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Targeting DNA repair for anti-cancer therapy

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Abbreviations:
ATM: Ataxia telangiectasia mutated
ATR: Ataxia telangiectasia mutated- and Rad3-related
BER: base excision repair
DSB: double strand break
HR: homologous recombination
NER: nucleotide excision repair
NHEJ: non-homologous end joining
MGMT: O6-methylguanine methyl transferase
MMR: mismatch repair
PARP: poly(ADP-ribose) polymerase
SSBR: DNA single strand break repair

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ABSTRACT | Chemotherapy often targets dividing cells by causing DNA damage that leads to replication-dependent toxic lesions. Cells possess several overlapping DNA damage repair pathways that allow them to survive these treatments. Inhibitors of DNA repair are therefore used in combination therapy to modulate the efficacy of DNA damaging drugs. Since DNA repair pathways are commonly altered during tumour development, cancer cells will depend on a remaining subset of DNA repair pathways for survival. These remaining pathways can be targeted by DNA repair inhibitors as monotherapy to selectively kill cancer cells. The advantage of DNA repair inhibition as a single agent therapy is that it selectively increases unrepaired endogenous DNA damage in tumour cells and therefore appears to have fewer side effects in non-cancerous cells. DNA damage response and repair inhibitors may also be used to amplify oncogene- or hypoxia-induced replication stress and convert these lesions into fatal replication lesions.

At a glance

- Several anti-cancer chemotherapy drugs work by causing excessive DNA damage that is converted into toxic lesions during DNA replication. Survival is promoted through repair of these lesions by a number of DNA repair pathways that have overlapping substrate specificities. The efficacy of anti-cancer drugs is therefore highly influenced by cellular DNA repair capacity. Inhibitors of DNA repair increase the efficacy of DNA damaging anti-cancer drugs in preclinical models. Small molecule inhibitors of DNA repair have been combined with conventional chemotherapy drugs in several phase I-II clinical trials.

- Tumour development is commonly associated with perturbed DNA damage response and repair pathways. This result in reduced DNA repair capacity and increased genetic instability of tumour cells. DNA repair pathways have overlapping specificities and defects in one pathway can be compensated for by other pathways. These compensating pathways can be identified in synthetic lethal screens and then specifically targeted for treatment of DNA repair-defective tumours.

- Inhibitors of DNA repair can work as single agents for targeted treatment of DNA repair-defective cancers. This hypothesis is currently being tested in phase II trials where patients with breast or ovarian cancers defective in homologous recombination are treated with a PARP inhibitor to target an overlapping pathway, DNA single-strand break repair (SSBR).

- Tumours often exhibit replication stress as a consequence of oncogene-induced growth signals or hypoxia-induced replication arrest. DNA repair inhibitors could be used to prevent the repair of replication lesions present in tumour cells and convert them into fatal replication lesions that specifically kill cancer cells.

Cancer therapy usually involves exposing the body to cytotoxic agents, administered with the aim of killing malignant cells more efficiently than normal tissue. The therapy must therefore exploit specific molecular and cellular features of the cancer it is aiming to eliminate. One fundamental characteristic of cancer cells is that they are rapidly proliferating and therefore most anti-cancer drugs target the cell cycle in various ways. Cell division can be targeted directly by inhibitors of the mitotic spindle, thus preventing equal division of DNA to the two daughter cells. The growth signals that result in entry into the cell cycle can be targeted by hormonal manipulation, therapeutic antibodies and drugs that inhibit growth signalling pathways. However, the most common means of targeting the cell cycle is to exploit the impact of DNA damaging drugs on DNA replication during S phase. When cells attempt to replicate the damaged DNA more severe lesions are generated, thus making DNA damaging treatments more toxic to replicating cells than non-replicating cells. The toxicity of DNA damaging drugs can however be reduced by the activities of several overlapping DNA repair pathways which remove lesions before the onset of DNA replication. DNA repair pathways thus modulate the efficacy of cancer therapy. In addition, they are frequently mutated in cancers. These two features make DNA repair a promising target for novel cancer treatments.

DNA damaging agents in cancer treatment

Many anti-cancer drugs employed in the clinic have been used for several decades and are highly efficient in killing proliferating cells by interfering with DNA replication through a range of different mechanisms (Figure 1). The principal mechanism by which toxicity is achieved is by obstruction of replication fork progression, which can lead to replication fork collapse, resulting in the formation of replication-associated DNA double-strand breaks (DSBs). DSBs are generally considered to be the main toxic DNA lesions that kill cells by induction of apoptosis [1,2].

Common types of DNA damage that interfere with replication fork progression are chemical modifications (adducts) of DNA bases, caused by reactive drugs that covalently bind DNA, either directly or after being metabolised in the body. These *alkylating agents* are grouped in two categories; mono-functional alkylating agents with one active moiety that modifies single bases, while bi-functional alkylating agents have two reactive sites and crosslink two bases within the same DNA strand (intra-strand crosslinks) or between opposite DNA strands (inter-strand crosslinks). Such inter-strand crosslinks pose a complete block to replication forks.

The DNA synthesis process itself is often targeted by chemotherapy, either through the use of replication inhibitors or by *anti-metabolites*. DNA replication inhibitors such as aphidicolin directly inhibit DNA polymerases [3], whereas the radical scavenger hydroxyurea inhibits ribonucleotide reductase, required for production of deoxyribonucleotides (dNTPs) that are used for DNA synthesis [4]. Replication inhibitors can be regarded as DNA damaging agents because, as explained above, impaired replication fork progression causes DNA lesions including DSBs [5,6]. Anti-metabolites resemble nucleotides or nucleotide precursors and act by inhibiting nucleotide metabolism pathways, thus depleting cells of dNTPs. They can also impair replication fork progression by becoming incorporated into the DNA [7]. In general, the biochemical mechanisms of cell death induced by anti-metabolites are poorly understood.

Another means of interfering with replication is to exploit DNA strand breaks that arise naturally during the process of DNA synthesis. Topoisomerases are a group of enzymes which resolve torsional strains imposed on the double helix during DNA replication. They induce transient DNA breaks to relax supercoiled DNA or allow DNA strands to pass through each other [8]. Topoisomerase inhibition, a common strategy for

anticancer treatment, prevents re-sealing of these breaks causing replication-associated DSBs [1,2].

Ionizing radiation and "radiomimetic" agents such as bleomycin cause replication-independent DSBs that efficiently kill non-replicating cells. However, radiation rapidly prevents replication by activation of cell cycle checkpoints to avoid formation of toxic DNA replication lesions [9]. These cell cycle checkpoints are regulated by effector kinases, such as ATM and ATR [10-12] which regulate the activities of downstream checkpoint proteins such as Chk1 and Chk2. . Defects in DNA damage checkpoint pathways result in sensitivity to a range of anti-cancer treatments, (e.g. loss of ATM results in sensitivity to ionizing radiation [13]) and inhibitors of these checkpoint pathways are being explored in the treatment of cancer, as discussed below.

