Long-term Consequences of Pelvic Irradiation: Toxicities, Challenges, and Therapeutic Opportunities with Pharmacologic Mitigators

Jung Wook Huh1,2, Jarred Tanksley2, Junzo Chino2, Christopher G. Willett2, and Mark W. Dewhirst2

ABSTRACT

A percentage of long-term cancer survivors who receive pelvic irradiation will develop treatment-related late effects, collectively termed pelvic radiation disease. Thus, there is a need to prevent or ameliorate treatment-related late effects in these patients. Modern radiotherapy methods can preferentially protect normal tissues from radiation toxicities to permit higher doses to targets. However, concerns about chronic small bowel toxicity, for example, still constrain the prescription dose. This provides strong rationale for considering adding pharmacologic mitigators. Implementation of modern targeted radiotherapy methods enables delivery of focused radiation to target volumes, while minimizing dose to normal tissues. In prostate cancer, these technical advances enabled safe radiation dose escalation and better local tumor control without increasing normal tissue complications. In other pelvic diseases, these new radiotherapy methods have not resulted in the low probability of normal tissue damage achieved with prostate radiotherapy. The persistence of toxicity provides rationale for pharmacologic mitigators. Several new agents could be readily tested in clinical trials because they are being or have been studied in human patients already. Although there are promising preclinical data supporting mitigators, no clinically proven options to treat or prevent pelvic radiation disease currently exist. This review highlights therapeutic options for prevention and/or treatment of pelvic radiation disease, using pharmacologic mitigators. Successful development of mitigators would reduce the number of survivors who suffer from these devastating consequences of pelvic radiotherapy. It is important to note that pharmacologic mitigators to ameliorate pelvic radiation disease may be applicable to other irradiated sites in which chronic toxicity impairs quality of life.

Introduction

Radiotherapy is frequently used in the treatment of pelvic cancers, including those of genitourinary, gynecologic, and anorectal origin. In locally advanced rectal cancer, preoperative chemo-radiotherapy (CRT) followed by radical surgery, is a standard-of-care (1). Pelvic CRT is frequently used in the treatment of cervical cancer (2). For localized prostate cancer, pelvic irradiation is often employed as a definitive or adjuvant treatment (3).

Unfortunately, irradiation of normal tissue within the radiation field is unavoidable. The use of three-dimensional conformal or intensity-modulated radiotherapy (IMRT), along with improvements in image guidance and patient immobilization, have reduced, but not eliminated, the incidence and severity of toxicities. In prostate cancer, modern radiotherapy methods have enabled dose escalation, leading to 95% local tumor control with <3% risk of chronic normal tissue complications (4). Utilization of extended field IMRT for cervix cancer, which included involved pelvic and para-aortic lymph nodes, led to a local control rate of 80%, with a 10% incidence of late grade 3 toxicity (5). Use of a simultaneous integrated boost, using IMRT, in 29 patients with gynecologic tumors led to a local control rate of 70%, with a 20%–30% incidence of grade 1–2 late toxicities, but no reported grade 3/4 toxicities (6). Further dose escalation in both scenarios is possible, but the risk of more severe late complications will also increase. Local control rates for rectal cancer are in the range of 90%–95% for modern conformal radiotherapy with surgery. The incidence of severe late complications is estimated to be between 5% and 10% (7, 8). In summary, dose escalation with conformal methods has improved local tumor control for prostate cancer, but did not increase normal tissue complication rates. In contrast, modern radiotherapy methods have improved local control for rectal and cervix cancer, but they have not lowered the complication rate to the level seen with prostate.

While the relative incidence of pelvic radiation disease is small, one has to consider the total number of patients affected. It is estimated that by 2030, the total number of patient survivors in the United States with irradiated pelvic tumors will be close to 1 million (9). This includes patients with cancers of the prostate (627,000), uterus (164,000), rectum (160,000), anus (49,000), and cervix (71,000). The total number of patients who could develop pelvic radiation disease is notable because of the number of projected survivors. For example, the incidence of chronic pelvic radiation injury for prostate cancer, assuming 627,000 survivors, is estimated to be between 6,300 and 19,000, assuming incidence rates between 1% and 3%. For the remaining cancers discussed above, the incidence of chronic pelvic radiation injury is projected to be 23,000–47,000, assuming a 5%–10% incidence rate. Furthermore, the incidence of these injuries will remain higher in resource limited countries where modern radiotherapy methods are not possible.

Organs-at-risk for chronic pelvic damage include the anus, rectum, prostate, gynecologic organs, bladder, pelvic bones, and small and large bowel. Damage to the small bowel, termed radiation enteropathy, or -enteritis, is the best-studied late effect. Concerns about small bowel toxicity commonly constrain the prescribed dose. Although there have
been a number of positive preclinical and clinical studies addressing treatment and prevention of chronic radiation injury, none have been translated to standard of care (10).

