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REVIEW

Improving the Efficacy of Chemoradiation with Targeted Agents

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ABSTRACT Chemoradiation is the standard therapy for the majority of inoperable, locally advanced cancers. Although there is a need to improve chemoradiation efficacy, normal-tissue toxicity limits our ability to give additional chemotherapy or higher doses of radiation. Thus, there is excitement about the addition of molecularly targeted agents, which tend to be less toxic than chemotherapy, to chemoradiation regimens. Unfortunately, initial empiric attempts have not been successful. This review will focus on the evidence that supports rational combinations of targeted agents with chemoradiation, with an emphasis on agents that target the DNA damage response and radiation-induced membrane signaling.

Significance: Too often, clinical trials are designed without comprehensive preclinical investigation. To design more rational trials of targeted agents with chemoradiation, it is necessary to understand the complex biology underlying the interactions between the targeted agent and chemoradiation. Thorough preclinical studies to evaluate key issues such as mechanisms of interaction, scheduling, selection of the appropriate patients through the use of biomarkers, and normal-tissue toxicity will lead to improved clinical trial designs and patient outcomes. *Cancer Discov; 4(3); 280–91. ©2014 AACR.*

OVERVIEW

The combination of radiation with cytotoxic chemotherapy has become a standard treatment option for the majority of locally advanced cancers, including brain, head and neck, lung, and gastrointestinal malignancies. Although chemoradiation is tolerable and cures the majority of patients with human papillomavirus (HPV)–positive cancers of the head and neck and anus, these high local control rates are the exception. Most patients with locally advanced solid tumors die of local disease, although metastatic disease resulting from uncontrolled local disease or preexisting metastatic disease is sometimes a cause. Numerous studies have documented that toxicity is the major limitation of concurrent chemoradiotherapy (1, 2). Clearly, there is a need to improve therapy efficacy in many cancer types (e.g., HPV-negative head and neck, pancreas, lung, brain, etc.) without increasing normal tissue toxicity.

Because standard chemoradiation regimens are already administered at the maximum-tolerated doses for normal tissues, strategies that selectively sensitize tumor cells to chemoradiotherapy are warranted. Indeed, in the last decade, several clinical trials have investigated the combination of chemoradiation with molecularly targeted agents. Unfortunately, these initial trials have not been successful. For example, a highly touted clinical trial combining cetuximab with cisplatin-based chemoradiation in patients with locally advanced head and neck cancer (RTOG 0522) was designed empirically based on prior clinical trials demonstrating the superiority of cetuximab–radiation (3) and cisplatin–radiation (4) over radiotherapy alone, and cetuximab–cisplatin (5) over cisplatin alone in the treatment of head and neck cancer. On the basis of these positive clinical trials, it was predicted that the three-agent combination of cetuximab and concurrent cisplatin-based chemoradiation, tested in RTOG 0522, would be more effective. However, there was no survival advantage afforded by the addition of cetuximab to cisplatin–radiation, and the triple combination was more toxic (6). Likewise, adding bevacizumab to the standard combination of capecitabine and radiation increased toxicity without improving survival for patients with pancreatic cancer (7). Although these negative results may have a mechanistic basis (e.g., antagonistic interactions, suboptimal scheduling), the lack of biomarker studies leaves us uncertain about whether these trials failed because the target was irrelevant or the target was not hit. Unfortunately, at this juncture, all we can conclude is that these combinations were unsuccessful. These trials have not helped us to understand where to go next.

To maximize the likelihood of clinical success for targeted agents in combination with chemoradiation, critical issues such as mechanisms of interaction, scheduling, biomarkers, efficacy, and normal-tissue toxicity must be investigated preclinically. In addition, the unique DNA lesions and membrane signaling

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KEY CONCEPTS

- Efficacy and toxicity are outstanding issues for chemoradiotherapy.
- Targeted agents have the potential to improve chemoradiation efficacy without excessive toxicity.
- Chemoradiation treatment produces more complex, difficult-to-repair DNA damage, making inhibitors of the DNA damage response, particularly homologous recombination repair, highly effective.
- Radiation induces several membrane-associated prosurvival pathways. Inhibition of these pathways may selectively increase chemoradiation sensitivity in tumor cells.
- The majority of phase III clinical trials in oncology fail. Thorough preclinical testing of mechanisms of interaction, scheduling, biomarkers, and the therapeutic index is required to increase the probability of successful clinical trials with targeted agents and chemoradiation.
- Dual-targeted inhibition in combination with radiation may reduce the need for standard chemotherapy and alleviate toxicity.

induced by chemoradiation need to be considered when choosing a targeted agent. Given that chemoradiation induces DNA double-strand breaks (DSB) in the context of chemotherapy-associated DNA aberrations/replication stress, it is logical to combine inhibitors of the DNA damage response, such as inhibitors of checkpoint kinase 1 (CHK1), WEE1, and PARP, with chemoradiation. Also, because radiation activates several membrane-associated signaling pathways such as EGF receptor (EGFR), phosphoinositide 3-kinase (PI3K), and TGFβ that promote DNA repair and survival in tumor cells, targeting these may also enhance chemoradiation efficacy. Thus, in this review, we will focus on rational approaches for combining agents directly targeted toward either the DNA damage response or radiation-induced membrane signaling with chemoradiation, while emphasizing critical preclinical issues. Finally, given the limitations of chemoradiotherapy (toxicity and limited efficacy), we will discuss combinations of targeted agents with radiation, which have the potential to eliminate conventional chemotherapy in chemoradiation regimens.

CHEMORADIATION

The combination of conventional chemotherapy with radiation introduces a unique set of DNA aberrations that differ from those induced by either chemotherapy or radiation alone (Fig. 1A). Unlike conventional chemotherapy, which exerts its cytotoxic effects on all replicating cells, conformal radiation is particularly effective at producing DNA DSBs specifically in tumor cells. When combined with chemotherapy, radiation produces DNA DSBs in an environment of replication stress and chemotherapy-associated DNA lesions (Fig. 1B and C). In this section, we discuss the unique DNA aberrations associated with the most commonly used chemoradiation regimens.

Antimetabolites, such as gemcitabine and 5-fluorouracil (5-FU), are commonly used in combination with radiation, especially in gastrointestinal malignancies. The cytotoxic activity of gemcitabine is mediated by its active metabolites, dFdCDP and dFdCTP, which inhibit ribonucleotide reductase (resulting in nucleotide depletion) and compete with dCTP for DNA incorporation, respectively. Cytotoxicity by 5-FU is mediated by its active metabolites, FdUMP and FdUTP, which inhibit thymidylate synthase and misincorporate into DNA, respectively, as well as by promoting misincorporation of uracil into DNA in place of thymine. Both gemcitabine and 5-FU are potent radiosensitizers, and this activity is thought to be mediated by redistribution of cells into S-phase of the cell cycle and depletion of nucleotide pools (8). Although S-phase is a radioresistant phase of the cell cycle in untreated cells, this is not the case in the presence of antimetabolites such as gemcitabine or 5-FU. The combination of these agents with radiation leads to the production of complex, slowly repaired radiation-induced DNA damage in S-phase cells, such as the 1-ended DSBs produced as radiation-induced single-strand breaks (SSB) collide with progressing replication forks (Fig. 1B; refs. 9, 10).

