Radiotherapy and Oncology 168 (2022) 75–82

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original Article

Use of angiotensin converting enzyme inhibitors is associated with reduced risk of late bladder toxicity following radiotherapy for prostate cancer

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ARTICLE INFO

Article history:
Received 30 July 2021
Received in revised form 10 January 2022
Accepted 12 January 2022
Available online 22 January 2022

ABSTRACT

Background and purpose: Genome-wide association studies (GWAS) of late hematuria following prostate cancer radiotherapy identified single nucleotide polymorphisms (SNPs) near *AGT*, encoding angiotensinogen. We tested the hypothesis that patients taking angiotensin converting enzyme inhibitors (ACEi) have a reduced risk of late hematuria. We additionally tested genetically-defined hypertension.

Materials and methods: Prostate cancer patients undergoing potentially-curative radiotherapy were enrolled onto two multi-center observational studies, URWCI (N = 256) and REQUITE (N = 1,437). Patients were assessed pre-radiotherapy and followed prospectively for development of toxicity for up to four years. The cumulative probability of hematuria was estimated by the Kaplan-Meier method.

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https://doi.org/10.1016/j.radonc.2022.01.014

Multivariable grouped relative risk models assessed the effect of ACEi on time to hematuria adjusting for clinical factors and stratified by enrollment site. A polygenic risk score (PRS) for blood pressure was tested for association with hematuria in REQUITE and our Radiogenomics Consortium GWAS.

Results: Patients taking ACEi during radiotherapy had a reduced risk of hematuria (HR 0.51, 95%CI 0.28 to 0.94, p = 0.030) after adjusting for prior transurethral prostate and/or bladder resection, heart disease, pelvic node radiotherapy, and bladder volume receiving 70 Gy, which are associated with hematuria. A blood pressure PRS was associated with hypertension (odds ratio per standard deviation 1.38, 95%CI 1.31 to 1.46, n = 5,288, p < 0.001) but not hematuria (HR per standard deviation 0.96, 95%CI 0.87 to 1.06, n = 5,126, p = 0.41).

Conclusions: Our study is the first to show a radioprotective effect of ACEi on bladder in an international, multi-site study of patients receiving pelvic radiotherapy. Mechanistic studies are needed to understand how targeting the angiotensin pathway protects the bladder.

 Radiotherapy and Oncology 168 (2022) 75–82

Radiotherapy is used with curative-intent in ~50% of prostate cancer cases [1], but late toxicities adversely impact quality-of-life. Hemorrhagic radiation cystitis, defined by gross hematuria, can be a severe and life-threatening complication [2]. The biologic mechanisms are poorly understood, hindering identification of molecular targets for protection or mitigation.

Inherited genetic variation, such as single nucleotide polymorphisms (SNPs), can affect risk of radiotherapy toxicity [3]. A genome-wide association study (GWAS) among prostate cancer radiotherapy cohorts (N = 3,871) identified SNPs near *AGT* associated with late hematuria, and these SNPs alter expression of *AGT* mRNA in various tissues [4]. *AGT* encodes angiotensinogen, which is converted to angiotensin by the angiotensin converting enzyme (ACE). The renin-angiotensin system (RAS) is involved in multiple normal and pathophysiologic processes like hypertension, inflammation, and fibrosis [5]. Studies suggest the RAS affects radiation-induced blood vessel injury and fibrosis [6], making it an attractive target for interventions aimed at preventing or mitigating radiation effects in the bladder.

Animal and human studies suggest ACE inhibitors (ACEi) are radioprotective in late responding normal tissues [7], though studies evaluating effects on the bladder are lacking. Some clinical studies reported an association between hypertension and risk of late toxicity bladder and/or rectal toxicity in patients receiving pelvic radiotherapy [8,9], but others reported none [10,11]. Others have reported an association with use of antihypertensives [8,12], but the mechanistic basis for this finding is unclear as the multiple classes of antihypertensive drugs were not analyzed separately. It is difficult to disentangle any direct effect of hypertension from the effect of ACEi, other anti-hypertensive medications, or co-occurring morbidities and health behaviors.

