Perspective on the pipeline of drugs being developed with modulation of DNA damage as a target

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Abstract

Inhibitors of various elements of the DNA repair pathways have entered clinical development or are in late pre-clinical stages of drug development. It was initially considered that agents targeting DNA repair would act to overcome tumour resistance to chemotherapy and radiotherapy. More recent data has shown that targeting DNA repair pathways can be effective in selected tumours via a synthetically lethal route – with single agent activity having been demonstrated with PARP inhibitors. An increased understanding of the biology and interaction of the DNA repair pathways also means that rationale combination of DNA repair inhibitors may also give great benefit in the clinic.
Introduction

Repair of DNA and thus preservation of the genetic code is critical for normal cellular function. To this end human cells have at least 5 recognised pathways which protect the genome by signalling specific types of DNA damage and carrying out repair (reviewed in (1-3)). In cancer cells these pathways represent a curious dichotomy – it is well recognised that mutations in the pathways can predispose to cancer and are hallmarks of many of the hereditary cancer syndromes (4-6). However, once an immortalised tumour cell has developed, the DNA repair pathways can be used by this cell to overcome many of our standard anticancer treatments and hence are a cause of treatment resistance. There is increasing evidence in the literature that tumour tissue has high levels of some elements of the DNA repair pathways (7, 8), being able to use these pathways to repair damage caused by many of our standard anticancer therapies. Hence inhibiting DNA repair may “level the playing field” and make the tumour more vulnerable to treatment.

The major DNA repair pathways are direct repair, mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), and double strand break recombinational repair which includes both non-homologous end joining (NHEJ) and homologous recombinational repair (HHR) (1, 2, 9). O^6^-alkylguanine-DNA alkyltransferase (MGMT, OGAT, ATase) is the main component of the Direct Repair Pathway, an efficient mechanism of DNA repair where the altered base is corrected without removal or disruption of the phosphodiester backbone. Over expression of ATase in mammalian cells confers resistance to DNA alkylating agents (reviewed in (10)), and is a major factor in tumour resistance to these drugs. NER is involved in the repair of UV damage and removal of bulky DNA adducts such as those caused by cross-linking agents. MMR repairs replication errors and is frequently mutated in cancer cells allowing tolerance of such lesions (11-13). BER is involved in the repair of single strand breaks, contributing to resistance to ionising radiation and alkylating agents. Recombinational repair has two pathways, the error-free HRR in dividing cells and error-prone NHEJ active in G1. These two pathways repair much of the damage caused by radiotherapy and chemotherapeutic agents such as cisplatin and mitomycin C (2).

There are compounds in the clinic or in late preclinical development which inhibit direct repair and elements of the base excision and double strand break repair pathways. The initial development of inhibitors of DNA repair pathways was designed to overcome chemo- or radio-resistance (14-19). However the increasing knowledge of the complexity and interactions of the DNA damage response pathways, as well as the entry of a range of compounds into the clinic has led to a fascinating area of drug development – where there are opportunities for improving on existing treatments but also for the design of rationale combinations of novel agents to improve treatment response. Table 1 demonstrates the range and variety of these DNA damage response modulators which have entered the clinic in recent years.

Chemo-potentiation

When considering the pipeline of novel agents which over the last 5 year have entered clinical development or are at a late preclinical phase it is worth briefly reviewing the earlier trials where blocking a DNA repair pathway was the primary aim. To date a common theme has emerged in the majority of trials which appears to be limiting the effectiveness of this strategy- potentiation of normal tissue toxicity. Depletion of MGMT and hence disruption of the direct repair pathway by O^6^-benzylguanine or lomeguatrib was successfully achieved more than 10 years ago. These agents
were combined with carmustine and temozolomide respectively and although pharmacodynamic assays confirmed depletion of the target this was achieved at the expense of the chemotherapeutic dose (14, 17). Enhanced normal tissue toxicity in the form of more profound myelosuppression meant that a significant reduction in chemotherapy dose was required and phase II studies did not demonstrate a benefit in terms of increased tumour response (15, 18). When the first PARP inhibitor in cancer treatment entered the clinic in 2003 it was also evaluated in combination with chemotherapy with the initial reports being that a PARP inhibitory dose of drug could be given with full dose temozolomide (19). This was not borne out in the subsequent phase II study where a 25% reduction in cytotoxic dose was needed for a tolerable regimen (20). Although this small study did suggest a possible clinical benefit of the combination this has yet to be confirmed in a randomised study.