Although antimetabolites are standard therapy for some malignancies, cisplatin is the most widely used chemotherapeutic agent in combination with radiation, especially in lung and head and neck cancers. Cisplatin interacts with cellular DNA to form cross-links that ultimately inhibit DNA replication, leading to DNA breaks and cytotoxicity. Radiosensitization by cisplatin is thought to directly involve these adducts and, unlike the antimetabolites, does not require cell-cycle redistribution. Cisplatin–DNA adducts in proximity to radiation-induced DSBs form complex (2-ended) DNA DSBs that are repaired with slow kinetics (ref. 11; Fig. 1C). In addition, cisplatin–DNA adducts are also thought to interact with radiation by enhancing the formation of radiation-induced DNA DSBs and/or by preventing non-homologous end-joining (NHEJ)–mediated DSB repair (reviewed in ref. 12).

The standard therapy for glioblastomas is concurrent temozolomide–radiation. Temozolomide is an alkylating agent that forms methyl adducts at the O^6 position of guanine (as well as at N^7-guanine and N^3-adenine). These adducts are often improperly repaired by mismatch repair, leading to DNA breaks and cytotoxicity. Radiosensitization by temozolomide involves inhibition of DNA repair (13) and/or an increase in radiation-induced DSBs due to radiation-induced SSBs in close proximity to temozolomide-induced O^6-methyl adducts (ref. 14; Fig. 1C). Like cisplatin, radiosensitization by temozolomide does not seem to require cell-cycle redistribution, and proximal temozolomide–radiation lesions likely represent complex, difficult-to-repair DNA DSBs.

The model proposed in Fig. 1 suggests that chemoradiotherapy induces complex DSBs that, depending on their nature (simple, complex 2-ended, or complex 1-ended) and context within the cell cycle, are repaired by either homologous recombination or NHEJ. The importance of cell-cycle context is reflected not only in the nature of the DNA DSBs formed, but also in the efficiency of their repair. For example, DSBs are repaired more efficiently in G_1 and less efficiently in the G_2 phase of the cell cycle (15). It should be noted that additional DNA DSB repair mechanisms such as alternative-NHEJ

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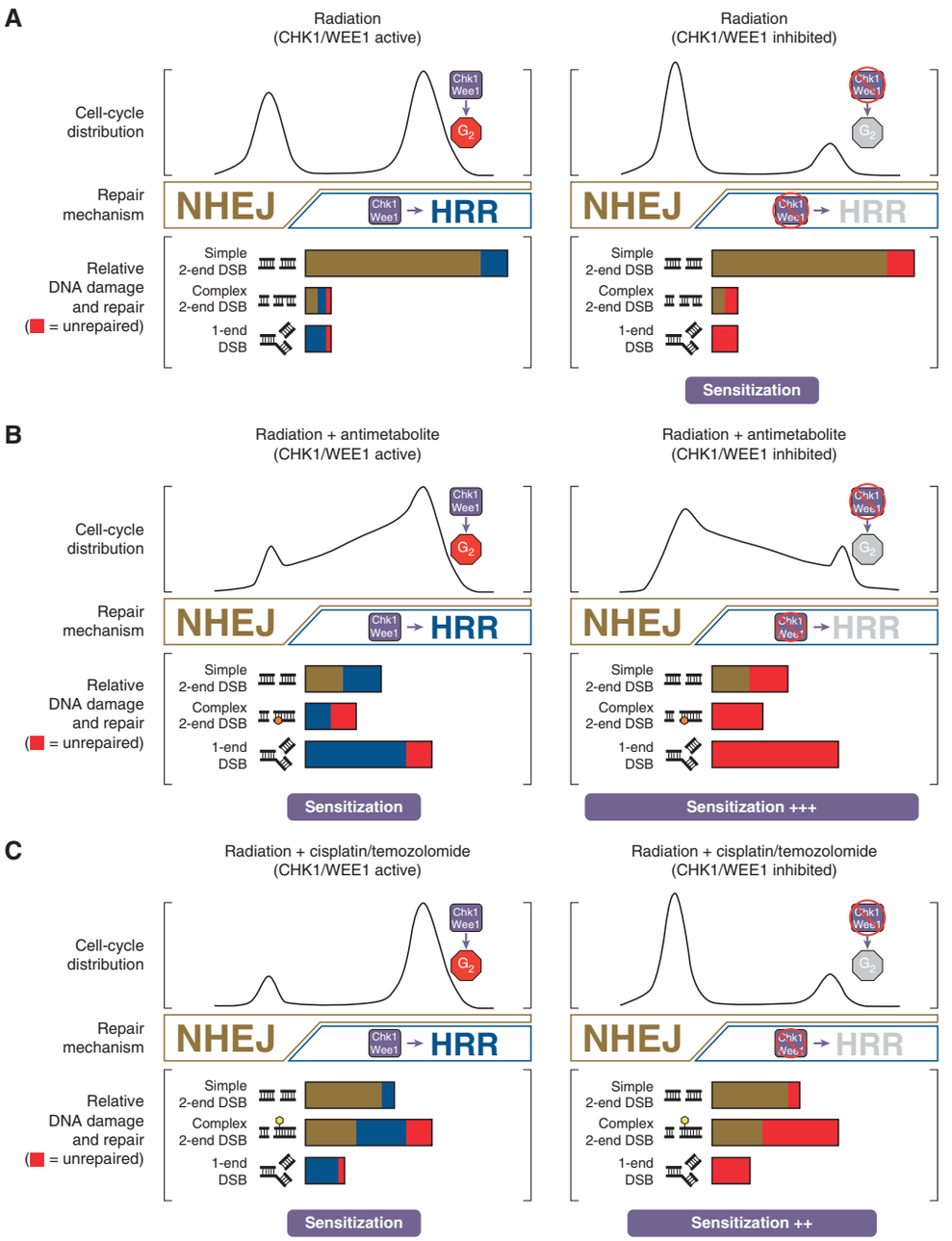