ACEi are a promising class of medication for radioprotection, but additional studies are needed to evaluate effects on bladder radiotoxicity and in relation to hypertension. We tested the hypothesis that ACEi protect against the development of late hematuria using data from two prospective longitudinal studies of prostate cancer radiotoxicity. We additionally undertook a Mendelian randomization study [13] to evaluate a direct role of hypertension, represented by genetic propensity, thereby minimizing confounding due to co-morbidities, health behaviors, and other factors, which are difficult to fully and accurately capture.

Materials and methods

Study population

The URWCI study recruited patients with prostate cancer between April 2016 and March 2019 from two hospitals in the United States (University of Rochester Wilmot Cancer Institute and the Medical College of Wisconsin). REQUITE [14] recruited patients with prostate cancer between April 2014 and October 2016 from eight countries: the United Kingdom (Manchester, Leicester), Spain (Santiago de Compostela), the Netherlands (Maastricht), Italy (Milan and Candiolo), Germany (Mannheim, Ludwigshafen, Karlsruhe, Freiburg), Belgium (Ghent, Leuven), and the United States (New York City). REQUITE also recruited participants from France, but data on ACEi use were not available and so they were excluded from the present analysis. Inclusion criteria for both cohorts were: prostate cancer diagnosis, curative-intent radiotherapy, no evidence of distant metastases, ability to provide a venous blood sample, ability to read/write in the primary language of the enrollment country and give informed consent. Exclusion criteria for both cohorts were: prior pelvic radiotherapy. URWCI additionally excluded patients with prior or concurrent bladder or colorectal cancer; REQUITE excluded patients with any other malignancy in the last 5 years except basal cell or squamous cell carcinoma of the skin as well as patients with limited life expectancy due to co-morbidity and/or known HIV infection/infectious hepatitis. No patients in either study received protons or High Intensity Focal Ultrasound. Both studies were approved by local ethics committees and all participants provided written, informed consent.

All participants were scheduled to receive external beam radiotherapy with or without brachytherapy boost or brachytherapy alone, according to local standard of care. Prior prostatectomy and/or hormone therapy was allowed. Participants were enrolled before or during the first week of radiotherapy and were assessed at the end of radiotherapy and annually (up to 3 years in REQUITE; up to 4 years in URWCI, which also included a 6-month assessment). URWCI collected patient reported outcomes (PROs) using the Expanded Prostate Index Composite (EPIC) [15] supplemented with questions on radiation-related symptoms and quality of life. REQUITE used a study-specific validated PRO questionnaire, described previously [14]. In both studies, PRO questionnaires were completed before/on the first day of radiotherapy and at each follow-up. Demographic, clinical, and treatment data were abstracted from electronic medical records. ACEi medication use was collected longitudinally, from the start of radiotherapy until end of study follow-up. Hypertension was determined by clinical diagnosis prior to or at the time of radiotherapy.

Radiation bladder dose was represented as the percent bladder volume receiving either 40, 50, 60, 65, 70, or 75 Gy. To account for variation in dose per fraction among patients, the dose-volume histogram cut-point representing the dose equal to the given threshold delivered in 2 Gy fractions was selected for each patient considering the linear-quadratic model in its formulation including treatment time correction as follows:

$$Y = (\text{D Gy} - \gamma^{*}\Delta t) * ((\alpha/\beta + 2\ \text{Gy})/(\alpha/\beta + X))$$

where Y is the dose-volume histogram cut-point that should be selected to represent the equivalent dose to D Gy (for each of 40,

50, 60, 65, 70, or 75 Gy) in 2 Gy fractions, Δt is the difference between the number of treatment days for a given patient and the number of treatment days for the average treatment plan among all patients, and X is the daily fraction size received. The present analysis used $\alpha/\beta = 3$ Gy and $\gamma = 0.7$ Gy/day (weighted mean of values reported in Fiorino et al [12] for the late urinary endpoints).

Data in URWCI were managed using REDCap electronic data capture tools hosted at the University of Rochester.[16,17] Study data in REQUITE were managed using an online centralized database system (OpenClinica, https://openclinica.com; LimeSurvey, https://limesurvey.org), as previously described [14].