Enhancement of normal tissue toxicity is emerging as a common theme with some of the other PARP inhibitors when combined with chemotherapy. Studies with ABT888 (veliparib) and AZD2281 (olaparib) with a range of cytotoxic agents have reported the need to reduce chemotherapy dose due to enhanced myelosuppression (21-23). The outlying data in this area is that from the combination of BSI-201 with carboplatin and gemcitabine in triple negative breast cancer where very encouraging evidence of increased activity was observed with no increase in toxicity (24, 25). It has been speculated that this may be due to the intermittent schedule of dosing of the PARP inhibitor allowing bone marrow recovery. This would argue for the increased activity being due to chemo-potentiation rather than single agent PARP activity acting through synthetic lethality on the proposed HRR deficient triple negative phenotype as other studies demonstrating single agent activity have required continuous and profound PARP inhibition (26-28). Other agents targeting DNA damage response in late preclinical development are entering the clinic (inhibitors of DNA PK, ATM, ATR, and RAD51 (29-33)). These agents have also demonstrated the ability to potentiate the activity of cytotoxic drugs in preclinical models, it remains to be seen whether this can be done without the increased toxicity and subsequent dose reductions which have been required in many of the previous studies. It may be that it is the area of radio-potentiation that we are able to use these powerful inhibitors to fuller potential. Radiation causes DNA single and double strand breaks, many of the DNA repair inhibitors have been shown to be radio-potentiating (29, 30, 34). The increasing use of highly technical radiotherapy techniques (IMRT, IGRT and tomotherapy) may allow radiation/inhibitor combination studies where tumour response is improved without consequent increase in normal tissue toxicity.

One of the very exciting developments in the field of DNA damage response research in the last few years has been the preclinical (35, 36) and subsequent clinical demonstration (26-28) of the ability to cause synthetic lethality in selected cell types using a DNA repair inhibitor without also using a DNA damaging agent. Although this has first been demonstrated with the use of PARP inhibitors in patients with familial breast and ovarian cancer carrying the BRCA genes it has opened up the possibility that this much less toxic strategy may be a benefit in patients with sporadic tumours if a predictive molecular phenotype can be identified. Many research groups are now working to develop functional assays for double strand break repair competence, or molecular signatures which will allow enrichment of patient populations within trials. An additional consequence of this pioneering research has been that DNA repair inhibitors have been recognised as potentially active anticancer agents in their own right. With the expanding knowledge of the DNA damage response pathways and the plethora of drugs entering clinical development targeting different elements of
these pathways it will be possible to design trials where novel combinations of repair inhibitors including the check point inhibitors (37-39) may be active, or where patients are selected based on the oncogenic mutation status of their tumour – for example mutations in ATR, ATM or the Fanconi proteins may also predict for sensitivity to PARP inhibitors (40, 41). Figure 1a summarises in simplified form the BER and DSB pathways emphasising the close interactions and cross talk between the BER and DSB repair pathways and cell cycle check point signalling. Figure 1b illustrates the currents DNA damage response modulating drugs targeting these pathways and related checkpoint signalling illustrating the fascinating potential for rationale combination of these novel agents.

