Targeting DNA damage response in cancer therapy

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Mechanism of DNA Damage Response

The genome DNA is constantly exposed to various genotoxic insults. Among the variety of types of DNA damage, the most deleterious is the DNA double-strand break (DSB). Double-strand breaks can be generated by endogenous sources such as reactive oxygen species produced during cellular metabolic processes and replication-associated errors, as well as by exogenous sources including ionizing radiation and chemotherapeutic agents. Double-strand breaks are also generated in a programmed manner during meiosis and during the V(D)J recombination and class switch recombination required for the development of lymphocytes. If left unpaired, DSBs can result in cell death. If accurately repaired, DSBs can result in survival of cells with no adverse effects. If insufficiently or inaccurately repaired, DSBs can result in survival of cells showing genomic alterations that may contribute to tumor development. In order to maintain genomic integrity, cells have evolved a well coordinated network of signaling cascades, termed the DNA damage response, to sense and transmit the damage signals to effector proteins, and induce cellular responses including cell cycle arrest, activation of DNA repair pathways, and cell death (Fig. 1).

Cancer chemotherapeutic agents and radiotherapy exert their cytotoxic effects by inducing DNA DSBs. As cancer cells often have specific abnormalities in the DNA damage response, therapeutic strategies based on such properties of cancer cells have been developed. Several inhibitors that block specific DNA damage responses or repair proteins have been tried not only as sensitizing agents in combination with DNA-damaging agents but also as single agents against cancers with defects in particular DNA repair pathways. The most prominent example of the latter is the killing effect of poly(ADP-ribose) polymerase (PARP) inhibitors on BRCA1- or BRCA2-defective tumors, which takes advantage of the defects in DNA repair in cancer cells.

In this review, we will first outline the mechanism of the DNA damage response. Next, we will describe the aberrations in DNA damage responses in human cancers. Finally, we will explain how different DNA damage response pathways can be targeted for cancer therapy.

Cancer chemotherapy and radiotherapy are designed to kill cancer cells mostly by inducing DNA damage. DNA damage is normally recognized and repaired by the intrinsic DNA damage response machinery. If the damaged lesions are successfully repaired, the cells will survive. In order to specifically and effectively kill cancer cells by therapies that induce DNA damage, it is important to take advantage of specific abnormalities in the DNA damage response machinery that are present in cancer cells but not in normal cells. Such properties of cancer cells can provide biomarkers or targets for sensitization. For example, defects or upregulation of the specific pathways that recognize or repair specific types of DNA damage can serve as biomarkers of favorable or poor response to therapies that induce such types of DNA damage. Inhibition of a DNA damage response pathway may enhance the therapeutic effects in combination with the DNA-damaging agents. Moreover, it may also be useful as a monotherapy when it achieves synthetic lethality, in which inhibition of a complementary DNA damage response pathway selectively kills cancer cells that have a defect in a particular DNA repair pathway. The most striking application of this strategy is the treatment of cancers deficient in homologous recombination by poly(ADP-ribose) polymerase inhibitors. In this review, we describe the impact of targeting the cancer-specific aberrations in the DNA damage response by explaining how these treatment strategies are currently being evaluated in preclinical or clinical trials.

Mechanism of DNA Damage Response

DNA-damaging agents induce various types of DNA damage including modification of bases, intrastrand crosslinks, interstrand crosslinks (ICL), DNA–protein crosslinks, single-strand breaks (SSBs), and DSBs. Each type of DNA damage is recognized and processed by proteins involved in the DNA damage response (Fig. 1).

In response to DSBs, the MRE11–RAD50–NBS1 (MRN) complex senses and binds to DSB sites, and recruits and activates the ataxia telangiectasia mutated (ATM) kinase through its autophosphorylation. Once activated, ATM

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phosphorylates a large number of downstream proteins. (6) Phosphorylation of Chk2 induces phosphorylation of the protein phosphatase CDC25A, leading to cell cycle arrest. Phosphorylation of BRCA1 leads to DSB repair as well as cell cycle arrest in the S phase, whereas activation of p53 triggers cell cycle arrest in the G1 phase or cell death. In the initiation of the response to SSBs or DNA replication fork collapse, the ataxia telangiectasia and Rad3-related (ATR) kinase is activated and recruited to the sites of DNA damage. (7) ATR phosphorylates and activates Chk1, (8) which plays a role in the S and G2/M cell checkpoints by regulating the stability of the CDC25 phosphatases. Activation of the 53BP1 protein, a mediator of the DNA damage response, contributes to the choice of the DSB repair pathways by promoting non-homologous end joining (NHEJ). (9)

The DNA repair pathways can either work independently or coordinately to repair different types of DNA damage (Fig. 1). Double-strand breaks are predominantly repaired by either NHEJ or homologous recombination (HR). (10) Non-homologous end joining is an error-prone repair pathway that is mediated by the direct joining of the two broken ends. (10) Factors involved in NHEJ include the Ku70/Ku80 complex, DNA-PK catalytic subunit (DNA-PKcs), the Artemis nuclease, XLF, XRCC4, and DNA ligase IV. Homologous recombination is an error-free repair pathway that requires a non-damaged sister chromatid to serve as a template for repair (Fig. 2). (10) Factors involved in HR include the MRN complex, CtIP, replication protein A (RPA), BRCA1, PALB2, BRCA2, and RAD51. In addition to NHEJ and HR, an alternative form of NHEJ, namely, alt-NHEJ, is also involved in DSB repair. (11) It exhibits a slower process than the classical NHEJ and can catalyze the joining of unrelated DNA molecules, leading to the formation of translocations as well as large deletions and other sequence alterations at the junction. Factors involved in this pathway include PARP-1, XRCC1, DNA ligase IIIα, polynucleotide kinase, and Flap endonuclease 1.

Single-strand breaks and subtle changes to DNAs are repaired using base-excision repair (BER) proteins, (12) which include PARP-1, XRCC1, DNA ligase IIIα, and apurinic/apyrimidinic endonuclease (APE1). Bulky DNA lesions such as pyrimidine dimers caused by UV irradiation are processed by the nucleotide excision repair (NER) pathway, (13) which requires the excision repair cross-complementing protein 1 (ERCC1). Base mismatches arising as a result of replication errors can be repaired by the mismatch repair pathway. (14) In the repair of ICL, ubiquitin-mediated activation of the Fanconi anemia (FA) pathway plays a key role. (15) The FA pathway is constituted by at least 15 FA gene products, whose germ-line defects result in FA, a cancer predisposition syndrome.
Activation of the FA core complex, which is comprised of eight FA proteins (FANCA/B/C/E/F/G/L/M) and associated proteins, leads to monoubiquitination of FANC D2 and FANCD1, which subsequently coordinates three critical DNA repair processes, including nucleolytic incision by XPF-ERCC1 and SLX4 endonucleases, translesion DNA synthesis, and HR.

**Aberrations in DNA Damage Responses in Human Cancers**

In sporadic cancers, both activation and inactivation of the DNA damage response are found in various cancers, reported. DNA-PK catalytic subunit is reported to be upregulated in radiation-resistant tumors or in tumors with poor survival. Overexpression of RAD51, BRCA1, ERCC1, APE1, and PARP1 is also observed in various cancers and is associated with resistance to chemotherapy.

However, inactivation of DNA damage response proteins is also observed in various cancers. The p53 gene is one of the most frequently mutated genes in human sporadic cancers. Although the reported frequencies of p53 mutation vary among the types of cancer, it is estimated that more than half of cancers might have inactivated p53 due to mutations, deletion, loss of heterozygosity of the gene, or decreased expression. Although inactivating mutations in ATM, BRCA1, or BRCA2 are less frequent than those in the p53 gene, decreased expression of ATM, the MRN complex, Chk2, RAD51, BRCA1, BRCA2, and ERCC1 is frequently observed, suggesting that aberration of the DNA damage response is common in sporadic cancers.