Chemotherapy-induced DNA lesions are efficiently repaired

DNA repair activity largely determines the efficacy of anti-cancer drugs in causing tumour regression. Direct DSBs are mainly repaired by *non-homologous end joining* (NHEJ)[14], whereas replication-associated DSBs are repaired by *homologous recombination* (HR)[15] and related replication repair pathways, as discussed below. DNA adducts, such as those created by alkylating agents, may be excised and repaired before they are confronted by the replication machinery. This is achieved by *base excision repair* (BER), excising a single damaged DNA base or a short strand containing the damaged base [16] or *nucleotide excision repair* (NER), which excises a single-stranded DNA molecule of approximately 24 to 30 base pairs containing the DNA lesion [17,18]. Damaged DNA can also be repaired without removal of the damaged base, in a process that directly reverses the DNA alkylation [19]. The O6-methylguanine methyl transferase (MGMT) is an *alkyltransferase* and removes alkylations on the O6 position of guanine produced from the anti-cancer drugs such as temozolomide [20], and the *DNA-dioxygenases* ABH2 and ABH3 revert 1-methyladenine and 3-methylcytosine back to adenine or cytosine respectively[21]. The repair of alkylated lesions is thought to be quick, with the majority of lesions appearing to be repaired within one hour [22]. If the lesions are removed before initiation of replication, the efficiency of alkylating agents in killing the tumour is significantly reduced. Thus, modulation of DNA repair clearly influences the efficacy of

alkylating agents, and resistance to alkylating agents is often explained by upregulation of DNA repair proteins.

Whereas most DNA repair pathways mediate resistance to DNA damage, *mismatch repair* (MMR) is actually required for the toxicity of several anti-cancer drugs (Figure 1). This has been explained by the "futile repair cycle" model in which mismatch repair removes the newly inserted intact base instead of the damaged base, triggering subsequent rounds of futile repair which might be deleterious [23]. It is also possible that mismatch repair might have an important role in triggering checkpoint signalling and apoptosis, which might mediate increased toxicity [24]. It has been established that a defect in mismatch repair is associated with resistance to many DNA damaging anti-cancer agents, such as mono- and bi-functional alkylators and antimetabolites [7,23,25]. It should be noted that mismatch repair acts directly at replication forks and can therefore not prevent them from encountering damage.

Collapse of replication forks during DNA synthesis can be avoided by bypassing DNA lesions in a process called *translesion synthesis* [26,27]. This process is carried out by switching the regular polymerases epsilon and delta, responsible for leading and lagging strand synthesis respectively [28,29], to polymerases with different substrate specificities, thus enabling them to bypass different types of damaged bases [30].

Once replication forks stall or collapse upon encountering DNA damage other repair pathways are required to permit resumption of replication. Collapsed replication forks are recognised by the checkpoint machinery, which will in turn trigger cell cycle arrest [12], DNA repair [31] or cell death through apoptosis or senescence [32-34]. Although we know very little of the nature of replication lesions, there is an increasing body of information concerning pathways that repair them. *Homologous recombination* plays a central role in the repair of most replication lesions formed by anti-cancer drugs [5,6,15,35]. There are several ways by which homologous recombination is utilised to restart replication. The sequence identity between two newly synthesised DNA molecules can be used to restart replication behind the replication block. Also, recombination can be used to bypass DNA lesions, in a process called template switching (see [36] for details). Other repair pathways active at replication forks involve the *Fanconi anaemia associated repair* [37], *endonuclease-mediated repair*, such as mediated by the Mus81 endonuclease[38],

and *RecQ-mediated repair*, involving DNA helicases such as BLM [39], WRN [40,41] and other members of the RecQ family of helicases [42]. Several of the proteins in these pathways have been found to be directly linked with homologous recombination [43] or the resolution of recombination products such as Holliday junctions [39,41,44,45]. However, cells that are defective in these pathways show distinct differences from recombination defective cells, indicating that they represent different but overlapping repair pathways [46].

Cells defective in a specific DNA repair pathway exhibit sensitivity to drugs producing DNA lesions that are normally repaired by this pathway. This sensitivity has been exploited to isolate hamster cell lines showing hypersensitivity to anti-cancer drugs (e.g. etoposide, mitomycin C) and ionizing radiation, and also to allow cloning of genes involved in DNA repair [47]. The DNA repair pathways involved in the repair of damage caused by various anti-cancer agents are summarised in Figure 1. These DNA repair pathways are often up-regulated in tumour cells, resulting in resistance to chemotherapeutic drugs [48]. Importantly, these DNA repair pathways can be inhibited pharmacologically to potentially increase the efficacy or specificity of anti-cancer agents (see below).

Current DNA repair inhibitors for cancer treatment

The basic understanding of DNA repair mechanisms, from the principles of the DNA lesions created and the pathways required to repair these lesions, has greatly increased during the past years. This permits a rational combination of cytotoxic agents and inhibitors of DNA repair to enhance tumour killing. Specific inhibitors of DNA repair that have been developed as clinical agents are discussed in this section (see Figure 2).

Sensitisers to alkylating agents. Despite the adverse side effects caused by alkylating agents on bone marrow and other normal tissues, drugs such as cyclophosphamide, ifosfamide, chlorambucil, melphalan and dacarbazine remain some of the most commonly prescribed chemotherapies in adults and children with various solid and haematological malignancies, particularly in multi-agent regimes combined with anthracyclines and steroids. More recently, a DNA alkylator and methylator developed in the 1980s, temozolomide (an oral prodrug which crosses the blood brain barrier) has

changed clinical practice in the treatment of high grade gliomas in adults and children [49,50]. The combination of PARP1 inhibition and temozolomide is currently in several clinical trials (see Figure 2). The rationale for this treatment strategy is that inhibition of PARP retards the repair of an intermediate damage lesion, the apurinic site, induced by temozolomide. However, this intermediate is not generally regarded as a major contributor to the cytotoxicity induced by temozolomide in the absence of PARP inhibition, as they are promptly removed by base excision repair in cells with abundant functional PARP1. The success of the treatment rationale adopted by current clinical trials of GPI-21016 (Guilford Pharmaceuticals, Baltimore, MD), INO-1001 (Inotek Pharmaceuticals, Beverly, MA) and AG014699 (Pfizer GRD, La Jolla, CA) therefore depends on the overall biological role of and necessity for PARP in cancer cells trying to repair this type of damage.

Another class of agents currently being tested in clinical trials in combination with temozolomide therapy consists of the pseudosubstrates for MGMT. The lead compounds in this class have been O(6)-benzylguanine and lomeguatrib (AstraZeneca, Lund, Sweden), the latter also known as O(6)-(4-bromothenyl)guanine or PaTrin-2. Resistance to O(6-)alkylating agents can be overcome in preclinical models by depletion of MGMT [51] and a relationship exists between MGMT activity and resistance to chloroethylating nitrosoureas and methylating agents in tumour cells grown in vitro and in xenograft models (reviewed in [52]). O(6)-benzylguanine and lomeguatrib have recently been tested in phase I-II clinical trials and biologically effective doses have been established for both agents [53]. However, results obtained so far indicate that, when used in combination with cytotoxic chemotherapy, myelosuppression is significantly enhanced, necessitating significant reductions in the doses of alkylating agents prescribed from those used in standard chemotherapy. On account of this lack of selectivity for malignant tissue versus normal bone marrow, no improvement in the *therapeutic index* has so far been demonstrated in clinical trials of these agents.

