Review

Novel Chemoradiosensitizers for Cancer Therapy

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Abstract. *Combined modality therapy using both chemotherapy and radiation has proved superior over radiation therapy alone for a variety of cancer types. While the locoregional control and survival benefits have been established, there is still much room for improvement both in terms of cancer control and normal tissue toxicity, i.e. the therapeutic ratio. Recently, the pace of research and development of both conventional cytotoxic and molecularly targeted radiosensitizers has been staggering. The aim of this paper is to bring the reader up to date on the clinical status of four promising new radiosensitizers: novel camptothecin analogs and inhibitors of poly(ADP-ribose) polymerase, histone deacetylase, and heat-shock protein 90.*

Radiation therapy (RT) has been an integral weapon in the arsenal against cancer since the early 20th century, and is the most common treatment modality for cancer (1). While chemotherapy has been used clinically since the 1940s, the application of chemoradiotherapy, or combined modality therapy (CMT), has only been more recently adopted. In 1979, Steel and Peckham (2) described a theoretical framework defining the mechanisms by which these modalities interact to improve therapeutic outcome: spatial cooperation, toxicity independence, protection of normal tissues, and enhancement of tumor response. More recently, Bentzen *et al*. (3) proposed a contemporary modification to this paradigm to account for the rational design of systemic agents and to account for the introduction of molecularly-targeted drugs: spatial cooperation, cytotoxic enhancement,

biological cooperation, temporal modulation, and normal tissue protection.

The locoregional control and survival advantages of combining RT with conventional cytotoxic agents is well-proven in phase III studies at various anatomic sites and CMT is now standard of care for many cancer types, either definitively or in conjunction with surgery. Examples for which CMT is used definitively include squamous cell carcinoma of the head and neck (HNSCC), esophageal cancer, locally advanced non small cell lung cancer (NSCLC), limited stage small cell lung cancer, and anal canal cancer. Examples in which CMT is used as a surgical adjunct include glioblastoma multiforme (GBM) and various carcinomas of the gastrointestinal tract such as those of the rectum, stomach, and pancreas, among others (4).

While these advances have been remarkable, there is much room for progress in most cancer types, and CMT can, and occasionally does, cause severe acute and late normal tissue toxicity. In a seminal phase III trial that established the survival superiority of CMT over RT alone in HNSCC, the CMT group experienced 89% grade 3-5 toxicity, compared with 52% in the radiation alone arm ($p<0.0001$) (5).

The key to effective local control is the 'therapeutic ratio', which is the difference at a given radiation dose between the sigmoidal curves for tumor control probability (TCP) and normal tissue complication probability (NTCP). These curves are plotted with increasing dose along the abscissa, and increasing probability along the ordinate. Methods for improving the therapeutic ratio include improvements in the delivery of radiotherapy, including three-dimensional conformal radiotherapy (3DCRT), intensity modulated radiotherapy (IMRT), image-guided radiotherapy (IGRT), and the use of particle therapy such as protons and carbon ions, which practically eliminates the exit dose of radiation by taking advantage of the so called 'Bragg peak'. Protecting normal tissues by agents such as amifostine can improve the therapeutic ratio by shifting the NTCP curve to the right. And finally, radiosensitizers that are preferential to tumor shift the TCP curve to the left to a larger degree than the NTCP curve.

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The purpose of this review is to provide an update of the clinical development of a few emerging radiosensitizers. The first section reviews new developments in a standard cytotoxic class of agents, the topoisomerase I inhibitors. The subsequent sections update the preclinical and clinical status of three intriguing molecularly targeted agents.

Topoisomerase I Inhibitors

Topoisomerase I (TopoI) is an essential enzyme in mammalian cells and is involved in the regulation of DNA topology during replication, recombination, and transcription. TopoI forms a phosphotyrosine bond with DNA, catalyzing a forward reaction in which DNA is cleaved to allow unwinding, and a reverse reaction in which DNA is ligated. Camptothecin and its derivatives interfere with this re-ligation step by binding to and stabilizing the enzyme/DNA complex. The resultant conversion of single-strand breaks (SSBs) into irreversible double-strand breaks (DSBs) results in cell death. TopoI inhibitors exhibit S-phase cytotoxicity and G_2/M cell cycle arrest. G_2/M is a relatively radiosensitive phase of the cell cycle and may in part explain the radiosensitization properties of TopoI inhibitors (6).