Promoter hypermethylation of the BRCA1 gene is frequently observed and may be one of the predominant mechanisms for deregulation of the BRCA1 gene. Furthermore, our group reported the functional inactivation of BRCA2 in cancer cells aberrantly expressing SYCP3, a cancer-testis antigen. Disruption of the FA pathway resulting from mutations or decreases or loss of expression due to promoter hypermethylation has been also described in various cancers.

As described above, both activation and inactivation of the DNA damage response are observed in cancers, and are expected to determine important properties of the DNA damage response machinery present in each cancer. The status of BRCA has been adopted as an important condition factor in current clinical trials, however, the status of other DNA damage response proteins have not yet been translated into clinical trials. In the next section, we will introduce various approaches for taking advantage of these cancer-specific properties of the DNA damage response in cancer therapy.

**How Can Different DNA Damage Response Pathways be Targeted for Cancer Therapy?**

Because the efficacy of cancer chemotheraphy and radiotherapy relies on generation of DNA damage that will be recognized and repaired by intrinsic DNA repair pathways, aberrant expression of a particular DNA damage response protein should be a biomarker of resistance or favorable response to therapies that induce the corresponding types of DNA damage. For example, patients with surgically treated non-small-cell lung cancer whose tumors lacked expression of ERCC1 were shown to benefit from cisplatin-based adjuvant chemotherapy in a clinical study. Another example is the case of RAD51, whose expression can serve as a marker of cisplatin resistance in non-small-cell lung cancer, which is consistent with the role of HR in the repair of ICL.

In contrast, many inhibitors of the DNA damage response have been developed and some of them have been tested for their potential to enhance DNA damage-induced tumor cell killing in preclinical studies and clinical trials. **Inhibitors of ATM/ATR and the MRN complex.** As ATM and the MRN complex play central roles as sensors or mediators in the DNA damage response machinery present in each cancer. The status of BRCA has been adopted as an important condition factor in current clinical trials, however, the status of other DNA damage response proteins have not yet been translated into clinical trials. In the next section, we will introduce various approaches for taking advantage of these cancer-specific properties of the DNA damage response in cancer therapy.
### Table 1. Examples of aberrations in DNA damage responses in human sporadic cancers

| Molecule | Activation or inactivation | Type of aberrations | Type(s) of cancer | Frequency | Phenotypes | Reference(s) |
|----------|---------------------------|---------------------|-------------------|-----------|------------|--------------|
| ATM      | Activation                | Increased autophosphorylation | Bladder, breast cancers | 30–68% | Cancer barrier function | (16,18) |
| ATM      | Activation | Increased copy number | Prostate cancers | ~2% | | (51) |
| ATM      | Inactivation | Mutation | Pancreatic, lung, colon, endometrial, prostate, skin, kidney, breast, central nervous system, ovarian cancers | 1–7% | | (49,50) |
| ATM      | Inactivation | Loss of heterozygosity, loss of copy number | Hematopoietic and lymphoid malignancies | ~11% | | (49) |
| ATM      | Inactivation | Decreased expression | Prostate cancers | ~5% | | (51) |
| ATM      | Inactivation | Decreased expression | Pancreatic cancers | 5% | | (51) |
| MRE11    | Inactivation | Decreased expression | Breast cancer | 7–31% | | (19,54,56) |
| MRE11    | Inactivation | Decreased expression | Colorectal, gastric, pancreatic cancers with microsatellite instability | 67–100% | Poor prognosis | (19) |
| RAD50    | Activation | Increased expression | Colorectal cancers | ~24% | | (21) |
| RAD50    | Inactivation | Decreased expression | Breast cancers | 3–28% | | (19,54,56) |
| RAD50    | Inactivation | Decreased expression | Colorectal, gastric cancers with microsatellite instability | 28–71% | | (19) |
| NBS1     | Activation | Increased expression | Esophageal, head and neck, non-small-cell lung cancers, hepatomas | 40–52% | Poor prognosis | (19,20) |
| Chk1     | Inactivation | Decreased expression | Breast cancers | 10–46% | | (19,54,56) |
| Chk1     | Activation | Increased phosphorylation | Cervical cancers | ~25% | Resistance to chemotherapy, poor prognosis | (22–27) |
| Chk1     | Activation | Increased expression | Lung, liver, breast, colorectal, ovarian, cervical cancers | 46–100% | | (22–27) |
| Chk1     | Inactivation | Decreased expression | Lung, ovarian cancers, hepatocellular carcinomas | 9–32% | | (22,23,26) |
| Chk2     | Activation | Increased phosphorylation | Bladder, colon, lung cancers, melanomas | 30–50% | Cancer barrier function | (16,17) |
| Chk2     | Inactivation | Increased expression | Ovarian cancers | ~37% | | (26) |
| Chk2     | Inactivation | Decreased expression | Breast, non-small cell lung cancers | 28–47% | | (57,58) |
| p53      | Inactivation | Mutation | Solid tumors | ~50% | | (47) |
| p53      | Inactivation | Decreased expression | Hematopoietic malignancies | ~10% | Resistance to chemotherapy, poor prognosis | (47) |
| p53      | Inactivation | Decreased expression | Solid and hematopoietic tumors | ~50% | | (48) |
| CDC25A   | Activation | Increased expression | Thyroid, breast, ovarian, liver, colorectal, laryngeal, esophageal cancers, non-Hodgkin’s lymphomas | 17–70% | | (28) |
| CDC25B   | Activation | Increased expression | Thyroid, breast, ovarian, liver, colorectal, laryngeal, esophageal, endometrial, prostate cancers, gliomas, non-Hodgkin’s lymphomas | 20–79% | | (28) |
| CDC25C   | Activation | Increased expression | Colorectal, endometrial cancers, non-Hodgkin’s lymphomas | 13–27% | | (28) |
| DNA-PKcs | Activation | Increased expression | Glioblastoma, prostate cancers | ~49% | Poor survival | (29,30) |
topoisomerase inhibitors. KU60019, an improved analog of KU55933, inhibits the DNA damage response and effectively radiosensitizes human glioma cells. Mirin is an inhibitor of the MRN complex, which prevents MRN-dependent activation of ATM without affecting ATM protein kinase activity and inhibits MRE11-associated exonuclease activity. Telomelysin is another inhibitor that inhibits the MRN complex through the adenoviral E1B-55 kDa protein. The therapeutic outcomes of these agents remain to be tested in clinical trials. Although the long search for selective inhibitors of ATR has not yet paid off, schisandrin B was recently identified as a modulator of ATR activity. Although the long search for selective inhibitors of ATR has not yet paid off, schisandrin B was recently identified as a modulator of ATR activity.

**Inhibitors of Chk1/Chk2 and CDC25.** As the triggering of cell cycle checkpoints is crucial in the DNA damage response, these checkpoints have also been widely investigated as a potential target for cancer therapy (Table 3). Among the inhibitors for Chk1 and/or Chk2, UCN-01 was the first to enter clinical trials, but it was discontinued due to toxicities such as symptomatic hypotension and neutropenia and a lack of convincing efficacy after phase II trials. Other Chk1/Chk2 inhibitors with improved specificities, including XL844 and AZD7762, also entered clinical trials but failed to achieve a good response. The selective Chk1 inhibitor SCH900776 has been used in phase I trials for acute leukemia in combination with cytarabine and for solid tumors in combination with gemcitabine, and showed some partial responses and stable disease. The Chk1 inhibitor LY2603618 and the dual Chk1/Chk2 inhibitor LY2606368 are also currently being tested in early clinical trials. CDC25 phosphatases, the key factors in cyclin-dependent kinase regulation crucial for cell cycle regulation, are also considered to represent promising novel targets in cancer therapy. CDC25 inhibitors have also been developed, and some have entered into clinical trials, although the clinical data is limited.

