DNA base excision repair: the Achilles' heel of tumour cells and their microenvironment?

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ABSTRACT

Our current understanding of cancer suggests that every tumour has individual features. Approaches to cancer treatment require thorough comprehension of the mechanisms triggering genomic instability and protecting cancer cells from therapeutic treatments. Base excision repair (BER) is a frontline DNA repair system that is responsible for maintaining genome integrity. The BER pathway prevents the occurrence of disease, including cancer, by constantly repairing DNA base lesions and DNA single strand breaks caused by endogenous and exogenous mutagens. BER is an important DNA repair system for cancer cell survival, as it can affect both chemo- and radio-resistance of tumours. Variations in BER capacity are likely responsible for a number of cases of sporadic cancer and may also modulate cancer sensitivity and resistance to therapeutic treatments. For these reasons, it is broadly accepted that targeting BER enzymes might be a promising approach to personalised anti-cancer therapy. However, recent advances in both treatment strategies and the comprehension of cancer development call for a better understanding of the consequences of BER inhibition. Indeed, the impact on both the tumour microenvironment and healthy tissues is still unclear. This review will summarise the current status of the approaches exploiting BER targeting, describing the most promising small molecule inhibitors and synthetic lethality strategies, as well as potential limitations of these approaches.

Genomic DNA is inherently unstable due to continuous damage from both exogenous and endogenous sources. Although substantial, the impact of DNA lesions of endogenous origin is often underestimated. A single human cell endures an astonishing gross estimate of 10^5 DNA-damaging events per day. In a whole human organism, this implies that DNA repair machineries have to process at a rate of 10^{16} - 10^{18} repair events per day in order to keep up with the amount of damage generated (1, 2). These endogenous lesions can be nucleobase modifications such as oxidation, alkylation and generation of baseless (AP) sites, or damage to the DNA phosphate backbone such as single and double-strand breaks. For the latter, endogenous DNA single-strand breaks (SSBs) occur spontaneously far more frequently than double-strand breaks (DSBs), by around two orders of magnitude (3). However, DSBs can arise due to conversion of SSBs during replication into their more toxic counterpart (4). This highlights the essential role of DNA repair systems in counteracting the accumulation of endogenous lesions to support cell survival. To achieve this goal, a number of different DNA repair pathways have co-evolved to promote stabilisation of the genomic information and, ultimately, to prevent the occurrence of mutations and disease, particularly cancer. Indeed, genomic instability has been listed as a central driver of tumour development and as one of the “emerging” hallmarks of cancer (5). Unfortunately, inaccuracy or deficiency in DNA repair sometimes occurs, leading to accumulation of mutations with wide consequences on human health, from neurodegeneration to cancer.

It is important to notice that the selective pressure that cancer cells endure during their clonal expansion often forces them to rely on one or several DNA repair pathways to sustain their survival and proliferation, or to resist to conventional anti-tumour therapies (6, 7). For these reasons, understanding DNA repair features in tumours, and how they are related to cancer development, has captivated the scientific community. Targeting DNA repair pathways has been pursued as an approach to cancer treatment, together with more innovative strategies such as the use of synthetic lethality (8). However, most of the research has concentrated on the targeting of DSB repair, neglecting the importance of SSBs. This review will focus on how the field of SSBs repair is catching up with the rest of DNA repair research and how targeting of a particular DNA repair pathway, namely DNA base excision repair (BER) is offering a wealth of new therapeutic opportunities. We will briefly introduce the pathway, depicting the relationship between BER status and cancer. This will lay the foundations to understand the rationale behind BER targeting for personalised medicine. We will then analyse the pathway step-by-step, illustrating some of the current approaches to personalised anti-tumour therapy using BER inhibition in cancer. The most promising small molecule inhibitors will be described. Limitations and drawbacks of BER targeting will eventually be discussed, focussing on critical and unexplored aspects of systemic administration of BER inhibitors. Modulation of BER will finally be analysed and we will discuss and propose alternative strategies to BER inhibition in the context of personalised medicine.

DNA base excision repair

The BER pathway is the major repair system dealing with endogenously generated DNA lesions. BER is responsible for the clearance of base modifications, AP sites and SSBs. These lesions arise spontaneously in eukaryotic cells, mainly due to the intrinsic chemical instability of DNA molecules and to the action of endogenous mutagens. Amongst these, reactive oxygen species (ROS) generated by cellular metabolism are the major component (1). Alkylated DNA bases are also eliminated by BER, giving the pathway particular relevance in the clinic as several chemotherapeutics are alkylating agents (9). BER has a crucial role in maintaining genomic stability as damaged DNA bases are potentially mutagenic if unrepaired: AP sites and SSBs – by-products of abortive BER – interfere with transcription and replication leading to cytotoxicity, to the formation of DSBs, and eventually to cell death or senescence (10, 11).

BER of damaged nucleobases usually involves five major steps (Figure 1).

- i) *Recognition and excision of the lesion.* This step is carried out by a damage-specific DNA glycosylase, which binds to the modified base and cleaves the C1'-N-glycosylic bond.
- ii) *AP site incision.* In mammalian cells the AP site resulting from base excision is processed by the major apurinic/aprimidinic endonuclease (APE1) which cleaves the DNA backbone 5' to the baseless site and generates a SSB.
- iii) *End trimming.* Most of the SSBs generated enzymatically or non-enzymatically do not bear the 5'-phosphate and the 3'-hydroxyl moieties suitable for gap-filling. For this reason, a set of end-processors can enzymatically restore “blocked” termini, generating a clean SSB. These include, but are not limited to, APE1, aprataxin (APTX), polynucleotide kinase 3'-phosphatase (PNKP) and the tyrosyl-DNA phosphodiesterases 1 and 2 (TDP1 and TDP2) (12).
- iv) *Gap filling.* The base gap generated by base excision is subsequently filled in by a DNA polymerase, which in mammalian cells can be either DNA polymerase β (Pol β) or, in mitochondria, DNA polymerase γ (13, 14).
- v) *Nick ligation.* A complex comprised of the scaffold X-ray repair cross-complementing protein 1 (XRCC1) and DNA ligase III (LigIII), or DNA ligase I (LigI) eventually seal the resulting nick in the phosphate backbone, restoring the intact DNA molecule (12, 15).

The canonical BER pathway as described above is frequently termed short-patch BER (SP-BER), as the DNA stretch affected by the repair is restricted to the damaged nucleotide. A second branch of the pathway, termed long-patch BER (LP-BER), has also been described. This usually involves strand displacement activity by a DNA polymerase and additional processing by structure-specific endonucleases such as the 5'-flap endonuclease 1 (FEN1), in order to remove a longer stretch of nucleotides (from 2 to 12) and replace them with the correct ones (10, 11) (Figure 1).