Topotecan/Irinotecan. There are currently two well-studied and FDA-approved TopoI inhibitors in clinical use. Topotecan is currently approved for use in ovarian and small cell lung cancer, and in cervical cancer in combination with cisplatin. Irinotecan is approved for use in metastatic colorectal carcinoma.

Preclinical studies demonstrated the radiosensitizing properties of irinotecan and topotecan, which have been confirmed in phase I and II trials in combination with radiotherapy for various disease sites, (including brain, lung, rectal, pancreas, esophageal, and cervical cancer) (7-9).

Phase III studies of chemoradiation, however, have so far not been as promising with these two FDA-approved drugs. A recent randomized trial from Greece in the postoperative treatment of rectal cancer randomized patients to bolus 5FU/LV or the same dose of bolus 5FU/LV and irinotecan (80 mg/m^2) with concurrent radiotherapy after the first chemotherapy cycle. There were no differences between the groups with respect to 3-year overall survival, disease-free survival, or local relapse-free survival. Grades 3 and 4 were similar between arms with the exception of leucopenia, neutropenia, and alopecia, all of which were higher in the irinotecan arm (10).

A phase III trial from Germany randomized patients with brain metastases (from either small cell or non-small cell lung cancer) to whole brain radiotherapy (40 Gy/2 Gy fractions) with or without topotecan (0.4 mg/m^2/day for 5 days over 4 weeks within 2 hours before RT). Due to slow accrual, an interim analysis conducted after only 95 of a

planned 320 patients showed that local response, progression-free survival, and overall survival did not differ between patients treated with chemoradiotherapy and those treated with radiotherapy alone. While nonhematologic toxicity did not differ between groups, 24 out of 25 (96%) of the hematologic toxicities occurred in the combined modality arm. On the basis of these results and because of the slow accrual, this study was not continued (11).

A randomized phase II study was also reported in abstract form at the 2008 ASCO annual meeting (12). RTOG 0247 compared neoadjuvant capecitabine (1200 mg/m^2 Monday through Friday) with irinotecan (50 mg/m^2 weekly x 4 doses) or capecitabine (1650 mg/m^2 Monday through Friday) with oxaliplatin (50 mg/m^2 weekly × 5 doses) and concurrent radiotherapy (50.4 Gy in 1.8 Gy fractions) in the setting of clinical stage T3 or T4 rectal cancer. Surgery was performed d 4-8 weeks following completion of chemoradiation. Adjuvant chemotherapy with oxaliplatin/5FU/leucovorin was administered every 2 weeks for 9 cycles, beginning 4-6 weeks after surgery. 101 patients were included in the analysis. Tumor and nodal downstaging and grade 3/4 nonhematologic toxicity were similar between the groups. Grade 3/4 hematologic toxicity was 8% in the irinotecan group and 4% in the oxaliplatin group (*p*-value not given). The pathologic complete response rate (pCR) was 10% in the irinotecan group and 21% in the oxaliplatin group (*p*-value not given). Because of the manageable toxicity and improved pCR rate in the oxaliplatin group, the capecitabine/ oxaliplatin regimen was chosen as the backbone for the next RTOG rectal cancer study evaluating the potential benefit of IMRT in reducing gastrointestinal toxicity.

A search of the National Cancer Institute's Clinical Trials database (13) shows 21 phase I or II studies in progress evaluating topotecan or irinotecan with radiotherapy in a number of disease sites, most notably primary brain tumors, lung and gastrointestinal cancers, and rhabdomyosarcoma. There is currently only one open phase III trial evaluating the efficacy of alternating irinotecan and vincristine chemotherapy with standard vincristine/dactinomycin/ cyclophosphamide chemotherapy in combination with radiation therapy in patients with intermediate-risk rhabdomyosarcoma (14).