This review highlights drugs that could target key cell signaling pathways involved in both acute and chronic radiation injury. We examine both preclinical and clinical trials with relevant drugs and conclude with a discussion of current and potential trials that may reduce incidence or severity of pelvic radiation disease, while not protecting tumor. We focus on the subject of pelvic radiation disease, but the principles apply to chronic toxicities that occur in other irradiated tumor sites, such as lung, head, and neck and brain. The total number of 5 year cancer survivors treated with radiotherapy is estimated to be over 4 million by the year 2030 (9). Many of these survivors of diseases outside the pelvis are also at risk for normal tissue injury that will reduce quality of life.

Pathophysiology of Radiation-induced Injury

Development of injury to tissues after radiotherapy follows sequential effects that begin with damage manifestation in rapidly proliferating cell populations, such as mucosa. Mucosal changes after radiotherapy can be seen within hours. In the small bowel, death of intestinal ating cell populations, such as mucosa. Mucosal changes after radiotherapy begin with damage manifestation in rapidly proliferating cell populations, such as mucosa. Mucosal changes after radiotherapy begin with damage manifestation in rapidly proliferating cell populations, such as mucosa. Mucosal changes after radiotherapy begin with damage manifestation in rapidly proliferating cell populations, such as mucosa. Mucosal changes after radiotherapy begin with damage manifestation in rapidly proliferating cell populations, such as mucosa.

Injury post radiotherapy can contribute to a chronic wound-healing response contributing to fibrosis. NFkB is a rapid acting prosurvival (27), proinflammatory (28), and proimmune (29) transcription factor that controls expression of many proinflammatory cytokines. It is rapidly upregulated by bacterial products, radiation, and oxidative stress, among other factors. Although HIF-1α regulation is primarily posttranscriptional, NFκB controls its transcription rate (30). Damage to nerves after radiotherapy could also contribute to late tissue damage. Chronic effects of radiotherapy on pelvic nerve function have been studied in a canine model of stereotactic radiotherapy for prostate cancer (31). Reduction in neurofilament content of penile nerves was observed along with axonal degeneration and reduced nerve conduction velocity. Such effects could contribute to erectile dysfunction after radiotherapy. It is also likely that nerve injury is at least partially the product of microvascular damage to vessels that supply these larger nerves (32). Oxidative stress plays a major role in this pathophysiology (33).

Consistent with the chronic wound-healing response discussed above, there is increased fibrosis of the bowel after radiotherapy (34). Fibrosis contributes to: (i) altered intestinal transit, (ii) nutrient malabsorption, (iii) impaired gut motility, (iv) fistulae formation, (v) rectal bleeding, (vi) partial or complete bowel obstruction due to strictures, and (vii) perforation with sepsis (35). These chronic toxicities are not currently preventable or reversible in the clinic. As will be discussed below, mitigators in development could be effective in prevention or reduction in severity of pelvic radiation disease.
Figure 1 summarizes the primary factors that contribute to pelvic radiation disease and the functional consequences of this chronic damage.

**Patient-related Factors that Contribute to Pelvic Radiation Disease**

Comorbidities may contribute to higher incidence of chronic normal tissue injury post radiotherapy (36–38). A few examples are discussed below. These and others are summarized in Supplementary Table S1.

Eifel and colleagues examined records of nearly 3,500 patients with cervix cancer, treated with definitive radiotherapy between 1960 and 1994 (36). Although these patients were not treated with modern conformal methods, the results revealed important information about patient-related factors that can contribute to chronic injury. Factors that were associated with overall risk included race, body mass index (BMI), smoking history, and prior histories of pelvic inflammatory disease or venereal disease. Patients who smoked >1 pack per day exhibited an overall 2.3-fold increase in risk of chronic injury in multivariate analysis. The associated incidence of small bowel complications increased from 2% for nonsmokers to 11.1% for patients who smoked more than 1 pack per day. Hispanic race was associated with decreased overall risk, whereas history of pelvic infection increased overall risk of injury by 1.7 fold. Patients with BMI <22 were 1.29-fold more likely to experience chronic injury.

Willett and colleagues examined the incidence of acute and chronic bowel injury in patients with rectal cancer who underwent radiotherapy for pelvic malignancies (39). Patient-related factors considered in this analysis included age, BMI, history of previous surgery, smoking history, diabetes, hypertension, history of inflammatory bowel disease, connective tissue disease, regular NSAID use, and whether or not the patients also received chemotherapy. 193 patients were eligible for analysis at one year. There was weak association between acute symptom scores and incidence of pelvic radiation disease, but no statistically significant association between any of the patient-related factors and toxicity was observed. The study included patients with any pelvic malignancy. The heterogeneity of treatment received and site of radiotherapy may have contributed to the inability to discern importance of patient-related risk factors.