Figure 1. Variation in types of DNA damage and repair as a basis for chemoradiosensitization. Each panel schematically represents the status of cell-cycle distribution and DNA damage/repair at a time point approximately 12 to 24 hours post-ionizing radiation in *P53* mutant cells. **A,** radiation ± CHK1/WEE1 inhibition. Cells exposed to radiation alone (left) contain mostly simple 2-end DSBs that are repaired soon after formation, predominantly by NHEJ in the early part of the cell cycle. A small fraction of damage comprises complex 2-end DSBs and 1-end DSBs, both of which are repaired more slowly and incompletely by NHEJ or homologous recombination (HRR), as indicated by brown or blue bars, respectively. The intact G_2 checkpoint promotes cell survival by delaying progression, allowing slow repair to proceed. Inhibition of CHK1/WEE1 (right) does not change the distribution of the types of damage formed, but compromises repair by preventing HRR and by abrogating the G_2 checkpoint, resulting in more unrepaired damage (red bars) than with radiation only, and in reduced cell survival (sensitization). **B,** radiation + antimetabolite ± CHK1/WEE1 inhibition. Antimetabolite treatment before radiation causes cells to accumulate in S-phase (making them more reliant on HRR than NHEJ) and stalls replication forks, increasing the frequency of 1-end DSB formation upon radiation treatment. Misincorporated nucleotides (dFdCTP or dFdUTP; shown in orange) also increase radiation-induced complex 2-end DSBs. Intact CHK1/WEE1 function (left) promotes substantial repair through HRR and G_2 checkpoint stimulation, but the increased burden of breaks that are difficult to repair (especially 1-end DSBs) results in more residual unrepaired damage and cell death than radiation alone (sensitization). Inhibition of CHK1/WEE1 (right) causes a large increase in unrepaired damage because of the heavy reliance of the cell on HRR and the G_2 checkpoint for repair in this situation, and, therefore, a high level of sensitization. **C,** radiation + cisplatin or temozolomide ± CHK1/WEE1 inhibition. Drug-induced DNA adducts (shown in yellow) increase the fraction of complex 2-end DSBs. Slower repair kinetics and partial NHEJ inhibition by the presence of adducts leads to increased reliance on HRR. With intact CHK1/WEE1 (left), most damage is repaired, although less completely than after radiation alone, due to its complexity. Inhibition of CHK1/WEE1 (right) compromises both HRR and the G_2 checkpoint; thus, more damage is left unrepaired and there is a concomitant increase in sensitization.

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(alt-NHEJ) or microhomology-mediated end joining (MMEJ) may be involved in the repair of chemoradiation-induced DSBs, especially when classical NHEJ is inhibited or when 1-ended DSBs are present (16, 17). In addition, chromatin complexity (heterochromatin) in the vicinity of the DSB may contribute to more difficult-to-repair DNA DSBs, and thus slower repair kinetics (18). Although our model is a simplified view of DNA DSBs and their repair, it illustrates the unique effects of chemoradiation on the cell cycle and DNA DSB induction and repair, as well as the proposed maximal efficacy of inhibitors of the DNA damage response when used in combination with chemoradiation.

SENSITIZING TO CHEMORADIATION BY DIRECTLY TARGETING THE DNA DAMAGE RESPONSE

CHK1 and WEE1

There are a number of drugs in various phases of clinical development designed to inhibit DNA damage response/repair pathways. Targeting these pathways attenuates repair of chemoradiotherapy lesions and enhances tumor cell killing. One such target is CHK1, which in response to DNA damage or replication stress mediates the S- and G_2-phase checkpoints (via inhibition of the CDC25 phosphatase family, leading to CDK1/2 inactivation) and homologous recombination repair, as well as stabilization of stalled replication forks (19). Although there is limited information about sensitization to chemoradiation, CHK1 inhibitors such as MK8776 (previously known as SCH900776), AZD7762, and LY2603618 exhibit chemosensitization and radiosensitization in a spectrum of cancer models (20). Although CHK1 inhibitors sensitize to a variety of chemotherapies, including platinum-containing drugs and topoisomerase inhibitors, the greatest chemopotentiation has been observed in response to antimetabolites, notably gemcitabine but also cytarabine and pemetrexed (21, 22). The ability of CHK1 inhibitors to maximally sensitize to antimetabolites is likely related to the importance of CHK1 in recovery from nucleotide pool depletion and/or misincorporation of cytotoxic antimetabolite nucleotides, ultimately leading to replication stress and S-phase perturbations. With regard to the ability of CHK1 inhibitors to sensitize tumor cells to chemoradiation, we have reported maximal sensitization of pancreatic cancer cells and tumors to gemcitabine–radiation (vs. radiation alone) by CHK1-targeted agents in association with homologous recombination inhibition (23, 24). By causing a temporary redistribution of cells into S-phase, antimetabolites synchronize cells and, thus, maximize the effects of CHK1 inhibitors on radiation-induced DNA damage (Fig. 1B). Given the cell-cycle dependence of DNA DSB repair pathways, with homologous recombination repair being most active in the S and G_2 phases, as well as the accumulation of 1-ended DSBs resulting from the collision of radiation-induced SSBs with progressing replication forks (which require homologous recombination for repair), there is a greater reliance on homologous recombination in the presence of antimetabolite-based chemoradiation. Thus, treatment with antimetabolite-based chemoradiation should render cancer cells highly vulnerable to homologous recombination inhibition by CHK1-targeted agents. In addition, having the majority of cells arrested in S or G_2 phases of the cell cycle promotes susceptibility to checkpoint abrogation by Chk1-targeted agents.

A second potential target for sensitizing tumors to chemoradiation is the cell-cycle kinase WEE1. WEE1 regulates the S- and G_2-phase checkpoints in response to DNA damage by directly phosphorylating and inactivating CDK1, leading to cell-cycle arrest. In addition, WEE1 may positively regulate homologous recombination via modulation of CDK1 and the BRCA2–RAD51 interaction (25). WEE1-targeted agents, such as AZD1775, a first-in-class WEE1 inhibitor currently in clinical development, sensitize to various chemotherapies including antimetabolites (gemcitabine, pemetrexed, 5-FU, and capecitabine), topoisomerase inhibitors (doxorubicin and camptothecin), and DNA cross-linking agents (mitomycin C, cisplatin, and carboplatin) as well as radiation (26–28). As with CHK1 inhibitors, there has been an emphasis on the development of WEE1 inhibitors as sensitizers to gemcitabine and 5-FU as well as radiation, in which cases AZD1775 seems to confer biologically (and potentially clinically) significant sensitization. Although the combination of WEE1-targeted agents with chemoradiation is currently under investigation, it is likely that maximal potentiation of chemoradiation will be associated with antimetabolites, given their effects on S-phase (described above) and the crucial function of WEE1 in S-phase (26). Despite the lack of published preclinical data about WEE1 inhibition in combination with cisplatin- or temozolomide-based chemoradiation, clinical studies are under way. Given the complex nature of the DNA DSBs associated with the combination of adduct-forming chemotherapies and radiation, synergy with WEE1 inhibition is a reasonable hypothesis (Fig. 1C). More interestingly, as CHK1 and WEE1 possess some nonoverlapping functions, differences between CHK1- and WEE1-targeted agents are emerging in terms of their efficacy and mechanisms of sensitization (29).