Novel camptothecin derivatives. In part because of the results of the aforementioned randomized trials, there is an apparent need for the development of more efficacious and less toxic camptothecin analogs. One limitation of existing topoisomerase I inhibitors lies in the equilibrium that exists between the intact lactone E ring and the E ring-opened carboxylate form, which has less than 10% the potency of the lactone form, and accounts for more of the toxic effects (15). Furthermore, topotecan and irinotecan are subject to multi-drug resistance (MDR) *via* P-glycoprotein (P-gp) efflux, as well as the breast cancer resistant protein (BCRP) pump (15, 16).

Several novel camptothecin derivatives have been developed (15) and have shown promise in phase I and II clinical trials (17-21), especially in cancer of lung, ovarian, and gastrointestinal origin. Few phase III studies, however, have so far been reported.

Exatecan is a water-soluble camptothecin analog that is a more potent TopoI inhibitor than camptothecin, topotecan, and SN38 (the active metabolite of irinotecan). Two phase III trials have now been completed evaluating exatecan in the setting of untreated, locally advanced pancreatic cancer, though with disappointing results. The U.S. trial compared exatecan (2 mg/m^2) plus gemcitabine (1000 mg/m^2) *versus* gemcitabine (1000 mg/m^2) alone. In 349 randomly assigned patients, no improvement in overall survival was seen in the exatecan arm, while grade 3 and 4 neutropenia and thrombocytopenia were higher (22). The European trial compared exatecan (0.5 mg/m^2) alone to gemcitabine (1000 mg/m^2) alone in 339 patients, and exatecan was found to be inferior in terms of response rate and quality of life (23).

There are currently two active phase III trials of novel camptothecin derivatives. NCT00477282 is being conducted largely in Russia and eastern Europe, evaluating karenitecan, 1.0 mg/m^2/day administered as a single daily IV infusion over 60 minutes for 5 consecutive days every 3 weeks, *versus* topotecan, 1.5 mg/m^2/day administered as a single daily *i.v.* infusion over 30 minutes for 5 consecutive days every 3 weeks, in stage III or IV, chemotherapy-resistant epithelial ovarian cancer. The study is currently recruiting patients, with an estimated enrollment of 500 (24). A second phase III trial in Korea (the COMBAT trial) is evaluating cisplatin with either belotecan or etoposide in patients with chemotherapy-naïve, extensive stage small cell lung cancer. Cisplatin, 60 mg/m^2 will be combined with either belotecan, 0.5 mg/m^2/day during days 1-4, or etoposide, 100 mg/m^2/day during days 1-3. 150 patients are currently being accrued for this trial (25).

A search of the literature reveals that one clinical study has been reported to date evaluating a novel TopoI inhibitor combined with radiotherapy. 9-Nitro-20(s)-camptothecin (9NC/orathecin/rubitecan) was an orally available semi-synthetic camptothecin analog that was created by adding a nitro group in the nine position of the A-ring of the parent camptothecin molecule. Because of reasonable efficacy seen in clinical studies of patients with advanced or refractory pancreatic cancer, as well as its preclinical radiosensitizing properties, investigators at Vanderbilt University initiated a phase I study to assess the maximum tolerated dose of 9NC combined with radiotherapy in patients with previously untreated locally unresectable adenocarcinoma of the pancreas (26). The drug was administered 5 days per week (Monday through Friday), taken 1-4 hours prior to radiation treatment, at a starting dose of 1 mg/m^2/day. Patients were advised to increase their oral hydration to at least three liters/day to prevent cystitis, which is a dose-limiting toxicity of 9NC. A radiotherapy dose of 45 Gy given in 1.8 Gy daily fractions was delivered to the gross tumor volume and regional draining lymph nodes. Dose-limiting toxicity (DLT) of grade 3 nausea and vomiting developed in one patient at the first dose level. At the second dose level of 1.25 mg/m^2/day, two of three patients developed DLT of grade 3 nausea, vomiting, and anorexia. Grade 3 dehydration, vomiting, and weakness, as well as grade 4 leukopenia, also were seen. Therefore the maximum tolerated dose (MTD) was found to be 1 mg/m^2/day. Six of eight patients at dose level 1 experienced periods of stable disease lasting at least 2.5 months, while two had progressive disease. Median time to progression for all 11 patients was 4.1 months, and median survival was 11.3 months. No patients were able to undergo successful resection. Six patients received additional gemcitabine-based chemotherapy at time of disease progression. It should be noted that, partly because of relatively disappointing results of a phase III trial of rubitecan *versus* ''best choice'' chemotherapy in 409 patients with refractory pancreatic cancer (27), SuperGen (Dublin, CA, USA) withdrew its FDA application and has since stopped manufacturing the drug.