Huscher and colleagues examined the incidence of chronic small bowel toxicity among 806 patients treated postoperatively for endometrial or cervix cancer (40). Factors associated with chronic injury in univariate analysis included radiotherapy dose per fraction (> vs. <1.8 Gy), patient age (< vs. ≥60 years) and whether or not the patients developed grade 3–4 acute toxicity. The RRs for these three factors in multivariate analysis were 2.81, 1.02 (continuous variable, per year), and 3.01, respectively.

In summary, although there is heterogeneity in reporting of patient-related factors and probability for pelvic radiation disease, smoking history (36, 39), BMI (36, 39), history of prior inflammatory disease in...
the irradiated site (36, 37), hypertension (41), and age (40) were linked to risk of pelvic radiation disease. Diabetes (38) and collagen vascular disease (42) have been associated with increased risk for late radiotherapy damage in some reports. In one report, hypertension was associated with lower risk for pelvic radiation disease after prostate radiotherapy (41). Severity of acute symptoms has also been reported to be associated with greater risk for chronic damage (39, 40). However, acute toxicity data could not be used for patient selection a priori.

Treatment methods, such as radiotherapy dose/fraction, total radiotherapy dose, addition of chemotherapy, and efforts to reduce radiotherapy dose to key normal tissues are linked to risks for pelvic radiation disease. This article focuses on patient-related factors and therefore, these treatment-related factors will not be discussed further.

Polymorphisms may also contribute to elevated risk for normal tissue injury.

**TGFβ**

Recently, the C-509T polymorphism (22%–55% incidence, depending on race) of TGFβ was studied prospectively as a secondary endpoint in a series of 184 patients with breast cancer who had been followed for a minimum of 3 years post radiotherapy. In multivariate analyses, the C-509T polymorphism was associated with a 4.47-fold higher risk of developing grade 2 or greater breast fibrosis, compared with the wild-type allele (Table 1; ref. 43). The role that this polymorphism might play in pelvic radiation disease has not been reported.

**Plasminogen activator inhibitor-1**

It has been reported that endothelial plasminogen activator inhibitor-1 (PAI-1) contributes to acute and chronic gastrointestinal injury after radiotherapy to intestine (44). Clinically, genetic polymorphisms of PAI-1 (Table 1) affect the incidence of acute injury after radiation to patients with rectal cancer (45). The rs1050955 variants were associated with lower risk of diarrhea, with ORs of 0.395 for GG versus AA. The rs2227631 variants of PAI-1 were associated with higher risk for anal incontinence with ORs of 2.079 and 3.064 for the AG and GG SNPs, respectively, compared with the AA wild-type. These results strongly support the evaluation of potential mitigators of PAI-1 as a means to protect against acute and chronic intestinal injury after radiation, but caution should be used for patients with rs1050955 variants because they appear to have lower risk for acute effects of radiotherapy.

### Genome-wide association analysis of SNPs

Oh and colleagues used genome-wide association methods of SNPs to predict for incidence of rectal bleeding and impotence, both of which can occur after prostate radiotherapy (46). They argued that evaluation of individual SNPs may lead to false positive or negative results, because single SNPs may have only a small influence by themselves. The investigators deleted rare SNPs from this analysis, keeping those that have a reasonable prevalence in the population. The analysis was based on a cohort of 368 patients with irradiated prostate cancer who had been enrolled previously into a GWAS analysis protocol. Clinical risk factors were included in these models. Interestingly, there was no overlap in the final identification of 198 and 90 SNPs associated with the development of rectal bleeding or impotence, respectively. Prominent processes associated with rectal bleeding included genes involved in potassium, metal, and calcium ion transport and transmembrane transport. Prominent processes associated with impotence included leukocyte chemotaxis and migration. In a separate report, this same group performed a similar analysis for urinary symptoms after radiotherapy for prostate cancer (47).

### Preclinical models of pelvic radiation disease

Despite the fact that patient comorbidities and SNPs influence the incidence and severity of normal tissue damage, few pre-clinical studies to evaluate impact of these comorbidities on normal tissue damage after therapeutic radiation have been published. Ettarh and colleagues examined acute effects of radiotherapy on small bowel of streptozocin-induced diabetic mice, but found no evidence for differences in damage at this early time point (48). We were unable to find any murine studies where pelvic radiation disease was studied in the context of comorbidities. Murine models of autoimmune disease offer a controlled environment for studying the effects of genetic variance on pelvic radiation toxicity. 