Radiotherapy toxicity

Patient-reported late hematuria was defined at each assessment using the PRO questionnaire. Participants in URWCI were asked (1) "Over the past 4 weeks, how often have you urinated blood?", and (2) "Have you needed medication or treatment for blood in urine?". A response of "More than once a week", "About once a day", or "More than once a day" to item 1, or a response of "Yes" to item 2 indicated the presence of hematuria. A response of "Rarely or never" to item 1 and a response of "No" to item 2 indicated the absence of hematuria. Participants in REQUITE were asked (1) "Have you had any blood in your urine during the past 2 weeks", and (2) "If yes, needed treatment for blood in urine?". A response of "Yes" to item 1, or a response of "Yes" to item 2 indicated the presence of hematuria. A response of "No" to both items indicated the absence of hematuria.

Rectal bleeding was evaluated as a secondary outcome, based on prior studies of hypertension [8,9,11,18,19] and ACEi [20], and was defined at each assessment using the PRO questionnaire. Participants in URWCI were asked (1) "How often have you had bloody stools during the last 4 weeks?", and (2) "Have you used medication or received treatment for bloody stools?". A response of "About half the time", "Usually", or "Always" to item 1, or a response of "Yes" to item 2 indicated the presence of rectal bleeding. A response of "Rarely" or "Never" to item 1 and a response of "No" to item 2 indicated the absence of rectal bleeding. Participants in REQUITE were asked (1) "Have you had any blood when you opened your bowels during the past 2 weeks", and (2) "If yes, needed treatment for blood in bowels?". A response of "Yes" to item 1, or a response of "Yes" to item 2 indicated the presence of rectal bleeding. A response of "No" to both items indicated the absence of rectal bleeding.

Toxicities were coded as time from radiation start to onset of first occurrence of gross bleeding and/or treatment for bleeding with time coarsened into discrete intervals used for study follow-up assessment. Acute symptoms occurring $\leq$3 months after starting radiotherapy were not considered, as the hypothesis pertained to late toxicity. Participants with gross hematuria (N = 32, 1.9%) or rectal bleeding (N = 46, 2.7%) present at the pre-radiotherapy assessment were excluded from analyses.

A secondary analysis used clinician-assigned CTCAEv4.0 grade of hematuria or rectal bleeding dichotomized to compare grade 2 or worse toxicity to grade 0 or 1. Assessments were made at the same follow-ups that PROs were collected.

Blood pressure polygenic risk score (PRS)

Mendelian randomization analysis tested a PRS comprising 882 of 901 SNPs previously associated with blood pressure [21] for association with radiotherapy toxicity in REQUITE and Radiogenomics Consortium GWAS cohorts (RAPPER N = 2,149 [3,4]; RADIOGEN N = 676 [4,22]; GenePARE N = 511 [4]; and UGhent N = 315 [4]). Of the published 901 SNPs, 19 were excluded during quality control filtering. The PRS was calculated for each study participant by multiplying the number of risk alleles for each SNP by the regression coefficient obtained from meta-analysis of systolic blood pressure in the UK Biobank [21], and then summing weighted values across all 882 SNPs. Late hematuria and rectal bleeding were previously defined in the Radiogenomics Consortium GWAS cohorts [4].

Statistical analysis

Descriptive statistics compared demographic and clinical factors by ACEi use. Time to report of toxicity was estimated by the Kaplan-Meier method, and the logrank test assessed differences in toxicity by ACEi. Bivariate grouped relative risk models assessed the effects of demographic and clinical factors on time to hematuria. Multivariable grouped relative risk models assessed the effects of ACEi use on time to hematuria, adjusting for demographic and clinical factors with $p < 0.1$ on bivariate analysis. Bladder volume receiving a given dose was derived from dose-volume-histograms capturing external beam radiotherapy dose and does not account for dose received from brachytherapy, thus, the bivariate model assessing the effect of the bladder volume receiving a given dose and the final multivariable model excluded patients who received brachytherapy alone (N = 76).