BER as a target for personalised medicine

As endogenous DNA damage is a constitutive threat to genomic instability, it is not surprising that BER is vital for higher eukaryotes. Indeed, homozygous knock-out of the core elements of the pathway, such as APE1, Pol β , LigIII and XRCC1, results in embryonic or early post-natal lethality in mice (10). Expression of BER proteins is pivotal to cell survival and imbalance in the steady-state levels of BER leads to genomic instability, mutations and eventually cancer. This is likely explained by a competition effect or an inadequate enzymatic activity when the equilibrium of BER components is lost. This is particularly well illustrated in the case of imbalance between APE1 and XRCC1 (16-18) or when LigI (19), and Pol β (20, 21) are dysregulated or mutated. On the other hand, coordination of BER is also required to maximise substrate channelling, therefore minimising the accumulation of potentially lethal reaction intermediates in the intracellular milieu. In fact, abortive BER substrates such as AP sites and SSBs with oxidised or blocked termini are paradoxically far more cytotoxic than the original DNA base lesion (22, 23). APE1 offers the perfect example of the relationship existing between BER and cancer. Overexpression of the protein is a feature of many different types of tumour (reviewed in (24)); this phenotype has been found to be associated with resistance to cisplatin in lung cancer (25) and to refractoriness to radiotherapy in glial (26) and cervical (27) cancers. Other BER components such as Pol β (21), LigIII (28, 29) and FEN1 (30-32) are also elevated in tumours. At the other end of the spectrum, low XRCC1 expression has been observed in a wide range of tumour types, including breast (33), cervical (34), gastric (35), and lung (36) carcinomas. XRCC1 under-expression is generally associated with increased tumour aggressiveness and poorer outcome (e.g. in triple negative breast cancer (33)), reduced survival (e.g. in gastric cancer (35)) and unresponsiveness to radiotherapy (e.g. in bladder cancer (37)). Moreover, low XRCC1 expression has recently been reported to affect prognosis in BRCA1-deficient breast cancers (38).

These examples provide a rationale for BER targeting in the context of anti-tumour therapy: in an ideal situation, we would like to achieve immediate cancer cell killing in the absence of systemic toxicity. An obvious way to induce cancer cell death exploiting BER is to force cells to accumulate persistent repair intermediates such as AP sites and SSBs, which would eventually be converted into more toxic DSBs. This would theoretically trigger a robust DNA damage response and apoptotic program (39). This basic notion suggests that while targeting specific BER factors, such as APE1, DNA polymerases, XRCC1 or DNA ligases could provoke accumulation of unrepaired DNA lesions, inhibition of DNA glycosylases could be potentially counterproductive, promoting the accumulation of less toxic modified nucleobases with unwanted mutagenic potential, therefore impeding the apoptotic programme triggered by the DNA damage response (40, 41). In this case, the design of synthetic lethality strategies might be more desirable.

At the same time, BER defects also affect the response to chemo- and radiotherapy and thus underline how BER could potentially be a useful target for personalised anti-cancer therapy. BER depletion studies have shown promising results when inactivation of BER enzymes is combined with treatment with DNA damaging drugs or ionising radiation (IR) (42). For this reason, BER targeting is expected

to affect DNA repair and to sensitise cancer cells to the therapeutic agent. Moreover, a number of chemotherapeutic agents have been shown to lead to accumulation of ROS and oxidative lesions (43-45). Therefore, BER inactivation could also increase the potency of chemotherapy in an indirect manner.

Although pre-clinical studies have been providing promising results, BER targeting strategies are still in their youth and potential limitations should be considered. In particular, most of the studies so far have been carried out using cancer cell lines. This suggests that the impact of BER inhibition *in vivo* could differ substantially, as we currently have very little information about the effect of BER targeting in healthy tissue, tumour microenvironment or the immune system. These issues will be discussed later on in this review. The following paragraphs will describe the current efforts aiming to develop selective inhibitors of the BER pathway. For each step of BER we will provide some examples of the molecules and strategies in current development.

Targeting DNA glycosylases

DNA glycosylases initiate BER by recognising DNA lesions. This class of enzymes encompasses at least eleven different proteins in human cells, classified into mono- or bi-functional DNA glycosylases. This separation is based on the enzymatic mechanism leading to the base excision. While mono-functional enzymes (such as the uracil-DNA glycosylase (UNG)) cleave the C1'-N-glycosidic bond and generate an AP-site, bi-functional DNA glycosylases (e.g. NEIL1) instead cleave the DNA backbone through a β - or β,δ -lyase mechanism, generating a SSB that requires end-tailoring for BER to proceed (15). DNA glycosylases display significant overlap in terms of damage specificity. Accordingly, although knock-out of individual DNA glycosylases in mice results in genomic instability and accumulation of oxidative DNA damage, animals are perfectly viable, contrasting with the lethal phenotype resulting from depletion of other BER components (15). The high degree of functional redundancy complicates specific targeting of DNA glycosylases and so far only a limited number of *in vitro*-tested inhibitors are available. Despite this, combining the inhibition of these enzymes with other DNA damaging agents has been suggested as a viable strategy. Indeed, commonly used antimetabolites have shown synergetic effects with NEIL1 ablation (46). Moreover, loss of 8-oxoguanine DNA glycosylase 1 (OGG1) has been suggested to sensitize cancer cells to chemo- and radio-therapy, as well as to poly(ADP-ribose) polymerase 1 (PARP1) inhibitors (47). Additionally, DNA glycosylase inhibition could be used within a synthetic lethality approach. Indeed, NEIL1 has previously shown to selectively kill Fanconi anaemia cells (48). A screening campaign by Jacobs and colleagues identified a set of small molecules targeting NEIL1 with IC_{50} in the low micromolar range. The hits were validated *in vitro* showing somewhat limited specificity (49) (Table 1). More recently, Donley and collaborators identified a few small molecules targeting OGG1 with

sub-micromolar IC₅₀. These inhibitors produced promising selectivity towards OGG1 and are the most potent DNA glycosylase inhibitors developed to date (47) (Table 1).

Inhibitors of the end-trimming step

SSBs resulting from the activity of DNA glycosylases and APE1 have to be sealed in order to restore an intact DNA molecule. APE1's AP-endonuclease function is generally sufficient to generate suitable DNA termini for the gap-filling and nick-ligation steps. However, modifications occurring as a result of ROS attack or abortive enzymatic processing can generate peculiar 3' and 5' termini, which are not ideal substrates for downstream repair factors. In addition to its endonuclease activity on AP sites, APE1 has been demonstrated to be capable of end-processing SSBs containing 3'-8-oxoguanine and 3'-phosphoglycolate ends (50, 51). Additional "end-processors" such as APTX, PNKP or TDP-1/2 are also essential to generate a clean SSB. Broadly speaking, end-tailoring enzymes recognise very specific substrates and this property can be exploited to target the enzyme and therefore induce accumulation of persistent DNA strand breaks. To the best of our knowledge, no specific inhibitors of APTX have been reported so far. PNKP and TDP-1 inhibitors will be briefly discussed in the following paragraphs.

PNKP

PNKP is a major end-tailoring enzyme responsible for 5'-phosphorylation and 3'-dephosphorylation of DNA termini (52, 53). In addition to its role in BER, PNKP has been shown to mediate end-processing at DSBs during nonhomologous end joining (NHEJ) (54). To the best of our knowledge, the only well characterised PNKP inhibitor is 2-(1-hydroxyundecyl)-1-(4-nitrophenylamino)-6-phenyl-6,7a-dihydro-1H-pyrrolo[3,4-b]pyridine-5,7(2H,4aH)-dione (A12B4C3) (Table 1). This molecule displays good specificity towards PNKP, compared to other phosphatases, and has been proven to radiosensitise cancer cell lines in a PNKP-dependent manner (55). Follow-up studies have shown that A12B4C3 acts as a non-competitive PNKP inhibitor by binding the PNKP-DNA complex. The compound has also been shown to sensitise cancer cells to camptothecin, a topoisomerase poison, without affecting either Pol β or LigIII activities (56) and to increase the toxicity of a radio-immunoconjugate, ¹¹¹In-NLS-7G3, in myeloid leukaemia cells (57).