### Table 1. Allele frequency of seven SNPs in four populations from the 1000 Genomes project and association with pelvic radiation disease.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Pos (hg38)</th>
<th>Gene</th>
<th>Ref Allele</th>
<th>Alt Allele</th>
<th>EUR Alt allele freq</th>
<th>AFR Alt allele freq</th>
<th>AMR Alt allele freq</th>
<th>EAS Alt allele freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2227631A</td>
<td>7</td>
<td>101126257</td>
<td>PAI-1</td>
<td>A</td>
<td>G</td>
<td>0.41</td>
<td>0.75</td>
<td>0.63</td>
<td>0.61</td>
</tr>
<tr>
<td>rs11718C</td>
<td>7</td>
<td>101137803</td>
<td>PAI-1</td>
<td>T</td>
<td>C</td>
<td>0.45</td>
<td>0.50</td>
<td>0.30</td>
<td>0.45</td>
</tr>
<tr>
<td>rs1050955A</td>
<td>7</td>
<td>101139179</td>
<td>PAI-1</td>
<td>G</td>
<td>A</td>
<td>0.21</td>
<td>0.25</td>
<td>0.48</td>
<td>0.47</td>
</tr>
<tr>
<td>rs32934A</td>
<td>5</td>
<td>76714881</td>
<td>PAI-1</td>
<td>C</td>
<td>T</td>
<td>0.06</td>
<td>0.06</td>
<td>0.09</td>
<td>0.41</td>
</tr>
<tr>
<td>rs2227744A</td>
<td>5</td>
<td>76714524</td>
<td>PAI-1</td>
<td>G</td>
<td>A</td>
<td>0.49</td>
<td>0.23</td>
<td>0.32</td>
<td>0.28</td>
</tr>
<tr>
<td>rs180179A</td>
<td>5</td>
<td>76734971</td>
<td>PAI-1</td>
<td>T</td>
<td>C</td>
<td>0.26</td>
<td>0.19</td>
<td>0.17</td>
<td>0.07</td>
</tr>
<tr>
<td>rs1800469A</td>
<td>19</td>
<td>41354391</td>
<td>TGFBI</td>
<td>G</td>
<td>A</td>
<td>0.31</td>
<td>0.22</td>
<td>0.46</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Abbreviations: AFR, Africans; Alt, alternative allele; AMR, admixed Americans; EAS, East Asians; EUR, Europeans; Pos, position; Ref, reference allele. Source: https://www.ncbi.nlm.nih.gov/snp/.

*Associated with lower risk of pelvic radiation disease.
*Associated with higher risk of pelvic radiation disease.
*Associated with pelvic radiation disease not reported.
Mitigating the Toxicities of Pelvic Radiation

Two important issues should be addressed when considering potential mitigators of radiotherapy damage. These two issues are relevant to all drugs: (i) scheduling of mitigators with radiotherapy and (ii) verifying that mitigators do not protect tumor against radiotherapy damage. Examples of these considerations are shown below.

Scheduling mitigators with radiotherapy

Scheduling of mitigators with radiotherapy depends upon the pharmacologic target and the pharmacokinetics/pharmacodynamics of the mitigator. The classical free radical scavenger, WR2721, had to be administered immediately before radiotherapy, because the targets were the free radicals generated by radiotherapy itself (61). Other agents target downstream tissue reactions to radiotherapy, such as chronic oxidative stress and activation of NFKB. Examples include an isoflavone, genistein, a salen-manganese (Mn) superoxide dismutase-catalase mimetic, and a redox-active Mn-porphyrin. Significant lung radioprotection was observed when genistein or the salen-Mn mimetic was administered concurrently or two weeks after whole thorax irradiation (68). Administration of the redox-active Mn-porphyrin for several days before radiotherapy was shown to achieve therapeutically active concentrations in tissue (69). This schedule of drug administration reduced neurocognitive loss (69, 70), mucositis, and xerostomia after radiotherapy to brain or head and neck (71), respectively. A similar compound partially ameliorated chronic radiation–induced lung damage, even when administered 8 weeks after radiation exposure (72).

Testing whether mitigators protect tumor from radiotherapy damage

Whenever radioprotective agents are used, one has to also determine whether the agents protect tumor. The redox-active Mn-porphyrin has been shown to protect normal tissue, but sensitize tumor to radiotherapy (70, 71). It has been hypothesized that the differences in tumor versus normal tissue response to this agent are related to differences in drug accumulation (much higher in tumors) and the effects of Mn-porphyrin on protein modifications that affect signal transduction downstream. Examples include NFKB, nuclear factor-like 2 (Nrf2), MAPK, and phosphatases (73).

Mitigation of oxidative stress and control of macrophage phenotype

Oxidative stress can promote the wound healing response by increasing HIF-1 transcriptional activity, as well as by directly contributing to tissue damage by oxidation. One of the major contributors to oxidative stress is the macrophage. The M1 phenotype contains activated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme that produces very high fluxes of superoxide (74). The M1 macrophage contributes to inflammation and tissue damage. The M2 macrophage, in contrast, is anti-inflammatory (75). RT increases infiltration of M1 macrophages into irradiated normal tissue in mice, contributing to radiation pathology (44). Isolavones (75) and the redox-active Mn-porphyrin downregulate M1 infiltration into tissue (70).