Logistic regression modeled hypertension (defined as current or past diagnosis at time of radiotherapy) as a function of the blood pressure PRS in REQUITE and the Radiogenomics Consortium cohorts. Grouped relative risk models assessed the distribution of time to late toxicity as a function of the PRS.

All models were stratified by enrollment site (defined by country). Grouped relative risk models used the Efron method to handle ties in event times. All statistical tests are two-sided. StataSE v15.1 was used for statistical analyses (StataCorp, LLC; College Station, Texas).

Results

Characteristics of the 1,693 study participants (N = 1,437 from REQUITE, N = 256 from URWCI) by ACEi use are in Table 1, and patients included/excluded from analysis are described in Supplemental Fig. 1. Compared with participants not taking an ACEi at the start of radiotherapy (N = 1,255; 74.1%), those on ACEi (N = 438; 25.9%) were older, had a higher BMI and were more likely to be diabetic, have heart disease, or have hypertension ($p < 0.05$; Table 1). Fewer patients on ACEi had a prior prostatectomy and fewer received a brachytherapy boost compared with those not on ACEi ($p < 0.05$; Table 1). Among those receiving external beam radiotherapy alone, the median bladder volume receiving 70 Gy was slightly lower in patients on vs. not on ACEi (8.9% vs. 10.3%, $p = 0.01$).

Patients were followed for a median of two years (maximum four years) after starting radiotherapy for development of hematuria. The estimated 4-year cumulative probability of developing hematuria among participants taking ACEi at the start of radiotherapy was 4.8% compared with 16.5% among those not taking ACEi (HR 0.52, 95% CI 0.30 to 0.93, site-stratified logrank p = 0.025; Fig. 1). Participants who started an ACEi after radiotherapy (N = 33) had a cumulative probability of hematuria similar to those who were not taking an ACEi during radiotherapy or follow-up (10.0% vs. 18.0%, site-stratified logrank p = 0.83), while those who were on an ACEi at the start of radiotherapy had similar protection from hematuria regardless of whether the ACEi was started >6 months (N = 253) or < 6 months (N = 144) prior to starting radiotherapy (2.8% vs. 6.0%, site-stratified logrank p = 0.80), though event numbers were small for these sub-group comparisons. There was insufficient evidence that ACEi affected rectal bleeding. The

cumulative probability of rectal bleeding was 17.2% among those taking vs 34.7% among those not taking an ACEi (HR 0.85, 95% CI 0.64 to 1.14, site-stratified logrank $p = 0.27$).

When toxicity was assessed using clinician-assigned CTCAE grades, ACEi showed a similarly protective association with grade 2 or worse hematuria (HR 0.13, 95% CI 0.02 to 0.94, $p = 0.043$) and lack of association with grade 2 or worse rectal bleeding (HR 1.03, 95% CI 0.77 to 1.39, $p = 0.82$). Patients taking ACEi during radiotherapy had a non-significant reduced risk of experiencing other bladder toxicity symptoms including urinary retention (HR 0.83, 95% CI 0.55 to 1.26, $p = 0.38$), dysuria (HR 0.74, 95% CI 0.45 to 1.25, $p = 0.27$), and increased urinary frequency (HR 0.93, 95% CI 0.63 to 1.36, $p = 0.70$) relative to patients not taking an ACEi.

On bivariate analyses, prior transurethral resection of the prostate (TURP), prior transurethral resection of the bladder (TURB), heart disease, and precent of the bladder volume receiving V70

Table 1
Patient demographic and clinical characteristics, overall and by ACEi use during radiotherapy.