**Inhibition of NHEJ by DNA-PK inhibitors.** Regarding NHEJ, inhibitors of DNA-PK, including NU7026 and NU7441, were found to induce extreme sensitivity to ionizing radiation as

### Table 1. (continued)

| Molecule | Activation or inactivation | Type of aberrations | Type(s) of cancer | Frequency | Phenotypes | Reference(s) |
|----------|----------------------------|---------------------|-------------------|-----------|------------|--------------|
| RADS1    | Activation                 | Increased expression| Breast, head and neck, non-small-cell lung cancer, pancreatic cancers, soft tissue sarcomas | 24-66%    | Resistance to platinum agents, poor outcome | (31-35)      |
| BRCA1    | Inactivation               | Decreased expression| Breast, colorectal cancers, lung cancers | ~10%      | Resistance to chemotherapy | (52,53)      |
|          | Activation                 | Increased expression| Breast, ovarian cancer | ~30%      | Resistance to chemotherapy | (52,53)      |
| BRCA2    | Inactivation               | Decreased expression| Breast, ovarian cancer, lung cancer | ~9-30%    | Resistance to chemotherapy | (60-62)      |
| ERCC1    | Activation                 | Increased expression| Colorectal, ovarian, gastric, head and neck, non-small-cell lung cancer | 14-70%    | Resistance to platinum agents | (31,37-43)   |
|          | Inactivation               | Decreased expression| Colorectal, gastric, non-small-cell lung cancer | 30-77%    | Resistance to chemotherapy | (37,38,42,43) |
| APE1     | Activation                 | Increased expression| Bladder, breast, cervical, head and neck, liver, non-small-cell lung cancer, ovarian cancer, medulloblastomas, gliomas, osteosarcomas, germ cell tumors | 19-99%    | Resistance to chemotherapy and/or radiation | (44)          |
| PARP     | Activation                 | Increased expression| Breast cancers, germ cell tumors | 5-47%     | Resistance to chemotherapy | (45,46)      |
| FANCA    | Inactivation               | Decreased expression| Acute myelogenous leukemias | ~4-40%    | Resistance to chemotherapy and/or radiation | (64,65)      |
|          | Mutation                   | Loss of expression  | Acute myelogenous leukemias | ~4-40%    | Resistance to chemotherapy and/or radiation | (64,65)      |
| FANCC    | Inactivation               | Mutation, loss of heterozygosity | Pancreatic cancers | ~7.6% | Resistance to chemotherapy and/or radiation | (64)          |
| FANCF    | Inactivation               | Decreased expression| Breast, cervical, head and neck, non-small-cell lung, ovarian cancers, acute myelogenous leukemias, germ cell tumors | 6.7-30%  | Resistance to chemotherapy and/or radiation | (64,65)      |
| FANCG    | Inactivation               | Loss of expression  | Acute myelogenous leukemias | 27%       | Resistance to chemotherapy and/or radiation | (65)          |

Expression has been confirmed at mRNA and/or protein levels. Studies using cultured cancer cells are excluded.
well as DNA-damaging agents in preclinical studies (Table 2). However, the therapeutic efficacy of DNA-PK inhibitors depends on the expression levels of DNA-PK in cancer cells versus normal cells, and their clinical application is currently restricted because of their toxicity to normal cells. The dual mTOR and DNA-PKcs inhibitor CC-115 is undergoing early clinical evaluation (Table 3). KU-0060648 is a potent dual inhibitor of DNA-PK and PI-3K, which has recently been reported to enhance etoposide and doxorubicin cytotoxicity (Table 2).

**Inhibition of NHEJ or alt-NHEJ by DNA ligase inhibitors.** DNA ligases are required for both NHEJ and alt-NHEJ pathways as well as other DNA repair pathways such as BER and NER. Small molecule inhibitors of human DNA ligases have been identified and shown to be cytotoxic and also to enhance the cytotoxicity of DNA-damaging agents. SCR7 is an inhibitor of DNA ligase IV, which is involved in the NHEJ pathway. SCR7 reduces cell proliferation in a DNA ligase IV-dependent manner and increases the tumor-inhibitory effects of agents that cause DSBs. L67 is an inhibitor of DNA ligases I and IIIα, which are involved in the alt-NHEJ pathway as well as BER and NER. The levels of the alt-NHEJ proteins such as DNA ligase IIIα and WRN are reported to be elevated in BCR-ABL-positive CML cell lines, so inhibition of alt-NHEJ factors may be an additional therapeutic approach in BCR-ABL-positive CML, which is usually treated by tyrosine kinase inhibitors. Indeed, CML cell lines with increased alt-NHEJ-positive CML, which is usually treated by tyrosine kinase inhibitors, may be an additional therapeutic approach in enhancing sensitivity to DNA crosslinking agents and ionizing radiation in cancer cells. Furthermore, targeting RAD51 was shown to overcome imatinib resistance in CML cells.

**Inhibitors of histone deacetylases, heat shock protein 90, and DSB repair.** Histone deacetylases (HDACs) are powerful regulators of the stability of the genome, and many HDAC inhibitors are shown to downregulate multiple components of the DNA damage response and repair, including HR, NHEJ, the MRN complex, and ATM. Thus, the use of HDAC inhibitors in combination with DNA-damaging agents may be an area of great interest with potential clinical utility. The HDAC inhibitor PCI-24781 caused increased apoptosis by inhibiting RAD51-mediated HR when used in combination with the PARP inhibitor PJ34 in a preclinical study. The inhibitor of heat shock protein 90, 17-allylamino-17-demethoxygeldanamycin, radiosensitizes human tumor cell lines by inhibiting RAD51-mediated HR. Curcumin is a natural product that has been tested for its chemosensitizing potential, and sensitizes cancer cells to PARP inhibitors by inhibiting NHEJ, HR, and the DNA damage checkpoint.

**Inhibitors of PARP and APE1 in combination with DNA-damaging agents.** Inhibitors of PARP, which inhibit the BER and SSB repair pathways, are the most advanced and promising drugs that target DNA repair. A number of clinical trials using PARP inhibitors are currently underway (Table 3). Inhibitors of PARP were first tried in combination with DNA-damaging agents. Some clinical responses were observed in the phase I and II trials of the PARP inhibitor rucaparib in combination with temozolomide. Further clinical trials of PARP inhibitors have been carried out in combination with various DNA-damaging agents and/or ionizing radiation (Table 3). Inhibitors of another BER protein APE1 are also being tested in combination with DNA-damaging agents in clinical trials (Table 3).

**Using PARP inhibitors as single agents in BRCA-deficient cancers based on the principle of synthetic lethality.** In 2005, PARP inhibitors were shown to selectively inhibit the growth of cells with defects in either the BRCA1 or BRCA2 genes, suggesting a new use of PARP inhibitors as single agents. A possible explanation for this lethality is as follows. The cancer cells with defects in the BRCA gene are defective in HR, as the wild-type BRCA allele is absolutely lost. However, HR is intact in normal cells of the same patients who carry one wild-type BRCA allele and one mutant BRCA allele. Inhibition of PARP1 results in the accumulation of SSBs, which are converted to lethal DSBs that require HR for their repair.