Mice with PAI-1 knockout of endothelial cells are less sensitive to RT, and experience reduced severity of late fibrosis (44). The protective effect of PAI-1 knockout is associated with a reduction in M1 macrophages and an increase in M2 content in irradiated bowel. These pre-clinical and clinical results strongly support the evaluation of potential mitigators of the PAI-1 response as a means to protect against acute and chronic intestinal injury after radiation.

However, one needs to insert a cautionary note. Infiltration of M1 macrophages into tumors is a desired outcome after radiation because...
Table 2. Summary of quantitative survival benefit of mitigators in preclinical studies.

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Agent</th>
<th>Class of agent</th>
<th>Mechanism of action</th>
<th>Species/strain</th>
<th>Drug dosing schedule</th>
<th>RT dose(s)</th>
<th>Results</th>
<th>Tumor effects/comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>Zhang (85)</td>
<td>Fibroblast growth factor (FGF)-2 peptide</td>
<td>Synthetic peptide</td>
<td>Promote stem cell renewal, progenitor cell differentiation, and epithelial proliferation</td>
<td>4 strains of mice (BALB/c, C57BL/6, C3H/NeN, and NIH Swiss)</td>
<td>10 min–4 hr post-RT and daily for 5 days; i.m. injection</td>
<td>10.5–16 Gy</td>
<td>0% survival for RT alone vs. 33–50% survival for RT+FGF-2 by 14 days after RT in all 4 strains of mice;</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Saha (86)</td>
<td>Bone marrow–derived adherent stromal cell transplantation (BMSCT)</td>
<td>Stem cell</td>
<td>Induce intestinal stem cell regeneration</td>
<td>C57BL/6 mice</td>
<td>24–72 hr post-RT; i.v. injection</td>
<td>10.4 Gy (whole body);</td>
<td>0% survival for RT alone vs. 100% survival for RT+BMSCT after 10.4 Gy whole body irradiation or 18 Gy abdominal irradiation beyond 25 days;</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Berbee (87)</td>
<td>Pentoxifylline (PTX) + Vitamin E γ-tocotrienol (GT3)</td>
<td>Xanthine derivative</td>
<td>Phosphodiesterase inhibitor</td>
<td>Male CD2F1 mice</td>
<td>GT3-22–24 hr pre RT; PTX-15–30 min pre-RT; s.c. injection</td>
<td>8.5, 10.5, 11.5 and 12.5 Gy</td>
<td>11 median survival times for GT3 alone vs. 30 for GT3+PTX in 12.5 Gy exposure; GT3+PTX did not reduce GI toxicity compared to GT3 alone.</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Fu (65)</td>
<td>SOM230</td>
<td>Somatostatin analogue</td>
<td>In cells with somatostatin receptors, inhibit cell proliferation and secretion of pancreatic enzymes</td>
<td>Male CD2F1 mice</td>
<td>24, 48, or 72 hr post-RT</td>
<td>8.5–11 Gy</td>
<td>15–18.5 day survival time for RT alone vs. 30 day RT+SOM230 in case of 48 hr post-RT at 9–9.5 Gy; 50% reduction in trypsin secretion by SOM230</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Li (88)</td>
<td>Mn (II) meso-tetrakis(N-ethylpyridinium-2-yi) porphyrin (MnTE)</td>
<td>Redox-active MnPorphyrin</td>
<td>Reduce oxidative stress Inhibit radiation-induced hematopoietic stem cell senescence through reactive oxygen species-p16 pathway</td>
<td>C57BL/6 male mice</td>
<td>6 hr post-RT and every day for 30 days; s.c. injection</td>
<td>6.5 Gy</td>
<td>No survival data Sister compound with similar properties is in human clinical trials - Mn (II) meso-tetrakis(N-n-butoxyethylpyridinium-2-yi)porphyrin (MnBuOE) No tumor radioprotection</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>Ajakaiye (89)</td>
<td>MFG-E8</td>
<td>Secreted glycoprotein</td>
<td>Upregulate p53, p21, and Bcl-2</td>
<td>Male Sprague-Dawley rats</td>
<td>6, 30, and 54 hr post-RT; s.c. injection</td>
<td>10 Gy</td>
<td>31% survival RT alone vs. 75% survival for RT+MFG-E8 by 21 days.</td>
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<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
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<th>Drug dosing schedule</th>
<th>RT dose(s)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>Rotolo (90)</td>
<td>2A2</td>
<td>Anti-ceramide mAb</td>
<td>BALB/c mice</td>
<td>15 min pre-RT; IV injection</td>
<td>15–16 Gy</td>
<td>0% 90-day survival for RT alone vs. 80% and 60% 90-day survival for RT+2A2 at 15 Gy and 16 Gy, respectively;</td>
</tr>
<tr>
<td>2012</td>
<td>Basile (91)</td>
<td>Recombinant IL12</td>
<td>Interleukin</td>
<td>Mice/C57BL6/rhesus monkey</td>
<td>24 and 72 hr post-RT</td>
<td>8 Gy-mice, 6.7 Gy-monkey (LD50/30)</td>
<td>Mice-14% survival RT alone vs. 87.5% for RT+IL12; EPO maintained 10X, gut stem cells (LGR5 marker).</td>
</tr>
<tr>
<td>2013</td>
<td>Wang (92)</td>
<td>Vanadate</td>
<td>Inorganic vanadium compound</td>
<td>C57BL/6 female mice</td>
<td>Immediately or 15–240 min post-RT; i.p. injection</td>
