumours.[44–47] For example, 100–120 mg/m$^2$ $O^6$-BG were effective in causing a complete depletion of MGMT in gliomas. The combination of $O^6$-BG at the dose of 120 mg/m$^2$ with BCNU at the reduced dose of 40 mg/m$^2$ showed no activity in resistant glioblastomas or melanomas.[48] These studies are difficult to interpret because the tumour levels of MGMT were not assessed. It may be that the dose reduction of BCNU, required to limit bone marrow tox-

icity, was the reason for the failure. In recurrent progressive gliomas, a loading dose of 120 mg/m$^2$ of O$^6$-BG over 1 h followed by a continuous infusion of 30 mg/m$^2$ for 48 h – effective in depleting tumour levels of MGMT – was combined with temozolomide at doses up to 472 mg/m$^2$, higher doses being too bone-marrow toxic. Although the results of the studies are not available yet, it appears that the bone-marrow toxicity represents the main drawback of the combination, not allowing the use of temozolomide at full doses.

O$^6$ (4-bromothenyl)guanine (lomeguatrib) appears more promising than O$^6$-BG. It has shown good bioavailability when given by an oral route. It works by transferring the bromothenyl group to the cystein residue in the active site of MGMT, thus inactivating it. The co-administration of lomeguatrib at the dose of 10 mg/m$^2$, which is effective in causing depletion of MGMT in tumour tissues, with temozolomide appears to be feasible with a dose reduction of temozolomide less than that required for O$^6$-BG. Lomeguatrib also increases the bone-marrow toxicity of temozolomide but, for unclear reasons, in a less severe way compared to other inhibitors and this justifies a significant clinical interest and phase II trials are ongoing.

Since methylating agents produce DNA breaks it has been suggested to combine them with inhibitors of PARP. Studies have been initiated to assess whether temozolomide activity can be enhanced by the concomitant use of PARP inhibitors. If this approach proves successful, as preliminary data seem to indicate, one can envisage the possibility of combining temozolomide with a MGMT depleting agent, e.g. lomeguatrib, and with a PARP inhibitor. These combinations should overcome the resistance mechanisms related to DNA repair, but presumably might increase the toxicity on normal tissues too, as discussed in previous sections there may be some selectivity in tumours exhibiting specific DNA repair defects.

### 3.2. *Targeting BER pathway*

As mentioned above, BER is able to recognise and repair the DNA damage caused by alkylating agents. Preclinical studies suggest that it can represent an attractive target for anticancer therapy as its inhibition has been shown to sensitise cells to the cytotoxic effects of different alkylating agents.[49] BER can be inhibited by small molecules that bind to the AP site preventing its processing by the AP endonuclease/redox effector factor-1 (APE1) enzyme, by molecules that directly target the function of APE1 enzyme and by interfering with Pol$\beta$.

Methoxyamine (MX) is a small molecule that binds avidly to the AP sites on the DNA reducing the APE cleavage of the backbone by more than 300-fold compared with the cleavage of normal AP sites. It was first introduced as a tool to study the BER pathway and then investigated therapeutically in the attempt to potentiate the antitumour effect of alkylating agents as well as other compounds causing AP sites both *in vitro* and *in vivo* systems.[50–52] These data not only proved that this type of inhibitor could be of potential therapeutic value, but also put forward the evidence that molecules binding to the AP sites could be developed to synergistically improve the therapeutic efficacy of DNA damaging agents. A phase I clinical trial with this compound is currently being undertaken in patients with solid tumours, but no data are available yet.