Much of what is known about the scheduling of CHK1 and WEE1 inhibitors with chemoradiation comes from early preclinical studies combining CHK1 inhibitors with chemotherapy. Although concurrent administration of antimetabolite chemotherapy and CHK1 inhibition produces some sensitization, maximal chemosensitization is observed when CHK1 inhibition occurs in S-phase–arrested cells. This schedule dependence is attributed to the roles of CHK1 in both initiating the intra-S and G_2 checkpoints and in stabilizing stalled replication forks that accumulate over time in antimetabolite-treated cells (30, 31). In terms of radiosensitization, as both CHK1 and WEE1 mediate DNA DSB repair as well as cell-cycle arrest, schedules in which an inhibitor is given immediately before radiation (to inhibit early repair) and for an extended time thereafter (to inhibit late repair and the S–G_2 checkpoints) are logical and effective (23). Thus, to maximize sensitization to chemoradiation, a schedule in which the CHK1/WEE1 inhibitor is administered after antimetabolite-based chemotherapy, and just before radiation, is most effective in preclinical models.

CHK1 and WEE1 inhibitors share a proposed mechanism of tumor cell selectivity conferred by *TP53* mutation. This model, supported by substantial evidence, suggests that normal cells are protected from CHK1 and WEE1 inhibition (in combination with chemotherapy or radiation) by an intact P53-mediated G_1 checkpoint, whereas *TP53*-mutant tumor cells are not (32). This model assumes that P53-induced G_1

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arrest protects normal cells from G_2 checkpoint abrogation following CHK1 or WEE1 inhibition. However, it is also possible that P53-mediated G_1 arrest protects normal cells indirectly by arresting cells in a phase of the cell cycle in which NHEJ rather than homologous recombination is the dominant DSB repair mechanism (33), as CHK1 and WEE1 inhibitors likely do not inhibit NHEJ. Thus, *TP53* mutation is expected to shift tumor cells toward homologous recombination–mediated DSB repair. This dependence on homologous recombination repair is likely to have greater consequence in the context of chemoradiation, given the increased reliance on homologous recombination for repair of chemoradiation-induced DSBs (Fig. 1). Consistent with this model, CHK1 inhibition does not sensitize the small intestine (the dose-limiting toxicity for chemoradiotherapy in patients with pancreatic cancer) to gemcitabine–radiation (24). Although *TP53* mutation is certainly a mechanism of tumor cell selectivity, this view is likely oversimplified, and other genetic aberrations in tumor cells, such as those occurring in *P16*, *KRAS*, and *RB*, may be involved. In addition, the P53 inactivation associated with HPV-positive head and neck, cervical, and anal cancers is another likely mechanism of tumor cell selectivity for sensitization by CHK1 and WEE1 inhibitors (34).

Taken together, preclinical data suggest that Chk1/Wee1 inhibitors are particularly effective sensitizers to antimetabolite-based chemoradiation in *TP53*-mutant cancers and that the most rational schedule would be to give the antimetabolite first, followed by CHK1/WEE1 inhibitors and radiation. Although we are unaware of plans for combining a CHK1 inhibitor with chemoradiation in the clinic, we and others are initiating clinical trials using the WEE1 inhibitor AZD1775 in combination with chemoradiation.

PARP

Among the agents currently being developed to target DNA damage response/repair pathways, inhibitors of PARP have advanced the furthest. Several PARP1/2 inhibitors are in clinical development, including rucaparib (PF-01367338), iniparib (BSI-201), veliparib (ABT-888), and olaparib (AZD2281). Preclinically, PARP inhibitors sensitize to platinum-based drugs, temozolomide, topoisomerase poisons, and radiation (35–37). Clinically, the efficacy of PARP inhibitors in combination with chemotherapy has been confounded by promising phase II clinical data, followed by a negative phase III clinical trial in which iniparib in combination with gemcitabine/carboplatin failed to produce a survival benefit in patients with triple-negative breast cancer (38). Subsequent to these trials, data have emerged which demonstrate that iniparib fails to exhibit key biologic properties associated with PARP inhibition (39). Thus, iniparib clinical data should be interpreted cautiously in the context of other *bona fide* PARP inhibitors, and determining the clinical efficacy of more-selective PARP inhibitors is a high priority. In combination with chemoradiation, PARP inhibition (by veliparib or olaparib) causes *in vitro* and *in vivo* sensitization to several chemoradiation regimens, including those containing irinotecan, oxaliplatin, and temozolomide (40, 41). These studies illustrate, similar to the model proposed in Fig. 1 for CHK1 and WEE1 inhibition, that PARP inhibition more effectively sensitizes to chemoradiation than to radiation alone. Currently, there is at least one active clinical trial combining a PARP inhibitor with chemoradiation (veliparib with capecitabine–radiation in locally advanced rectal cancer; clinicaltrials.gov).

The cytotoxicity of PARP inhibitors is mediated by both the catalytic inhibition of PARP, which inhibits base excision repair, and the trapping of PARP to DNA, both of which lead to replication-associated DNA DSBs (42). Radiosensitization or chemosensitization by PARP inhibitors involves inhibition of base excision repair of chemotherapy- or radiation-induced SSBs, which subsequently are converted to DSBs during replication (43). Existing preclinical data primarily support the use of PARP inhibitors in combination with DNA-damaging chemotherapeutic agents that produce DNA adducts, such as cisplatin and temozolomide. Although there are multiple proposed mechanisms for the interaction between cisplatin and temozolomide with radiation, data suggest that these agents act in part by attenuating the repair of radiation-induced DSBs (12, 13). Because PARP inhibitors are more effective sensitizers in DSB-defective cells (44, 45), synergy between PARP inhibitors and platinum- or temozolomide-based chemoradiation is expected.