Platinum chemotherapies. Cisplatin, carboplatin and oxaliplatin have become three of the most commonly prescribed chemotherapeutic drugs used to treat solid cancers in patients [54]. Platinum resistance, either intrinsic or acquired during cyclical treatment, is a

major clinical problem since additional agents that can be added to therapy in order to circumvent tumour resistance do not currently exist.

Currently, platinum chemotherapy is being tested with PARP inhibition in 2 clinical trials (Figure 2). The rationale for combining PARP inhibition with platinum chemotherapy is based on preclinical observations that PARP inhibitors preferentially kill neoplastic cells and induce complete or partial regression of a wide variety of human tumor xenografts in nude mice treated with platinum chemotherapy [55-57]. For example, ABT-888 (Abbott Laboratories, Chicago, IL), a potent inhibitor of PARP-1 and PARP-2, has been shown to potentiate regression of established tumours induced by temozolomide, cisplatin, carboplatin, or cyclophosphamide therapy in rodent orthotopic and xenograft models [58]. In monotherapy, ABT-888 exhibits no significant anticancer activity in these preclinical models.

DNA demethylating agents such as 2′-deoxy-5-azacytidine (decitabine; MGI Pharma, Bloomington, MN) have been combined with cisplatin or carboplatin to reverse drug resistance caused by hypermethylation silencing of mismatch repair genes. The toxicity of agents such as cisplatin depends at least partly on functional mismatch repair (Figure 2). Preclinical data from xenograft models and translational studies from drug-resistant cells and tissues that are mismatch repair deficient owing to *MLH1* hypermethylation have demonstrated increased chemotherapeutic efficacy when a demethylating agent is combined with platinum chemotherapy [59,60]. Decitabine is currently being tested in combination with carboplatin in a phase II clinical trial in patients with ovarian cancer.

Attenuators of checkpoint signalling. An alternative approach to modulate DNA repair activity and potentially improving therapeutic index is to interfere with cell cycle checkpoint signalling. XL844 (EXEL-9844) is a small-molecule inhibitor of the checkpoint kinases 1 and 2 (Chk1 and Chk2). It causes inhibition of cell cycle arrest, progressive DNA damage, inhibition of DNA repair, and, ultimately, tumour cell apoptosis in cancer cells grown *in vitro* [61]. XL844 is currently being tested in a clinical trial in combination with the deoxycytidine analogue, gemcitabine, which normally

causes cell cycle arrest and apoptosis by incorporation into DNA. The treatment efficacy of the inhibitor of ATM kinase, KU55933 (AstraZeneca, Lund, Sweden), is currently in late preclinical development.

Radiosensitisers. DNA-dependent protein kinase (DNA-PK) is highly important for DSB repair by non-homologous end joining/NHEJ following ionizing radiation, and cells defective in DNA-PK are highly sensitive to ionizing radiation [62]. Wortmannin is a fungal product that irreversibly inhibits PI-3 protein kinases, such as DNA-PK, at low nanomolar concentrations, resulting in antiproliferative effects and radiosensitisation in preclinical models [63]. Unfortunately, it has been found to be unsuitable for clinical applications due to its inherent toxicity and instability in cells [64]. Other small molecule inhibitors of DNA-PK have been synthesised which reversibly inhibit the kinase activity at low micromolar concentrations, and these are currently in transition from late preclinical development to early clinical trials. In particular, NU7441 (AstraZeneca, Lund, Sweden), has demonstrated chemosensitization of topoisomerase II poisons and radiosensitization in a manner consistent with DNA-PKcs inhibition [65].

DNA repair inhibitors as single agent treatment for DNA repair defective cancer

As discussed above, most of the current small molecule inhibitors of DNA repair have so far been tested in early clinical trials as sensitisers of tumour cells to chemotherapy. However, DNA damage also occurs spontaneously in cells in the absence of treatments and DNA repair pathways are therefore essential for the survival of untreated cells. As many cancers are defective in DNA damage response and repair pathways (Table 1), *synthetic lethal* interactions can be utilised to advocate DNA repair inhibitors as monotherapy (Figure 3). DNA repair is an ideal target for inhibition in cancer cells, as the inhibitors should be exclusively toxic to cancer cells and therefore be associated with minimal side effects for patients (see BOX1 for a summary of advantages and limitations with DNA repair inhibitors as single treatment).

Indeed, DNA repair inhibitors have been demonstrated to work as single agents to treat cancer, particularly in DNA repair defective tumours. The most notable example so far is a novel treatment for inherited breast and ovarian cancers that arise from cells

which have lost the wild-type copy of the *BRCA1* or *BRCA2* genes [66,67]. *BRCA1*- and *BRCA2*-mutated cells are defective in homologous recombination repair [68,69] and show extensive replication-associated lesions [70,71]. These recombination defective cells are 100-1000 fold more sensitive to PARP inhibitors used as monotherapy than are the heterozygote or the wild-type cell lines, indicating the potential to be exploited to specifically treat *BRCA1* or *BRCA2* defective tumours [66,67]. The molecular explanation for this extreme sensitivity is the overlapping roles of DNA single strand break (SSB) repair, which is dependent on PARP1 [72], and homologous recombination in repair at replication forks [67,73,74]. Translation of this hypothesis has led to phase II clinical trials of monotherapy using the PARP inhibitor, AZD2281 (AstraZeneca, Lund, Sweden), currently recruiting patients with breast and ovarian cancer who harbour mutations in *BRCA1* or *BRCA2* genes. A separate phase II trial with the PARP-1 inhibitor AG014699 (Pfizer GRD, La Jolla, CA) is due to open to recruitment in the near future in known carriers of BRCA1 or BRCA2 mutations with locally advanced or metastatic breast or ovarian cancers.

Another synthetic lethal interaction has recently been discovered between the Fanconi anaemia repair pathway and the ATM checkpoint kinase [75] by the demonstration that two pancreatic tumour lines defective in the Fanconi anaemia pathway were more sensitive to the ATM inhibitor, KU-55933, than isogenic control lines. This finding provides a rationale to explore ATM inhibitors in the treatment of Fanconi anaemia repair-defective pancreatic cancer.

Mutations in DNA damage response and repair pathways are commonly associated with cancer (Table 1). Thus, it should be straightforward to exploit DNA repair inhibitors for the treatment of tumours carrying specific defects in DNA repair or damage signalling. We have compiled a list of reported cancer mutations in DNA repair genes and present synthetic lethal interactions demonstrated in *S. cereviseae* (Table I). Proteins encoded by the synthetic lethal-interacting genes may represent good targets for specific treatment of cancers carrying a mutation in DNA repair genes.