Conclusion

As the DNA repair inhibitors continue to move forwards in clinical development we need to be able to learn from and build on the lessons of history so that the true potential of these drugs is realised. It is likely that they will be ultimately be used in combination regimens, the response rates in the BRCA population are not at the levels seem in CML and GIST with imatinib. There is, therefore, much work to be done exploring scheduling to avoid increased toxicities. When a DNA damage modulating agent is to be used to prevent repair and so potentiate the activity of the cytotoxic agent they need to be given concurrently, it may be that pulsed schedules of the modulator would, in this situation, cause the desired tumour cell kill but allow normal tissue toxicity to recover. If the modulator is predicted to have single agent activity in a particular disease setting then scheduling apart from the cytotoxic with longer duration of coverage might be the optimal route. The interplay between the increasing knowledge of the biology of these pathways and the increasing ability to explore the molecular profile of our patients’ tumours using array and circulating tumour cell technologies means that this is an exciting, fast moving and potentially very beneficial area of cancer treatment research.

References


Figure legends

Figure 1 Simplified schematic of signalling and repair of single and double DNA strand breaks to illustrate the interactions between pathways and potential rational inhibitor combinations.

Figure 1a Damaged DNA bases are excised involving APE-1, and single strand break activates PARP which recruits other components of BER. In a dividing cell unrepaired SSB will become DSB. The presence of DS breaks is signalled via the ATM/ATR pathways. In non-dividing cells G1 arrest is signalled via Chk2 and NEHJ pathways repairs the break. In dividing cells G2 arrest allows error free repair using HR.

Figure 1b Simplified schematic of signalling and repair of single and double DNA strand breaks showing the points of action of current inhibitors in the clinic or in late preclinical development. Additionally it is highlighted that Chk1 inhibitors have entered the clinic, presenting an opportunity to combine these agents with DNA damage response modulators.
Table 1

DNA damage response modulating drugs in clinical development – grouped by repair pathway targeted. Data taken from [www.clinicaltrials.gov](http://www.clinicaltrials.gov), compounds thought to be in late preclinical development also included for completeness.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Company</th>
<th>Administration</th>
<th>Single/combination therapy</th>
<th>Disease indications</th>
<th>Clinical status</th>
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<tbody>
<tr>
<td><strong>Direct repair (MGMT)</strong></td>
<td></td>
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<tr>
<td>O$^6$benzyguanine</td>
<td></td>
<td></td>
<td>Combination BCNU</td>
<td>GBM</td>
<td>Phase II complete</td>
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<tr>
<td>Lomeguatrib</td>
<td>KuDos</td>
<td>Oral</td>
<td>Combination with TMZ</td>
<td>Melanoma</td>
<td>Phase II complete</td>
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<tr>
<td><strong>Single strand break repair (PARP inhibitors, PARPi)</strong></td>
<td></td>
<td></td>
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<tr>
<td>PF0367338 (AG014699)</td>
<td>Pfizer</td>
<td>IV</td>
<td>Combination ++ single agent</td>
<td>Solid tumours, melanoma</td>
<td>Phase I and II complete and others on going</td>
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<td>Olaparib (AZD2281)</td>
<td>AztraZeneca (KuDos)</td>
<td>Oral</td>
<td>Combination ++ single agent</td>
<td>BRCA defective, solid tumours various</td>
<td>Phase I and II studies completed and on going</td>
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<td>Veliparib (ABT888)</td>
<td>Abbott</td>
<td>Oral</td>
<td>Combination ++</td>
<td>Various solid tumours</td>
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<tr>
<td>Iniparib (SAR240550, BSI 201)</td>
<td>Sanofi Aventis (Bipar)</td>
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<td>Combination</td>
<td>Triple negative breast</td>
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<td>Single agent</td>
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<td></td>
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<td>Methoxyamine</td>
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<tr>
<td>CP466722</td>
<td>Pfizer</td>
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Damaged base DNA SSB

Signaling G1 arrest to allow repair

Error prone NHEJ in G1

Rad51, DNA-PK, XRCC4, Ligase IV

Unrepaired SSB at replication

DNA SSB

Damage base

APE-1, APE-1

PARP, PARP

Base Excision Repair

Retract