Characteristic, *N* (%)	Total *N* = 1,694	Using ACEi at RT start *N* = 438	Not using ACEi at RT start *N* = 1,255	*P*-value[a]
Age (years) at radiotherapy, median [range]	70 [42 to 86]	71 [51 to 86]	69 [42 to 86]	<0.001
Race				0.11
White	1,622 (95.8)	419 (95.7)	1,203 (95.9)	
Black/African American	42 (2.5)	15 (3.4)	27 (2.2)	
Other/not specified	29 (1.7)	4 (0.9)	25 (2.0)	
BMI[b]				<0.001
Median [Range]	27 [17 to 59]	28 [19 to 50]	27 [17 to 59]	
Underweight	6 (0.4)	0	6 (0.5)	
Normal/healthy weight	390 (23.3)	73 (17.0)	317 (25.5)	
Overweight	816 (48.8)	196 (45.6)	620 (49.9)	
Obese	460 (27.5)	161 (37.4)	299 (24.1)	
Smoking status[c]				0.12
Never	702 (41.6)	165 (37.8)	537 (43.0)	
Former	808 (47.9)	219 (50.1)	589 (47.1)	
Current	177 (10.5)	53 (12.1)	124 (9.9)	
Diabetes				<0.001
Yes	240 (14.2)	102 (23.3)	138 (11.0)	
No	1,453 (85.8)	336 (76.7)	1,117 (89.0)	
Autoimmune disease[d]				0.42
Yes	88 (5.2)	26 (5.9)	62 (4.9)	
No	1,605 (94.8)	412 (94.1)	1,193 (95.1)	
Hypertension				<0.001
Yes	870 (51.4)	395 (90.2)	475 (37.9)	
No	823 (48.6)	43 (9.8)	780 (62.1)	
Heart disease				<0.001
Yes	363 (21.4)	139 (31.7)	224 (17.8)	
No	1,330 (78.6)	299 (68.3)	1,031 (82.2)	
Prior abdominal surgery[e]				0.29
Yes	602 (35.7)	165 (37.8)	437 (35.0)	
No	1,085 (64.3)	272 (62.2)	813 (65.0)	
Prior transurethral bladder resection[f]				0.11
Yes	28 (1.7)	11 (2.5)	17 (1.4)	
No	1,655 (98.3)	426 (97.5)	1,229 (98.6)	
Prior transurethral prostate resection[g]				0.88
Yes	98 (5.8)	26 (5.5)	72 (5.7)	
No	1,592 (94.2)	411 (94.1)	1,181 (94.3)	
NCCN risk group[h]				0.06
Very low/Low	99 (5.9)	35 (8.1)	64 (5.2)	
Intermediate	1,314 (78.4)	338 (77.9)	976 (78.5)	
High/Very high	263 (15.7)	61 (14.1)	202 (16.3)	
Prior prostatectomy[i]				0.002
Yes	475 (28.1)	97 (22.2)	378 (30.2)	
No	1,214 (71.9)	339 (77.8)	875 (69.8)	
Hormone therapy[j]				0.08
Yes	1,140 (67.4)	310 (70.8)	830 (66.2)	
No	552 (32.6)	128 (29.2)	424 (33.8)	
Length of hormone therapy[j]				0.87
≤3 months	23 (2.4)	7 (2.8)	16 (2.3)	
4–6 months	255 (27.0)	63 (25.2)	192 (27.7)	
7–12 months	93 (9.9)	25 (10.0)	68 (9.8)	
>12 months	572 (60.7)	155 (62.0)	417 (60.2)	
Inclusion of pelvic nodes in radiotherapy field[k]				0.06
Yes	455 (28.3)	106 (24.8)	349 (29.5)	
No	1,155 (71.7)	322 (75.2)	833 (70.5)	
Inclusion of seminal vesicles in radiotherapy field[l]				0.77
Yes	800 (51.3)	213 (50.7)	587 (51.5)	
No	759 (48.7)	207 (49.3)	552 (48.5)	
Brachytherapy				0.03