The mechanisms of tumor cell selectivity for PARP-targeted agents have been established in the context of *BRCA1/2*-mutant cancers (36). As single agents, PARP inhibitors selectively kill *BRCA1/2*-mutant/homologous recombination–deficient cancers and, in combination with chemotherapy, exhibit enhanced sensitization in homologous recombination–deficient cancers (45, 46). Likewise, with regard to radiosensitization, cancers with defects in homologous recombination or other DSB repair pathways (i.e., alt-NHEJ) are more radiosensitized by PARP inhibition as compared with cells with proficient DSB repair pathways (44, 45). On the basis of the efficacy of PARP inhibitors in these preclinical models, it would be logical to select tumors with similar defects in DSB repair for clinical trials. However, PARP inhibitors are currently under clinical investigation in combination with chemotherapy/radiation in unselected (in terms of DSB repair proficiency/deficiency) patient populations. Although it is likely that PARP inhibitors will produce some tumor sensitization even in the absence of obvious DSB repair defects, it is not clear that these tumors will be more sensitized than normal tissues. In homologous recombination–proficient cancers, radiosensitization by PARP inhibition is thought to require replication-dependent conversion of SSBs to DSBs, and thus is predicted to affect rapidly proliferating tumors more than normal tissues (37). Although PARP inhibitors sensitize homologous recombination–proficient colon cancer xenografts to chemoradiation (40), it is not clear whether there is a therapeutic index associated with this combination, as normal-tissue toxicity has not been investigated. In summary, PARP inhibition represents a promising strategy for sensitizing tumor cells to chemoradiation. Most preclinical studies, however, have focused on the tumor cell selectivity conferred by defects in DSB repair, leaving the outstanding issue of whether PARP inhibitors will selectively sensitize cancers without obvious DSB repair defects to chemoradiation. In addition, little work has been done to determine the best type of chemotherapy to combine with PARP inhibitors and radiation, as well as their optimal sequence of administration. These issues need to be addressed to design more rational clinical trials.

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SENSITIZING TO CHEMORADIATION BY TARGETING RADIATION-INDUCED MEMBRANE SIGNALING

EGFR

In contrast to agents targeting the DNA damage response, in which their interactions with the unique DNA aberrations induced by chemoradiation are critical to their sensitizing efficacy, drugs targeting membrane receptor signaling pathways, often aberrant in cancer, counteract radiation-mediated activation of these prosurvival pathways (Fig. 2). The best characterized of these targets is EGFR. In response to radiation, EGFR is rapidly activated (in a ligand-independent manner), inducing several downstream signaling pathways including MAP–ERK kinase (MEK) and PI3K/AKT (47, 48). Activation of these pathways promotes cellular proliferation (and apoptosis evasion), as well as repair of radiation-induced DNA damage through homologous recombination and NHEJ (47, 49, 50). The ability of several clinically available inhibitors of EGFR (cetuximab, erlotinib, panitumumab, etc.) to sensitize tumor cells to chemotherapy or radiation has been established both preclinically and clinically (3, 5, 51, 52). Although studies investigating the combination of EGFR-targeted therapy with chemoradiation are limited, some studies have demonstrated significant sensitization to chemoradiation by EGFR inhibitors (53–55), while others have not (56). More importantly, despite some promising phase II studies, the addition of EGFR-targeted therapy to chemoradiation has not as yet produced clinical benefit in randomized clinical trials (6, 57). Notably, in a recent, randomized phase III clinical trial in patients with head and neck cancer (RTOG 0522), the addition of cetuximab to cisplatin–radiation did not improve survival, but increased toxicity compared with patients treated with cisplatin–radiation (6). Similar results were obtained in a phase II/III esophageal cancer trial combining cetuximab with concurrent cisplatin, capecitabine, and radiotherapy (SCOPE1; ref. 58). One possible explanation for the clinical findings that cetuximab sensitizes to either cisplatin or radiation, but not to the combination of cisplatin and radiation, is that cetuximab and cisplatin may have overlapping mechanisms of radiosensitization. Because cisplatin and EGFR inhibitors share the ability to inhibit both radiation-induced DSB repair and repopulation between radiation fractions, the combination of cetuximab with cisplatin may not further inhibit these processes beyond the inhibition produced by either agent alone. Another possible issue is the treatment schedule. Preclinical data suggest that the optimal schedule is chemotherapy, followed by concurrent EGFR inhibitor and radiation. The RTOG 0522 trial, however, used a loading dose of cetuximab before cisplatin–radiation (6), a schedule that preclinical data suggest may actually be antagonistic (52). A major shortcoming in the preclinical studies leading up to RTOG 0522 was a lack of investigation of the triple combination of cetuximab, cisplatin, and radiation in animal models of head and neck cancer. Completion of these preclinical studies would have been informative in terms of efficacy, sequence of administration, and possible antagonistic interactions between agents.

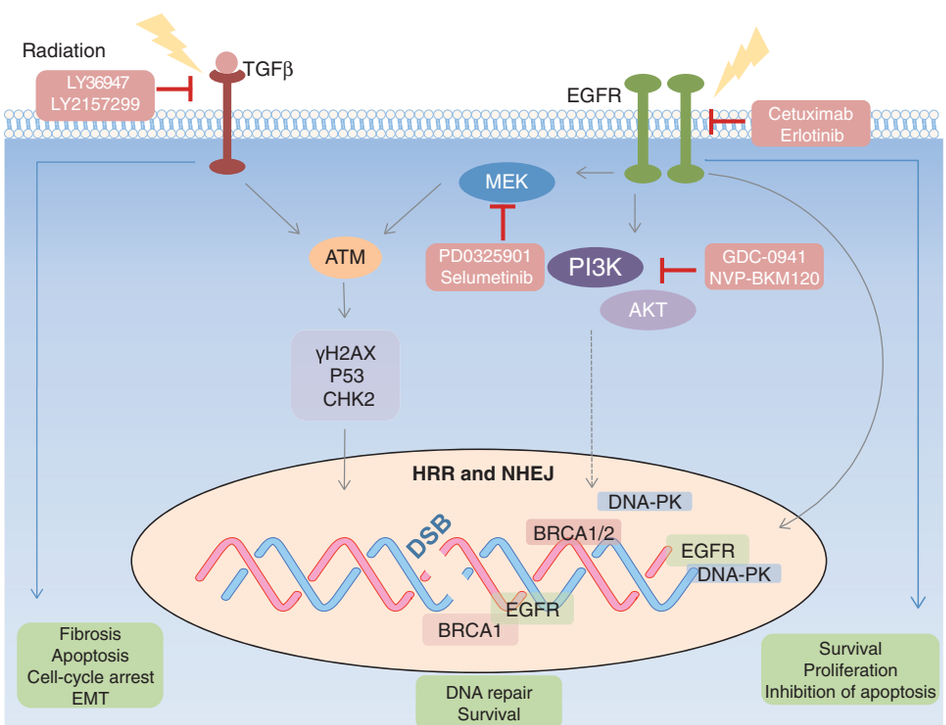

Figure 2. Activated membrane signaling promotes survival in response to radiation. Radiation activates a series of cellular signaling pathways that promote repair of radiation-induced DNA damage as well as other cellular processes (green boxes). Many of these radiation-induced prosurvival pathways can be targeted with small-molecule inhibitors or antibodies. Inhibition of these radiation-induced, prosurvival pathways potentially results in maximal potentiation of chemoradiation. EMT, epithelial-to-mesenchymal transition.