<td>7.5 Gy</td>
<td>No survival data</td>
</tr>
<tr>
<td>2014</td>
<td>Taniguchi (77)</td>
<td>DMOG</td>
<td>Prolyl hydroxylase domain inhibitor protein</td>
<td>C57BL/6 mice</td>
<td>24 hr pre-RT and daily post-RT</td>
<td>16–20 Gy</td>
<td>None of control mice survived beyond 10 days vs. 67% of mice treated with DMOG survived beyond 60 days after 20 Gy; DMOG mitigates death from GI syndrome</td>
</tr>
<tr>
<td>2014</td>
<td>Alexeev (79)</td>
<td>RTA 408</td>
<td>Triterpenoid</td>
<td></td>
<td>24 hr pre, 1 hr pre, 24 and 48 hr post-RT; i.p. route</td>
<td>8 Gy</td>
<td>8 Gy alone-no survivors by 24 days; 8 Gy+RTA 408—100% survival; crypt proliferation maintained; no apoptosis in drug group;</td>
</tr>
<tr>
<td>2014</td>
<td>Coleman (80)</td>
<td>Mn macrocyclic complex (GC4401)</td>
<td>Superoxide dismutase mimetic</td>
<td>C57BL/6</td>
<td>30 min before each fraction; i.p. route</td>
<td>2 × 2 Gy</td>
<td>No survival data Recently received FDA approval as a radioprotectant for mucositis</td>
</tr>
<tr>
<td>2015</td>
<td>Ramou (44)</td>
<td>PAI-1</td>
<td>Anti-fibrinolytic and profibrotic protein</td>
<td>C57BL/6/J female mice</td>
<td>7 days pre-RT</td>
<td>19 Gy</td>
<td>40% survival for RT alone vs. 75% survival for RT+deletion of PAI-1 within two weeks;</td>
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<table>
<thead>
<tr>
<th>Year</th>
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</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>Wang (93)</td>
<td>Ghrelin</td>
<td>Amino acid peptide</td>
<td>Stimulate pituitary growth hormone secretagogue receptor (GHSR 1α)</td>
<td>Male Sprague-Dawley rat</td>
<td>24 or 48 hr post-RT and daily for 6 days; s.c. injection</td>
<td>10 Gy</td>
<td>30% 30-days survival for RT alone vs. 61% and 63% 30-days survival for RT+Ghrelin starting at 48 hr and 24 hr post-RT, respectively;</td>
<td>30% 30-days survival for RT alone vs. 61% and 63% 30-days survival for RT+Ghrelin starting at 48 hr and 24 hr post-RT, respectively;</td>
</tr>
<tr>
<td>2015</td>
<td>Krivokrysenko (94)</td>
<td>Entolimod</td>
<td>Recombinant protein</td>
<td>Toll-like receptor 5 agonist, activate NF-kB</td>
<td>Macaca mulatta</td>
<td>1-48 hr post-RT; i.m. injection</td>
<td>11 Gy</td>
<td>37% 40-day survival RT alone vs. 83% 40-day survival for RT+Entolimod at 25 hr post-RT.</td>
<td>37% 40-day survival RT alone vs. 83% 40-day survival for RT+Entolimod at 25 hr post-RT.</td>
</tr>
<tr>
<td>2016</td>
<td>Xu (95)</td>
<td>Adult bone marrow stromal stem cells (ABMSCs)</td>
<td>Stem cell</td>
<td>Increase IL10, but inhibit IL17</td>
<td>Female beagle</td>
<td>48 hr post-RT; arterial perfusion</td>
<td>14 Gy</td>
<td>All dogs in control group died within 16–23 days vs. 100% survival beyond 35 days in ABMSC-treated group; significant reduction of diarrhea and bloody stools in treated group</td>
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</tr>
<tr>
<td>2016</td>
<td>Shainer (96)</td>
<td>Preimplantation factor (PIF)</td>
<td>Embryo-secreted 15-amino acid peptide FGF</td>
<td>Activate macrophages and T cell proliferation</td>
<td>C57BL/6 and F1 mice</td>
<td>2 hr post-RT and daily for 14 days; i.v. injection</td>
<td>8 Gy</td>
<td>0% survival for RT alone vs. 100% survival for RT+PIF by day 29;</td>
<td>0% survival for RT alone vs. 100% survival for RT+PIF by day 29;</td>
</tr>
<tr>
<td>2017</td>
<td>Castillo (97)</td>
<td>Protected graft copolymer formulated FGF4 and 7 (PF4/7)</td>
<td>Embryo-secreted 15-amino acid peptide FGF</td>
<td>Activate macrophages and T cell proliferation</td>
<td>Male C57BL/6 and female CD1 mice</td>
<td>24 hr post-RT and daily for 7 days; s.c. injection</td>
<td>14.5-16.5 Gy</td>
<td>60% survival for sham (untreated) vs. 76.7% PF4/7 vs. 93.9% PF4 or PF7 vs. 96.6% Amifostine (positive control) Treatment with PF4/7 had no survival benefit over control group due to dose-related lack of sustained receptor saturation</td>
<td>60% survival for sham (untreated) vs. 76.7% PF4/7 vs. 93.9% PF4 or PF7 vs. 96.6% Amifostine (positive control) Treatment with PF4/7 had no survival benefit over control group due to dose-related lack of sustained receptor saturation</td>
</tr>
<tr>
<td>2017</td>
<td>Shi (98)</td>
<td>Seabuckthorn pulp and seed oils</td>
<td>Natural herb</td>
<td>Reduce intestinal apoptosis</td>
<td>C57BL/6 mice</td>
<td>8 am, once per day for 7 days pre-RT; orally administered</td>
<td>7.5 Gy</td>
<td>4.5 median survival days for control olive oil group vs. 8 days for seed oil group vs. 10 days for pulp oil group, respectively;</td>
<td>4.5 median survival days for control olive oil group vs. 8 days for seed oil group vs. 10 days for pulp oil group, respectively;</td>
</tr>
</tbody>
</table>