### Table 2. Examples of DNA damage response inhibitors in preclinical studies

| Pathway | Target(s) | Name(s) | Preclinical evidence |
|---------|-----------|---------|---------------------|
| DNA damage sensors and mediators | MRE11 | Mirin, telomelysin | Sensitization to ionizing radiation |
| | ATM | KU59933, KU60019, CP466722 | Sensitization to ionizing radiation and topoisomerase inhibitors |
| | ATR | Schisandrin B | Sensitization to UV treatment |
| | | NU6027, VE-821 | Sensitization to ionizing radiation and a variety of chemotherapy |
| | | SAR-020106 | Sensitization to irinotecan and gemcitabine |
| | | VRX0466617 | Sensitization to ionizing radiation |
| | | NU7026, NU7441 | Sensitization to ionizing radiation and topoisomerase II inhibitors |
| | DNA-PK and PI3K | KU-0060648 | Sensitization to etoposide and doxorubicin |
| | DNA ligase IV | SCR7 | Sensitization to ionizing radiation and etoposide |
| Alternative non-homologous end joining | DNA ligases | L67 | Sensitization to ionizing radiation and methyl methanesulfonate |
| Homologous recombination (HR) | RADS1 | B02, A03, A10 | Identified by high-throughput screenings of RADS1 inhibitors |
Table 3. Examples of DNA damage response inhibitors in clinical trials

| Pathway                  | Target(s) | Name            | Combination       | Type of cancer                                                                 | Clinical trial number | Stage     | Trial periods                  |
|--------------------------|-----------|-----------------|-------------------|--------------------------------------------------------------------------------|-----------------------|----------|--------------------------------|
| Cell cycle checkpoints   | Chk1      | UCN-01          | Combination therapy | Advanced solid tumor Metastatic or unresectable solid tumor, triple negative breast cancer | NCT00036777, NCT00031681 | Phase I  | Completed, Completed          |
|                          |           |                 | Carboplatin       |                                                                                   |                       |          |                                |
|                          |           |                 | Irinotecan        |                                                                                   |                       |          |                                |
|                          |           |                 | Cytarabine        | Refractory or relapsed acute myelogenous leukemia, myelodysplastic syndrome       | NCT00004263           | Phase I  | Completed                      |
|                          |           |                 | Perifosine        | Relapsed or refractory acute leukemia, chronic myelogenous leukemia, high risk myelodysplastic syndrome | NCT00301938           | Phase I  | Completed                      |
|                          |           |                 | Gemcitabine       | Unresectable or metastatic pancreatic cancer                                       | NCT00039403           | Phase I  | Completed                      |
|                          |           |                 | Topotecan         |                                                                                   |                       |          |                                |
|                          |           |                 | Cisplatin         |                                                                                   |                       |          |                                |
|                          |           |                 | Fluorouracil      |                                                                                   |                       |          |                                |
|                          |           |                 | Prednisone        |                                                                                   |                       |          |                                |
|                          |           |                 | Irinotecan        |                                                                                   |                       |          |                                |
|                          |           |                 | Fluorouracil,     |                                                                                   |                       |          |                                |
|                          |           |                 | leucovorin        |                                                                                   |                       |          |                                |
|                          |           |                 | Topotecan         |                                                                                   |                       |          |                                |
|                          |           |                 | Fludarabine       |                                                                                   |                       |          |                                |
|                          |           |                 | Fluorouracil      |                                                                                   |                       |          |                                |
|                          |           |                 | Cisplatin         |                                                                                   |                       |          |                                |
|                          |           |                 | Topotecan         |                                                                                   |                       |          |                                |
|                          |           |                 | Fludarabine       |                                                                                   |                       |          |                                |
|                          |           |                 | Monotherapy       |                                                                                   |                       |          |                                |
| SCH900776                |           |                 | Combination therapy | Relapsed or refractory T-cell lymphoma                                              | NCT00082017           | Phase II | Completed                      |
|                          |           |                 | Cytarabine        |                                                                                   | NCT0072189, NCT0001444 | Phase I  | Completed, Completed          |
|                          |           |                 | Metastatic melanoma |                                                                                   |                       |          |                                |
|                          |           |                 | Breast cancer, lymphoma, prostatic neoplasm |                                                                                   |                       |          |                                |
|                          |           |                 | Leukemia/lymphoma / unspecified adult solid tumor |                                                                                   |                       |          |                                |
|                          |           |                 | Advanced or metastatic kidney cancer |                                                                                   | NCT00030888           | Phase II | Active, not recruiting         |
|                          |           |                 | Hydroxyurea       | Advanced solid tumors                                                              | NCT00779584, NCT01521299 | Phase I  | Completed, Withdrawn           |
| Pathway                        | Target(s)          | Name                | Combination | Type of cancer                                                                 | Clinical trial number | Stage               | Trial periods                      |
|-------------------------------|--------------------|---------------------|-------------|---------------------------------------------------------------------------------|-----------------------|---------------------|-----------------------------------|
| LY2603618                     |                    | Combination therapy | Desipramine, pemetrexed, gemcitabine          | Cancer                | NCT01358968         | Phase I             | Completed                        |
|                               |                    |                     | Pemetrexed, gemcitabine                        | Advanced or metastatic solid tumor | NCT01296568         | Phase I             | Completed                        |
|                               |                    |                     | Pemetrexed, cisplatin                          | NSCLC                 | NCT01139775         | Phase I, II         | Until March, 2014               |
|                               |                    |                     | Gemcitabine                                     | Pancreatic cancer     | NCT00839332         | Phase I, II         | Completed                        |
|                               |                    |                     | Gemcitabine                                     | Solid tumor           | NCT01341457         | Phase I             | Until December, 2014             |
|                               |                    |                     | Pemetrexed                                      | Cancer                | NCT00415636         | Phase I             | Completed                        |
|                               |                    |                     | Pemetrexed                                      | NSCLC                 | NCT00988858         | Phase II            | Until April, 2014               |
| Chk1 and Chk2                 |                    | Combination therapy | Gemcitabine                                     | Advanced cancer, lymphoma | NCT00475917         | Phase I             | Terminated                       |
|                               |                    |                      | Monotherapy                                     | Advanced cancer, lymphoma | NCT00475917         | Phase I             | Terminated                       |
|                               |                    |                      |                                                   | Chronic lymphocytic leukemia | NCT00234481         | Phase I             | Terminated                       |
| AZD7762                       |                    | Combination therapy | Gemcitabine                                     | Solid tumor           | NCT00413686         | Phase I             | Completed                        |
|                               |                    |                      | Gemcitabine                                     | Solid tumor           | NCT00937664         | Phase I             | Terminated                       |
|                               |                    |                      | Irinotecan                                      | Solid tumor           | NCT00473616         | Phase I             | Terminated                       |
| PF-00477736                   |                    | Combination therapy | Gemcitabine                                     | Advanced solid tumor  | NCT00437203         | Phase I             | Terminated                       |
| Non-homologous end joining    | DNA-PK and mTOR    | CC-115               | Monotherapy                                     | Multiple myeloma, non-Hodgkin’s lymphoma, glioblastoma, squamous cell carcinoma of head and neck, prostate cancer, Ewing’s osteosarcoma, chronic lymphocytic leukemia | NCT01353625         | Phase I             | Until April, 2015               |
| Base excision repair          | APE1               | TRC102               | Combination therapy                            | Neoplasm              | NCT00692159         | Phase I             | Completed                        |
|                               |                    |                     | Pemetrexed                                      | Lymphoma, solid tumor | NCT01851369         | Phase I             | Until February, 2015             |
|                               |                    |                     | Temozolomide and Fludarabine                    | Relapsed or refractory hematologic malignancy | NCT01658319 | Phase I | Until January, 2015 |
|                               |                    |                      | Lumactantone                                    | Combination therapy   | NCT02014545         | Phase II            | Until December, 2017             |
|                               |                    |                      | Radiotherapy                                    | Brain metastases from NSCLC | NCT01587144 | Phase II | Terminated                 |
|                               |                    |                      | Temozolomide and radiation                      | Glioblastoma multiforme | NCT01074970         | Phase II            | Until May, 2014                 |
|                               |                    |                      | Combination therapy                            | Triple negative breast cancer | NCT01009190         | Phase I             | Until December, 2013             |
|                               |                    |                      | Rucaparib (AG014688)                            | Platinum-sensitive, relapsed, high-grade epithelial ovarian, fallopian tube, or | NCT01891344 | Phase II | Until December, 2015 |