TDP1

TDP is involved in the repair of SSBs requiring removal of topoisomerase I (TOPI)-DNA complexes resulting from abortive cleavage. It acts by cleaving the covalent bond between DNA and the catalytic tyrosine residue of TOPI. Additionally, TDP1 can trim 3'-phosphoglycolate termini at DSBs as well as a variety of chain-terminating adducts (58). Examples of DNA damage that promote TOPI trapping

are AP sites and oxidised bases, but also adducts created by anti-cancer TOPI inhibitors. Therefore, inhibition of TDP1 in combination with existing anti-cancer drugs might be a viable option in order to improve the efficacy of current chemotherapy. Indeed, some evidence supports the benefit of combining TDP1 and TOPI inhibitors, such as camptothecin (59). The TDP1 inhibitors that have been reported so far have been identified through high-throughput screening (HTS) campaigns and optimised using medicinal chemistry (59-61), or derived from natural compounds (62, 63). For most of these inhibitors a selective effect has been reported towards TDP1 rather than TDP2. Nevertheless, further investigation into systemic toxicity after TDP1 inhibition is warranted (Table 1).

In addition, recent studies have been focussing on the development of dual inhibitors targeting simultaneously TDP1 and TOPI. Evidence that TDP1 depletion might increase sensitivity to topoisomerase poisons is the rationale behind these research efforts. A limited amount of research has been completed so far, but the resulting compounds have already shown promising results when used for *in vitro* enzymatic assays (see for instance (64)).

APE1 Inhibitors

APE1 is a key protein within the BER pathway as it processes the vast majority of AP sites in the cell (65). Amongst BER components, APE1 has been intensively studied for its extensive range of cellular functions. In fact, in addition to its known role as the major AP site-endonuclease, APE1 has transcriptional regulatory activity involving redox-mediated modulation of transcription factors (66), as well as multiple roles in RNA metabolism, which have yet to be fully elucidated (67).

APE1 has long been considered an appealing target for anti-cancer therapy for a number of reasons. Firstly, overexpression of the APE1 protein is frequently observed in cancer. Elevated APE1 levels have been associated with increased resistance to chemo- and radiotherapy, poorer survival and higher angiogenesis (66, 68). Secondly, APE1-mediated modulation of transcription factors is important both within the tumour and in its microenvironment (69). Additionally, impairment of APE1 function potentiates the cytotoxicity of clinical genotoxic agents such as streptozotocin, temozolomide (TMZ), bleomycin, cisplatin and radiation (70, 71). These effects are also likely to be extended to other alkylating agents such as procarbazine and dacarbazine (72). Therefore, synthesis of small molecule inhibitors of APE1 is currently a very active research focus. As APE1's redox and AP-endonuclease functions are carried out by structurally distinct protein domains, selective depletion of either function is theoretically possible. The following paragraphs will describe the molecules currently available to target the endonuclease function of APE1. As APE1's redox activity is not strictly connected to BER, this review will not describe the small molecule inhibitors of this APE1 function. The reader is referred to excellent reviews on the subject (such as (69)).

As mentioned earlier, complete APE1 knock-out is lethal in mice (73), suggesting that systemic inhibition of the protein might have potentially deleterious effects on normal tissues. For this reason,

alternative approaches to systemic inhibition are currently under evaluation and will be briefly described in a separate paragraph.

Inhibitors of the APE1 endonuclease activity

Targeting APE1's endonuclease function is a very appealing option to impede BER. As more than 95% of the AP-endonuclease activity in a whole cell extract is exerted by APE1 (74, 75), APE1 down-regulation results in accumulation of AP sites, which can block DNA replication resulting in cell toxicity (76). Several inhibitors of the APE1 DNA repair activity are currently being developed. The archetype inhibitor is methoxyamine (MX), a small compound binding the aldehyde form of AP sites (77). Given the somewhat unspecific effect of the molecule however, MX should be considered a broad BER inhibitor (78). Despite this fact, combination with MX has been demonstrated to potentiate the cytotoxicity of alkylating agents (79, 80). MX recently underwent a phase I clinical trial under the trademark TRC102. The compound was well tolerated and showed favourable pharmacokinetic and pharmacodynamic properties as well as potentiation of the cytotoxicity of the folate anti-metabolite pemetrexed (81). Additional clinical trials using MX are currently underway for patients with advanced refractory cancer in combination with alkylating agents, antimetabolites, platinum compounds and radiotherapy (*ClinicalTrials.gov*) (Table 1).

The relative lack of selectivity of MX has prompted additional HTS campaigns to try to identify more specific APE1 inhibitors. Most of the studies have generated series of compounds selected for their ability to sensitise cancer cells to alkylating agents (82-84). Amongst the inhibitors developed, Lucanthone (Miracil D) has shown promising effects when combined with MMS or TMZ. However, as the molecule is also known to inhibit topoisomerase II, the effect observed might be not entirely APE1-dependent (85, 86). One of the first reported APE1-specific inhibitors is the CRT0044876 compound (7-nitroindole-2-carboxylic acid) (83). This molecule has since been used in multiple studies and demonstrates low inherent cytotoxicity and a synergistic effect with MMS and TMZ, even though the compound has not yet been tested in animal models (24). Additional APE1 inhibitors have been identified during other HTS campaigns (82, 87). One example is AR03 (2,4,9-trimethylbenzo[b][1,8]-naphthyridin-5-amine), which has been reported to inhibit APE1 in whole cell extracts, leading to accumulation of AP sites in cancer cell lines and potentiating the effects of MMS and TMZ (82). Finally, it is worth noting that a number of recently designed APE1 inhibitors (i.e. compound #3 and #52 see (88)) have recently been tested *in vivo* showing desirable pharmacological properties for translational applications (Table 1).

In addition to chemically designed APE1 inhibitors, naturally occurring compounds have also been considered. Gossypol, a plant-derived molecule has been recently observed to inhibit both the APE1 endonuclease and redox functions (89). However, Gossypol's anti-tumorigenic activity is probably related to its ability to target a broad spectrum of cellular factors. Nevertheless, the molecule has been

extensively assessed in a number of clinical trials. Gossypol is currently being tested in combination with cisplatin and docetaxel in a phase III trial in non-small cell lung cancer patients bearing high APE1 expression (*ClinicalTrials.gov*) (Table 1).

In summary, despite the availability of multiple molecules targeting the endonuclease activity of APE1, further optimisation will be needed in order to reduce unwanted toxicity and to improve cellular delivery. Thorough animal testing will also be required for most of the molecules before any translational application. Finally, it is worth mentioning that APE1's endonuclease domain has been shown to exert additional catalytic functions on DNA termini and on RNA (reviewed in (66)). To the best of our knowledge the impact of APE1 inhibitors on these functions has been poorly investigated. Therefore, caution should be used when interpreting the results obtained with these molecules.

Strategies complementary to APE1 inhibition

As APE1 is essential to cell survival, systemic inhibition of the enzyme might cause toxicity to normal tissue. In order to overcome this issue, several laboratories have proposed different strategies that, while still targeting APE1, exploit features and weaknesses specific to cancer cells. This should increase the effectiveness of APE1 inhibitors and ultimately allow clinicians to significantly reduce drug dosage.

Cardoso and colleagues have proposed a dual targeting strategy for pancreatic cancer, whereby simultaneous pharmacological inhibition of APE1 redox activity and the transcription factor STAT3 results in a synergistic killing of cancer cells through apoptosis (90). Synthetic lethality is yet another strategy that has been explored in order to potentiate APE1 inhibitors. Two studies from the same laboratory have shown how combination of APE1 endonuclease inhibitors with either DSB repair or PTEN deficiency could induce cell death in several cancer cell lines (91, 92).