Reliable *biomarkers* are critical for selection of patients that will respond to treatments in clinical trials. This is particularly important for treatments with DNA repair

inhibitors that exploit specific cancer defects for treatments, as cancers in patients without the DNA repair or damage response defect will not respond to treatment. Thus, the lack of reliable assays to measure biomarkers in accessible malignant tissues is an important barrier to the success of DNA repair inhibitors in the clinic. The most reliable markers are likely to be those that identify loss of specific post-translational modifications present in the DNA damage response and repair pathways, or upregulation of the activity of the targeted pathway (Figure 3).

Exploiting tumour specific replication stress for targeted cancer treatment

Current chemotherapy clearly proves that production of excessive replication lesions represents a highly successful means of killing cancer cells. It has been observed that tumour cells themselves exhibit a high level of endogenous replication lesions that result in genetic instability [33,76]. Ideally, DNA repair inhibitors could be used to impair the repair of replication lesions present in tumour cells and covert them into fatal replication lesions that specifically kill cancer cells.

Oncogene-induced replication stress. The transformation of normal cells to a cancerous state is often initiated by the activation of oncogenes, which provide excessive growth signals [77]. Oncogene-induced growth signals often mimic the growth signals that are used by the body to transfer cells from quiescent into proliferative states. Early on during neoplastic transformation, the pre-cancerous cells are often recognised by checkpoint proteins (e.g. p53, Chk2), which stop cell proliferation by initiating apoptosis or senescence [78,79], cell inactivating processes termed the tumour barrier [80] It was recently shown that oncogene activation induces replication-associated DNA lesions, and that these lesions are responsible for triggering the cell cycle checkpoint response that activates the tumour barrier[33,34,81,82] (Figure 4).

Genes encoding proteins in the checkpoint pathways (e.g. the p53 pathway) are often mutated during cancer development [83], allowing cells to evade the tumour barrier and continue to proliferate (Figure 4). A key feature of cancer cells which express oncogenes and have managed to evade the tumour barrier, is that they have a higher level of endogenous replication-associated lesions than normal cells. This in turn contributes

to genetic instability [84] that will assist the tumour to induce the genetic changes required for continued transformation to malignancy [76]. More importantly, the replication lesions caused by oncogene activation resemble those produced by anti-cancer treatments [33] which need to be repaired for the cancer cells to survive. We therefore suggest that future DNA repair inhibitors should be used to make existing cancer-specific replication lesions more toxic, resulting in fatal replication lesions selectively killing oncogene-expressing cancer cells.

Hypoxia-associated replication stress. More advanced cancers are exposed to another source of replication stress, owing to the tumour microenvironment. Tumours are often *hypoxic*, which have been shown to disrupt DNA synthesis [85]. These conditions cause replication lesions that activate the ATM/ATR mediated checkpoint response [86-88]. Furthermore, DNA repair is down-regulated in hypoxic cells [89], which cumulatively contributes to the genetic instability observed in these cells [90,91]. In this case, inhibitors of the checkpoint response might be more efficient than inhibitors of DNA repair [92].

In summary, cancer cells are potentially exposed to unusually high levels of replication stress and endogenous DNA damage during cancer development. A future challenge will be to characterise forms of replication lesions occurring during different stages of carcinogenesis, which may be exploited for therapy.

Conclusions

The potential of DNA repair inhibitors in future cancer therapy is starting to be realised. Although selective inhibition of DNA repair pathways can be used to enhance current chemotherapy and radiotherapy, the most attractive use of DNA repair inhibitors may be in utilising cancer defects for more selective cell killing. DNA repair inhibitors that exploit tumour mutations in DNA repair pathways to convert spontaneous DNA lesions into fatal replication lesions may represent the most straightforward means to find selective treatments. This type of therapy is highly advantageous when compared to current chemotherapy as it is likely to produce minimal side effects whilst resulting in highly toxic replication lesions that will actively trigger cell death in cancer cells. A

potential limitation of this approach is that it is likely confined to DNA repair-defective tumours and that resistance mechanisms may develop. A more challenging treatment strategy is the inhibition of the repair of tumour-specific replication lesions and conversion of these into fatal lesions. Replication stress appears to be present in a majority of tumours, during at least one stage of carcinogenesis. Thus, the conversion of replication stress into fatal replication lesions could potentially be used to target a wide range of tumours. As we are still unaware of the exact nature of the replication lesions formed by many traditional chemotherapies, there is still considerable work to be done in characterising tumour-specific lesions to target cancers. Basic research into understanding the nature of toxic replication lesions as well as obtaining a more complete picture of all DNA repair pathways and their interplay is critical for the future of DNA repair inhibitors as single agents in cancer therapy.

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BOX 1. Advantages and limitations using DNA repair inhibitors as single agents in treatment of cancers:

(1) DNA repair inhibitors can exploit tumour-specific defects in checkpoint signalling and DNA repair to convert endogenous DNA lesions into fatal replication lesions that selectively kill tumour cells.

(2) A general problem for novel cancer therapies is that they are not sufficiently efficient to replace current therapy. As a result many enzyme inhibitors, that are not targeting DNA repair, have failed at the phase III or IV stage during clinical trials owing to a general lack of efficacy. Inhibition of DNA repair amplifies toxic replication-associated DNA lesions that directly result in cell death. DNA repair inhibitors should therefore be highly efficient at killing tumours.

(3) Extensive cross-talk between DNA repair pathways minimizes side effects in normal cells during inhibition of a single DNA repair pathway.

(4) Tumour inactivation of DNA damage signalling and DNA repair are often relatively early events during carcinogenesis, suggesting that non-toxic DNA repair inhibitors may be considered in the treatment of patients with pre-malignant or early neoplastic lesions (e.g. ductal carcinoma-in-situ in patients with BRCA1 and BRCA2 inherited breast and ovarian cancer; intestinal lesions in patients with hMLH1 and hMSH2 hereditary non-polyposis colorectal cancer).

(5) Extensive crosstalk between DNA repair pathways likely results in acquisition of resistance mechanisms in tumours, which is a limitation for killing late stage tumours.

Figure 1. Overview of DNA repair pathways involved in repairing toxic DNA lesions formed by cancer treatments. DNA damaging agents used in cancer treatment induce a diverse spectrum of toxic DNA lesions. These lesions are recognised by a variety of DNA repair pathways which are lesion-specific but highly overlapping. **(A)** Ionising radiation and radiomimetic drugs are the only agents to directly induce double strand breaks (DSBs), which are toxic independently of replication, and predominantly repaired by non-homologous end joining. **(B)** Mono-and **(C)** bi- functional alkylators induce DNA base modifications, which interfere with DNA synthesis and are processed into toxic lesions in a mismatch repair dependent manner. The base and nucleotide excision repair pathways are, together with alkyltransferases, major repair pathways, whereas other repair pathways repair toxic replication lesions, such as those produced following interstrand crosslinks. **(D)** Anti-metabolites interfere with nucleotide metabolism and DNA synthesis, causing mismatch repair mediated, but poorly characterised replication lesions. The repair pathways involved in repair of anti-metabolite-induced lesions are, apart from base excision repair, poorly characterised. **(E)** Topoisomerase inhibitors trap topoisomerase I or II in transient cleavage complexes with DNA, thus creating indirect DNA breaks and interfering with replication. **(F)** Replication inhibitors induce replication fork stalling and collapse, resulting in indirect DSBs. The relative contributions of the major repair pathways to the respective types of DNA damage outlined are indicated by the sizes of the boxes. Abbreviations used: AT, alkyltransferases; BER, base excision repair; O_2G, DNA dioxygenases;

ENDO, endonuclease-mediated repair; FA, Fanconi anaemia-mediated repair; HR, homologous recombination; NER, nucleotide excision repair; NHEJ, non-homologous end joining; RecQ, RecQ-mediated repair; SSBR, DNA single-strand break repair; TLS, trans lesion synthesis.