While further clinical trials evaluating novel TopoI inhibitors with concurrent radiotherapy are eagerly awaited, none are currently in progress. The Authors' laboratory has recently developed a novel camptothecin analog, TLC388, that is significantly less toxic to animals than topotecan and was also shown to be an effective radiosensitizer of H23 human NSCLC cells (28). In this preclinical study, the combination of TLC388 and therapeutic doses of radiation produced a significantly higher percentage of both apoptotic and necrotic cells, with a sensitizer enhancement ratio (SER) of 1.91. Additionally, using γ-H2AX foci as a marker of DNA DSBs, it was noted that the formation of γ-H2AX foci increased significantly with 30 nM TLC388 plus 2 Gy radiation, when compared to either TLC388 or 2 Gy radiation alone. Furthermore, it was found that the percentage of M phase cells exhibiting γ-H2AX foci increased significantly with 30 nM TLC388 plus 0.5 Gy radiation compared to either modality alone, suggesting that TLC388 may be particularly radiosensitizing to mitotic cells. TLC388 is currently undergoing a phase I trial in patients with advanced solid tumors.

Novel Targeted Agents

Due to the nonspecificity of traditional chemotherapeutic agents, tumor control efficacy has largely been limited by normal tissue toxicity. This naturally led to investigation into and development of molecularly targeted agents that theoretically would have less severe normal tissue adverse effects due to relative tumor selectivity. An example of an agent which has been clinically successful in this regard is cetuximab, an EGFR inhibitor shown in a landmark phase III

trial to significantly improve locoregional control and overall survival in squamous cell carcinoma of the head and neck (HNSCC) when combined with radiotherapy, compared with radiotherapy alone. This benefit was achieved with little increase in toxicity (other than acneiform rash) over that associated with radiotherapy alone (29).

Several targeted agents have now been proven to be clinically useful, either as monotherapy or in combination with chemotherapy or radiation for a variety of tumor types. Besides EGFR inhibitors, other examples of targeted agents with proven and FDA-approved clinical utility include trastuzumab in HER-2/neu overexpressed breast cancer, gefitinib and other small molecule tyrosine kinase inhbitors (TKIs) in well-defined subsets of patients with NSCLC, and vascular endothelial growth factor (VEGF) inhibitors such as bevacizumab. Most of these agents are either already approved or are in advanced clinical trials as radiosensitizers. This review will focus on three newer, less proven, though equally exciting and potentially as effective radiosensitizers: inhibitors of poly (ADP-ribose) polymerase (PARP), histone deacetylase (HDAC), and heat-shock protein 90 (Hsp90).

PARP inhibitors. PARPs are a family of enzymes that catalyze the transfer of ADP-ribose from nicotinamide adenine dinucleotide (NAD+) to acceptor proteins, resulting in the creation of long, negatively charged, branched polymers on the acceptor proteins. While there are currently at least 17 known members of the PARP family, PARP1 and PARP2 are the two family members known to participate in the DNA repair process, and most is known about PARP1.

PARP1 is important in the repair of DNA SSBs *via* the base excision repair (BER) pathway, binding with high affinity to DNA strand breaks. This binding activates the enzyme, and the resulting auto-poly (ADP-ribosyl)ation creates a negatively charged target at the SSB which recruits BER effector proteins such as XRCC1 (30, 31). Furthermore, PARP1 appears to modify local histone proteins and relaxes the chromatin structure, resulting in improved access for repair proteins (32).

