

DNA Repair Modulators as Anticancer Agents

Yves Pommier, M.D., Ph.D.

Laboratory of Molecular Pharmacology, Center for Cancer Research, NCI-NIH, Bethesda. Tel: 301-496-5944; Fax: 301-402-0752; email: pommier@nih.gov

Summary: DNA damaging agents constitute a large fraction of the anticancer armamentarium (including radiation and small molecules). It is also becoming increasingly clear that DNA repair defects and defects in DNA damage response (DDR) cause cancer and are common in cancer cells. Those defects probably account for the selectivity of systemically administered anticancer agents toward cancer cells. Here, we summarize the DNA repair and DDR defects most commonly associated with human cancer. We also summarize the various DNA repair pathways elicited by the anticancer agents, and the inhibitors currently available to interfere with those pathways. Finally, we discuss the rationale approaches for using DNA repair and DDR inhibitors based on the specific tumor defects (conditional/synthetic lethality), and examples for rational development of combination therapies.

Abbreviations and glossary (in alphabetic order): AGT: O6-alkylguanine transferase (polypeptide which transfers O6 alkyl guanine adducts to itself); AT: ataxia telangiectasia (a rare genetic disease with cancer predisposition); ATM: ataxia telangiectasia mutated (the gene mutated in AT; it encodes a PIKK); AP site: apyrimidinic/apurinic site; BER: base excision repair; Aptx: Aprataxin (a repair protein that act as cofactor for ligases during BER, SSB and DSB repair); BLM: Bloom syndrome helicases (a RecQ helicase cofactor for Top3 α); DDR: DNA damage response; DNA-PK: DNA-dependent protein kinase (a PIKK); PIKK: phosphatidylinositol kinase-like kinase; DSB: DNA double-strand break; Et743: Ecteinascidin 743 (an anticancer agent which targets NER); FA: Fanconi anemia (a rare genetic disease with cancer predisposition); FANC: Fanconi anemia factor; GG-NER: Global genome NER; HNPCC: Hereditary nonpolyposis colorectal cancer; HR: homologous recombination; ISC: Interstrand crosslinks (produced by alkylating agents and platinum derivatives); MMR: mismatch repair; Nbs1: Nijmegen Breakage syndrome (a rare genetic disease with cancer predisposition); NER: nucleotide excision repair; NHEJ: Non-homologous end joining; PARP: poly(ADPribose) polymerase; SSB: DNA single-strand break; PNKP: polynucleotide kinase phosphatase (involved in BER); RPA: Replication protein A (an heterotrimeric complex that binds single-stranded DNA; involved in NER and HR); TC-NER: Transcription-coupled NER; Tdp1: tyrosyl DNA phosphodiesterase (involved in BER and repair of Top1cc); Top1: DNA topoisomerase I; Top1cc: Topoisomerase I cleavage complex; Top2: DNA topoisomerase II; Top2cc: Topoisomerase II cleavage complex; WRN: Werner syndrome helicases (a RecQ helicase with nuclease activity).

Importance of DNA repair in oncology

DNA REPAIR DEFECTS PREDISPOSE TO AND ARE ASSOCIATED WITH CANCERS:

DNA repair is essential as DNA is highly susceptible to spontaneous damage (thousands of lesions occur in a normal cell per day as a result of oxidative radical generation, spontaneous chemical modifications and replication errors). Cellular DNA is also highly susceptible to carcinogens, and the target of a broad range of anticancer agents. It is therefore not surprising that a number of cancer susceptibility genes encode for DNA repair and DNA damage response (DDR) factors. Oncogenic defects in such genes enable the generation of cells with a mutator phenotype, which gives rise to transformed cells that escape the normal homeostatic processes. A large number of hereditary cancers are rooted in genetic defects of DNA repair factors (see below). Germ line mutations in the XP nucleotide excision repair factors lead to Xeroderma Pigmentosum with high incidence of skin cancer and visceral tumors; defects in mismatch repair to HNPCC (human nonpolyposis colorectal cancer); defects in crosslink repair to Fanconi anemia with increased risk of acute leukemia and squamous cell carcinoma; and defects in DSB repair (BRCA2, BRCA1) to breast and ovarian cancers. Defect in replication and repair RecQ helicases (BLM and WRN) lead to Bloom and Werner syndrome with an early incidence of broad range of cancers.