Figure 2. Ongoing clinical trials of small molecule inhibitors of the DNA damage response and related signalling pathways. The recent or current stage of development of clinical trials is indicated for individual compounds, which are grouped by molecular target. For details of specific agents, see main text.

Figure 3. Synthetic lethal interactions to identify molecular targets and biomarkers for inhibitors of DNA repair. Proteins that interact are often within functional modules involved in catalysing checkpoint and repair pathways. A mutation in a single tumour suppressor gene **(A)** normally impairs the full functional module. Such loss of a checkpoint or repair pathway results in genetic instability, which would lead to cell death unless a DNA repair salvage pathway **(B)** is upregulated. As the two pathways collaborate to maintain survival, targeting pathway B in monotherapy will specifically kill tumour cells and be non-toxic to normal cells, as they can use pathway A for survival. An additional mutation **(C)** upstream of the targeted pathway B causes complete resistance to the treatment. For instance, if pathways A+B are required for resolving a certain type of recombination intermediate, a mutation in a protein C involved in the formation of this recombination intermediate (e.g. BRCA2, which is involved in early stages of recombination) will make pathways A+B redundant. In the absence of C, the **(D)**+**(E)** pathways would be used to rescue replication, independently of recombination. A novel monotherapy targeting pathways D+E would then be needed to kill B resistant tumour cells. Proteins are indicated by circles, protein interactions with red lines, functional modules with blue boxes. Black boxes indicate mutated pathways. Red boxes indicate salvage DNA repair pathways.

Figure 4. Oncogene-induced replication stress as a target for DNA repair inhibitors. Oncogene expression results in unscheduled replication origin firing, which decreases the distance between origins [34] and causes replication forks to collapse [33]. Such replication lesions activate the tumour barrier, including the ATM-mediated checkpoint pathway, to trigger apoptosis or senescence and to prevent tumour outgrowth [81,82]. Inactivation of checkpoint pathways (for instance by *p53* mutation) results in cancer cells evading apoptosis and senescence, which allows continued proliferation. Collapsed replication forks need to be repaired to allow cell survival. Tumour defects in checkpoint and repair pathways will result in collapsed forks that are often incorrectly repaired, resulting in

genetic instability that will drive future mutations. Here, we suggest that tumour-specific replication lesions can be converted into fatal replication lesions through inhibition of DNA repair. Such therapy is likely to be tumour specific as normal cells should not have oncogene-induced replication stress.

Definitions

Alkylating agents
Electrophilic compounds that are reactive either directly or following metabolism and bind covalently to electron rich atoms in DNA bases (i.e. oxygen and nitrogen).

Alkyltransferases
Class of enzymes that directly reverse DNA base modifications induced by alkylating agents by transferring the alkyl group from the base on to the protein.

Antimetabolites
Compounds with similar chemical structures to nucleotide metabolites that interfere with nucleotide biosynthesis or are incorporated into DNA.

Base excision repair
A repair pathway that replaces missing or modified DNA bases, such as those produced by alkylating agents or in spontaneously degraded DNA, with the correct DNA base.

Biomarkers
A molecule or substance whose detection indicates a particular disease state or treatment response.

DNA-dioxygenases
Class of enzymes that directly reverse DNA base methylations via an oxidation mechanism. The human DNA-dioxygenase ABH2 is believed to act at replication forks.

Endonuclease-mediated repair
A repair pathway that introduces a DNA single-strand break in a DNA structure to facilitate continuous repair.

Fanconi anemia-associated repair
A repair pathway with largely unknown function active at damaged replication forks.

Homologous recombination
A process that can copy a DNA sequence from an intact DNA molecule (often the newly synthesised sister chromatid) to repair or bypass replication lesions.

Hypoxia
A shortage of oxygen. In cancer this is often the result of insufficient vasculature.

Mismatch repair
Acts during DNA replication to correct base-pairing errors made by the DNA polymerases.

Non-homologous end joining
Connects and re-seals the two ends of a DNA double strand break without the need for sequence homology between the ends.

Nucleotide excision repair
Removes large DNA adducts or base modifications which distort the double helix and use the opposite strand as template for repair.

RecQ-mediated repair
A repair pathway that unwinds complex DNA structure to facilitate repair.

Synthetic lethality
Genetic phenomenon where the combination of two non-lethal mutations results in lethality because the second mutation inactivates a backup mechanism allowing for tolerance of the first mutation and vice versa.

Trans-lesion synthesis
Mechanism during DNA replication where the standard DNA polymerase is temporarily exchanged for a specialised polymerase which can synthesise DNA across base damage on the template strand.

Therapeutic index
The therapeutic index describes the ability of a treatment strategy to kill cancer cells in preference to cells in normal tissues.

Table I. Synthetic lethal interations in DNA repair and cell cycle checkpoint genes implicated in cancer. Abbreviations used: BER, base excision repair; FA, Fanconi anaemia-mediated repair; HR, homologous recombination; NER, nucleotide excision repair; NHEJ, non-homologous end joining; MMR, mismatch repair; RecQ, RecQ-mediated repair