If the DNA SSBs are not properly repaired they can be converted to potentially lethal DSBs during DNA replication (32). Importantly, it has also been found that homologous recombination repair mechanisms are engaged in cells in response to DNA damage when PARP1 is absent (30). It is reasonable to assume, therefore, that cells with defects in homologous recombination repair would be sensitive to loss of SSB repair capability through inhibition of PARP activity, a concept defined as 'synthetic lethality'. Indeed, this theory has been confirmed in both preclinical and clinical studies. The *BRCA* gene product is a critical protein involved in DNA DSB repair by the homologous recombination repair pathway. It has been demonstrated *in vitro* that BRCA-deficient cells are highly sensitive to PARP inhibitors (30). Furthermore, a recent phase I study from Europe evaluated the orally available

PARP1 inhibitor olaparib in 60 patients, 22 of whom were known *BRCA1* or *BRCA2* mutation carriers (33). The maximum tolerated dose was determined to be 400 mg twice daily. PARP was found to be inhibited by more than 90%, even at low doses, and sustained induction of DNA DSBs was seen 6 hours after treatment with olaparib, as evidenced by formation of γH2AX foci. Most important clinically, 12 out of 19 (63%) of *BRCA* carriers with ovarian, breast, or prostate cancers had radiologic or tumor marker responses, or meaningful disease stabilization. These patients also did not appear to have an increased risk of adverse effects. Unfortunately, no objective antitumor responses were seen in patients without known *BRCA* mutations.

Based on the above results demonstrating the real-world applicability of synthetic lethality, it is possible that this concept may be applied in a broader range of tumors than those deficient in *BRCA1* or *2*. Homologous recombination is dependent on other proteins such as RAD51, ATM/ATR, and Chk1/Chk2, and loss of these proteins sensitizes cells to PARP inhibition (33). Defects in these (or other) repair proteins may be common in certain sporadic cancers, and therefore it is reasonable to propose that PARP inhibition may be useful in a variety of tumor types, either alone or with chemotherapy or radiotherapy.

PARP inhibitors have demonstrated radiosensitizing effects in preclinical studies. Among others, Dungey *et al*. in the UK demonstrated that KU-0059436, a PARP1 inhibitor, increased the radiosensitivity of four human glioma cell lines, and that radiosensitization was S-phase dependent. Radiosensitization was maintained with a clinically-relevant fractionated radiation schedule. The observed radiosensitization was consistent with the hypothesis that PARP inhibition increases the incidence of collapsed replication forks after exposure to ionizing radiation, generating persistent DNA DSBs (34). Russo *et al*. at the NCI investigated the *in vitro* and *in vivo* radiosensitizing effects of another PARP inhibitor, E7016, on U251 human glioblastoma cells. They demonstrated a dose enhancement factor of 1.6 at a surviving fraction of 0.1 when E7016 was combined with 2 Gy of radiation, and the data were consistent with the hypothesis that E7016 inhibits the repair of radiation-induced DNA DSBs. Furthermore, in a clinically relevant test, the investigators found that the combination of 40 mg E7016 with temozolomide (3 mg/kg) and radiation produced a tumor growth delay on mouse xenografts that was significantly longer (*p*=0.03) than that produced by the standard of care treatment of temozolomide and radiotherapy alone (32). In an even more recent study, investigators at the University of Pennsylvania showed that yet another PARP inhibitor, GPI-15427, significantly reduced tumor volumes in a xenograft model of human head and neck squamous cell carcinoma (35)

There are currently at least 24 phase I or II trials involving PARP inhibitors, either alone or with traditional chemotherapy

drugs, for a variety of indications (13). There are at least two phase III trials in progress. The first trial is evaluating the role of BSI-201, another PARP1 inhibitor, in addition to gemcitabine/carboplatin chemotherapy in patients with triple negative (estrogen, progesterone, and Her-2/neu negative) breast cancer (36). This trial has completed accrual. Another trial, which recently opened, is evaluating the same chemotherapy agents with and without BSI-201 in advanced squamous cell lung cancer (37). Furthermore, a phase I/II trial is currently recruiting patients with newly diagnosed malignant glioma, assessing the safety and tolerability of BSI-201 when added to standard temozolomide and radiation therapy, as well as the efficacy, with overall survival as the primary outcome measure (38).