DNA repair is coupled with DNA damage responses that are commonly referred to as checkpoint response. Those checkpoints enable cell cycle arrest, which provides time for repair and avoids further damage until the DNA damaging agent is cleared from the cell. Hereditary defects in DDR are exemplified by ataxia telangiectasia. Inactivation of the ATM gene confers high risk of tumors, in particular lymphomas. Genetic inactivation of p53 is the cause of Li-Fraumeni syndrome. Defects in Chk2, the downstream effector kinase from ATM leads to Li-Fraumeni syndrome with

normal p53, and defects in Mre11 and Nbs1 (both ATM cofactors) in ATLD (Ataxia-like-disorder) and Nijmegen breakage syndrome, which both predispose to cancers.

Somatic mutations of the cancer predisposing genes listed above, especially in patient heterozygote for those genes, is likely to contribute to oncogenesis. For instance, defects in NER genes has a high incidence in ovarian and colorectal cancers (1), and defects Mre11 and mismatch repair has a high incidence in colorectal cancers (2). p53 is mutated/inactivated in approximately 50% of sporadic tumors. Thus, it seems that characterization of tumors should include genetic status of the DNA repair and DDR genes in order to stratify tumors and rationalize therapy (see below).

CHECKPOINT DEFECTS AND GENOMIC INSTABILITY IN CANCER CELLS MAKES THEM DEPENDENT UPON DNA REPAIR:

DNA repair and DDR are tightly coupled. Indeed, DNA repair requires cell cycle checkpoints to arrest cell cycle progression and enable DNA repair to take place without interference from replication of the damaged DNA template. For instance, p53 (and its downstream target p21^{CIP1/WAF1}) is a key factor for cell cycle arrest in G1, while ATM, BRCA1, Mre11 and Nbs1 arrest S-phase progression. Inactivation of ATM, BRCA1, Mre11 and Nbs1 result in radioresistant DNA synthesis (RDS) (3), and leads to oncogenic and mutagenic DNA lesions. In addition, DDR can act as death effector and induce apoptosis in case of failure to repair DNA accurately. This is a well-known function of p53 in addition to its cell cycle arrest function. Thus, DNA repair and DDR are functionally linked and combination of agents that modulate DNA repair and DDR is likely to yield potent antiproliferative regimens (see last section).

DNA REPAIR AND DDR STATUS DETERMINE RESPONSE TO ANTICANCER AGENTS:

At the same time that DNA repair and DDR defects contribute to the malignant phenotype, they also are the tumor's Achilles' heel for DNA damaging agents. For instance, cells with defective NER are hypersensitive to platinum derivatives (4) and enhanced NER is one of the mechanisms of resistance to platinum derivatives (5). Conversely, defective NER tends to confer resistance to ecteinascidin 743 (Yondelis, Trabectedin) (6, 7).

One of the landmark characteristics of Fanconi anemia cells is their exquisite sensitivity to mitomycin C (8) and platinum derivatives. BRCA2-deficient and ATM-deficient cells tend to be hypersensitive to agents that produce DSB and topoisomerase inhibitors (9). Thus, defects in DNA repair and DDR increase the susceptibility of cancer cells to DNA damaging agents.

Because of the importance of knowing the DNA and DDR status of tumors to guide therapeutic choice (see below), it might be important to systematically evaluate the functional status of DNA repair and DDR genes in sporadic tumors. However, some of those genes are large (such as ATM, BRCA2 and BRCA1), which poses a technical and financial challenge to those determinations.