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PI3K/AKT and MEK

In addition to EGFR, radiation activates other prosurvival pathways that may affect DNA repair, such as PI3K/AKT and MEK (47, 59). Several agents designed to directly inhibit these pathways (e.g., NVP-BKM120 and selumetinib) are in various stages of clinical development (for review on PI3K/AKT, ref. 60). Preclinically, inhibitors of PI3K/AKT and MEK sensitize tumor cells to both radiation and chemotherapy (59, 61), supporting the development of clinical trials combining PI3K/AKT or MEK inhibitors with chemoradiation. Thus, it is important to consider the underlying biology of these combinations. Inhibition of PI3K causes downregulation of BRCA1/2, suggesting a role in homologous recombination (62), and inhibition of radiation-induced DNA damage repair (63). The MEK pathway has also been implicated in regulating both homologous recombination repair and the ATM-mediated DNA damage response (64). In one study, the MEK inhibitor selumetinib (AZD6244; administered after chemotherapy but before radiation) sensitized to 5-FU–based chemoradiation in gastrointestinal tumor models (65). This sensitization was associated with induction of apoptosis and mitotic catastrophe. Subsequent studies have confirmed that, as with EGFR inhibitors, the efficacy of MEK inhibitors in combination with chemotherapy is highly schedule-dependent; chemotherapy given first followed by MEK inhibition is superior (61). Inhibition of PI3K and MEK is logical in gastrointestinal malignancies given the high frequency of mutations in their upstream regulators, *KRAS/RAF*. Likewise, based on the finding that PI3K signaling is frequently aberrant (via *PTEN* mutation) in glioblastomas, inhibitors of PI3K or its downstream target, MTOR, are under clinical investigation in combination with temozolomide-based chemoradiation (66). These trials were designed on the basis of the preclinical observations that PI3K or MTOR inhibition can sensitize to either temozolomide or radiation.

In summary, deregulation of EGFR, PI3K, and MEK signaling occurs frequently in tumor cells. As these prosurvival, membrane-signaling pathways are further activated by radiation, targeting these pathways represents a promising approach for tumor cell–selective sensitization to chemoradiation. However, given the cytostatic properties of agents targeting these pathways, it is crucial to determine the optimal sequence of administration to achieve maximal efficacy in combination with chemoradiation and to avoid chemotherapy antagonism.

TGFβ

Elevated TGFβ signaling is a common feature of many types of cancer and is associated with progression of established tumors. TGFβ signaling is further activated in response to radiation or chemotherapy and promotes therapy resistance. In response to radiation, TGFβ1 activation has been shown to promote repair of radiation-induced DNA damage by activating P53 and ATM (67). TGFβ1 inhibitors attenuate this ATM/P53 response, resulting in accumulation of unrepaired DNA damage and radiosensitization (67, 68). Enormous effort has gone into the development of TGFβ inhibitors for multiple diseases, including cancer, and currently there are more than 15 TGFβ–targeted agents in various phases of clinical development. Because TGFβ inhibitors alone are not cytotoxic, there is an emphasis on combined treatment strategies, especially in glioma, melanoma, breast cancers, and pancreatic cancers. Currently, there is at least one clinical trial combining the TGFβ receptor inhibitor LY2157299 with temozolomide–radiation in gliomas (69).

SENSITIZING TO CHEMORADIATION BY ALTERNATIVE STRATEGIES

One alternative approach to sensitize tumor cells to chemoradiation involves inhibition of the ubiquitin proteosome system, which is commonly aberrant in cancer (70). Several agents targeting the ubiquitin proteosome are in various phases of development (e.g., MLN9708 and carfilzomib) with bortezomib the first to receive U.S. Food and Drug Administration (FDA) approval. Bortezomib acts in part by inhibition of NF-κB via accumulation of its negative regulator IκB. Because the NF-κB pathway is activated in response to radiation and chemotherapy, and promotes survival via upregulation of genes such as *COX2*, *Survivin*, and *BCL2*, it is an appealing target for sensitization to chemoradiation (71). Although the ubiquitin proteosome system has also been implicated in modulating components of the DSB repair system (e.g., BRCA1, MDC1, KU80; ref. 72), there is currently no clear evidence that bortezomib affects DNA repair. Unfortunately, initial attempts to combine bortezomib with chemoradiation have produced increased toxicity with no apparent benefit in terms of tumor reduction (70). Toxicity associated with bortezomib may be related to the deleterious effects of broadly inhibiting ubiquitin-mediated protein degradation. Thus, agents that more selectively target subsets of E3 ubiquitin ligases important in cancer are being developed. One such agent, MLN4924, inhibits the NEDD8-activating enzyme, thus preferentially inhibiting the cullin-RING type E3 ubiquitin ligases that require neddylation for activity. MLN4924 exhibits tumor cell–specific radiosensitization in association with enhanced radiation-induced DNA damage (73). Preclinical evidence suggests that several genes involved in the DNA damage response (e.g., *ATM*, *MDC1*, and *CDKN1A*) are important in MLN4924-induced cell death (74). Although MLN4924 is currently under clinical investigation as a single agent or in combination with chemotherapy, based on positive preclinical data, it is likely that radiation and chemoradiation combinations will emerge in the future.

One other noteworthy approach for sensitizing tumor cells to chemoradiation is via modulation of tumor oxygen levels and aberrant tumor vasculature. Bevacizumab, an antibody recognizing VEGF, can transiently "normalize" tumor vasculature, increase tumor oxygen levels, and sensitize to radiation given within this window of oxygenation (75). The vascular effects of bevacizumab, however, may also antagonize chemotherapy efficacy by decreasing the perfusion of chemotherapeutic agents to tumors (76). These preclinical results suggest that bevacizumab should be administered just before radiation, but after chemotherapy. Although phase II clinical studies of bevacizumab in combination with chemoradiation (such as in brain and head and neck cancers) have suggested some activity (77), two recently completed, major randomized phase III clinical trials (AVAglio and RTOG 0825) combining bevacizumab with standard temozolomide–radiotherapy in patients with glioblastoma failed to demonstrate an overall survival advantage,

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despite increased toxicity (78, 79). Although progression-free survival seemed to be extended in the bevacizumab treatment arm, the consensus is that bevacizumab reduced vascular imaging rather than actual tumor growth. Taken together, these studies again underscore the importance of preclinical testing that might better determine the optimal clinical endpoints, treatment schedules, and biomarkers of response.