HDAC inhibitors. Histones play a critical role in regulating chromatin structure and function. Post-translational epigenetic modifications of histone proteins through acetylation, methylation, and phosphorylation determine how these proteins control chromatin remodeling. Histone acetylation is regulated through the opposing actions of histone acetyltransferases (HAT) and histone deacetylases (HDACs). While HAT activity relaxes chromatin and promotes transcription by allowing access of transcription factors to DNA, HDAC activity condenses chromatin, leading to transcriptional repression (39). Nonhistone proteins of potential importance in tumorigenesis, such as p53, E2F, Hsp90, and Ku70, are also substrates of HDACs (40). Both HAT inactivity and HDAC overactivity have been associated with tumorigenesis, presumably because of transcriptional repression of tumor suppressor genes (41). Because it is easier to inhibit an enzyme than induce one, HDAC inhibition has been of clinical interest.

There are four structural classes of HDAC inhibitors: hydroxamate, cyclic peptide, aliphatic acids, and benzamide. At least 16 HDAC inhibitors have been developed and evaluated in clinical trials (40).

As monotherapy, HDAC inhibitors have shown efficacy in hematologic malignancies, and two phase II clinical trials reported in 2007 led to the FDA-approval of the hydroxamate HDAC inhibitor vorinostat for use in relapsed or refractory cutaneous T-cell lymphoma (CTCL). Romidepsin, a cyclic peptide HDAC inhibitor, was also approved for use in CTCL in November 2009.

HDAC inhibitors as monotherapy in solid tumors have not been as successful, though they have demonstrated efficacy in combination with standard chemotherapy agents such as epirubicin and carboplatin/paclitaxel in phase I and II clinical trials. There are currently around 100 phase I and II trials involving HDAC inhibitors in progress, both as monotherapy and combined with other agents, in a variety of solid and hematologic malignancies. There are also five phase III trials in progress (13).

Preclinical studies have also shown that HDAC inhibitors exhibit radiosensitizing effects in a variety of malignancies, such as glioblastoma, non-small cell lung cancer, colorectal cancer, prostate cancer, and metastatic breast cancer. While the mechanism of radiosensitization is not well understood, accumulating evidence suggests that it is at least in part due to inhibition of DNA DSB repair as evidenced by prolonged expression of γH2AX (41). Furthermore, in preclinical studies HDAC inhibitors appeared to radiosensitize tumor cells without an increase in radiosensitization of normal cells, potentially improving the therapeutic ratio. Although the reason for this specificity is not yet clear, a reasonable assumption is that the aberrant histone deacetylase activity in tumor cells relative to normal cells plays an important role.

The first clinical trial reporting on the combination of an HDAC inhibitor with radiotherapy was recently published. The Pelvic Radiation and Vorinostat (PRAVO) trial assessed the safety and dose limiting toxicity, and determined the MTD of orally administered vorinostat in combination with palliative pelvic radiotherapy for gastrointestinal carcinomas (42). Sixteen patients received vorinostat in escalating doses administered 3 hours prior to radiotherapy, 30 Gy in 3 Gy fractions. The MTD of vorinostat was determined to be 300 mg. The DLTs were grade 3 fatigue and anorexia. Grade 1 and 2 fatigue and gastrointestinal events were observed in all patients. Histone hyperacetylation of tumor biopsy specimens was detected, indicating biological activity of vorinostat. In 14 evaluable patients, a mean reduction in tumor volume of 26% was observed, which was felt to be consistent with outcomes observed with palliative radiation alone. Overall, this study demonstrated that vorinostat can be safely combined with short-term palliative pelvic radiation.

Currently, at least 9 clinical studies are in progress evaluating the combination of HDAC inhibitors and radiotherapy in a variety of solid tumor types, the results of which are eagerly anticipated (13).