Main DNA repair pathways elicited by anticancer agents and inhibitors

Because DNA damaging agents target DNA similarly in normal and cancer tissues, the effects of those clinically approved chemotherapeutic agents is likely to result from tumor-specific defects in DNA repair and DDR pathways. Here we will briefly summarize the different repair pathways elicited by the main classes of DNA damaging agents used in cancer treatment, and for each of those pathways, we will discuss the available repair inhibitors. We will not address DNA replication inhibitors, which have been reviewed elsewhere¹ (10) although it is obvious they have a major impact on DNA repair. The main repair pathways can be grouped as: base repair, which includes guanine alkylation reversal by AGT, BER, NER and MMR; SSB repair, which includes BER and DNA-PK-mediated ligation; DSB repair, which includes NHEJ and HR; Interstrand crosslink repair, which involves the FA factors; and DPC repair, which is a less well characterized repair pathway, and which we will detail for topoisomerase inhibitors.

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<http://discover.nci.nih.gov/pommier/Replication.inhibitors.htm>

GUANINE O6 ALKYLATION, AGT AND INHIBITION BY O6-BG:

DNA alkylating agents including chloroethylating nitrosoureas [carmustine (BCNU) and lomustine (CCNU)] and methylating agents [dacarbazine (DTIC) and temozolomide (TMZ), procarbazine and streptozotocin] alkylate DNA preferentially at guanine N2 and O6 and adenine N3. The cytotoxicity of O6-methyl guanine is mediated by the mismatch repair (MMR) pathway. During replication, DNA polymerase stalls at the O6-methylguanine sites and incorporates thymine opposite to O6-methylguanine. That mismatch is recognized by the MMR, which removes the normal thymine instead of the O6-methylguanine. Reincorporation of thymine generates futile circles of MMR, leading to the formation of SSB, recombinations, chromosomal aberration and cell death.

AGT (O6-alkylguanine transferase; also referred to as methylguanine methyl transferase [MGMT]) efficiently removes alkyl substitutions (methyl-, ethyl-, benzyl-, 2-chloroethyl, and pyridyloxobutyl-) on guanine O6 by transferring it to an active cysteine (Cys145) acceptor site within the AGT. Thus, this process has been referred to as a suicide reaction since accepting the alkylating group from the DNA irreversibly inactivates AGT. AGT is an important determinant of response to therapy as Mer- (Methylguanine repair deficient) human cells, which lack AGT are extremely sensitive to alkylating agents. The response of brain tumors has been attributed to their Mer- (AGT-deficient) phenotype (11).

Inhibitors of AGT have been developed and evaluated clinically. O6-benzylguanine (O⁶-BG) is the paradigm for such inhibitors. The main critical question regarding the use of AGT inhibitors is whether they increase the therapeutic index of alkylating agents. Combinations of alkylating agents with O⁶-BG lower the bone marrow tolerance to the alkylating agents, indicating O⁶-BG acts both on normal and tumor tissues, and therefore may not provide a significant increase in selectivity toward tumor tissues (therapeutic index). Recently, O⁶-BG has been shown to enhance the activity of platinum derivatives independently of AGT depletion (12). Analogs of O⁶-BG such as o6-benzyl-2-deoxyguanine (B2dG) are being evaluated.

BER, METHOXAMINE (MX) AND PARP INHIBITORS:

Base excision repair corrects a variety of base damages resulting from oxidation, methylation, deamination or spontaneous base loss. DNA alkylating agents produce such lesions. These alterations are highly mutagenic following replication and misincorporation. BER is subdivided into short and long patch BER depending on whether a single or several nucleotides are incorporated to replace the damaged DNA strand. In both cases, the reaction starts by conversion of the damaged base into an AP site. DNA glycosylases remove the damaged bases by hydrolyzing the base N-glycosidic bond with the deoxyribose sugar. The base can also be eliminated by spontaneous hydrolysis of the N-glycosidic bond. In either case, the AP site is converted into a SSB by APE1 (the main AP endonuclease), which cleaves the DNA backbone immediately 5' to the AP site, resulting in a 3'-hydroxyl group and a transient 5'-abasic deoxyribose phosphate (dRP). For short patch BER, beta polymerase ($\text{pol-}\beta$) removes the 5'-dRP by its AP lyase activity and adds back a base at the 3'-hydroxyl end of the SSB. Finally, ligase III joins the new base with the 5'-hydroxyl of the SSB. Short patch BER represents the most prevalent BER reaction.