Another way to inhibit the BER pathway is through the targeting of APE1. APE1 is a multifunctional enzyme playing a role in BER and DNA strand break repair unrelated to the processing of the AP site.[53] In fact, it has been described to have a role in the protection of the toxic effects caused by bleomycin and IR treatments through its 3′phosphoglycolate diesterase activity. The protein is also endowed with a so-called redox activity regulating the binding activity of different DNA transcriptional factors. In addition, APE1 protein has been shown to be over-expressed in some human tumours and this expression pattern seems to be correlated with poor response to therapy. CRT0044876 was isolated by screening a chemical library as a specific and potent APE1 inhibitor, lacking inherent toxicity to human cell lines but able to potentiate the cytotoxic effects of different DNA damaging drugs, including alkylating agents.[54] These preliminary data provided the proof of principle that APE1 can be targeted, even if further development is required to identify a clinical lead compound. Recently, E3330, able to inhibit the redox activity of APE1, has been isolated and shown to dramatically increase the cytotoxic activity of alkylating agents in ovarian cancer cell lines.[55]

Pol$\beta$ has a crucial role in creating an intact DNA strand after the removal of the damaged base.[56] Many natural products are non-specific inhibitors of Pol$\beta$ including glycoglycerolipids and triterpenoids. It has been shown that the synthetic compound palmoic acid inhibits the DNA polimerase and lyase activities of the enzyme *in vitro* sensitising wild-type but not Pol$\beta$-null fibroblast to the cytotoxic activity of alkylating agents.[57] It is, however, still to be defined the degree to which inhibition of Pol$\beta$ would interfere with normal DNA synthesis and therefore further studies are indeed needed.

### 3.3. *Targeting PARP*

PARP1 is the best characterised, most abundant and active member of the PARP family, comprising of as many as 18 related proteins. It has an important role in regulating cell death and cellular response to DNA damage.[58] When activated this multifunctional enzyme transfers ADP-ribose unit from NAD+ to nuclear target proteins and itself, forming long and branched polymers of poly (ADP-ribose) (PAR) at the expense of cellular NAD pools. The controversial reported results on the role of the enzyme in the DNA signalling and DNA-damage induced cell death have been unified by Virag et al.[59] who suggested that when DNA is moderately damaged, PARP1 participates in the DNA repair and the cell survives; in the presence of severe DNA damage, apoptosis is activated and caspases inactivate PARP1 by cleavage and finally, in the case of extreme damage, PARP1 is overactivated and induces a decrease in NAD+ and ATP levels leading to cell dysfunction or even to necrosis. The pleiotropic involvement of the enzyme in such different cellular responses to damage makes it amenable for therapeutical intervention in different pathophysiological conditions such as cancer, inflammation, stroke, and hypertension. We will here consider the inhibition of its DNA repair functions as a tool to potentiate the activity of chemoradiotherapy.

PARP1 has been shown to have a key role in BER pathway, stimulating and facilitating the process.[60] It does in fact interact with DNA ligase III, the adaptor factor XRCC1, DNA polymerase and other components of the single–strand break repair. In addition, PARP1 cooperates with Cockayne syndrome B protein, Werner syndrome nuclear protein and DNA topoisomearse I, proteins involved in other DNA repair processes, favouring their activities. It has been proposed that the negatively charged polymers of poly (ADP-ribose) catalysed by activated PARP1 help the opening of the damaged DNA to allow the access of other components of the repair process. In addition, it is likely that the polyADP ribosylation serves as a scaffold for the recruitment of different repair proteins at the sites of damage with a local amplification of the response. The data from the literature suggest that PARP1 has a more important role in the repair of DNA under stress conditions (such as after IR and certain anticancer agent exposure) than in normal (non-stressed) conditions. PARP knock out mice are more susceptible to the cytotoxic activity of different anticancer agents and IR. These data, together with the cumulating evidence that PARP1 inhibitors were able to potentiate *in vitro* the cytotoxic activity of the same agents, made PARP1 a target for the clinical development of specific PARP1 inhibitors as potential chemo- and radio-sensitisers.[4]

Inhibitors were largely designed on the structure of nicotidamine, and were competitive of the catalytic domain of the enzyme. The first nicotidamine analogues were the benzamides (3-amino-benzamide) developed in the 1980s and mainly used for *in vitro* studies.[61] These molecules had, however, a weak inhibitory activity and possessed many other side effects unrelated to PARP inhibition. During the last decade, structure-based drug design studies and a better understanding of the molecular details of the active site of PARP have facilitated the discovery of inhibitors with increased potency, increased specificity and better pharmacokinetic and toxicological properties.[60,62] All these compounds have been shown to potentiate the *in vitro* and *in vivo* anti-tumour activity of anticancer agents, in particular temozolomide, topoisomerase I poisons, platinum compounds and IR. In most cases the observed potentiation has been correlated with the ability of PARP inhibitors to inhibit the repair of the DNA lesions caused by the treatment of the anticancer agents.