Another strategy is to target APE1's interaction partners. A recent publication described an HTS campaign aiming to identify small molecules affecting the interaction between APE1 and its protein partner nucleophosmin 1 (NPM1). This approach was based on previous observations where the APE1/NPM1 interaction had been identified as potentially important to cancer cell growth (reviewed in (67)). In their study, the authors identified three biologically active compounds with previously unrecognised anti-tumoral properties (93). This area of research is relatively young and therefore more work is needed in order to identify molecules with suitable selectivity towards cancer cells. Yet, preliminary results seem to indicate that alternative strategies are worth pursuing.

Targeting XRCC1

XRCC1 is a scaffold protein central to BER. Although, XRCC1 has no enzymatic activity, it plays a substantial role in organising, recruiting and stimulating BER, as well as other DNA repair pathways (94). Currently, no existing drug can directly target XRCC1. However, synthetic lethality approaches

have been proposed. Recently, Chakrabarti and colleagues showed that β -lapachone (ARQ761), an inhibitor of the NAD(P)H:Quinone oxidoreductase 1 (NQO1), can synergise with XRCC1 depletion and enhance cancer cell death. The synergistic effect is probably triggered by the release of ROS and the generation of oxidative DNA damage upon NQO1 inhibition. In line with this hypothesis, depletion of OGG1 protects cells from β -lapachone cytotoxicity, likely by preventing the accumulation of DNA strand-breaks (95). Even though the drug is not currently used in combination with BER deficiency, ARQ761 is undergoing two phase I clinical trials testing its performance in advanced solid tumours, as well as in combination with gemcitabine and paclitaxel in pancreatic cancer (*ClinicalTrials.gov*).

Targeting FEN1

The structure-specific endonuclease FEN1 exerts enzymatic activity during LP-BER. The 5'-flap structure, however, is a very common intermediate that can occur during DNA recombination, replication and DSB repair (96). For these reasons, targeting FEN1 could potentially have consequences broader than BER impairment. To date, a number of low molecular weight inhibitors of FEN1 have been reported. The first molecules to be identified derived from structure-activity relationship optimisation studies on N-hydroxyurea analogues (Table 1). The small inhibitory molecules have been shown to display selectivity over other FEN1-related endonucleases and IC_{50} in the nanomolar range. Consistent with inhibition of DNA repair, potentiation of MMS and TMZ cytotoxicity in combination with these molecules has been reported (97). An additional inhibitor (NSC-281680) has successfully been shown to impair LP-BER *in vitro* through inhibition of FEN1's endonuclease activity. Treatment of cancer cells with NSC-281680 potentiates TMZ cytotoxicity (98). Other more recent HTS campaigns have identified additional proof of concept compounds displaying micromolar or sub-micromolar potency (99, 100) (Table 1). In conclusion, current FEN1 inhibitors are still at the early stages of development and most have been only tested *in vitro*. Additional efforts will be required to take these molecules from benchtop to bedside.

Targeting Pol β

Pol β is a major DNA polymerase in BER. The protein is a bifunctional enzyme equipped with polymerase (or nucleotidyl-transferase) activity and 5'-dRP lyase activity. This second function allows Pol β to conduct an essential gap-tailoring step in BER, removing the 5'-deoxyribose phosphate (5'-dRP) moiety from the nick generated by APE1 (101).

Several strategies have been explored in order to target Pol β . These include: i) inhibition of its polymerase activity, ii) inhibition of its 5'-dRP lyase activity, iii) inhibition of its DNA binding activity, iv) inhibition of its interaction with binding partners and v) RNA interference-mediated depletion (reviewed in (101)). Among a wide variety of compounds proposed to target the protein,

only a handful have so far shown real clinical potential in terms of IC₅₀, specificity and delivery. Only the most promising molecules will be addressed in this review.

Lithocholic acid (LCA) is a bile acid that has been identified, together with its derivatives, as a potent inhibitor of both Pol β 's nucleotidyl-transferase and 5'-dRP lyase activities (102-104) (Table 1). Stachelek and colleagues demonstrated that LCA can synergise with TMZ to kill BRCA2-deficient cells, possibly in a Pol β -dependent manner (104). However, subsequent studies have questioned the specificity of LCA, showing that the molecule can affect other DNA polymerases (102) and DNA processing enzymes such as topoisomerase II (105). Furthermore, LCA has been shown to bind the vitamin D receptor and to alter NF- κ B signalling (106). Synergy with TMZ is often considered indicative of Pol β inhibition. This criterion has been employed to identify NSC-666715 as a small molecule targeting Pol β (107) (Table 1). Further analyses confirmed that this compound inhibits Pol β 's DNA binding ability (107, 108). Pol β inhibition is known to display synthetic lethality with MMR-deficiency (i.e. loss of MSH2) (109). Interestingly, NSC-666715 showed a similar synthetic lethal effect on MMR-deficient colorectal cancer cells in combination with TMZ (107). NSC-666715 has also been shown to target the 5'-dRP lyase activity of Pol β (107). However, a recent report showed that this effect was present only when Pol β was engaged in LP-BER, likely by interfering with FEN1-dependent strand-displacement activity (108). An additional compound, NSC-124854, displayed Pol β inhibitory activity in both SP- and LP-BER assays (Table 1). NSC-124854 showed synergy with TMZ and selective killing of MMR-deficient cancer cells (110). Pamoic acid (PA) is another molecule that has been identified as a specific 5'-dRP lyase inhibitor by NMR chemical shift mapping (111). PA has also been shown to inhibit the nucleotidyl-transferase activity of Pol β *in vitro* (111). Its specificity and dual action suggest that PA might be a promising Pol β inhibitor.

Overall, available data suggest that Pol β inhibitors are more efficient when they target the 5'-dRP lyase domain. A recent study by Quiñones and colleagues addresses the importance of this activity in Pol β -dependent cell toxicity. Their work demonstrated that Pol β can be trapped onto DNA when trying to process oxidised AP sites. Interestingly, the cytotoxic effect of Pol β trapping is dependent on its 5'-dRP lyase domain (112). It is tempting to speculate that binding of a compound to this domain will not inhibit only the activity of the enzyme, but will also mimic the cytotoxic effect of Pol β trapping. Consistent with this idea, novel and specific irreversible 5'-dRP lyase inhibitors (i.e. “compound 3a” and an additional bisacetate analogue), have been designed to mimic oxidised AP sites. Interestingly, these compounds display intrinsic cytotoxic effects in prostate cancer cells and potentiate the cytotoxicity of MMS between two and five-fold (113).

Targeting DNA ligases

DNA ligases are enzymes devoted to the sealing of nicks in the DNA phosphate backbone. These proteins have an important role in the repair of SSBs as well as DSBs but are also crucial for DNA replication. Human cells have three different DNA ligases (114). The design of specific DNA ligase

inhibitors is additionally complicated by the three enzymes displaying overlapping functions. Despite this, structure-based approaches have identified a number of inhibitors that have shown promising effect in cancer cell lines and mouse xenograft models (115-117). Currently, most of the available inhibitors display intrinsic cytostatic activity and are able to potentiate the cytotoxicity of DNA damage-inducing agents such as IR (115-117) (Table 1). Additional compounds have been identified through an *in silico* approach, showing varying degrees of selectivity towards the three different human DNA ligases. Among these, compounds L67, L82 and L189 have demonstrated cytostatic and cytotoxic activity and preferentially sensitise cancer cells to IR and MMS (29). Notably, combination of L67, inhibiting LigIII, with PARP1 inhibitors shows cytotoxicity in chronic myeloid leukaemia cell lines due to the role of LigIII in NHEJ (118). Recently, SCR7, an improved derivative of L189, has been shown to display selectivity towards LigIII and IV, but not LigI, potentiating the effects of IR and etoposide in xenograft models (119) (Table 1).