In long patch BER, the AP lyase activity of $\text{pol-}\beta$ is unable to remove abnormal 5'-termini, and $\text{pol-}\beta$ is replaced by the replicative polymerase ($\text{pol-}\delta/\epsilon$) in association with its processivity factor PCNA. Consequently, several bases (up to 10) are incorporated, which displaces the 5'-end of the broken DNA. The resulting 5'-flap (with its blocking 5' terminus) can then be excised by FEN-1 (the flap endonuclease), and ligase I seals the break.

In addition to the factors mentioned above, BER complexes can involve additional cofactors such as XRCC1 (a scaffolding protein), Tdp1 (which can process blocking 3'-end lesions), PNKP (a dual DNA phosphatase and kinase, which can further process the ends of the SSB), Apx (a DNA-binding protein that resolves abortive ligation intermediates) (13) and PARP. Thus, the PARP inhibitors, which are currently under intense development, are potent inhibitors of BER. Besides PARP inhibitors, the BER inhibitor in clinical trials is methoxamine (MX).

MX potentiates the activity of alkylating agents including temozolomide in human tumor xenograft models (14). MX reacts with the C1' atom of the abasic site rendering it refractory to APE1, so that BER is interrupted. Recently, a natural peptide, indolicidin has been shown to act similarly as MX (15).

NER AND ITS INHIBITION BY ET743:

Nucleotide excision repair acts on a wide range of DNA lesions including UV-induced cyclobutane pyrimidine dimers and 6,4-photoproducts, carcinogenic adducts, platinum adducts and intrastrand crosslinks, and some forms of oxidative damage. The common feature of these lesions is the presence of distorting lesions originating from the covalent modification of one strand of the DNA duplex. NER is relatively well understood and proceeds in highly conserved sequential steps. It consists in two main pathways (TC-NER and GG-NER) depending on whether the damaged strand is being transcribed. Those two pathways only differ by their initial DNA damage recognition step.

In transcription-coupled NER (TC-NER), the RNA polymerase II complex encounters the DNA lesion and is remodeled (displaced?) by CSA and CSB, which then recruit the downstream NER factors. In GG-NER, the XPC/HHR23B protein complex is responsible for the initial detection of the DNA lesion and recruits the common downstream NER factors. The following steps are common to TC-NER and GG-NER. XPA binds to the damaged sites and recruits the DNA single-strand binding protein complex RPA, which keeps the two strands of the DNA duplex separated. Then the helicases of the TFIIH complex, XPB and XPD open the DNA duplex over a region of approximately 30 base pairs. The endonucleases XPF/ERCC1 and XPG cleave the damaged strand at the junction of the single- and double-stranded DNA (at the 5' and 3' junctions, respectively). The oligonucleotide containing the lesion can then be removed making way for gap repair synthesis (performed by the replicative polymerase, $\text{pol-}\delta/\epsilon$). Finally, the newly synthesized strand is ligated back.

The marine alkaloid, ecteinascidin 743 (Et743; Yondelis; Trabectedin) is an extremely potent NER inhibitor. Et743 has recently been approved for the treatment of soft tissue sarcomas and is in clinical trials for ovarian cancers. Et743 exhibits a unique mechanism of action, which was discovered after

Et743 had been identified as a potent anticancer agent. Following its sequence-specific binding to DNA in the minor groove, Et743 forms a covalent bond with the exocyclic residue N-2 of a guanine (16). Such bonding distorts the DNA by inducing a bend toward the major groove, opposite from the Et743 adduct, which probably recruits NER. Attempts by the TC-NER to repair the Et743-DNA adduct leads to the trapping of the NER complex following incision of the damaged strand (6). Recent evidence suggests that Et743 binds at the interface of the XPG-DNA complex (17), and that the molecular interaction takes place between XPG and the C-ring of Et743 that protrudes from the DNA minor groove (18). Accordingly, Et743-resistant cells have XPG mutation (6) and NER deficiencies confer resistance to Et743 (6, 7). As expected from the mechanisms of action of Et743 and platinum derivatives, combinations between Et743 and platinum derivatives produce synergistic effects (our unpublished data). Results of clinical trials combining those agents in ovarian cancers are awaited.