Abbreviations: hr, hours; i.m., intramuscularly; i.p., intraperitoneally; min, minutes; PTX, pentoxifylline; RT, radiotherapy.
Mitigating the Toxicities of Pelvic Radiation

Table 3. Summary of clinical trials of putative mitigators of radiation toxicity in human studies.

<table>
<thead>
<tr>
<th>Protocol no.</th>
<th>Therapeutic drug target &amp; primary outcome variable</th>
<th>Trial type</th>
<th>Status</th>
<th>Disease being studied</th>
<th>Accrual</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00583700</td>
<td>Pentoxifylline (PTX) + Vitamin E γ-tocotrienol (GT3)</td>
<td>Phase II</td>
<td>Completed</td>
<td>Breast cancer</td>
<td>2007-2010</td>
<td>SOMA score between control arm and PTX-GT3 arm is 1.59 and 1.0, respectively.</td>
</tr>
<tr>
<td>NCT00813592</td>
<td>SOM230</td>
<td>Phase II</td>
<td>Terminated</td>
<td>Meningioma</td>
<td>2008-2015</td>
<td>Only 2 patients enrolled - Reported toxicities Hyperglycemia, foot pain and pneumonia</td>
</tr>
<tr>
<td>NCT03386500</td>
<td>BMX-001 Modify oxidative stress</td>
<td>Phase 1</td>
<td>Recruiting</td>
<td>Anal cancer</td>
<td>2017-2020</td>
<td>No results reported</td>
</tr>
<tr>
<td>NCT02990468</td>
<td>BMX-001 Modify oxidative stress</td>
<td>Phase 1 and 2</td>
<td>Active, not recruiting</td>
<td>Head and neck cancer</td>
<td>2017-2020</td>
<td>No results reported</td>
</tr>
<tr>
<td>NCT02655601</td>
<td>BMX-001 Modify oxidative stress</td>
<td>Phase 2</td>
<td>Recruiting</td>
<td>High-grade glioma</td>
<td>2018-2021</td>
<td>No results reported</td>
</tr>
<tr>
<td>NCT03608020</td>
<td>BMX-001 Modify oxidative stress</td>
<td>Phase 1, followed by randomized phase 2</td>
<td>Recruiting</td>
<td>Patients with multiple brain metastases</td>
<td>2018-2022</td>
<td>No results reported</td>
</tr>
<tr>
<td>NCT02142995</td>
<td>RTA 408 lotion</td>
<td>Randomized phase II</td>
<td>Completed</td>
<td>Breast cancer</td>
<td>2014-2015</td>
<td>Results not reported</td>
</tr>
<tr>
<td>NCT01921426</td>
<td>Mn macrocylic complex (GC4419)</td>
<td>Phase 1</td>
<td>Completed</td>
<td>Head and neck cancer</td>
<td>2013-2015</td>
<td>Recently received FDA approval as a radioprotectant for mucositis</td>
</tr>
<tr>
<td>NCT01728480</td>
<td>Entolimod Toll-like receptor 5 agonist, activate NF-κB</td>
<td>Phase 1</td>
<td>Withdrawn</td>
<td>Head and neck cancer</td>
<td>2014-2015</td>
<td>Results not reported</td>
</tr>
<tr>
<td>NCT01472432</td>
<td>Vildagliptin upregulates HIF-1 and VEGF Promote diabetic ulcer healing</td>
<td>Randomized completed</td>
<td>Diabetic foot ulcer</td>
<td>2008-2011</td>
<td>Drug facilitated diabetic ulcer healing by 2-fold HIF-1 &amp; VEGF upregulated</td>
<td></td>
</tr>
</tbody>
</table>