**Table 3. (continued)**

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Table 3. (continued)

| Pathway                        | Target(s)                                | Name              | Combination                        | Type of cancer                                                                 | Clinical trial number | Stage | Trial periods                     |
|--------------------------------|------------------------------------------|-------------------|------------------------------------|-------------------------------------------------------------------------------|-----------------------|-------|----------------------------------|
|                                |                                          |                   |                                    | primary peritoneal cancer                                                      |                       |       |                                  |
|                                |                                          |                   |                                    | Solid tumor (Phase I), ovarian cancer with germline BRCA mutations (Phase II)   | NCT01482715           | Phase I, II | Until March, 2014               |
|                                |                                          |                   |                                    | Platinum-sensitive, high-grade serous or endometrioid epithelial ovarian, primary peritoneal or fallopion tube cancer | NCT01968213           | Phase III | Until November, 2016            |
|                                |                                          |                   |                                    | BRCA-mutated locally advanced or metastatic breast cancer or advanced ovarian cancer | NCT00664781           | Phase II | Until September, 2014           |
| Olaparib (AZD2281)             | Combination therapy                      | Cediranib         |                                    | Recurrent ovarian, fallopian tube, peritoneal cancer or recurrent triple-negative breast cancer | NCT01116648           | Phase I, II | Until May, 2014                |
|                                |                                          |                   |                                    | Metastatic castration-resistant prostate cancer                                | NCT01972217           | Phase II | Until July, 2018                |
|                                |                                          |                   |                                    | Recurrent triple-negative breast cancer or recurrent high-grade serous ovarian cancer | NCT01623349           | Phase I | Until Dec, 2014                |
|                                |                                          | Radiotherapy      |                                    | Esophageal cancer                                                              | NCT01460888           | Phase I | Until August, 2018 Completed    |
|                                |                                          | Paclitaxel        |                                    | Recurrent or metastatic gastric cancer                                          | NCT01063517           | Phase II |                               |
|                                |                                          | Radiotherapy with or without cisplatin | Irinotecan, cisplatin, mitomycin C | locally advanced NSCLC                                                        | NCT01562210           | Phase I | Until March, 2015               |
|                                |                                          | Temozolomide      |                                    | Advanced pancreatic cancer                                                       | NCT01296763           | Phase I, II | Until January, 2016           |
|                                |                                          | Paclitaxel        |                                    | Relapsed glioblastoma                                                           | NCT01390571           | Phase I | Until September, 2015           |
|                                |                                          | Carboplatin and paclitaxel | Stage III, stage IV relapsed ovarian cancer or uterine cancer | Advanced squamous cell carcinoma of the head/neck with heavy smoking histories | NCT01650376           | Phase I, II | Until February, 2015           |
|                                |                                          | Radiation therapy and cetuximab |                                    |                                                                                       | NCT01758731           | Phase I | Until July, 2016               |
|                                |                                          | Gefitinib         |                                    | EGFR mutation-positive advanced NSCLC                                            | NCT01513174           | Phase I, II | Until June, 2015               |
|                                |                                          | Temozolomide      |                                    | Advanced Ewing's sarcoma                                                        | NCT01858168           | Phase I | Until July, 2017               |
|                                |                                          | Carboplatin       |                                    | Mixed muellerian cancer, cervical cancer, ovarian cancer, breast cancer, primary peritoneal cancer, fallopian tube cancer, endometrial cancer, carcinosarcoma | NCT01237067           | Phase I | Until September, 2014           |
|                                |                                          | Carboplatin and paclitaxel | Advanced ovarian cancer |                                                                                       | NCT01081951           | Phase II | Until June, 2013               |
|                                |                                          | Cisplatin-based chemoradiotherapy | Locally advanced squamous cell carcinoma of the head and neck |                                                                                       | NCT01491139           | Phase I | Withdrawn                       |
| Pathway                        | Target(s)                                      | Name                                        | Combination                                                                 | Type of cancer                                                                                              | Clinical trial number | Stage     | Trial periods                     |
|-------------------------------|------------------------------------------------|----------------------------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|------------------------|-----------|-----------------------------------|
| Carboptatin and/or paclitaxel | Irinotecan                                     | Tripe-negative metastatic breast cancer, advanced ovarian cancer | NCT00516724 Phase I Until December, 2014                                     |                                                                                                             |
|                               |                                                | Locally advanced or metastatic colorectal cancer | NCT00535353 Phase I Until December, 2013                                     |                                                                                                             |
|                               | Dacarbazine                                    | Advanced melanoma                            | NCT00516802 Phase I Completed                                                 |                                                                                                             |
|                               | Paclitaxel                                     | Metastatic triple negative breast cancer      | NCT00707707 Phase I Until December, 2012                                     |                                                                                                             |
|                               | Liposomal doxorubicin                          | Advanced solid tumor                         | NCT00819221 Phase I Until August, 2013                                      |                                                                                                             |
|                               | Topotecan                                      | Advanced solid tumor                         | NCT00516438 Phase I Completed                                                 |                                                                                                             |
|                               | Gemcitabine                                    | Pancreatic cancer                             | NCT00515866 Phase I Completed                                                 |                                                                                                             |
|                               | Bevacizumab                                    | Advanced solid tumor                         | NCT00710268 Phase I Completed                                                 |                                                                                                             |
|                               | Cisplatin                                      | Advanced solid tumor                         | NCT00782574 Phase I Until December, 2014                                     |                                                                                                             |
|                               | Carboplatin                                    | Breast and ovarian cancer with BRCA mutations or family histories | NCT01445418 Phase I Recruiting                                                |                                                                                                             |
|                               |                                                | Advanced solid tumor with normal or impaired liver function | NCT01894256 Phase I Until December, 2015                                     |                                                                                                             |
|                               |                                                | Metastatic breast cancer with germline BRCA1/2 mutations | NCT02000622 Phase III Until February, 2021                                    |                                                                                                             |
|                               |                                                | Advanced castration-resistant prostate cancer | NCT01682772 Phase II Until July, 2015                                        |                                                                                                             |
|                               |                                                | Advanced solid tumor BRCA-mutated ovarian cancer after a complete or partial response following platinum-based chemotherapy | NCT01813474 Phase I III Until November, 2014                                   |                                                                                                             |
|                               |                                                | BRCA-mutated advanced cancer                  | NCT01078662 Phase II Until December, 2013                                     |                                                                                                             |
|                               |                                                | BRCA-mutated advanced ovarian cancer following first line platinum based chemotherapy | NCT01844986 Phase III Until January, 2022                                     |                                                                                                             |
|                               |                                                | Advanced Ewing’s sarcoma                      | NCT01583543 Phase II Completed                                                 |                                                                                                             |
|                               |                                                | Stage IV colorectal cancer with microsatellite instability | NCT00912743 Phase II Completed                                                  |                                                                                                             |
|                               |                                                | BRCA-deficient ovarian, peritoneal, fallopian tube cancer | NCT01661868 Phase II Withdrawn                                                |                                                                                                             |
|                               |                                                | Advanced NSCLC                                | NCT01788332 Phase II Until May, 2015                                         |                                                                                                             |
|                               |                                                | BRCA-positive advanced breast cancer          | NCT00494234 Phase II Until December, 2013                                     |                                                                                                             |
|                               |                                                | BRCA-positive advanced ovarian cancer         | NCT00494442 Phase II Until December, 2013                                     |                                                                                                             |
|                               |                                                | Platinum-sensitive relapsed serous ovarian cancer | NCT00753545 Phase II Completed                                                  |                                                                                                             |
|                               |                                                | Advanced solid tumor                         | NCT00572364 Phase I Completed                                                 |                                                                                                             |