Pathway	**Protein**	**Syndrome**	**Primary cancers**	**Biomarker**	**Synthetic lethality**	**Homolog *S. cerevisiae***	**Synthetic lethality *S. cerevisiae*** [93-172]
HR							
	BRCA1		breast, ovarian [173]		PARP1 [66]	-	-
	BRCA2	Fanconi's anemia	breast, ovarian [174]		PARP1 [66,67]	-	-
	RAD54B		non-Hodgkin lymphoma, colon cancer [175]			rdh54	cla4, bim1, rad27, ctf4, ctf8, ctf18, dcc1, tof1, pol32, srs2, ulp1, elg1, nup133, nup120, ccr4, cik1, ctk1, ctk2, ctk3, lsm7, pop2, rnr4, rrm3, sod1, swi6, tsa1.
	RAD51B		lipoma, uterine leiomyoma [176]			rad51	rad27, ctf4, ctf8, ctf18, tof1, pol32, elg1, orc2, orc5, nup133, nup120, ctk1, ctk2, ctk3, rnr4, sod1, swi6, tsa1, ubc9
	CtIP		colorectal cancer [177]			sae2	sgs1, rad27, rrm3, dia2, pph3
NHEJ							
	MRE11	Ataxia-telangiectasia-like disorder (ATLD)	colorectal cancer [178]			mre11	rad27, bim1, ctf4, ctf18, dcc1, top1, chs1, chs5, kre9, rm3, sap30, elg1, srs2, yku80, ulp1, xrs2, rad50, nup133, nup120, hsp82, orc6, cdc6, ccr4, dia2, ccs1, cik1, ctk1, ctk2, ctk3, mdm12, pop2, rnr4, sod1, swi6, tsa1, vid22, pph3, gcs1, dna2
	LIG4	LIG4 syndrome	Leukemia [179]			lig4	-
	Artemis	Omenn syndrome	Lymphoma [180]			pso2	-
MMR							
	hMSH2		hereditary nonpolyposis colorectal cancer (HNPCC) [181]	microsatelite instability (MSI)		msh2	pol3
	hMLH1		HNPCC [182]	MSI		mlh1	cdc7, pol3, mms4
	hMSH6		HNPCC [183]	MSI		msh6	pol3
	hPMS1		HNPCC [184]	MSI		pms1	pol3
	hPMS2		HNPCC [184]	MSI		pms1	pol3
	hMLH3		HNPCC [185]	MSI		mlh3	none
RecQ homologues							
	BLM	Bloom's syndrome	Various [186]	Elevated SCE		sgs1	srs2, dcc1, mrc1, cdc7, cdc8, hst3, dna2, est2, slx5, slx8, wss1, yku70, rnr202, elg1, ccs1, nup133, nup120, dia2, slx1, sae2, slx4, pol31, siz1, nfi1, asf1, rnr1, rrm3, mgs1, csm3, esc2, rtt107, top1, swe1, pub1, rpl24a, sis2, sod1, pby1, ctf18, ctf4, mms4, mus81, rad50
	WRN	Werner's syndrome	Various [187]			sgs1	srs2, dcc1, mrc1, cdc7, cdc8, hst3, dna2, est2, slx5, slx8, wss1, yku70, rnr202, elg1, ccs1, nup133, nup120, dia2, slx1, sae2, slx4, pol31, siz1, nfi1, asf1, rnr1, rrm3, mgs1, csm3, esc2, rtt107, top1, swe1, pub1, rpl24a, sis2,

							sod1, pby1, ctf18, ctf4, mms4, mus81, rad50
	RECQL4	Rothmund-Thomson syndrome	skin basal and sqamous cell, osteosarcoma [187]			sgs1	srs2, dcc1, mrc1, cdc7, cdc8, hst3, dna2, est2, slx5, slx8, wss1, yku70, rnr202, elg1, ccs1, nup133, nup120, dia2, slx1, sae2, slx4, pol31, siz1, nfi1, asf1, rnr1, rrm3, mgs1, csm3, esc2, rtt107, top1, swe1, pub1, rpl24a, sis2, sod1, pby1, ctf18, ctf4, mms4, mus81, rad50
Damage signaling							
	ATM	Ataxia-telangiectasia	Leukemia [188]		PARP1 [189,190], FANC [75]	tel1	mec1, dna2
	NBS1	Nijmegen breakage syndrome	Various [191]			xrs2	ctk2, ctk3, dia2, mdm12, nup133, pop2, rnr4, sod1, swi6, tsa1, vid22, rad27, cdc73, kar3, mrc1, pol32, cdc45, mms4, srs2, rrm3, mre11, elg1, nup120, orc6, cdc6, ccr4, ccs1, mms22, cik1, ctk1
	p53	Li-Fraumeni	Various [192]			-	-
	CHEK2	Li-Fraumeni	Various [193]			dun1/rad53	chk1, bmh1, nat1, rad9, ubx7, bsc4, cdc7, mec1, pol3, clb5, rnr4, rmi1, elg1, orc6, cdc6, ccr4, cdc73, clb5, ctk3, eaf5, htz1, ies2, lsm1, mrc1, npl3, pep3, pep5, pop2, puf4, rad27, snf8, eaf1, vps34, yaf9, dia2, dbf4, pap2
NER							
	XPA	Xeroderma pigmentosum (XP)	skin cancers [194]			rad14	gmh1, ntg1, ntg2
	XPB	XP, Cockayne syndrome (CS)	skin cancers [195]			rad25	rad3, sti1
	XPC	XP	skin cancers [196]			rad4	ric1, ypt6, csm3, hsp82, ctf4, ctf18, dcc1, tof1, rad23, mad2
	XPD	XP, CS, Trichothiodystrophy	skin cancers [197]			rad3	act1, nip7, nop1, rad50, rad52, kin28, ssl2
	XPE/ DDB2	XP	basal cell carcinomas [198]			-	
	XPF	XP	skin cancers [199]			rad1	ntg1, ntg2, apn2, apn2, rad27, tdp1, mec1
	XPG	XP	squamous cell carcinoma, head and neck [200]			rad2	none
	XPV	XP	skin cancers [201]			rad30	msh6, pms1
	ERCC1	cerebro-oculo-facio-skeletal syndrome	squamous cell carcinoma, head and neck [200]			rad10	cla4, gim4, mec1, mad2, apn1, apn2
Crosslink repair							
	FANCA	Fanconi's anemia	Various [202]	FANCD2 Ubiquitination [75]	ATM [75]	-	-
	FANCB	Fanconi's anemia	Various [202]			-	-
	FANCC	Fanconi's anemia	Various [202]	FANCD2 Ubiquitination [75]	ATM [75]	-	-
	FANCD2	Fanconi's anemia	Various [202]		ATM [75]	-	-
	FANCE	Fanconi's anemia	Various [202]	FANCD2 Ubiquitination [75]	ATM [75]	-	-

	FANCF	Fanconi's anemia	Various [202]			-	-
	FANCG/ XRCC9	Fanconi's anemia	Various [202]	FANC D2 Ubiquti nation [75]	ATM [75]	-	-
	FANCJ	Fanconi's anemia	Various [202]			-	-
	FANCL	Fanconi's anemia	Various [202]			-	-
BER							
	PolB		Various [203]			pol4	-
	FEN1		Various [204]			rad27	lcd1, sgs1, mms4, mus81, sae2, rad50, srs2, ddc1, cac2, exo1, mre11, rad6, rad9, rad17, rad24, rad52, xrs2, ctf4, rpl27a, rps30b, doc1, esc2, hst1, hpc2, csm3, ccs1, sis2, sod1, ydj1, hst3, ylr352w, ypr116w, bud27, ctf18, dcc1, chl1, mrc1, tof1, pol32, cdc8, exo1, mre11, pol3, rad1, cln1, cln2, rad51, rad53, rad54, rad55, rad57, rad59, rfc1, xrs2, ulp1, elg1, rnh201, rnh202, rnh203, mec3, rad6, slx8, slx9, top3, asf1, rlf2, pap2, rpn4, doa4, bro1, grr1, nup84, nup120, nup133, nat3, sfp1, thp1, tef4, aat2, gas1, pep5, pmr1, ume6, bre1, slx5, ige1, mec1, mec3, mms22, arp8, dia2, hur1, lrs4, lsm7, lte1, npl3, rmd9, rtf1, uaf30, eaf1, sae2, pph3, ulp1, nup60