Table 1 summarises the main PARP1 inhibitors in cancer clinical development. Most of the PARP1 inhibitors are in early stage clinical development and the data from these clinical studies are awaited.[63,64] As much of the pre-clinical work has been focused on the potentiation of temozolomide, this was the first combination with PARP inhibitors introduced in the clinic for cancer patients in 2003. The phase I of AG014699 + temozolomide (TMZ) showed that a full dose of TMZ could be given in the presence of a profound inhibition of PARP.[65] No toxicity specific to the PARP inhibitor was observed, even if an enhancement of TMZ mielo-toxicity was observed with the higher doses of AG014699. Recently, the final report of a phase II study of AG014699 in combination with TMZ in patients with malignant melanoma was presented at the 2006 ASCO meeting showing an encouraging activity of the combination. Still awaited are the data on the correlation between clinical outcome and PARP expression and activity in blood cells and tumour.[66]

Even if pre-clinical data showed that PARP inhibitor treatment strengthen the effect of radiotherapy both in cell lines and in tumour xenografts and, as mentioned before, PARP1 knocked out mice are hypersensitive to IR, the combination trials of PARP1 inhibitors and radiotherapy are still challenging as regards to the definition of end-points to define a benefit. Radiotherapy is generally given in an adjuvant setting to improve local tumour control. This implies not only the need to investigate dose reduction of established regimens, but also long follow-up times. For these reasons, these combination trials, even if potentially interesting, are still at a design stage.

While these PARP inhibitors have been thought to be used in combination with IR and with other different anticancer agents, two exciting papers have been published on the possible application of PARP inhibitors as single agents in tumour cells harbouring defects in recombination repair, due to mutations of the genes BRCA1 and BRCA2.[67,68] It was reported that both BRCA-1 and -2 homozygous mutant cells lines and tumour xenografts were hypersensitive to PARP inhibitor treatment, while wild type and heterozygous cell lines showed a normal sensitivity. As PARP is a non-redundant component of BER that repairs SSBs normally formed during cell cycle, the treatment with PARP inhibitors in normal cells will lead to persistent SSBs that are converted to double strand breaks (DSB) when they meet the replication fork. In the presence of a functional recombination repair, these DSBs are efficiently repaired by Rad51-mediated homologous recombination. BRCA1/2 proteins have been shown to physically interact with Rad51 and play a pivotal role in recombination repair. In a BRCA-1 and –2 homozygous setting background, the DSBs originated by the treatment with a PARP inhibitor cannot be properly processed and repaired due to the lack of the functional recombination repair with the collapse of the replicative fork and illegitimated DNA ends joining leading to cell growth

**Table 1 – PARP inhibitors in development**

| Name (Company) | Phase of development | Single/combination | Tumour type |
|---|---|---|---|
| AG-014699 (Pfizer/University of Newcastle) | Phase II | Single agent/Combination with temozolomide | Malignant Melanoma |
| AG-01400361 (Pfizer/University of Newcastle) | Preclinical | Combination therapy | |
| KU 59436 (KuDOS/Astra Zeneca) | Phase I | Single agent | Solid tumours |
| GPI 210216 (Guilford Pharmaceutical Inc) | Preclinical | Combination | |
| INO-1001 (Inotek Corp) | Phase I, II | Combination | Glioblastoma Malignant Melanoma |
| BSI 201 (BiPar Science) | Phase I | Single | Advanced cancer |

arrest and apoptosis. As germ line mutations of *BRCA1* and *BRCA2* have been found to contribute to most of the familial breast cancer cases, the above mentioned exciting preclinical results open new chemo-preventive and therapeutic strategies for BRCA1/2-associated breast cancers. These data have indeed prompted the evaluation of PARP1 as mono-therapy for BRCA-deficient tumours.[69,70]