Hsp90 inhibitors. Because it is becoming clearer that chemo- and radiosensitivity reflects the end result of a complex process involving multiple signaling and effector molecules and that single molecules may play greater or lesser roles in a cell type-dependent manner, the effectiveness of targeted radiation sensitizers may be limited by intertumor and intratumor genetic and epigenetic heterogeneity. As such, it would seem logical to attempt to inhibit multiple molecular determinants simultaneously, or aim at the final result of a signaling cascade.

Hsp90, the 90 kDa heat-shock protein, is a chaperoning protein that modulates a diverse set of oncogenic proteins involved in cell-signaling, proliferation, and survival. Examples of client proteins of Hsp90 are mutant p53, HER-2/neu, Raf-1, c-kit, and Bcr-Abl (43, 44). Hsp90 therefore is an attractive target for inhibition, as it would theoretically

target multiple oncogenic proteins at once. A number of these client proteins have been associated with radioresponsiveness, as demonstrated in preclinical studies with the Hsp90 inhibitor geldanamycin and its clinically relevant analogues, 17AAG and 17DMAG, in a wide variety of tumor types (45-48).

Some of these preclinical studies elucidated that a major mechanism of radiosensitization appeared to be due to a decrease in the levels of ErbB2, one of the client proteins of Hsp90. ErbB2 is one of the members of the family of EGFR, which also include ErbB1 and ErbB3. ErbB1 can be activated by heterodimerization with ErbB2 or ErbB3, initiating a cascade of downstream signaling events which affect cellular growth, differentiation, and proliferation. When levels of ErbB2 were decreased due to Hsp90 inhibition, cells were radiosensitive, unless tumor cells expressed ErbB3, which could activate ErbB1 in the absence of ErbB2. When ErbB3 was knocked down by small interfering RNA (siRNA), these same cell lines were radiosensitized by Hsp90 inhibition (49). Subsequent studies indicate that, while ErbB1 deactivation is necessary, it is not sufficient to explain the entire mechanism of radiosensitization by Hsp90 inhibitors. A separate direct interaction was found between Hsp90 and the MRE11/Rad50/NBS1 (MRN) complex, a vital component in the DNA damage response/cell cycle checkpoint pathway (50). Exposure to 17DMAG was found to inhibit the interaction of MRN with the ataxia telangiectasia mutated (ATM) gene product, leading to abrogation of the G_2/S cell cycle checkpoint and increasing tumor radiosensitivity. Discoveries such as these are important for several reasons: (i) understanding the mechanisms of radiosensitization drives further development of novel therapies and helps to tease out the proper temporal sequencing of targeted therapies with radiation; (ii) this knowledge emphasizes the need for targeting multiple pathways in order to inhibit tumor growth due to inter- and intratumor variability, and to take advantage of synthetic lethality as described above and (iii) discovery of potential biomarkers of radiosensitivity (such as the lack of ErbB3) can help the clinician appropriately select potentially active therapies and exclude patients who lack these biomarkers from potentially futile yet toxic treatments.

The clinical applicability of Hsp90 inhibitors will depend on the relative selectivity of tumor cells over normal tissue cells (*i.e.*, the therapeutic ratio). Preclinical studies have thus far demonstrated that the radiosensitivity of normal human fibroblasts is not increased when exposed to 17DMAG or 17AAG, in spite of the fact that the radioresponse-associated protein levels were reduced to a similar degree. While the mechanism of selective radiosensitization of tumor cells by Hsp90 is unclear, these results do support the existence of a useful therapeutic ratio for these drugs, with the ultimate proof being awaited in clinical trials.

A number of phase I and II trials of Hsp90 inhibitors have been conducted in a variety of malignancies, either as monotherapy or with other chemotherapeutic or targeted agents such as irinotecan and trastuzumab. Many of these trials demonstrated the safety and tolerability of Hsp90-containing regimens, and overall efficacy was variable. The MTD varied with the dosing schedule, and DLTs were varied, including cardiac toxicity (elevated troponin, myocardial infarction), pancreatitis, fatigue, peripheral neuropathy, and renal dysfunction. Unfortunately, the two phase II trials in patients with renal cell carcinoma and melanoma showed no objective responses (51, 52).