DSB REPAIR, DNA-PK, ATM INHIBITORS AND PARP INHIBITORS:

Double-strand breaks are perhaps the most serious form of DNA damage. A single DSB is probably sufficient to kill a cell as chromosome breakage can result in imbalanced transmission of the genetic material during mitosis. DSB can be generated by ionizing radiation (1 DSB for 20 SSB), radiomimetic agents such as bleomycin, and Top2 inhibitors. DSB can also be generated by the replication of DNA templates containing preexisting SSB or Top1cc. Those DSB are referred to as Rep-DSB (for replication-mediated DSB) (19).

The two main pathways for DSB repair are homologous recombination (HR) and non-homologous end joining (NHEJ). In normal cells, the choice of which pathway to use appears to be largely influenced by the stage of cell cycle at the time of the DNA damage. Because HR utilizes undamaged sister chromatids, it requires cells to be in S- and G2-phase of the cell cycle. In contrast, NHEJ does not utilize a homologous template for DNA repair and thus, can take place in G1. However, it is likely that NHEJ can also operate in S- and

G2-phase and complement for defects in HR, which are the hallmark of BRCA2-deficient cells.

HR corrects DSB in an error-free manner using mechanisms that retrieve genetic information from a homologous, undamaged DNA segment. To that effect, the first step of HR requires the formation of protruding 3'-ends following resection of the 5'-end of the DSB and coating of the protruding 3'-ends with RPA. BRCA2 then promote the loading of Rad51 (eukaryotic orthologs of RecA) and the formation of Rad51 coated DNA filaments that can invade the homologous, undamaged DNA segment. This process is referred as DNA strand exchange. Rad52 is essential for completion of HR and proper synapsis between the various DNA strands.

NHEJ is error-prone and dependent upon DNA-PK and its cofactors, the Ku heterodimer (Ku70/Ku80). Ku heterodimers initiate NHEJ by binding the free DNA ends as a hollow ring, and recruiting DNA-PK, XRCC4 and ligase IV. DNA-PK then becomes activated and phosphorylates a number of substrates including p53, Ku, XRCC4 and the endonuclease Artemis, which processes the ends prior to joining. XRCC4 promotes ligation of the ends by ligase IV, and Apx, which binds XRCC4, can reactivate ligase IV in case ligase IV fails to complete DNA rejoining (13, 20). Recently, an additional NHEJ has been identified, Cernunnos-XLF, which promotes NHEJ in unknown ways.

In parallel to their repair by HR and NHEJ, DSB activate the DSB response pathway consisting primarily of ATM and Chk2 (21). Although DNA-PK is also activated by DSB, the cross talks between ATM and DNA-PK remain to be clarified. A number of ATM, Chk2, and DNA-PK inhibitors have been identified and are in preclinical development. Finally, PARP is also an important regulatory factor of DSB repair as poly(ADPribosyl)ation of Ku suppress the NHEJ pathways.

SSB REPAIR:

DNA single-strand breaks are among the most promiscuous DNA lesions. Ionizing radiations produce approximately 5000-1000 SSB per Gray per cell, and \approx 20 SSB for each DSB. Abasic sites, which can form by spontaneous depurination and as BER intermediates are readily converted to SSB by β -elimination. Alkylating agents also promote the formation of abasic sites and SSB, and Top1 inhibitors (topotecan and irinotecan) generate high

number of SSB as the drugs trap Top1cc (22) (The repair of Top1cc will be discussed below).