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| Pathway | Target(s) | Name | Combination | Type of cancer | Clinical trial number | Stage | Trial periods |
|---------|-----------|------|-------------|----------------|-----------------------|-------|--------------|
| Advanced or metastatic solid tumor | Ovarian cancer | | | NCT00633269 | Phase I | Completed |
| Advanced solid tumor | High grade ovarian cancer, triple-negative breast cancer, BRCA-mutated breast cancer or ovarian cancer | | | NCT00516373, NCT00777582, NCT00679783 | Phase I, Phase I, Phase II | Until December, 2014, Until March, 2014, Until December, 2012 |
| BRCA-positive advanced ovarian cancer | | | | NCT00628251 | Phase II | Until December, 2013 |

Veliparib (ABT-888) | Combination therapy | Gemcitabine, cisplatin | Locally advanced or metastatic pancreatic cancer with BRCA or PALB2 mutations | NCT01585805 | Phase II | Until July, 2017 |
| Temozolomide or combination with carboplatin and paclitaxel | Radiotherapy and temozolomide | Newly diagnosed childhood diffuse pontine glioma | NCT01514201 | Phase I, II | Until August, 2019 |
| Radiotherapy | Advanced solid malignancies with peritoneal carcinomatosis | NCT01264432 | Phase I | Until April, 2014 |
| Bendamustine, rituximab | Advanced lymphoma, multiple myeloma, or solid tumors | NCT01326702 | Phase I, II | Until November, 2015 |
| Topotecan | Relapsed epithelial ovarian, primary fallopian tube, or primary peritoneal cancer with negative or unknown BRCA status | NCT01690598 | Phase I, II | Until April, 2015 |
| Gemcitabine and radiotherapy | Locally advanced, unresectable pancreatic cancer | NCT01908478 | Phase I | Until July, 2019 |
| Dinaciclib with or without carboplatin | Advanced solid tumors with BRCA mutations | NCT01434316 | Phase I | Until January, 2016 |
| Radiotherapy, carboplatin, paclitaxel | Stage III NSCLC that cannot be removed by surgery | NCT01386385 | Phase I, II | Until December, 2016 |
| Doxorubicin, carboplatin, bevacizumab | Recurrent ovarian cancer, primary peritoneal cancer, or fallopian tube cancer | NCT01459380 | Phase I | Until August, 2015 |
| Cisplatin, gemcitabine | Advanced biliary, pancreatic, urothelial, NSCLC | NCT01282333 | Phase I | Terminated |
| Cisplatin, vinorelbine | Recurrent and/or metastatic breast cancer with BRCA mutations, triple-negative breast cancer | NCT01104259 | Phase I | Until September, 2014 |
| Mitomycin C | Metastatic, unresectable, or recurrent solid tumor | NCT01017640 | Phase I | Until June, 2014 |
| Capecitabine, radiotherapy | Locally advanced rectal cancer | NCT01589419 | Phase I | Until June, 2015 |
| Cyclophosphamide | Locally advanced or metastatic HER2-negative breast cancer | NCT01351909 | Phase I, II | Until May, 2015 |
| Pathway | Target(s) | Name | Combination | Type of cancer | Clinical trial number | Stage | Trial periods |
|---------|-----------|------|-------------|----------------|------------------------|-------|--------------|
| Docetaxel, cisplatin, fluorouracil, radiotherapy, hydroxyurea, paclitaxel | | | | Stage IV head and neck cancer | NCT01711541 | Phase I, II | Until October, 2014 |
| Temozolomide | Cisplatin, etoposide | | | Solid tumor | NCT01193140; NCT01642251 | Phase II | Completed | Until January, 2018 |
| | | | | Extensive stage small-cell lung cancer, metastatic large cell neuroendocrine NSCLC, small-cell carcinoma of unknown primary or extrapulmonary origin | | | |
| Paclitaxel, carboplatin | | | | Metastatic, unresectable solid tumor with liver or kidney dysfunction | NCT01366144 | Phase I | Until July, 2015 |
| Oxaliplatin, capecitabine | | | | BRCA-related malignancy, metastatic colorectal cancer, metastatic ovarian cancer, metastatic gastrointestinal malignancies in which oxaliplatin has shown some activity | NCT01233505 | Phase I | Until July, 2014 |
| Carboplatin | | | | Stage III or stage IV breast cancer with BRCA mutations | NCT01149083 | Phase II | Until June, 2014 |
| Temozolomide | | | | Acute leukemia | NCT01139970 | Phase I | Until October, 2013 |
| Carboptin | Carboplatin | paclitaxel | Topotecan | Solid tumor | NCT016317928 | Phase I | Completed | Until June, 2018 |
| | | | | Recurrent ovarian epithelial cancer, primary peritoneal cavity cancer, unspecified solid tumor | NCT01012817 | Phase I, II | |
| Carboplatin, paclitaxel | | | | Advanced NSCLC | NCT01560104 | Phase II | Until September, 2014 |
| | | | | HER2-negative metastatic or locally advanced breast cancer | NCT01251874 | Phase I | Until September, 2013 |
| Paclitaxel, cisplatin | | | | Advanced, persistent, or recurrent cervical cancer | NCT01281852 | Phase I, II | Until March, 2020 |
| Topotecan with or without carboplatin | | | | Relapsed or refractory acute leukemia, high-risk myelodysplasia, or aggressive myeloproliferative disorders | NCT00588991 | Phase I | Until December, 2012 |
| Abiraterone, prednisone | | | | Metastatic hormone-resistant prostate cancer | NCT01576172 | Phase II | Until February, 2014 |
| Topotecan and filgrastim or pegfilgrastim | | | | Persistent or recurrent cervical cancer | NCT01266447 | Phase II | Until November, 2016 |
| Gemcitabine | | | Modified FOLFOX6 | Solid tumor | NCT01154426 | Phase I | Until October, 2013 |
| | | | | Metastatic pancreatic cancer | NCT01489865 | Phase I, II | Until December, 2014 |
| FOLFIRI | Temozolomide | | | Advanced gastric cancer | NCT01123876 | Phase I | Until December, 2014 |
| | | | | Recurrent or refractory childhood central nervous system tumor | NCT00946335 | Phase I | Until October, 2011 |
| Temozolomide | Carboplatin, paclitaxel, doxorubicin, cyclophosphamide | | | Hepatocellular carcinoma | NCT01205828 | Phase II | Until December, 2013 |
| | | | | Advanced solid tumor | NCT012881150 | Phase I | Until December, 2013 |
| | | | | Stage Iib-Ilic triple-negative breast cancer | NCT01818063 | Phase II | Until April, 2018 |
| Pathway                  | Target(s)                                           | Name                                      | Combination                       | Type of cancer                                                                 | Clinical trial number   | Stage               | Trial periods       |
|-------------------------|-----------------------------------------------------|-------------------------------------------|-----------------------------------|-------------------------------------------------------------------------------|------------------------|---------------------|---------------------|
| Floxuridine             |                                                     |                                           | Metastatic epithelial ovarian, primary peritoneal cavity, or fallopian tube cancer | NCT01749397                  | Phase I             | Until March, 2016 |
| Liposomal doxorubicin   |                                                     |                                           | Recurrent ovarian cancer, fallopian tube cancer, or primary peritoneal cancer or metastatic triple-negative breast cancer | NCT01145430                  | Phase I             | Until March, 2014 |
| Bortezomib, dexamethasone| Temozolomide                                        |                                           | Relapsed refractory multiple myeloma | NCT01495351                  | Phase I             | Until October, 2013 |
|                        |                                                     |                                           | Recurrent small-cell lung cancer   | NCT01638546                  | Phase II             | Until June, 2017   |
| Cyclophosphamide, doxorubicin |                         |                                           | Metastatic or unresectable solid tumor, non-Hodgkin's lymphoma | NCT00740805                  | Phase I             | Until December, 2013 |
| Whole brain radiation   |                                                     |                                           | Brain metastases from NSCLC        | NCT01657799                  | Phase II             | Until November, 2014 |
| Temozolomide            |                                                     |                                           | Recurrent high grade serous ovarian, fallopian tube, or primary peritoneal cancer | NCT01113957                  | Phase II             | Completed          |
| Temozolomide            |                                                     |                                           | Metastatic or locally advanced breast cancer and BRCA1/2-associated breast cancer | NCT01009788                  | Phase II             | Until December, 2014 |
| Carboplatin, paclitaxel |                                                     |                                           | Advanced cancer with liver or kidney problems | NCT01419548                  | Phase I             | Withdrawn          |
| Whole brain radiation   |                                                     |                                           | Cancer with brain metastases       | NCT00649207                  | Phase I             | Completed          |
| Radiotherapy            |                                                     |                                           | Inflammatory or locally recurrent breast cancer | NCT01477489                  | Phase I             | Until December, 2016 |
| Carboplatin, paclitaxel, bevacizumab |                     |                                           | Newly diagnosed ovarian epithelial cancer, fallopian tube cancer, or primary peritoneal cancer | NCT00989651                  | Phase I             | Until July, 2014   |
| Carboplatin, paclitaxel |                                                     |                                           | Advanced solid tumor or BRCA1/2-associated advanced solid tumor | NCT00535119                  | Phase I             | Until October, 2012 |
| Temozolomide            | Cyclophosphamide                                    |                                           | Refractory BRCA-positive ovarian, primary peritoneal or ovarian high-grade serous carcinoma, fallopian tube cancer, triple-negative breast cancer, and low-grade non-Hodgkin's lymphoma | NCT01051596, NCT01306032    | Phase II             | Until December, 2013 |
|                         |                                                     |                                           | Metastatic or unresectable solid tumor, lymphoma | NCT00576654                  | Phase I             | Until December, 2013 |
| Temozolomide            |                                                     |                                           | Recurrent or refractory childhood central nervous system tumor | NCT00994071                  | Phase I             | Completed          |
| Cyclophosphamide        |                                                     |                                           | Refractory solid tumor or lymphoma | NCT01445522                  | Phase I             | Completed          |
| Temozolomide            |                                                     |                                           | Recurrent high-grade glioma         | NCT01026493                  | Phase I, II         | Until February, 2014 |
| Pathway                  | Target(s)                                 | Name                        | Combination                  | Type of cancer                                                                 | Clinical trial number | Stage                        | Trial periods                  |
|-------------------------|-------------------------------------------|-----------------------------|------------------------------|--------------------------------------------------------------------------------|-----------------------|------------------------------|--------------------------------|
| Cyclophosphamide        |                                           | Solid tumor or lymphoma     | NCT00810966                  | Phase I                                                                         | Active, not recruiting|
| Radiotherapy,            |                                           | Grade IV astrocytoma        | NCT00770471                  | Phase I, II                                                                     | Completed             |
| Temozolomide            |                                           | Metastatic prostate cancer  | NCT01085422                  | Phase I                                                                         | Completed             |
| Temozolomide            |                                           | Advanced non-hematologic    | NCT00526617                  | Phase I                                                                         | Completed             |
| Topotecan               |                                           | Refractory solid tumor or   | NCT00553189                  | Phase I                                                                         | Completed             |