### 3.4. *Targeting double strand break repair*

A DSB is a lethal lesion that needs to be repaired to avoid chromosomal rearrangement and aneuplody. This requires a coordinated cellular response that not only involves the recruitment of proteins that physically repair the damage (effector proteins), but also the activation of signal transduction pathways leading to cell cycle arrest necessary to perform the repair (sensing and transducer molecules).[11] Even if this division is likely to be too simplistic as many proteins have redundant and overlapping functions, it helps to understand the different classes of molecules developed to inhibit this repair process. In particular, two kinases of the phosphatidylinositol 3-kinase (PI3K) super-family, ataxia-teleangectasia mutated (ATM) and ATM-RAD3-related (ATR), play a crucial role in sensing DSBs and in activating transduction pathways with phosphorylating events on target proteins leading to cell cycle arrest and facilitation of the repair process.[71–73] ATM-mutated cells show hypersensitivity to DBS inducers suggesting that ATM inhibition could sensitise to IR and other chemotherapeutic agents.[71] The first selected inhibitors, as caffeine, wortmannin and LY294002, were rather unspecific being able to target multiple members of the PI3 kinase super-family and too toxic for clinical exploitation. Recently, an ATP-competitive inhibitor KU-55933 has been screened for its greater specificity toward ATM than the other components of the PI3K super-family and it has been shown to chemo-sensitise and radio-sensitise wild type ATM cells to IR, etoposide, doxorubicin and camptothecin.[74] However, more data are necessary to fully develop this class of compound in a clinical setting.

The other approach undertaken to inhibit DSB repair has been focus on inhibiting NHEJ. Cells deficient in components of the NHEJ pathway are highly sensitive to IR and topoisomerase II poisons, such as etoposide, doxorubicin and mAMSA. Over-expression of DNA-PKcs correlated with increased repair of IR-, etoposide- and doxorubicin-induced DNA DSBs and with resistance to these agents.[75] DNA-PK, as ATR and ATM, is a member of the PI3K-related protein kinase. A number of compounds have been developed as potent and specific inhibitors of DNA-PK with 100-fold selectivity for the enzyme as compared with other PI3K family members. One of the first novel LY294002-based compounds with an increased activity toward DNA-PK was NU7026.[76] This compound enhanced the cytotoxicity of both IR and topoisomerase II poisons and retarded DSB repair. Further compound elaboration lead to the identification of NU7441 as a yet more potent and specific inhibitor for the DNA-PK and able to enhance the antitumour activity of etoposide in the human colon cancer xenograft model.[77] However, even these data provided excellent proof of principle of the *in vitro* and *in vivo* chemo-sensitisation and radio-sensitisation potential of this class of compounds; the limited aqueous solubility and oral bioavailability restrict NU7441's further development. Other compounds have been shown to inhibit NHEJ pathway such as vanillin, a plant-derived natural compound that sensitises cells to cisplatin[78] or a chemically synthesised inhibitor of DNA-PKcs, SU11752, that competitively inhibits DNA-PK binding to the ATP pocket.[79]

### 3.5. *Targeting Fanconi anaemia/BRCA pathway*

Data from the literature suggest that cells deficient in this pathway are hypersensitive to cross-linking agents. Attempts to identify small molecule inhibitors of the FA/BRCA pathway that could sensitise cancer cells to DNA damaging cross-linking agents have recently been published using a cell-based strategy.[80] Four inhibitors (three protein kinase inhibitors and one natural compound) have been identified. This targeting approach is still very much in its infancy though and further biochemical and preclinical work needs to be done.

## 4. Exploiting DNA repair defects for cancer therapy

With the implementation of microarray and proteomic technologies, the molecular characterisation of human tumours has been cumulating in the past years. This information, together with the unravelling of both the mechanisms of action of the anticancer agents and the pathways involved in the repair of the lesions they cause, have opened up the possibility to tailor the treatment of cancer, with a potential increase of the therapeutic index. In particular, the definition of a sub-set of tumours with specific defect in DNA repair pathways has envisaged the possibility to differentially treat the patients whose tumours harbour such defects.