SSB repair can utilize the BER pathways described above. Additional enzymes are also important for SSB processing. They include Tdp1, PNKP and Aptx. Tdp1 and PNKP process 3'-ends by removing remaining atoms from the processed deoxyribose that was associated with the abasic site to convert them to 3'-hydroxyl ends, which are proper substrates for DNA polymerases and ligase. PNKP and Aptx process the 5'-ends of SSB into 5'-phosphate ends, which are proper substrates for ligases. PNKP is an efficient DNA kinase and adds a phosphate to 5'-hydroxyl ends. Aptx binds the BER scaffolding factor XRCC1 and specifically removes 5'-adenylates that arise from abortive ligation reactions, resulting in the production of 5'-phosphate termini that can be efficiently rejoined (13, 20).

INTERSTRAND CROSSLINK REPAIR:

Interstrand crosslinks are produced by DNA alkylating anticancer agents. Platinum derivatives can produce guanine-guanine interstrand crosslinks in addition to the more toxic intrastrand crosslinks described above in the NER section. Nitrogen mustards (melphalan, chlorambucil, cyclophosphamide and carboxyphosphamide) and mitomycin C also produce such G-G interstrand crosslinks whereas nitrosoureas (BCNU and CCNU) produce G-C interstrand crosslinks (10)². To our knowledge, the repair mechanisms for interstrand crosslinks remain poorly understood, in spite of the recent progress in understanding the molecular events associated with FA-associated repair complexes (23). In addition, the FA factors are at the interface of several pathways as FANCD1 corresponds to BRCA2 and several FANC elements interact with well-known DDR proteins, including BRCA1, ATM and Nbs1 (8). Nevertheless, inactivation of FA genes may be associated with a broad range of sporadic tumors, which may have implications for the predicting the sensitivity of tumors to widely

used anticancer DNA crosslinking agents (cisplatin, mitomycin C and melphalan) (23).

REPAIR OF DNA-PROTEIN CROSSLINKS AND TOPOISOMERASE CLEAVAGE COMPLEXES:

Human cells contain several topoisomerases, which are essential for cell survival. Top1 and Top2 are the targets of some of the most commonly used anticancer agents. Camptothecin derivatives (topotecan and irinotecan) selectively target Top1 (22), whereas etoposide (VP-16), etoposide (VM-26), anthracyclines (doxorubin, daunorubicin, epirubicin, idarubicin), and mitoxantrone target Top2 (24). Both Top1 and Top2 inhibitors act as topoisomerase "poisons" rather than catalytic inhibitors. Indeed they act by trapping the key catalytic intermediates by which the topoisomerase regulates DNA supercoiling. Those intermediates are referred to as cleavage complexes because the DNA breakage requires the topoisomerase to form a covalent linkage with its catalytic tyrosine. Thus, each break is associated with the formation of a topoisomerase covalent complex. In the case of Top1 the covalent linkage is with the 3'-end of the break, whereas it is with the 5'-end for Top2. Normally, those cleavage complexes are transient and topoisomerase-mediated religation of the DNA releases the topoisomerase. All the topoisomerase inhibitors used clinically act similarly by trapping cleavage complexes (22, 24). The differences between Top2 inhibitors are mostly related to the sequences where the drugs trap the Top2cc, and to the stability of such drug-trapped Top2cc (24).

The repair of Top1cc has recently been reviewed (9)³. It involves redundant pathways, which might be explained by the fact that Top1cc forms under physiological conditions and need to be efficiently removed (25). Our current view is that two main pathways can remove the Top1-DNA adduct. The first is by way of Tdp1, which hydrolyzes the tyrosyl-phosphodiester bond (26). However, Tdp1 can only hydrolyze that bond if Top1 is reduced to a small denatured polypeptide. Thus, this first pathway implies a proteolytic degradation of Top1 prior to Tdp1 action (27). The second pathway to remove Top1-DNA complexes is by way of endonuclease that can excise the DNA strand covalently attached to Top1. Several endonuclease

² see Figure 5 in

<http://discover.nci.nih.gov/pommier/Replication.inhibitors.htm>

³ <http://discover.nci.nih.gov/pommier/pommier.htm>

have been implicated in this pathway: Mus81/Eme1 or XPF/ERCC1 (9)³. Most remarkably the choice of which pathway is used to excise the Top1cc appears to be regulated by the DDR response (Rad9 in yeast, which might be the ortholog of human BRCA1) (see below).