M1 macrophages contribute to the innate immune response (74). In contrast, M2 macrophages contribute to immune tolerance and promote tumor angiogenesis and metastasis. Thus, the desired balance of M1/M2 after RT is opposite for tumor vs. normal tissue. Future studies should evaluate both M1/M2 populations in tumor and normal tissue RT responses when combined with drugs to ameliorate the PAI-1 response.

HIF-1 levels increase in tumors after radiation and this increase protects tumors from radiation damage (76). The story with irradiated normal tissue may be quite different from tumor. Taniguchi and colleagues reported that inhibition of the prolyl hydroxylases that are responsible for priming HIF-1 and HIF-2 for proteasomal degradation, protect bowel against the acute effects of radiation (77). HIF-2 was found to be the primary transcription factor responsible for gastrointestinal mortality. Dimethyloxalylglycine (DMOG), an oxoglutarate analogue, inhibits the prolyl hydroxylases, stabilizing both HIF-1α and HIF-2α. DMOG exhibited profound radioprotection of acute injury to bowel after radiation exposure via a HIF-2 mediated mechanism. In contrast, DMOG did not protect tumors from RT.

In Table 2, we indicate which agents have been studied for radioprotective effects preclinically in normal tissues and which have also been evaluated for tumor radioprotection. In Table 3, we used clinicaltrials.gov to identify: 1) clinical trials that have tested potential mitigators in combination with RT. 2) other trials where relevant drugs were used, but not in conjunction with RT. We have included trials that are currently open as well as some that are now closed. Listed below are a few examples of potentially promising agents.

**Pentoxifylline and Vitamin E:** NCT00583700: This combination was studied in a randomized trial of women with breast cancer who were treated with radiotherapy. Results have not been reported.

**RTA 408 (omaveloxolone):** NCT02142959: This drug is an inhibitor of NFκB (79). It was studied in a randomized trial for its protective effect in mitigating radiation-induced dermatitis in women with breast cancer who were treated with radiotherapy. Results have not been reported.
BMX-001: BMX-001, a redox-active Mn porphyrin, is currently in four trials. A phase I trial (NCT03608020) is a lead in trial to a randomized phase II trial of patients with multiple brain metastases, undergoing radiotherapy. A phase II trial (NCT02655601) examines whether BMX-001 protects against neurocognitive loss after temozolomide + radiotherapy for the treatment of primary glioblastoma. Two other trials test whether BMX-001 can protect mucosa and salivary glands from acute and chronic damage, respectively, after CRT for head and neck cancer (NCT02990468) or mucosal radioprotection for anal cancer (NCT03386500).

GC4419: GC4419 is a Mn–macrocyclic complex and a superoxide dismutase mimetic (80). This drug selectively converts superoxide anion to peroxide. The initial phase I trial of this agent was completed (NCT01921426). GC4419 was recently approved by the FDA for the prevention of mucositis patients with in head and neck cancer undergoing CRT.

Considerations for Clinical Trial Designs to Test Mitigators of Pelvic Radiation Disease

As discussed above, there are several new agents that may prevent or reduce severity of both acute and chronic injury. These could be tested in clinical trials relatively easily, given that several are being or have been studied in human patients in other contexts already. As chronic radiation toxicities can develop over longer time periods, and occur in a minority of patients, designing and funding a clinical trial that shows therapeutic benefit in all-comers is challenging. One option would be to identify patients at greater risk for developing chronic injury. The previously discussed polymorphisms of PAI-1 and TGF
eralds the prevalence of SNPs for PAI-1 and TGF

Conclusions

Technological improvements in radiotherapy have decreased the risk of long-term side effects, however, not eliminated pelvic radiation disease in all patients. It is hoped that many of the approaches discussed in this article will move into well-designed clinical trials of mitigators of pelvic radiation disease, with an ultimate goal of allowing for optimal dose to targets with a concurrent further reduction of chronic toxicity risk.

Disclosure of Potential Conflicts of Interest

M. W. Dewhirst reports receiving commercial research grants from Biomimetix and is a coinventor for the use of BMX-001 for oncology applications (this patent application has been filed with the U.S. Patent Office). Duke University holds the patent for BMX-001, a redox-active Mn porphyrin, discussed in this paper. No potential conflicts of interest were disclosed by the other authors.

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References

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