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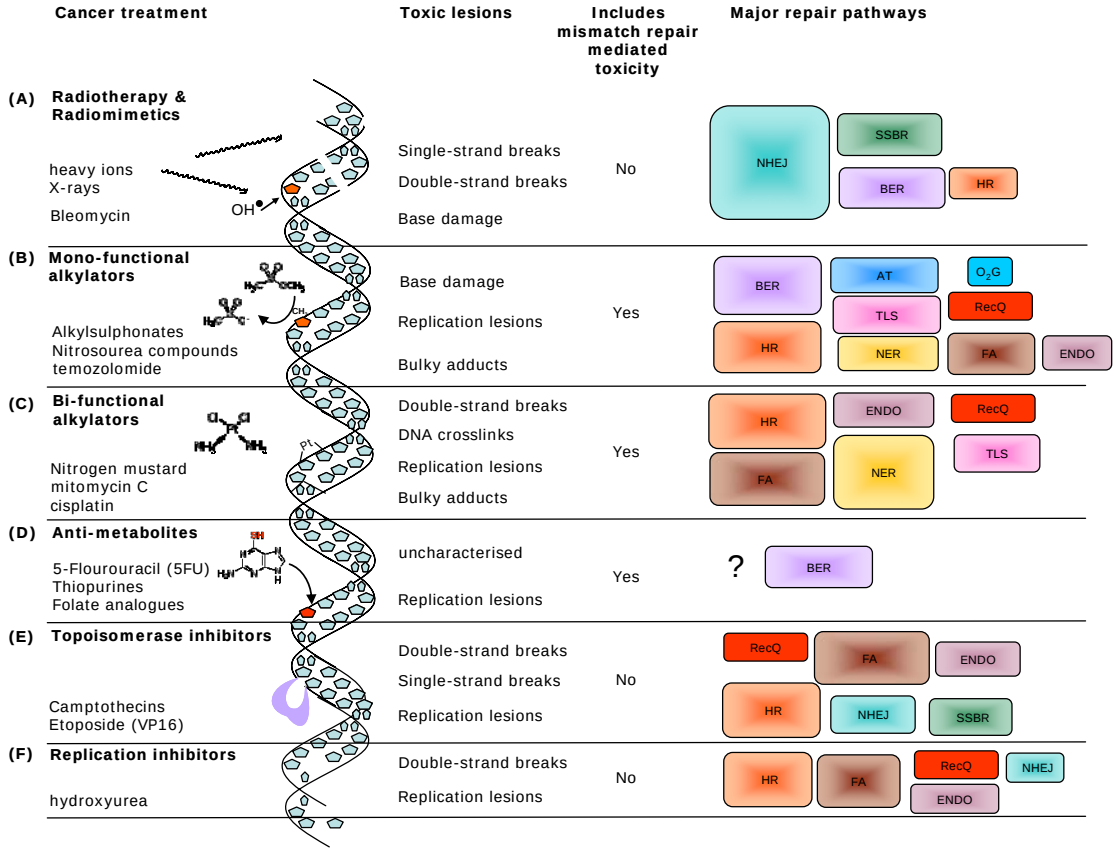
Figure 1


Cancer treatment
Toxic lesions
Includes mismatch repair mediated toxicity
Major repair pathways
(A) Radiotherapy & Radiomimetics
heavy ions
X-rays
Bleomycin
OH
Single-strand breaks
Double-strand breaks
Base damage
No
NHEJ
SSBR
BER
HR
(B) Mono-functional alkylators
Alkylsulphonates
Nitrosourea compounds
temozolomide
Base damage
Replication lesions
Bulky adducts
Yes
BER
AT
O2G
TLS
RecQ
HR
NER
FA
ENDO
(C) Bi-functional alkylators
Nitrogen mustard
mitomycin C
cisplatin
Double-strand breaks
DNA crosslinks
Replication lesions
Bulky adducts
Yes
HR
ENDO
RecQ
FA
NER
TLS
(D) Anti-metabolites
5-Flourouracil (5FU)
Thiopurines
Folate analogues
uncharacterised
Replication lesions
Yes
?
BER
(E) Topoisomerase inhibitors
Camptothecins
Etoposide (VP16)
Double-strand breaks
Single-strand breaks
Replication lesions
No
RecQ
FA
ENDO
HR
NHEJ
SSBR
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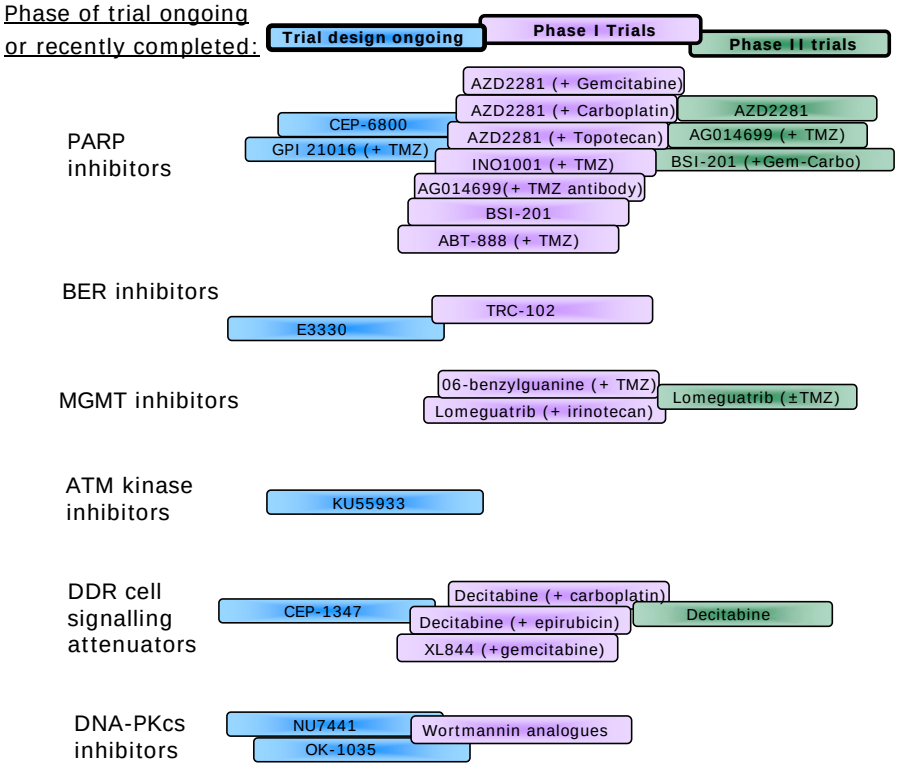
hydroxyurea
Double-strand breaks
Replication lesions
No
HR
FA
RecQ
NHEJ
ENDO