A broad range of anticancer agents besides topoisomerase inhibitors can also form dNA-protein crosslinks. In such case, the proteins involved in the crosslinks have not been fully characterized. Those anticancer agents include DNA crosslinking alkylating agents (platinum derivatives), aminoflavone (28), which has just started clinical trials, and DNA demethylating agents (decitabine) (29).

Rationale for using DNA repair and DDR modulators in cancer therapy

CONDITIONAL (SYNTHETIC) LETHALITY:

A powerful concept for therapeutic combinations and rationale administration with DNA repair and DDR inhibitor is based on conditional (synthetic) lethality. In yeast genetic, synthetic lethality is rooted in the fact that knocking out one gene (for instance gene X in Fig. 1B) in a normal strain has no biological effects, whereas knocking out that same gene X in another strain bearing an alteration of another gene (Y) (Fig. 1B) functioning in a redundant pathway is lethal (Fig. 1C). This simple concept has several important implications. First, it underlies the importance of dissecting out the various redundant pathways involved in repairing specific lesions. Second, it demonstrates the value of having a variety of inhibitors whose use needs to be tailored to the particular tumor defects. Applying the conditional lethality principle should overcome a main pitfall for DNA repair inhibitors, which is the overall amplification of DNA damage both in tumor and normal cells, thereby providing no or only limited increase of selectivity of the DNA damaging agent for the tumor. Such pitfall has been observed with O6-BG, which increases bone marrow toxicity and forces dose reduction without obvious therapeutic benefit.

The inhibitors of DNA repair and DDR provide several examples of rationale use and/or combinations based on the conditional lethality principle. One of the most striking examples is for the PARP inhibitors (30, 31), which are selectively active in BRCA2-deficient tumors. Going back to Figure 1, this would place HR in one of the two pathways and PARP in the other. The exact mechanism of the conditional activity of PARP inhibitors has been attributed to the fact that cells deficient for HR rely on NHEJ and that PARP inhibition stimulates HR. Another example may concern the use of DNA-PK inhibitors in ATM-deficient tumors. Indeed, knocking out ATM or DNA-PK is not lethal, whereas DNA-PK inactivation kills cells when ATM is also inactivated (32). Thus, ATM-deficient tumors (for instance lymphoma; see first section) might be the preferred indication for the DNA-PK inhibitors in development (33). They may also be preferentially sensitive to PARP inhibitors (31). Another example of rationale drug combination is for association of checkpoint and Top1 inhibitors. UCN-01 (7-hydroxystaurosporine), which acts as a Chk1 (and Chk2) inhibitor produces a remarkable synergistic activity in association with Top1 inhibitors in p53-deficient cells. This synergism might be due to the fact that those cells are defective in checkpoint pathways, and that targeting Chk1 (and Chk2) in those cells has a more profound effect than in normal cells, which have intact redundant pathways besides Chk1 and Chk2.

We are currently using the conditional lethality principle to rationalize the development of Tdp1 inhibitors in combination with Top1 inhibitors. Indeed, as mentioned above, redundant pathways repair Top1cc. Thus, knocking out Tdp1 fails to confer sensitization to camptothecin in yeast unless the experiments are performed in checkpoint-deficient (Rad 9-defective) strains. This has been interpreted as the fact that the checkpoints channel the repair away from the Tdp1 pathway, and that Tdp1 becomes essential in the absence of checkpoints. Since a large fraction of tumors are checkpoint-deficient, a working model is that Tdp1 inhibitors should synergize with camptothecins and non-camptothecin Top1 inhibitors in checkpoint-deficient tumors (for instance, in BRCA1-, ATM- or Mre11-deficient tumors), while having minimal impact on the toxicity of Top1 inhibitors toward normal tissues.