PRECLINICAL STUDIES TO MAXIMIZE THE LIKELIHOOD OF CLINICAL SUCCESS

To improve the likelihood of clinical success when combining targeted agents with chemoradiation, preclinical models (each with unique advantages and limitations) are required to investigate issues such as mechanisms, scheduling, biomarkers, and therapeutic index. Although a thorough discussion of preclinical oncology models is beyond the scope of this review, important considerations specifically related to chemoradiation are discussed below.

In vitro studies are beneficial in terms of providing a broad understanding of the potential for schedule-dependent efficacy, mechanism, and cellular context for a given therapy combination. Clonogenic survival is the standard for assessing the ability of a targeted agent to increase the sensitivity of tumor cells to chemoradiation. This assay reflects a variety of types of cell death mechanisms and importantly captures mitotic catastrophe and/or reproductive death, common forms of radiation-induced cell death that require multiple cell doublings (80). Although clonogenic survival is particularly useful for assessing sensitization to chemoradiation in cancer cells, it may not be ideal for measuring sensitization of normal cells, as many normal cell lines proliferate poorly in culture, thus resulting in inherent protection from radiosensitizing agents.

There are several types of *in vivo* tumor models routinely used, of which cell line–derived xenografts (usually implanted in mice) are the most malleable and commonly used system. Cell line–derived xenografts can provide proof-of-principle evidence of sensitizing activity as well as the optimal sequence of administration. Because animals can be treated with a fractionated course of radiation similar to that used clinically, agent scheduling studies in animals have a greater potential for direct translation to humans. More recently, genetically engineered mouse models (GEMM) or patient-derived xenografts (PDX) have been used. GEMMs are advantageous in that tumors initiate in the correct tissue of origin in nonimmunocompromised animals, but disadvantageous in that tumors may arise asynchronously following long latency periods without recapitulating the heterogeneity or radiation responsiveness of human tumor cells (81). Allografts of GEMMs can circumvent the problems of long latency and variable tumor initiation times, permitting cohorts of animals with similarly matured tumors to begin treatment together. Ultimately, however, given that the goal of most studies combining targeted agents with chemoradiation is translation to humans, mouse-derived tumors may be limited by their inability to fully recapitulate human tumor characteristics and therapeutic responses. Orthotopic PDX models are considered to be a clinically relevant system for studying new targeted agents, because they capture human tumor heterogeneity and microenvironment in an appropriate body site. However, in the context of radiation, orthotopic mouse models are limited by the technical difficulty of delivering targeted irradiation to small anatomy, which is not reflective of the human clinical setting. Other disadvantages of orthotopic PDXs include the need for imaging to monitor tumors and the difficulty of biological/genetic manipulation. Thus, for chemoradiation studies, ectopic PDX models coupled with normal-tissue toxicity studies in the relevant organs of dose-limiting toxicity are appealing and offer a technically feasible option, especially for cancers that would be difficult to irradiate in the orthotopic setting.

The standard clinical paradigm for the development of novel targeted agents is to add the new drug to standard therapy (i.e., chemoradiation), with the rationale that while the targeted agent may increase toxicity, it should not decrease chemoradiation efficacy. Without thorough preclinical evaluation to determine the optimal schedule of administration, however, this strategy has the potential to antagonize standard therapy. For example, the G_1 arrest induced by EGFR inhibitors protects cells from the replication-dependent effects of chemotherapy and blocks chemotherapy-induced phosphorylation and subsequent degradation of EGFR (51, 82). Thus, defining the optimal sequence of administration of a targeted agent in combination with chemoradiation is an important preclinical consideration. Unfortunately, scheduling information obtained from preclinical studies is sometimes lost in the translation to clinical trials, in part due to the realities of treating patients. If made a priority, however, it is feasible to adopt preclinical scheduling information into a practical clinical trial design by, for example, giving cytostatic targeted agents after chemotherapy but concurrent with radiation or on alternating cycles with chemotherapy.

Another important consideration in the preclinical development of a targeted agent with chemoradiation is the disease site to be treated and whether a patient will benefit from the radiosensitizing or chemosensitizing properties of a given agent. For example, tumors such as those of the head and neck, in which the need for local control dominates, may benefit from daily targeted agent to maximize radiosensitization, whereas in pancreatic tumors, where the need for systemic control dominates, the chemosensitizing properties of a drug may be more important. The radiosensitizing versus chemosensitizing properties of a targeted agent can be leveraged by giving either a low dose with each daily fraction of radiation or a higher dose only with chemotherapy, respectively. In addition, the dose-limiting toxicities for a targeted agent in combination with chemoradiation are also dependent upon the disease site. Relevant model systems for assessing dose-limiting toxicities in normal tissues are necessary and include, for example, duodenum and lung for pancreatic and lung cancers, respectively.

A final consideration for the preclinical development of targeted agents with chemoradiation is to identify biomarkers that can be used for patient selection as well as to ensure effective target inhibition and predict cytotoxicity against tumor cells. Static biomarkers such as the mutational status of *KRAS, TP53, EGFR,* and *PIK3CA* have demonstrated utility for patient selection in certain scenarios. Pharmacodynamic biomarkers such as poly-ADP-ribose (PAR) and phosphorylated EGFR are promising in the context of monitoring target inhibition by PARP and EGFR inhibitors, respectively, and pharmacodynamic biomarkers of DNA damage such as γH2AX, 53BP1, phosphorylated CHK1, and RAD51 may predict tumor response to therapy.

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On the basis of these considerations, preclinical studies addressing the issues of mechanism, scheduling, biomarkers, efficacy, and toxicity in the most clinically relevant model systems should increase the likelihood of successful clinical trials. It is imperative, however, that preclinical data are analyzed rigorously and that only the most robust/synergistic combinations of targeted agents with chemoradiation are chosen for clinical development, in a carefully selected patient population, and with the best available agent for the given target.

ELIMINATING CHEMOTHERAPY BY A DUAL-TARGETED APPROACH WITH RADIATION

Given the nonselective nature of chemotherapy, chemoradiation is associated with substantial toxicity. Targeted agents, in contrast, have been developed with the goal of providing greater tumor cell selectivity and fewer side effects. Unfortunately, as monotherapy, targeted agents have shown only modest efficacy in solid tumors. Given the number of targeted agents in development, it is increasingly feasible to consider combinations of targeted agents as therapy. This type of dual-targeted approach is being tested as an alternative to standard chemotherapy and is based on three fundamental concepts. First, because inhibition of a single pathway can lead to potentiation of a second pathway, which promotes acquired resistance, targeting both pathways may increase efficacy. Second, inhibition of a single pathway by two different agents may increase overall pathway inhibition and efficacy. Third, simultaneous inhibition of two different (strategically selected) pathways may produce synthetic lethality in tumor cells. Although precedence for these dual-targeted strategies is just emerging, initial clinical studies in breast cancer have demonstrated responses to a dual-targeted approach against HER2 that are approaching those obtained by chemotherapy (83).