Altogether, these results from preclinical studies support and encourage further optimisation of existing DNA ligase inhibitors for use in personalised medicine.

Targeting PARP1

PARP1 is a member of the large family of ADP-ribosyltransferase enzymes involved in cell signalling. The main activity of PARP1 is to attach poly(ADP-ribose) onto target proteins using nicotinamide adenine dinucleotide (NAD⁺) as a substrate (120). This peculiar post-translational modification (PTM) is found on a multitude of proteins, including PARP1 itself, and can be used as a signal for the recruitment of DNA repair machineries to sites of DNA strand breaks (120).

PARP1 has not only been one of the most successfully targeted molecules but has also been the starting point for a new era in personalised medicine (121). The discovery of synthetic lethality between PARP inhibitors (PARPi) and homologous recombination (HR)-deficient cells (122-124) has led to tremendous improvements in the treatment of hereditary breast and ovarian cancer (125). Subsequently, other synthetic lethal interactions involving PARP1 have been characterised. Examples include Mre11 (126), CDK5 (127) and PTEN (128) deficiencies, and ETS overexpression (129-131). The mechanisms of action of PARPi are not yet fully understood even for the well-studied synthetic lethality interaction with BRCA deficiency (132). It has been suggested that inhibition of PARP1 could cause SSB accumulation, due to its putative role in BER. Unrepaired SSBs would then also be converted into cytotoxic DSBs during DNA replication (132). However, our laboratory has demonstrated that PARP1 is not essential to BER (133). In fact the protein appears to be necessary only for SSB protection and to prevent SSB conversion into DSBs during acute damage (134). Therefore, PARP1 inhibition alone should not be sufficient to induce SSB accumulation, as BER capacity would virtually be unaffected (133). Consistently, further studies have shown that neither PARPi nor PARP1 depletion are sufficient to induce accumulation of DNA damage (135). It seems more likely in fact, that PARPi act by trapping PARP1 on DNA repair intermediates, in particular

during BER; this would then hinder replication fork progression, therefore requiring HR intervention to resolve the damage (132, 135). Indeed, such collisions between BER and replication forks have been observed following MMS treatment, particularly upon PARP1 inhibition (136). In addition to this, other mechanisms of action have been proposed, such as the role of PARP1 in preventing Mre11-dependent degradation of stalled replication forks (132, 137).

Among the multitude of PARPi designed so far, olaparib, talazoparib, niraparib, rucaparib and velaparib have shown remarkable success and are now widely employed in clinical trials on breast, ovarian, prostate, central nervous system cancers, just to name a few (138) (Table 1).

Olaparib recently obtained FDA approval for the treatment of BRCA-deficient tumours. These molecules perfectly exemplify the importance of biomarkers in personalised therapy and the benefits arising from patient stratification. The reader is referred to recent and extensive reviews on the topic (121, 125, 138-140).

Targeting BER: all that glitters is not gold

Recent advances in the development of BER inhibitors and synthetic lethality strategies suggest that approaches aiming to target BER are potentially promising for translational medicine. Nonetheless, some considerations are worth being assessed.

- i) *Tumour microenvironment.* There is currently very little information regarding the role of BER in tumour stroma. As the microenvironment plays a major role in cancer development and resistance, further assessment of the impact and the efficacy of BER targeting strategies are needed in this context.
- ii) *Healthy tissue.* As for the tumour microenvironment, studies assessing the impact of BER inhibition in non-cancerous cells are still lacking. Additional efforts are clearly required to understand the effect of systemic BER targeting, together with potential side effects for patients.
- iii) *Redundancy in DNA repair systems.* DNA repair pathways display significant overlap and this may trigger backup mechanisms leading to reduced efficacy of BER inhibitors.
- iv) *Emergence of resistance.* It is still very early to predict the occurrence of resistance in tumours treated with BER inhibitors. However, potential pitfalls have to be taken into consideration. PARPi have given us a very valuable lesson and have highlighted potential scenarios that could occur with other BER inhibitors.

In the following paragraphs we will discuss the potentially overlooked consequences of BER targeting. We will summarise current knowledge regarding BER inhibition in healthy tissue and tumour stroma, with a glimpse at the existing backup DNA repair systems and mechanisms likely to impact the efficacy of BER targeting-strategies. We will then conclude by describing how BER is modulated, offering novel potentially interesting approaches aiming to target BER.

BER and tumour microenvironment

Most studies addressing the role of BER in tumorigenesis, as well as BER targeting, have been carried out on cancer cells. It is important to bear in mind that although extremely valuable models, cancer cell lines have limitations and very often do not fully recapitulate the biology of a tumour. In particular, the role of BER in the tumour microenvironment has been so far poorly investigated. The tumour microenvironment is composed of both non-cancerous epithelial cells adjacent to the tumour and stromal cells from different origin such as fibroblasts, infiltrating immune cells and vascular cells. Cancer cells thrive within a microenvironment, which provides them protection and promotes their survival, growth and migration (141).

Although we can state that BER inhibitors show promising effects on cancer cells, very little is known about their action on the tumour microenvironment. A study on APE1 suggests that the protein could be essential for survival of tumours within an acidic microenvironment. In this study, suppression of APE1 or XRCC1 led to unrepaired oxidative DNA damage, cell-cycle arrests, increased DSBs and eventually cancer cell death (142). These results support APE1 inhibition as a strategy to target cancer cells within a microenvironment. APE1 has been shown to modulate the activity of transcription factors involved in angiogenesis, such as HIF1 α (69, 90); therefore, inhibition of APE1's redox activity could affect not only cancer cells, but also reduce angiogenesis within the tumour microenvironment (69). Despite this evidence, in a recent unpublished study the APE1 redox inhibitor APX3330 showed cytotoxicity against cancer cells, but not against cancer-associated fibroblasts (CAFs) in an *ex vivo* 3D co-culture system (143).

Another study investigating the consequences of hypoxia on tumour microenvironment observed that hypoxic tumours down-regulate BER components, sensitising cells to genotoxins such as H₂O₂ and MMS (144). Thus, hypoxic tumours might be more sensitive to BER inhibitors. Nevertheless, the authors also concluded that a persistent down-regulation of BER components in the microenvironment facilitates a mutator phenotype, increasing genetic instability and cancer progression (144). Should downregulation of BER generate a pro-tumorigenic microenvironment, the balance between benefits and risks will clearly have to be assessed when using BER inhibitors in situations where the microenvironment is not already hypoxic. In fact, DNA damage is both an Achilles' heel and a driving force for the tumour microenvironment. Oxidative stress is an important trigger for the formation of CAFs (145) and tumour-associated macrophages (146). Common chemotherapeutic drugs, such as cisplatin and carboplatin, have been shown to have similar effects, promoting CAF formation (147). In principle, BER inhibition could have the same impact, resulting in persistent genomic instability and stimulating CAF formation, rather than inducing an anti-tumorigenic effect. Consistently, a recent study from our laboratory showed that XRCC1 depletion in fibroblasts determines a proteomic switch resembling a cancer-like phenotype (148).