Perspective

DNA damaging agents were the first anticancer drugs introduced approximately 60 years ago, starting with the alkylating agents. The number of drugs and targeted pathways has increased remarkably since. The DNA repair mechanisms have also become better understood and it is known that most tumor cells require DNA repair and DDR deficiencies for survival.

Our challenges are to continue our detailed investigations of DNA repair and DDR pathways and to integrate this expanding wealth

of knowledge (in DNA repair, pharmacology, tumor genetic, and drug discovery) to achieve cancer cure. It is plausible that detailed characterization of individual tumors for DNA repair and DDR factors will be required to achieve this goal. This will require the development of molecular diagnostic tools. The availability of a broad spectrum of drugs with well-defined molecular targets will provide the rationale to use those drugs in relation to the tumor specific defects, and to combine DNA damaging agents with the appropriate DNA repair and DDR modulators.

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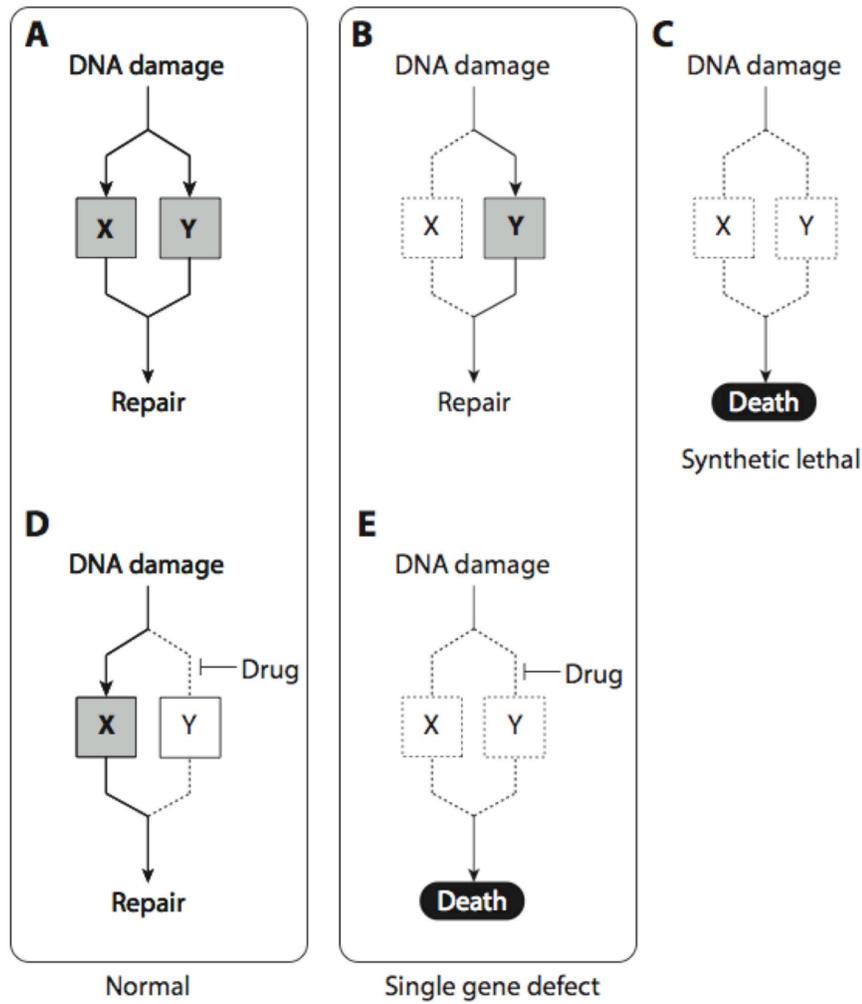


Figure 1: Concept of synthetic lethality for cancer treatment. **A.** DNA repair in normal cells relies on two redundant pathways (X and Y). **B.** Single defect (as in tumor cells) for pathway/gene X has no functional impact as pathway Y overcome X defect. **C.** Double defect is lethal as no pathway is left to repair the DNA damage. **D** and **E.** Rationale for selective enhancement by drug in cells with single gene defect.