The best example of a dual-targeted therapy designed to block emerging resistance following primary target inhibition is the combined inhibition of EGFR and MET (84). Although *EGFR*-mutant lung cancers are particularly sensitive to EGFR inhibitors, *MET* amplification is a common resistance mechanism. For this reason, the efficacy of combined inhibition of EGFR and MET is under investigation. Although preclinical and phase II clinical studies showed promising activity and tolerability of the combination of EGFR and MET inhibitors, a phase III trial (MARQUEE) combining erlotinib with the MET inhibitor ARQ197 in lung cancer was discontinued at interim analysis due to an inability to meet the overall survival endpoint, despite a significant effect on progression-free survival (85). A randomized phase II clinical trial has been established to compare this combination with single-agent chemotherapy in patients with locally advanced and metastatic non–small cell lung cancer (clinicaltrials.gov). Although it is still unknown whether combined EGFR and MET inhibitor therapy will be effective in combination with radiation, it is of interest to consider whether radiation might inhibit acquired resistance to targeted agents by eliminating resistant subpopulations, which would otherwise expand and cause therapy resistance. Specifically, in the context of radiotherapy and MEK inhibition, given that PI3K/AKT mediates compensatory signaling and thus, resistance, the combination of MEK and AKT inhibitors has been evaluated. Because both of these pathways are activated and promote survival in response to radiation (described above), combined inhibition of MEK and AKT produces radiosensitization greater than either agent alone (59). Similarly, dual inhibition of PI3K and MTOR has demonstrated potent radiosensitizing activity in *KRAS*–mutant lung cancers (86).

A second potential strategy for combining targeted agents is to use two different agents against a single target or a single pathway to achieve more complete pathway inhibition. This approach has been investigated in the case of EGFR, in which inhibition by two agents, erlotinib or gefitinib (small molecules) and cetuximab (a monoclonal antibody), results in more complete pathway inhibition than either agent alone (87). Combined inhibition of HER2 in breast cancer has progressed to several clinical studies, and the combinations of trastuzumab with lapatanib or pertuzumab show promising clinical activity in HER2-positive breast cancers (83). In addition, a strategy to inhibit mitotic checkpoints by combined inhibition of CHK1 and WEE1, which converge on CDK1, was shown to cause unscheduled (or premature) mitotic entry and tumor cell killing similar to that observed with the combination of chemotherapy and CHK1 inhibitor (26). This combination also produces dramatic radiosensitization and cytotoxicity in tumor cells (Table 1). It is still unclear, however, whether the combination of CHK1 and WEE1 inhibitors will afford any tumor cell selectivity (29).

A final approach for combining targeted agents is to choose agents that inhibit separate pathways, which when inhibited simultaneously produce synthetic lethality. In particular, agents that produce synthetic lethality in combination with PARP inhibitors are currently an intense area of investigation. Given the known efficacy of PARP inhibitors as single agents in homologous recombination–defective cancers (i.e., *BRCA1/2*-mutant cancers) as well as their ability to maximally radiosensitize DSB repair–defective cells (44, 45), strategies to induce homologous recombination defects with targeted agents in otherwise homologous recombination–proficient cancer cells are under way. Several agents have been found to inhibit homologous recombination, including those which target HSP90 (88), CHK1 (89), WEE1 (25, 90), PI3K (62), PP2A (91, 92), and EGFR (49). These agents synergize with PARP inhibitors alone and in combination with radiation (Table 1). Unlike the selectivity afforded by *BRCA1/2* mutation in tumor cells, systemic inhibition of homologous recombination by any of these targeted agents requires an additional tumor cell–selective mechanism. In the case of combined CHK1 and PARP inhibition, for example, tumors harboring *KRAS/TP53* mutation are preferentially radiosensitized by combined CHK1/PARP inhibition that is likely attributable to the selectivity of CHK1 inhibitors toward *KRAS/TP53*–mutant tumors (Table 1; ref. 89).

In summary, the concept of using dual-targeted therapy *in lieu* of cytotoxic chemotherapy in chemoradiation regimens is attractive, especially for the treatment of locally advanced cancers that are well-controlled by standard chemoradiation and where there is a need to reduce toxicity, such as in HPV-positive head and neck, cervical, and rectal cancers. For tumors that are poorly controlled by chemoradiation, such as those of the lung, pancreas, and brain, eliminating chemotherapy is not

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Table 1. Summary of combined molecularly targeted agents with radiation

Targets	Agents	Cell type	Cytotoxicity	Radiosensitization	Ref.
CHK1 and PARP1	AZD7762, olaparib	MiaPaCa-2 (pancreas)	–	+++	89
	"	M-Panc96 (pancreas)	–	+++	"
	"	H460 (lung)	–	++	"
	"	H460 (*P53*dn; lung)	+	+++	"
	"	HCT116 ($TP53^{+/+}$; colon)	+	++	"
	"	HCT116 ($TP53^{-/-}$; colon)	–	+++	"
	"	CCL-241 (normal intestine)	+	+	"
	"	DLD1 ($KRAS^{wt/-}$; colon)	+	+	[a]
	"	DLD1 ($KRAS^{mt/-}$; colon)	–	+++	"
CHK1 and WEE1	MK8776, AZD1775	MiaPaCa-2 (pancreas)	+++	+++	[a]
HSP90 and PARP1	17-AAG, olaparib	U87-MG (glioma)	nd	–	88
	"	T98G (glioma)	nd	+++	"
PARP1 and WEE1	Olaparib, AZD1775	MiaPaCa-2 (pancreas)	–	++	90
	"	AsPC-1 (pancreas)	–	++	"
AKT and MEK	API-2, PD0325901	MiaPaCa-2 (pancreas)	nd	++	59
MTOR and PI3K	NVP-BEZ235	H460 ($KRAS^{mt}$; lung)	–	+++	86
	"	H23 ($KRAS^{mt}$; lung)	–	+++	"

NOTE: The degree of cytotoxicity and radiosensitization are denoted by +, ++, and +++, indicating modest, intermediate, and maximal effects, respectively, and –, indicating no effect.
Abbreviation: nd, not determined.
[a]Engelke and colleagues, unpublished data.

currently a feasible option. Because phase I clinical trials have demonstrated acceptable toxicity profiles for some combinations of targeted agents (e.g., c-Met and EGFR inhibitors), the notion of adding radiation to these dual-targeted therapies is appealing. Although it is possible that radiation may add to the complexity of toxicity issues, modern highly conformal radiation can usually be safely added to maximum-tolerated doses of systemic therapies. Whether combining dual-targeted therapies with radiation will provide a greater therapeutic index relative to conventional chemoradiotherapy will be the subject of future investigations.

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No potential conflicts of interest were disclosed.

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