Individuals with heterozygous, germ line mutations in either *BRAC1* and *BRCA2* have an increased life time risk of developing breast and others tumours.[81] These tumours have generally lost the wild type allele resulting in a non expressing *BRAC1* or *BRCA2* protein. Normal tissues of the patients harbouring *BRAC1* and *BRCA2* defective tumours do not seem to show haplo-insufficiency, suggesting a difference in the capacity of DNA DSBs repair between normal and tumour cells.[82] The *in vitro* data clearly show that cell lines lacking these genes are selective and exquisitely sensitive to agents causing DNA interstrand cross-links, such as cisplatin, carboplatin, mitomycin C, and recently to PARP inhibitors.[67,68] Even if these agents are not generally used in metastatic sporadic breast cancer, the great difference in the HR repair between the normal cells and tumour cells of *BRCA1* and *BRCA2* mutation carrier patients suggests the possibility of a potent and selective tumour cell killing by these agents in familiar breast cancer. To corroborate this hypothesis, a clinical study has been implemented randomising BRCA1 or BRCA2 mutation carriers with metastatic breast cancer to either the DNA cross-linking agent carboplatin or docetaxel, the current gold standard treatment.[83]

Recent data emerging from the literature suggest that BRCA-associated pathways seem to be inactivated in a substantial fraction of sporadic cancers. These tumours display a phenotype similar to the BRCA germ line mutation in the absence of a mutation in BRCA genes, a phenomenon named

'BRCA-ness'.[84–86] For example, epigenetic silencing of the critical gene involved in HR through methylation of promoter region has been described for BRCA1 and components of the FA pathways (FANCF, FANCC, FANCG) and by amplification of a novel gene, called EMSY, that negatively regulates the transcription of BRCA2. These BRCA mutant tumours should also be very sensitive to the treatment with PARP inhibitors, used as mono-therapy, as already discussed in the PARP inhibition section.

Beside the BRACness phenotype mentioned above, disruption or altered expression of other DNA repair pathways have been reported in sporadic human tumours, accounting, at least in part, for the specific drug and radiation sensitivity of these tumours. NER is compromised in testicular cancer which is fairly sensitive to cisplatin.[87,88] Recently, it has been published that patients with completely resected non-small cell lung ERCC1-negative tumours appear to benefit from adjuvant cisplatin-based chemotherapy expressed as longer survival, whereas patients with ERCC1-positive tumour do not. ERCC1 is one of the limiting factors in NER, involved in the removal of platinum-DNA adducts.[89] Finally, MR genes involved in MR (*MLH1*, *MSH2*, *MLH3*, *MSH6*, *PMS1*, *PMS2*) are either mutated in the germ-line or inactivated by hyper-methylation of the promoter as a somatic epigenetic phenomenon.[85] The products of these genes are indeed important for the cellular response to different anticancer agents. It has in fact been shown that MR deficiency is associated with resistance to antimetabolites, methylating agents, platinum compounds and some DNA minor groove binders.[15] Considering the high incidence of defects in this pathway in colorectal cancer, the different sensitivity to chemotherapeutic agents may be relevant to the treatment of this neoplasia. Prospective clinical trails will have to assess whether patients with MR deficient tumours would benefit from adjuvant chemotherapy as compared with MR proficient tumours.[90]

There is emerging literature focused on the polymorphisms of DNA repair genes that, even if they have a less dramatic functional impact, might also influence the patient's cancer risk and tumour response to therapy.[91,92] Polymorphisms in DNA repair genes have been described to confer suboptimal DNA repair capacity leading to an increase in the cancer predisposition, to influence the natural biology and progression of the tumour and to affect both the toxicity and response to the therapy.