In contrast, an open-label phase II/III study of tanespimycin (17AAG) plus bortezomib in relapsed/refractory multiple myeloma (TIME-2) has been completed, with initial results in 22 patients reported in abstract form at the 2009 American Society of Hematology Annual Meeting (53). These heavily pretreated patients were randomized to 1 of 3 treatment arms in combination with bortezomib 1.3 mg/m^2: (1) tanespimycin 340 mg/m^2, (2) tanespimycin 175 mg/m^2, or (3) tanespimycin 50 mg/m^2. All drugs were given on days 1, 4, 8, and 11 of each 21-day cycle, and treatment continued for at least 4 cycles and then until progression. Overall response rate was 14%, with one minimal response, one partial response, and one very good partial response. In addition, 10 patients had a best response of stable disease. Moreover, the treatment was well tolerated, with grade 3 neutropenia in 18% of patients, and grade 3/4 thrombocytopenia in 27%. Only one grade 3 peripheral neuropathy was encountered, a toxicity known to be associated with bortezomib. Final results of this study are eagerly awaited.

There are currently 23 open phase I or II clinical trials involving Hsp90 inhibitors in hematogenous or solid malignancies (13). Most are testing Hsp90 inhibitors alone, though two involve the combination of Hsp90 inhibitors with bortezomib (a proteasome inhibitor) in patients with multiple myeloma (54, 55), and an additional trial is evaluating three different schedules of 17-AAG in combination with gemcitabine in patients with metastatic pancreatic cancer (56). No phase III studies are currently in progress. Furthermore, there are not yet any human clinical trials investigating the radiosensitizing effects of Hsp90 inhibitors.

Conclusion

Winning the war against cancer is a painstaking and incremental process. Between 1990 and 2005, death rates due to cancer have decreased by 19.2% among men and 11.4% among women (57). Reasons for this success have been multifactorial, including improved cancer screening and early detection, public awareness and lifestyle changes, and more effective treatments.

As treatments provide more cancer-free years for our patients, one must address how these treatments affect the quality of life-years gained by long-term survivors. New and improved cancer treatments must not only improve cure and

survival rates, but should also increase the therapeutic ratio by being less toxic, especially with respect to long-term adverse effects. The last few decades have provided exciting advances in the arsenal of anticancer modalities. More precise radiation targeting limits damage to normal tissues and/or allows increased tumor dose. Systemic therapies are becoming more effective, less toxic, and more molecularly targeted, and toxicity management interventions such as hematopoietic support and nausea management have vastly improved. Combining more powerful and selective radiation sensitizers with more precisely focused radiotherapy will further the efforts to cure more patients, while empowering them with an improved quality of life.

This review has detailed only a few of the exciting new systemic therapies that exhibit the potential to provide improved radiosensitization of a variety of tumors. Agents such as irinotecan and topotecan are relatively mature and successful as chemotherapeutic agents, but have not yet proven as successful as radiosensitizers, although more clinical trials are in progress. It is hoped that some of the novel camptothecin derivatives will convert their theoretical efficacy as powerful radiosensitizers into clinical use. The targeted PARP, HDAC, and Hsp90 inhibitors are still in their infancy with respect to clinical evaluation as radiosensitizers, but their rational design is intelligent and intriguing, and prior targeted agents have shown remarkable efficacy in specific clinical situations. The PARP1 inhibitor olaparib is already showing exciting promise in the setting of BRCA-deficient breast cancer, and the HDAC inhibitors vorinostat and romidepsin received accelerated FDA approval for refractory CTCL. Their usefulness as radiosensitizers remains to be evaluated in clinical trials. These new agents should be viewed with cautious optimism, as history tells us that only a very small minority of these drugs will prove themselves to be clinically useful, but success of only a few of these agents could make outcomes substantially better for thousand of individual cancer patients.

Acknowledgements

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