Altogether, these studies suggest that caution should be used when targeting BER *in vivo*, as cancer cells and the tumour microenvironment might respond differently to BER inactivation. However, as information regarding BER and the tumour microenvironment is very limited, more efforts are necessary to have a complete picture of the response to BER targeting.

Targeting BER in normal tissue

As our comprehension of BER progresses, the availability of efficacious strategies targeting BER for personalised medicine draws nearer. This highlights the need to understand the consequences of these strategies on normal tissue. Although BER inhibitors are far from being a “magic bullet” alternative to traditional chemotherapy, we can at least aim for a significant reduction in the dosage of chemotherapeutic drugs required when BER inhibitors are used in combination therapy. This should theoretically decrease cytotoxicity to normal tissue and reduce side effects common to traditional anti-cancer agents.

Differently from cancer cells, normal cells show better adaptation to BER deficiency. Normal fibroblasts and epithelial cells have recently been demonstrated to adjust APE1 protein levels to match XRCC1 amount through a p53/Sp1-dependent mechanism (16). Coordination between BER components avoids deleterious accumulation of DNA damage and therefore genomic instability during DNA replication (16, 17, 149). Furthermore, in the presence of unrepaired DNA damage normal cells have been shown to delay S phase entry through an ATM/p21-dependent mechanism (41). Despite the existence of robust mechanisms buffering genomic stability in normal cells, some studies suggest that BER depletion in normal cells can have negative consequences (148, 150). Our laboratory showed that depletion of XRCC1, PNKP or TDP1 in normal fibroblasts determines proteomics changes resembling those found in cancer cells. This is accompanied by profound alterations in tissue homeostasis, serine biosynthesis, and one-carbon- and amino acid metabolism, all of which have been identified as features typical of cancer cells (148). These observations suggest that although normal cells can withstand BER deficiency despite accumulation of endogenous DNA damage (16, 41), their response to stress might make them more likely to promote tumorigenic behaviour in surrounding cancer cells through a “reverse Warburg effect”-like mechanism (151). Another study showed that epithelial cells display strong defects in both BER and the DNA damage response when entering senescence. Worryingly, the authors observed that SSB repair deficiency leads to the emergence of post-senescent precancerous cells and suggested that SSB accumulation during ageing could be one of the very first steps of carcinogenesis in epithelial cells (150). In accordance with this work, exhaustion of BER capacity has been observed during ageing, where Pol β levels and DNA ligase activity have been shown to decline in an age-dependent manner in rodents (152), with defects in the regulation of APE1 (153), and an overall reduction of BER capacity (154). Similar observations have been made in human cells (155, 156). Furthermore, BER deficiency during differentiation has been documented. XRCC1 levels decrease during terminal differentiation of

muscle cells (157) and neurons (158). XRCC1 loss has also been observed during monocyte maturation (159). Overall, it seems that undifferentiated cells have greater BER capacity than their differentiated counterparts. This paradigm holds true also for fibroblasts, as embryonic stem cells have been shown to display higher expression of BER proteins than primary fibroblasts (160). Thus, there is a reasonable concern regarding BER inhibition in normal tissues as it might interfere with physiological processes such as ageing, differentiation and senescence. Even worse, BER inhibition could promote tumorigenesis in normal cells. These observations highlight the need for further studies, as at the moment it is difficult to predict the consequences of BER inhibition on normal tissue.

Backup DNA repair pathways and issues in BER targeting

As explained earlier in this review, BER is a vital pathway. It is therefore expected that cancer cells will have difficulties compensating for a complete loss of BER capacity. Unfortunately, DNA repair pathways are not an isolated array of reactions acting independently of one another; some complex DNA lesions might require the intervention of multiple enzymes in order to be resolved. This becomes particularly relevant during anti-tumour treatment, as DNA damage generated by exogenous sources such as radiotherapy or chemotherapy is often diverse and composite. In the case of BER, several DNA repair pathways have been suggested to overlap and to establish cross-talks.

Nucleotide excision repair (NER), canonically thought to deal with DNA cross-links and bulky base adducts, has been shown to compete with BER for the repair of oxidative lesions and cisplatin-induced interstrand crosslinks (161, 162). The relationship between BER and NER is again highlighted by evidence that the XRCC1/LigIII complex is essential for NER completion in quiescent cells upon UV-induced damage (163). Additionally, interaction of several BER components with the Cockayne Syndrome group B (CSB) protein further hints at the existence of cross-talk between the NER and the BER pathways (164). These observations show an intricate connection between the two systems. The toxicity of cisplatin also implies an epistatic relationship between the BER and mismatch repair (MMR) pathways for the same reason (165). Consistent with these observations, BER and MMR epistatic interactions have also been discovered in the model organism *C. elegans*, where the two pathways were suggested to play a role in determining sensitivity to the antimetabolite 5-fluorouracil (166).

Another example of cross-talk, provided by the work of Sultana and colleagues, is the synthetic lethality relationship between APE1 inhibition and deficiency of HR repair factors such as BRCA1 and BRCA2 (92). This observation echoes the synthetic lethality existing between PARP1 and BRCA deficiency (122-124), suggesting that PARP1 might be the connection between BER and HR (167). Genetic interactions between DSB repair and BER are not completely unexpected, as LigIII has previously been shown to act as a backup DNA ligase for the NHEJ pathway (168). Moreover, Kiraly

and colleagues provided evidence of a crosstalk between DSB repair via HR and the alkyladenine DNA glycosylase AAG (22). Finally, the same work by Sultana and colleagues also identified synthetic lethality between APE1 and DNA damage response (DDR) signalling components, ataxia-telangiectasia mutated (ATM) and DNA-PK (92), a relationship that was later confirmed with XRCC1 (33).

In summary, BER and other DNA repair pathways should not be considered as rigid pipelines of biochemical reactions but instead as an extensive and plastic network. This notion highlights the impact of backup systems on BER targeting, as overlaps in DNA repair specificity will likely reduce cell sensitivity to BER inhibitors. Extensive cross-talk, for instance, suggests that formation of DSBs upon BER targeting could be rescued by the HR pathway. Therefore, this and other genetic interactions will have to be taken into account when designing strategies to target BER in personalised medicine. These considerations might well explain why synthetic lethality strategies seem to be particularly successful.

Resistance to BER inhibitors

As only a very limited number of BER inhibitors have made their way into clinical trials, it is too early to predict the occurrence of resistance to BER targeting. However, PARPi have been extensively tested over the past ten years (125) and although PARP1 is not essential to canonical BER (134), the occurrence of resistance with PARPi can provide valuable information regarding other BER inhibitors. As discussed earlier, PARPi have been mainly employed in synthetic lethality strategies against BRCA-deficient tumours (125). Resistance has been observed through a number of different mechanisms. Firstly, cancer cells can reverse BRCA deficiency through acquisition of secondary mutations thus invalidating the synthetic lethal interaction (169). The selective pressure applied by PARPi has been shown to stabilise BRCA1 mutants in an Hsp90-dependent way, ultimately reversing BRCA deficiency (170). Additionally, it has been observed that HR deficiency can be overcome by the activation of alternative pathways. In BRCA-deficient cells PARPi lead to accumulation of DSBs; however, loss of 53BP1 has been shown to decrease cell death and partially restore HR activity by avoiding DNA end resection and switching to unconventional DNA repair systems (171). Similarly, loss of REV7 in mouse and human cell lines re-establishes CtIP-dependent end resection of DSBs in BRCA1-deficient cells, leading to resistance to PARPi (172). With respect to BER inhibition, it is possible to envision a similar scenario for the synthetic lethality interaction between Pol β and MMR-deficiency. Secondary mutations in the *MSH2* gene, or demethylation of the *MLH1* promoter could theoretically reverse the synthetic lethality and give rise to resistance mechanisms.