| Temozolomide            |                                           | Metastatic melanoma         | NCT00804908                  | Phase II                                                                        | Until March, 2014     |
| Carboplatin, gemcitabine|                                           | Advanced solid tumor        | NCT01063816                  | Phase I                                                                         | Until September, 2014|
| Radiotherapy            |                                           | Breast cancer               | NCT01618357                  | Phase I                                                                         | Until April, 2016     |
| Monotherapy             |                                           | Solid tumor                 | NCT01199224                  | Phase I                                                                         | Completed             |
|                        |                                           | Locally advanced or         | NCT01585805                  | Phase II                                                                        | Until July, 2017      |
|                        |                                           | metastatic pancreatic      |                              |                                                                                  |                      |
|                        |                                           | cancer                     |                              |                                                                                  |                      |
|                        |                                           | Metastatic, unresectable,   | NCT01017640                  | Phase I                                                                         | Until June, 2014      |
|                        |                                           | or recurrent solid tumors   |                              |                                                                                  |                      |
|                        |                                           | Stage III or Stage IV       | NCT01149083                  | Phase II                                                                        | Until June, 2014      |
|                        |                                           | breast cancer with BRCA     |                              |                                                                                  |                      |
|                        |                                           | mutations                   |                              |                                                                                  |                      |
|                        |                                           | BRCA-mutated metastatic     | NCT01853306                  | Phase I                                                                         | Until January, 2015   |
|                        |                                           | or unresectable malignancy, |                              |                                                                                  |                      |
|                        |                                           | high grade serous ovarian,  |                              |                                                                                  |                      |
|                        |                                           | fallopian tube, or          |                              |                                                                                  |                      |
|                        |                                           | peritoneal cancer           |                              |                                                                                  |                      |
|                        |                                           | BRCA-mutated epithelial     | NCT01540565                  | Phase II                                                                        | Until April, 2014     |
|                        |                                           | ovarian, fallopian tube, or |                              |                                                                                  |                      |
|                        |                                           | primary peritoneal cancer   |                              |                                                                                  |                      |
|                        |                                           | Advanced solid tumor        | NCT02009631                  | Phase I                                                                         | Until December, 2014  |
|                        |                                           | BRCA-related malignancy,    | NCT00892736                  | Phase I                                                                         | Until December, 2013  |
|                        |                                           | platinum-refractory ovarian,|                              |                                                                                  |                      |
|                        |                                           | fallopian tube, or          |                              |                                                                                  |                      |
|                        |                                           | primary peritoneal cancer   |                              |                                                                                  |                      |
|                        |                                           | or basal-like breast cancer,|                              |                                                                                  |                      |
|                        |                                           | advanced solid tumor        |                              |                                                                                  |                      |
|                        |                                           | Relapsed epithelial ovarian,| NCT01472783                  | Phase I, II                                                                     | Until December, 2015  |
|                        |                                           | ovarian, primary fallopian  |                              |                                                                                  |                      |
|                        |                                           | or primary peritoneal cancer|                              |                                                                                  |                      |
|                        |                                           | with BRCA mutations         |                              |                                                                                  |                      |
|                        |                                           | Chronic lymphocytic         | NCT00387608                  | Phase I                                                                         | Completed             |
|                        |                                           | leukemia, follicular        |                              |                                                                                  |                      |
|                        |                                           | lymphoma, unspecified       |                              |                                                                                  |                      |
|                        |                                           | solid tumor                 |                              |                                                                                  |                      |
|                        |                                           | Invasive breast cancer      | NCT01042379                  | Phase II                                                                        | Until November, 2014  |
|                        |                                           | Advanced solid tumor        | NCT01827384                  | Phase II                                                                        | Until March, 2017     |
| INO-1001                | Combination therapy                       | Unresectable melanoma       | NCT00272415                  | Phase I                                                                         | Terminated            |
| MK4827                  | Combination therapy                       | Advanced solid tumor,       | NCT01227941                  | Phase I                                                                         | Terminated            |
|                        |                                           | platinum-resistant high     |                              |                                                                                  |                      |
|                        |                                           | grade serous ovarian cancer |                              |                                                                                  |                      |
|                        |                                           | Advanced solid tumor        | NCT01294735                  | Phase I                                                                         | Completed             |
Although such lesions would be repaired by HR in normal cells, they are not repaired in BRCA1- or BRCA2-deficient cancer cells because these cells are defective in HR repair, and thus the tumor cells are led to death. This concept is termed synthetic lethality, namely, the process by which defects in two different genes or pathways together result in cell death while defects in one of the two different genes or pathways do not affect viability (Fig. 3). This attractive new therapeutic strategy based on the principle of synthetic lethality relies on the frequent defects in the DNA damage response observed in cancer as summarized in the previous chapter and Table 1, in which alternative DNA damage response pathways may be activated to allow cancer cells to survive in the presence of genotoxic stress. Because this strategy targets the cancer-specific aberrations in the DNA damage response, it will cause few or no toxicities on normal cells. The first report of a clinical trial of a PARP inhibitor as a single agent in patients with BRCA mutations was the phase I study of the oral PARP inhibitor olaparib. It established the safety of olaparib as a single agent, and good responses were observed in patients with BRCA-mutated breast, ovarian, or prostate tumors. In subsequent phase II studies, approximately one-third of the patients with breast or ovarian cancer with germline BRCA mutations showed a favorable response to the drug with no severe toxicities. Several other PARP inhibitors are currently being investigated in patients with germline BRCA mutations as single agents (Table 3). It is likely that PARP inhibitors have significant benefit to at least a subpopulation of cancer patients with defects in BRCA-mediated HR pathways. Using PARP inhibitors as single agents in cancers with no BRCA mutations. The potential for PARP inhibitors as single agents has also been tested in clinical trials of cancers with no germline BRCA mutations, such as high-grade serous ovarian cancers and triple-negative breast cancers. Inhibitors of PARP were also effective in a subset of cancers with no germline BRCA mutations, suggesting that there may be a subset of sporadic cancers that show features of “BRCAness,” which may show good response to PARP inhibitors. Indeed, cancer cells expressing the cancer-testis antigen SYCP3, in which BRCA2 is functionally inactivated, as described above, show extreme hypersensitivity to a PARP inhibitor. Defects in other HR-related proteins such as RAD51, RAD54, and RPA also confer selective sensitivity to PARP inhibition. Moreover, defects in the DNA damage response proteins, such as NBS1, MRE11, ATR, ATM,
BRCA1, and PALB2. This lethal effect may be due to the hyperdependence of the FA pathway-deficient cells on the FA pathway. (a) In the absence of the pathway B inhibitor, cancer cells can survive, because the defect in pathway A is compensated by the alternative pathway B. (b) When the cells are treated with the pathway B inhibitor, both pathways will be blocked in cancer cells, which will result in cell death. However, normal cells will not be affected, because inhibition of pathway B will be compensated by pathway A.