None	1,461 (86.3)	386 (88.1)	1,075 (85.7)	
HDR	146 (8.6)	40 (9.1)	106 (8.5)	
LDR	86 (5.1)	12 (2.7)	74 (5.9)	
Bladder V40 Gy %, median [range][m]	54.1 [4.4 to 100]	53.1 [4.6 to 100]	54.4 [4.4 to 100]	0.14
Bladder V50 Gy %, median [range][m]	36.6 [3.0 to 100]	34.4 [3.5 to 100]	37.0 [3.0 to 100]	0.01
Bladder V60 Gy %, median [range][m]	25.5 [0.4 to 100]	24.2 [1.6 to 95.0]	26.2 [0.4 to 100]	0.007
Bladder V65 Gy %, median [range][m]	20.4 [0 to 100]	18.6 [0.1 to 88.4]	21.1 [0 to 100]	0.007
Bladder V70 Gy %, median [range][m]	9.9 [0 to 100]	8.9 [0 to 71.1]	10.3 [0 to 100]	0.01
Bladder V75 Gy %, median [range][m]	1.5 [0 to 88.9]	1.8 [0 to 81.5]	1.3 [0 to 89.9]	0.74

Abbreviations: ACEi, angiotensin converting enzyme inhibitor; RT, radiotherapy; BMI, body mass index; NCCN, National Comprehensive Cancer Network; EBRT, external beam radiotherapy.
[a]p-value is from a chi-square test for categorical variables and from a Wilcoxon rank-sum test for continuous variables comparing patients using vs not using an ACEi at RT start. Fisher's exact test was used for categorical variables if any cell within a contingency table had counts < 5.
[b]BMI was not available for 21 participants.
[c]Smoking status was not available for 6 participants.
[d]Autoimmune diseases included inflammatory bowel disease (N = 43), rheumatoid arthritis (N = 29), ankylosing spondylitis (N = 1), systemic lupus erythematosus (N = 3), scleroderma (N = 1), and other collagen vascular disease (N = 13). Participants could report more than one autoimmune disease.
[e]Prior abdominal surgery included appendectomy (N = 182), cholecystectomy (N = 59), and/or others (N = 349). Participants could report more than one type or prior abdominal surgery. Prior abdominal surgery was not available for 6 participants.
[f]Prior bladder resection was not available for 10 participants.
[g]Prior prostate resection was not available for 3 participants.
[h]NCCN risk group was not available for 17 participants.
[i]Prior prostatectomy was not available for 4 participants.
[j]Hormone therapy was not available for 1 participant, and length of hormone therapy was not available for 197 of the 1,140 participants who received hormone therapy.
[k]Pelvic node radiotherapy was not available for 83 participants.
[l]Seminal vesicle radiotherapy was not available for 134 participants.
[m]Bladder volume receiving the stated dose was derived from dose-volume-histograms capturing EBRT dose and does not account for dose received from brachytherapy. Data were available for 1,398 patients who received EBRT alone.

Gy (in units of 5%) were associated with time to hematuria; receipt of pelvic node radiotherapy showed a trend (Table 2). There was insufficient evidence that any of the other factors associated with ACEi use (age, BMI, diabetes, hypertension, prior prostatectomy, or brachytherapy boost) were associated with time to hematuria (all $p > 0.05$, Table 2), and the ACEi-hematuria association remained when adjusting for each of these individually (all $p < 0.05$). Use of anti-coagulants during radiotherapy did not affect risk of hematuria among participants in URWCI, in which these data were available ($p = 0.08$), and none of the 19 participants from this cohort who developed hematuria were taking anticoagulants during follow-up. ACEi use remained protective in a multivariable model including prior TURP and/or TURB, heart disease, pelvic node radiotherapy, and percent of the bladder volume receiving 70 Gy (in units of 5%) (Table 3). The effect of ACEi on time to hematuria was similar (HR 0.51, 95%CI 0.28 to 0.94, $p = 0.030$) when the model was additionally adjusted for receipt of brachytherapy.

A PRS for blood pressure was strongly associated with hypertension in our GWAS cohorts, which include REQUITE (Table 4). There was insufficient evidence that this PRS was associated with late hematuria (HR per standard deviation 0.96, 95%CI 0.87 to 1.06, $p = 0.41$) or rectal bleeding (HR per standard deviation 1.03, 95%CI 0.95 to 1.11, $p = 0.51$) following radiotherapy in these patients (Table 4).