Secondly, a genetic screen showed that mutations of PARP1 itself act as a major trigger of resistance to PARPi (173). BER genes display polymorphic variants (see below); therefore, the occurrence of similar resistance mechanisms is conceivable under the selective pressure provided by BER inhibitors.

A third, and more difficult to predict, mechanism of resistance has also been observed. In a recent publication the c-Met receptor – a well-known oncogene – has been shown to phosphorylate PARP1, increasing its enzymatic activity and decreasing the binding of catalytic inhibitors (174). With this in mind, it is hard to foretell whether oncogenic mutations could affect BER capacity, therefore desensitising cancer cells to the effects of BER inhibitors.

Altogether, these examples indicate the importance of understanding the mechanism underlying BER modulation, as well as the relationships between BER and other cellular pathways. A thorough understanding of BER and its regulation will certainly help to predict potential challenges to BER inhibition.

Modulators of BER: new targets for personalised medicine?

As continuous efforts to develop potent and specific BER targeting agents will keep providing the clinical field with new molecules, the benefits and drawbacks of BER targeting will soon become clearer. In our opinion, future studies aiming to target BER should take advantage of our ever-growing comprehension of the pathway. In particular, we feel that the concept of BER targeting should be brought to a new level, including rigorous patient stratification criteria and exploiting the numerous systems maintaining BER coordination in order to improve targeting of the pathway.

BER is far from being a static pathway, and substantial variation in BER capacity has been measured in different individuals. Curiously, the capacity of the pathway does not appear to be proportional to the amount of BER components (175). This suggests that if we want to stratify patients on the basis of their response to BER inhibitors we will need to develop reliable assays to assess inter- and intra-individual BER variability (176). A large number of polymorphisms have been detected for BER genes. Interestingly, when considering cancer development, some polymorphisms have been suggested to promote higher risk than others. BER variants have been extensively reviewed (see (177-179)); polymorphisms in the *OGG1*, *MUTYH* and *POLB* genes have been detected. The *APEX1* and *XRCC1* genes also bear numerous polymorphisms, even though their role in cancer development is still inconclusive. Some polymorphic variants have been shown to hamper or decrease protein activity. This has been proven for the majority of *POLB* mutations, observed in 30% of tumours (180). Other polymorphisms have been predicted to affect gene expression, such as the T77C polymorphism in the *XRCC1* gene, which impacts promoter activity, leading to decreased protein expression and increased risk of non-small cell lung cancer (181). We propose that information regarding inter-individual variations in BER capacity should be exploited in order to stratify patients and select those who could benefit more from BER targeting. This would allow safer administration of BER inhibitors and minimisation of side effects on normal tissue.

The BER pathway is a highly coordinated system. BER is subject to temporal and spatial modulation and these dynamics promote genomic stability. Importantly, normal cells have been shown to

modulate BER protein levels in order to coordinate the pathway, while cancer cells display impaired control mechanisms, often with deleterious consequences (16). To maintain BER coordination, different mechanisms have been evolutionarily selected and act by adjusting the relative amount and activity of the enzymes. This is achieved by PTMs as well as modulation of protein-protein interactions and gene expression (16, 182, 183). XRCC1, for instance, regulates BER by establishing different multiprotein DNA repair complexes, thus channelling BER through distinct sub-pathways (184). Furthermore, BER capacity can be adjusted to the cellular load of DNA damage. Such regulation requires DNA damage sensors and pathway modulators, which are able to detect and transmit the signal originating from DNA lesions. Amongst these auxiliary proteins PARP1, the ATM kinase and ubiquitin ligases such as carboxy-terminus of Hsc70 interacting protein (CHIP) and Mule (also known as HUWE1) are worth to be mentioned (Figure 2).

In the case of BER, PARP1 has been shown to accelerate SSB repair and to protect exposed strand-breaks (134, 185, 186). Moreover, the protein has been shown to enhance BER of uracil- and AP site-containing DNA (187). Therefore, it is commonly assumed that PARP1 acts as an ancillary protein that modulates BER by taking part in a subset of BER events (184, 188).

ATM is an important DNA damage sensor that has only recently been linked to BER. While the role of ATM in DSB signalling and DDR is well established (189), little is known about its ability to detect and signal SSBs and its relationship with BER. ATM is a kinase known to respond to DNA damage by phosphorylating a plethora of substrates. A series of very recent publications shed light on ATM's role in BER regulation (41, 190). In particular, the observation that some BER-eliciting agents, such as methyl methanesulfonate (MMS) and H₂O₂, are able to activate ATM has sparked surprise and interest in a potential interaction between ATM and the BER pathway (40, 41, 190, 191). For instance, ATM activation in response to MMS has been shown to be prevented by depletion of methylpurine-DNA glycosylase (MPG) (40, 41). This indicates that DNA strand-breaks and not DNA base lesions are able to induce ATM auto-phosphorylation. Moreover, our laboratory recently showed that ATM activation occurs in presence of persistent SSBs (41). In this context, ATM activation has been demonstrated to trigger a p53/p21-dependent cell-cycle delay, thus allowing more time for SSB repair before replication (41). These observations led us to hypothesise that ATM might be a previously overlooked SSB signalling factor that allows fine tuning of the cell-cycle checkpoint, therefore preventing conversion of SSBs into DSBs during replication (41). Unfortunately, the enzymology and the mechanistic leading to SSB-dependent ATM activation have still to be clarified. Besides the simple signalling of SSBs, signals from PARP1 and ATM might also be used to modulate BER capacity and adjust it to the cellular load of DNA damage. However, BER components are believed to be relatively abundant proteins endowed with a long half-life. A reasonable hypothesis to understand BER modulation is to assume that the amount of BER factors oscillates constantly around their steady-state level. This would allow continuous adjustment of BER capacity to the load of DNA damage, simply by modulating the amplitude of the oscillations. While long-term adjustments in

protein levels have been demonstrated to require transcriptional regulation (16), short-term modulation of BER generally involves stabilisation of components engaged in DNA repair and degradation of excessive factors. The ubiquitin-proteasome system plays a major role in the modulation of BER, whereby interplay between mono- and poly-ubiquitylation has been shown to affect protein levels of APE1, XRCC1, PNKP, Pol β , LigIII (reviewed in (192)). As an example, Pol β turnover appears to be the result of a balance between Mule-mediated mono-ubiquitylation and CHIP-mediated poly-ubiquitylation. These reactions can be reversed by ubiquitin-specific proteases such as USP47, therefore allowing for continuous adjustments in the amount of Pol β (193).

Interestingly, one of the modulators of Mule's activity is the p14 alternative reading frame (p14^{Arf}) tumour suppressor (193). Our laboratory showed how p14^{Arf} expression can be regulated by PARP1 activation and NAD⁺ depletion in the presence of persistent DNA strand-breaks (194). p14^{Arf} upregulation in response to DNA damage has a dual effect. On one hand, it affects DNA repair through inactivation of Mule and stabilisation of BER components (193, 195). On the other hand, it leads to a cell-cycle delay through stabilisation of p53 (196) (Figure 2). In principle, this mechanism could be complementary to ATM activation and could contribute to providing additional time for SSB repair before replication.