Figure 2


Phase of trial ongoing or recently completed:
Trial design ongoing
Phase I Trials
Phase II trials
PARP inhibitors
AZD2281 (+ Gemcitabine)
AZD2281 (+ Carboplatin)
AZD2281
CEP-6800
AZD2281 (+ Topotecan)
AG014699 (+ TMZ)
GPI 21016 (+ TMZ)
INO1001 (+ TMZ)
BSI-201 (+Gem-Carbo)
AG014699(+ TMZ antibody)
BSI-201
ABT-888 (+ TMZ)
BER inhibitors
TRC-102
E3330
MGMT inhibitors
06-benzylguanine (+ TMZ)
Lomeguatrib (±TMZ)
Lomeguatrib (+ irinotecan)
ATM kinase inhibitors
KU55933
DDR cell signalling attenuators
Decitabine (+ carboplatin)
CEP-1347
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Decitabine
XL844 (+gemcitabine)
DNA-PKcs inhibitors
NU7441
Wortmannin analogues
OK-1035

Figure 3

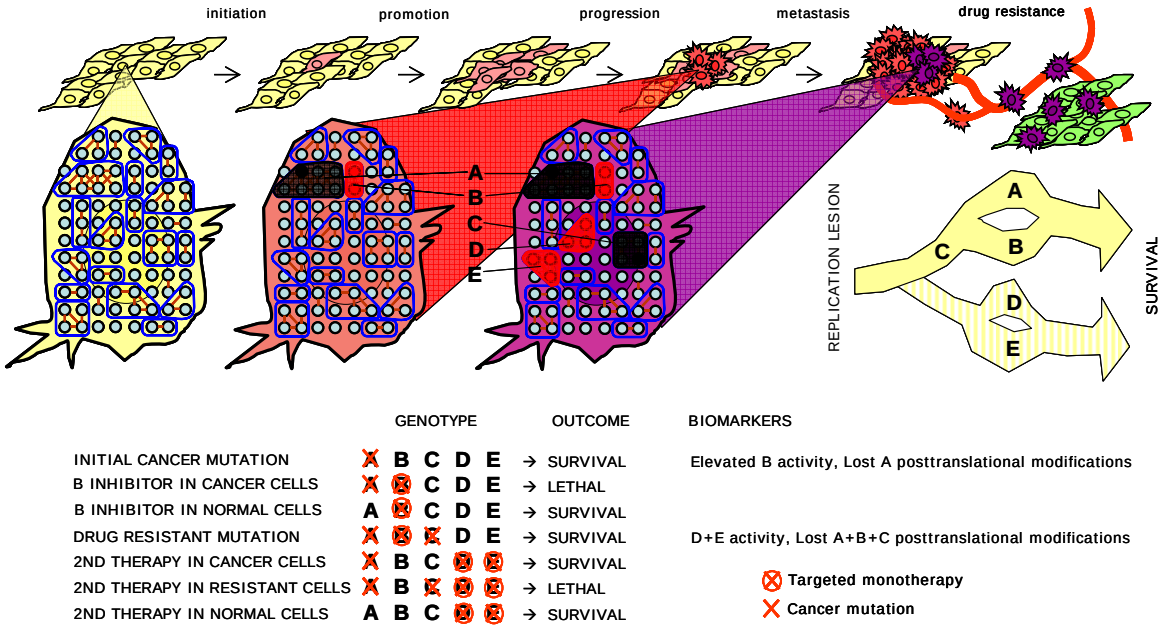
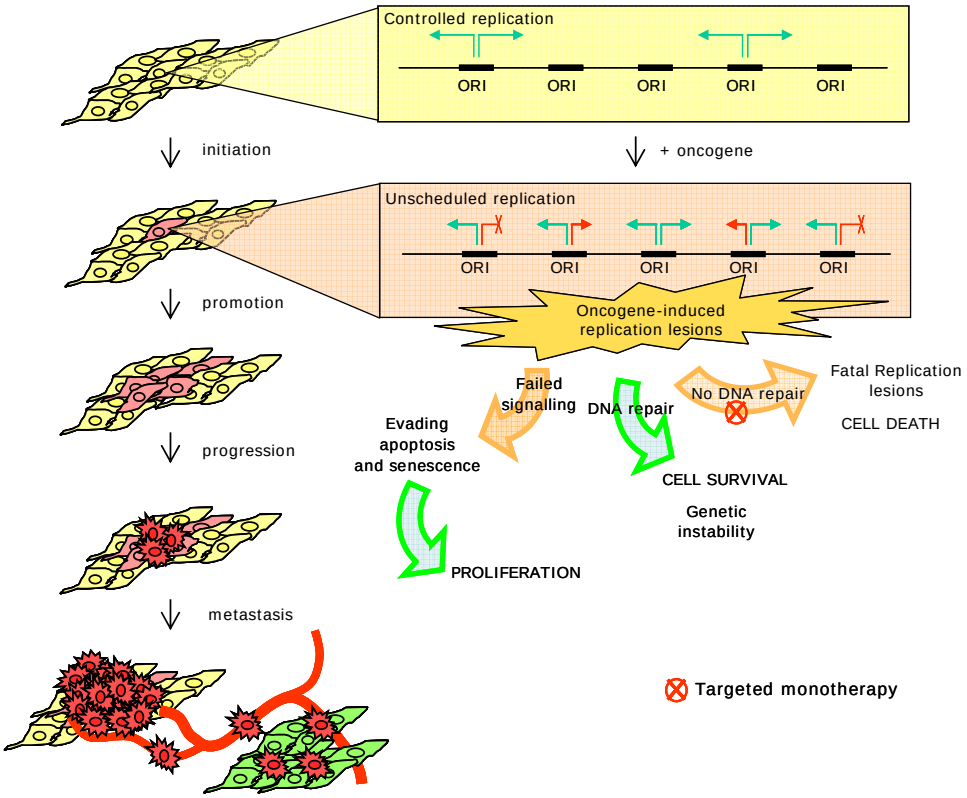

initiation
promotion
progression
metastasis
drug resistance
A
B
C
D
E
REPLICATION LESION
SURVIVAL
GENOTYPE
OUTCOME
BIOMARKERS
INITIAL CANCER MUTATION → SURVIVAL
B INHIBITOR IN CANCER CELLS → LETHAL
B INHIBITOR IN NORMAL CELLS → SURVIVAL
DRUG RESISTANT MUTATION → SURVIVAL
2ND THERAPY IN CANCER CELLS → SURVIVAL
2ND THERAPY IN RESISTANT CELLS → LETHAL
2ND THERAPY IN NORMAL CELLS → SURVIVAL
Elevated B activity, Lost A posttranslational modifications
D+E activity, Lost A+B+C posttranslational modifications
Targeted monotherapy
Cancer mutation

Figure 4


Controlled replication
ORI
ORI
ORI
ORI
ORI
initiation
+ oncogene
Unscheduled replication
ORI
ORI
ORI
ORI
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promotion
Oncogene-induced
replication lesions
progression
Failed
signalling
DNA repair
No DNA repair
Fatal Replication
lesions
CELL DEATH
Evading
apoptosis
and senescence
CELL SURVIVAL
Genetic
instability
PROLIFERATION
metastasis
Targeted monotherapy