Exploitation of other synthetic lethalities by DNA damage response. Taking advantage of the dysregulated DNA damage response in cancer using the synthetic lethality approach may be one of the most promising prospects for the future of cancer treatment. From this point of view, many efforts have been made to identify defects of two different DNA damage response genes or pathways that are synthetically lethal when combined. For example, ATM inhibition is shown to be synthetically lethal with FA pathway deficiency. The suggested explanation for this lethality is as follows. The FA pathway-deficient cancer cells are defective in the repair of DNA replication fork stalling, which is normally repaired by ATR and the FA pathway. In FA pathway-deficient conditions, the stalled fork will collapse and form a DSB that will alternatively activate an ATM-dependent DNA damage response. Inhibition of ATM in such FA pathway-deficient cells will leave no alternative mechanism for repair, leading to cell death. The FA pathway-deficient cells are also hypersensitive to Chk1 silencing, which may be explained by the hyperdependence of the FA pathway-deficient cells on G2/M checkpoint activation mediated by Chk1 for viability. Because defects in the FA pathway are frequently observed in a number of different types of cancer (Table 1), the use of ATM inhibitors or Chk1 inhibitors in FA pathway-deficient tumors will be a promising approach that should be evaluated in clinical trials in the future. In another example, RAD54B deficiency is shown to be synthetically lethal in cells with reduced Flap endonuclease 1 expression, but the mechanisms of this lethality remain to be elucidated. Recently, inhibition of APE1 was shown to be synthetically lethal in BRCA- and ATM-deficient cells, presenting a novel model for APE inhibition as a synthetic lethal strategy in cells deficient in DSB repair. Briefly, APE1 inhibition leads to AP site accumulation and results in indirect generation of SSBs that are eventually converted to toxic DSBs, which cannot be repaired in cells deficient in DSB repair. The APE1 inhibitors are being tested in combination with DNA-damaging agents in current clinical trials, and they may be evaluated further as a synthetic lethal strategy. More recently, inactivation of the HR protein RAD52 was shown to be synthetically lethal with deficiencies in BRCA2, BRCA1, and PALB2. This lethal effect may be due to the loss of RAD51-dependent HR function mediated by the BRCA1–PALB2–BRCA2 complex, because human RAD52 is suggested to function in an independent and alternative repair pathway of RAD51-dependent HR when deficiencies exist in BRCA1, PALB2, or BRCA2. As no inactivating mutations of RAD52 have been documented in human sporadic cancers, inhibition of RAD52 could be an attractive strategy for improving cancer therapy in the BRCA- or PALB2-defective subgroup of cancers. Although no inhibitors of RAD52 have been developed yet, it would be of great interest to assess the effects of inhibition of RAD52 on cancer-specific killing of the cancers with "BRCAneness" profiles and compare them with those of PARP inhibitors in future clinical trials. There might be additional synthetic lethalities to be discovered and exploited in future.

Current Limitations and Future Perspectives

Although the data from clinical trials of the inhibitors of DNA damage response, including PARP inhibitors, seem encouraging, we should note that the use of PARP inhibitors also faces significant limitations.

The first limitation is the evolution of resistance. In the case of using PARP inhibitors in cancer cells carrying mutations in BRCA1 or BRCA2, the drug resistance can be caused by secondary mutations in the BRCA1 or BRCA2 gene that restore the open reading frame of the gene and enable the generation of functional BRCA proteins possessing the ability to repair DNA damage caused by PARP inhibitors. Other suggested mechanisms underlying the resistance to PARP inhibitors include the loss of 53BP1 expression in BRCA-deficient cells and the upregulation of genes that encode P-glycoprotein efflux pumps, although the importance of these factors in clinical resistance to PARP inhibitors has not been elucidated. In future clinical trials, it would be desirable to periodically monitor the sequences of BRCA1 and BRCA2 and the expression levels of the key proteins such as 53BP1 or P-glycoprotein efflux pumps.

The second limitation is the lack of reliable biomarkers of response or resistance to the inhibitors. There is a pressing need to identify biomarkers to predict the response to the inhibitors. Regarding the sensitivities to PARP inhibitors, elevated levels of PARP and CDK12 deficiency are suggested to be possible biomarkers for favorable responses. We should also keep in mind that many factors might affect the DNA damage response and take into account the complexity of the networks regulating DNA repair. For instance, most cancer cells grow under hypoxia, a condition that activates hypoxia inducible factor-1 (HIF-1). Because HIF-1 contributes to therapy resistance, it is considered an attractive target molecule for cancer therapy. Diverse functional interactions occur between HIF-1 and PARP1, and these interactions have been shown to be crucial for the survival of cancer cells. It has been suggested that HIF-1 might act as a synthetic lethal partner with PARP1 in cancer cells, which might explain the synergistic effect of PARP inhibitors and HIF-1 inhibitors in cancer treatment. Further studies are needed to clarify the role of HIF-1 in the resistance to PARP inhibitors and to identify new biomarkers that can predict the response to these drugs.
between HIF-1 and the DNA damage response have also been described,\(^{113}\) so the efficacy of the combination of HIF-1 inhibitors and inhibitors of the DNA damage response proteins should be examined in the future.

**Conclusions**

Defects or upregulation of the proteins involved in DNA damage response and repair are common in cancers, and may be induced by both genetic and epigenetic causes. Inhibition of the DNA damage response proteins can be used to enhance chemotherapy and radiotherapy, and also to selectively kill cancer cells showing deficiencies in particular DNA repair pathway(s) based on the principle of synthetic lethality. Inhibition of PARP in BRCA-defective cancers seemed effective in early clinical trials. Better understanding of the basic biology underlying the DNA damage response and the mechanisms responsible for its dysregulation in cancer will provide exciting opportunities for new and efficient cancer therapy targeting the DNA damage response.

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