Further illustrating this interconnection, the ubiquitin-specific protease USP7 has been shown to play an important part in signalling the DNA damage load back to components of BER. In a series of studies from our laboratory, we showed that ATM activation upon DNA damage can trigger a signalling cascade that ultimately leads to destabilisation of USP7 and Mule, with consequences on BER capacity and a p53-dependent cell-cycle delay (195-197) (Figure 2).

In conclusion, extensive cross-talk and modulation clearly exists beyond the canonical illustration of BER presented in Figure 1. It appears that BER is tightly interconnected with DNA damage signalling pathways and cell-cycle regulators. For this reason, we would suggest that targeting BER for personalised therapy should be considered from a broader perspective. For example, targeting modulators of BER separately, or together with the inhibition of core BER components could in principle lead to better therapeutic benefits. From this perspective, several strategies must be devised in order to tackle BER from different angles, including regulation of BER gene transcription, PTMs and signalling of DNA damage (Figure 3). A detailed comprehension of the mechanism modulating BER will therefore be required in order to tailor BER targeting to the physiological status of each individual.

Concluding remarks

This review summarises the current approaches aiming to target BER in order to personalise cancer therapy. Our current knowledge has just scratched the surface of the rich PTM landscape, which introduces a myriad of subtle alterations tuning BER function and capacity (198). Moreover, our

understanding of the mechanisms governing BER coordination is still fairly limited. It is clear that a number of signalling pathways converge on BER in an intricate fashion. A thorough comprehension of this cross-talk is of the utmost interest in order to exploit BER modulation as an additional target to improve personalised medicine. Despite these limitations, there is a growing body of evidence suggesting that BER might be a vulnerable point for cancer cells, as their ability to coordinate the pathway is clearly different from normal cells. Research aiming to generate small molecules targeting BER has yielded interesting inhibitors with appealing features for translational applications. Several of them, inhibiting for instance APE1, Pol β and PARP1 already support the validity of BER targeting as a therapeutic strategy.

Nevertheless, the importance of BER for healthy tissue has to be considered. BER has been proven to play a role in cellular differentiation and ageing. There is still a gap regarding the impact of BER inhibition in this context. It is therefore imperative for future strategies aiming for systemic BER targeting to consider healthy tissue as well as the differential expression levels of BER components between cancer cells and tumour microenvironment. For these reasons, strategies exploiting synthetic lethality or patient stratification on the basis of their BER capacity have to be explored as well. This will minimise the risk of systemic toxicity, while maximising the therapeutic benefits.

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Figure Legends and Tables

Figure 1. Crucial steps in the DNA base excision repair pathway.

Canonical BER is reported, together with the crucial steps of the pathway. Each one of the indicated steps of BER can theoretically be targeted. The scheme shows the division of BER into long and short patch.

Figure 2. The intricate modulation of BER and cell-cycle.

Schematic representation of the intricate network promoting BER coordination with the load of DNA damage. DNA strand breaks are constantly detected by sensors such as ATM and PARP1. PARP1 activation and enzymatic poly(ADP ribosylation) of target proteins affects SSB repair capacity. Persistence of DNA damage and NAD^+ depletion lead to transcriptional activation of p14^{Arf} and modulation of BER capacity and cell-cycle through Mule activity. Additionally, ATM activation and auto-phosphorylation trigger a checkpoint response that, through degradation of USP7, leads to modulation of Mule stability and ultimately regulation of BER. At the same time, the USP7-mediated effect on p53 enforces a cell-cycle delay together with p53-dependent transcriptional regulation of DNA repair genes. The combined effect of cell-cycle response and BER modulation promotes genomic stability and adjustment of BER capacity to the cellular load of DNA strand breaks.

Figure 3. Targeting BER: more than enzymatic inhibition.

Examples of BER targeting strategies are reported in this scheme. In addition to the classical approach aimed at interfering with enzymatic activities of the pathway, BER can in principle be targeted at the level of transcription, through modulation of the activity of crucial transcription factors. Synthetic lethality approaches can be exploited. PTMs, as well as the enzymes responsible for these modifications are potential targets for BER modulation, together with BER interacting partners. An additional targeting strategy could involve modulation of the BER DNA damage response through inhibition of DNA damage sensors such as ATM or PARP1.

Table 1. List of currently available small molecules targeting BER. For each of the BER targets the better characterised inhibitors are listed, along with their chemical structure, IC₅₀ (calculated using *in vitro* assays), status of validation and, where appropriate, clinical trials. Note that when more than one analogue is listed only a representative chemical structure is reported.

Target	Inhibitor	Chemical structure	<i>In vitro</i> IC ₅₀ (μmol/l)	Validation	Ongoing clinical trials
NEIL1	NCGC00188618 NCGC00188616 NCGC00188617 NCGC00188619		4-10	<i>In vitro</i>	
OGG1	O8, O154, O167, O151, O158		0.2-0.6	<i>In vitro</i>	
PNKP	A12B4C3		0.06	<i>In vitro</i> Cell lines	
TDP-1	NCGC00183674 JLT048		7.7-148	<i>In vitro</i> Cell lines	
	Benzopentathiepin		0.22-6.03	<i>In vitro</i> Cell lines	
	JBIR-21		18	<i>In vitro</i> Cell lines Xenografts	
	CD00509		<1.0	<i>In vitro</i> Cell lines	
APE1	Methoxyamine		>1000	<i>In vitro</i> Cell lines Xenografts	Phase I/II
	Lucanthone		5	<i>In vitro</i> Cell lines Xenografts	Phase II - suspended
	CRT0044876		3.06	<i>In vitro</i> Cell lines	
	AR03		<10	<i>In vitro</i> Cell lines	
	Compound #3 Compound #52		<10	<i>In vitro</i> Cell lines In vivo	
	Gossypol		0.036	<i>In vitro</i> Cell lines Xenografts	Phase III

FEN1	Compound #17		0.011	<i>In vitro</i> Cell lines	
	NSC-281680		1.2	<i>In vitro</i> Cell lines	
	NSC-13755		0.93	<i>In vitro</i>	
DNA polymerases	Compound 3a		21	<i>In vitro</i> Cell lines	
	Lithocholic acid		47	<i>In vitro</i> Cell lines	
	NSC-124854		5.3	<i>In vitro</i> Cell lines	
	NSC-666715		4.1	<i>In vitro</i> Cell lines Xenografts	
	Pamoic acid		100	<i>In vitro</i> Cell lines	
DNA ligases	L67, L82, L189		5-12	<i>In vitro</i> Cell lines	
	SCR7		0.04	<i>In vitro</i> Cell lines Xenografts	
PARP1	Olaparib		0.005	<i>In vitro</i> Cell lines Xenografts	FDA-approved
	Veliparib		0.005	<i>In vitro</i> Cell lines Xenografts	Phase III
	Talazoparib		0.0006	<i>In vitro</i> Cell lines Xenografts	Phase III
	Niraparib		0.004	<i>In vitro</i> Cell lines Xenografts	Phase III
	Rucaparib		0.0008	<i>In vitro</i> Cell lines Xenografts	Phase II

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DNA STRAND-BREAK

**PPM1G-
DEPENDENT
DEGRADATION**

**MODULATION OF
REPAIR CAPACITY**

**NAD⁺
DEPLETION**

p14^{Arf}

**CELL-CYCLE
DELAY**

**TRANSCRIPTIONAL
REGULATION OF
DNA REPAIR GENES**