## 5. Concluding remarks

In the last decade much research has been focused on the discovery and development of novel drugs that interact with signal transduction pathways found to be overexpressed or aberrantly regulated in cancer cells. It was anticipated by many authoritative scientists that the new therapeutic approaches would lead to the development of non-toxic drugs that would rapidly make obsolete the standard anticancer treatments based mostly on DNA–directed drugs.[93–95] This prediction was essentially incorrect. There is no question that DNA-damaging agents such as platinum coordination complexes, alkylating agents, topoisomerase I and II and antimetabolites that inhibit DNA synthesis still represent the most effective drugs for a large fraction of human malignancies. This consideration suggests that to use the current available knowledge to increase the selectivity and efficacy of conventional treatments is a potentially useful approach that can realistically lead to significant therapeutic improvements. In this respect, much work is currently in progress to exploit the very recent knowledge on cell cycle checkpoints and on the cell death pathways to potentiate the activity of conventional chemotherapeutics.[96–98] At least in preclinical systems, evidence exists that combining DNA damaging agents with inhibitors of cell cycle checkpoints, or with proapototic compounds, results in a selective antitumour advantage, and clinical studies pursuing this approach are currently in progress. Another approach, overviewed in the present paper, is related to inhibitors of DNA repair to be used in combination anticancer agents or radiotherapy.

As explained in detail within this article, definition of the molecular pathways involved in the repair of the lesions, induced by the anticancer agents commonly used in the clinic, has envisaged the possibility to inhibit such pathways. This approach should certainly improve the efficacy of radiotherapy with relatively low risk of increased toxicity. In addition, in metastatic melanoma, a phase II study combining temozolomide and a PARP inhibitor showed a 18% confirmed CR/PR rate with 40% of patients remaining on treatment for 6 months or more.[70] What appears extremely attractive is the ability to potentiate systemic anticancer treatment of human tumours exhibiting DNA repair defects that can be intelligently exploited, as highlighted in the ongoing clinical studies testing the PARP inhibitors as single agents in BRCA1/2 deficient tumours.

In exploiting this opportunity however, it is worth bearing in mind that this approach, as it was discussed, is still too far away to be specific for the tumour and has two major potential drawbacks: an increased toxicity and an increased tumourigenic risk of the concomitant antitumour treatment. DNA repair inhibitors are unlikely to be toxic by their own, but as they are expected to potentiate cytotoxicity of IR or chemotherapeutic agents, a greater effect on normal proliferating tissues (bone marrow and gastrointestinal tract) is to be seen, as the clinical studies with MGMT inhibitors suggest. This means that greater caution has to be put in the design of clinical investigations that should be driven by appropriate laboratory studies aimed at establishing the most rational dosage-schedules of the combination, the optimisation of the antitumour activity and reduction of the treatment toxicity.

A further anticipated drawback is related to the expected increased tumourigenic risk as the repair process is needed for normal cells to counteract mutagenic lesions of anticancer agents. It has been proved that IR, alkylating agents and topoisomerase II inhibitor treatments induce secondary tumours several years after the initial exposure and this is related to the potentially mutagenic lesions they induced in normal tissues. The addition of an inhibitor of DNA repair is likely to potentiate the mutagenic risk and hence the risk of secondary cancers of such treatments. Until long-term safety follow-ups to prove the safety of such an approach are available, these combinations should be limited to refractory cancers for which no standard therapy exists and not applied for the therapy of potentially curable tumours occurring in children or young adults.

One relevant aspect of medical oncology that cannot be ignored is that most human tumours, at the diagnosis, have multiple genetic aberrations involving the abnormal regulation of many pathways, and the use of relatively unspecific DNA damaging agents seems to be one of the few realistic ways to achieve tumour regressions in an effective way. Nevertheless, the growing knowledge on cell response to DNA damage and DNA repair is providing the rationale for new therapies based on the combination of DNA damaging agents with modulators of cell response including DNA repair inhibitors. We anticipate that this approach cannot be pursued in all cancer patients and using the same regimes, but it should be tailored according to the specific tumour DNA repair pattern. Therefore, as already clearly demonstrated for other target therapies, it seems essential to develop reliable markers and imaging techniques to be used for an appropriate patient's selection and as predictors of response.

## Conflict of interest statement

None declared.

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