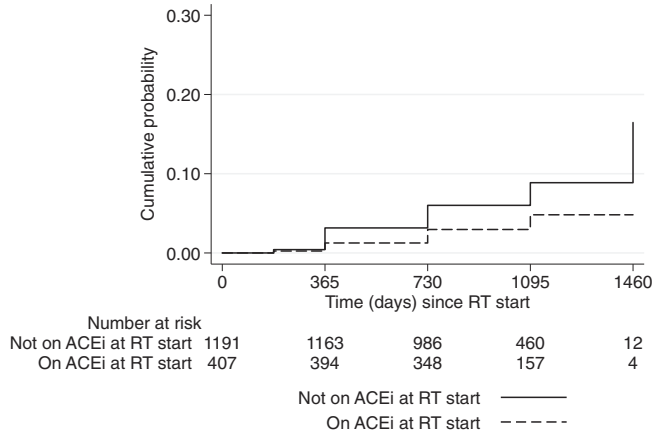

Fig. 1. Kaplan-Meier estimates of the distribution of time to hematuria by ACEi use during radiotherapy.

Discussion

Our multi-site clinical study is the first to show a radioprotective effect of ACEi on bladder, and is consistent with findings for kidney, lung, gastrointestinal, and brain radiotoxicity [23]. The clinical findings indirectly support the GWAS-identified risk locus for hematuria [4], though functional studies are needed to understand how risk SNPs tagging *AGT* act in different cell and tissue types. Other risk factors for hematuria in this study, such as bladder V70 Gy and prior TURP/TURB, are consistent with prior findings [24–26]. We did not find an increased risk of hematuria among patients taking anticoagulants during radiotherapy as has been reported [25], though these data were only available for a minority of study participants, and our analysis was not powered to test anticoagulants. No patients reported a diagnosis of coagulopathy, though it is possible that a small number of patients who developed radiation-induced hematuria have an underlying coagulopathy that was not captured and/or undiagnosed. Such disorders are relatively rare (von Willebrand disease being the most common, with approximately 1% prevalence [27,28]) and are unlikely to account for most instances of hematuria. It is plausible that a genetic predisposition to coagulopathy might increase risk of developing radiation-induced hematuria, and future studies should investigate a potential shared heritability. The protective effect of heart disease is interesting and could reflect the action of other medications that, like ACEi, act on the vascular system. Prior studies suggest statins may be radioprotective [19], however use of statins during radiotherapy was not associated with time to hematuria among participants in REQUITE, in which these data were available (HR 0.98, 95% CI 0.60 to 1.59, $p = 0.93$). Other medications for heart disease could be involved and warrant further investigation. While a prior study reported an association of ACEi with reduced proctitis [20], the hematuria-associated SNPs near *AGT* were not associated with rectal bleeding in our GWAS [4], con-

Table 2
Hazard ratios from separate grouped relative risk models, each assessing the effect of a demographic or clinical characteristic on time to hematuria, stratified by study enrollment site.

Characteristic	HR (95% CI)	*P*-value
ACEi use at start of radiotherapy		
No	Ref.	–
Yes	0.53 (0.30 to 0.93)	0.029
Age at radiotherapy	1.00 (0.97 to 1.02)	0.75
Race		
White	Ref.	–
Non-White	0.44 (0.11 to 1.87)	0.27
BMI	0.97 (0.93 to 1.02)	0.29
Smoking status		
Never	Ref.	–
Former	1.13 (0.73 to 1.74)	0.59
Current	1.10 (0.54 to 2.22)	0.8
Diabetes		
No	Ref.	–
Yes	0.66 (0.33 to 1.31)	0.23
Autoimmune disease[a]		
No	Ref.	–
Yes	0.64 (0.23 to 1.76)	0.38
Hypertension		
No	Ref.	–
Yes	0.87 (0.58 to 1.31)	0.51
Heart disease		
No	Ref.	–
Yes	0.35 (0.17 to 0.69)	0.003
Prior abdominal surgery		
None	Ref.	–
Any[b]	1.23 (0.81 to 1.88)	0.33
Prior transurethral bladder resection		
No	Ref.	–
Yes	3.52 (1.28 to 9.70)	0.015
Prior transurethral prostate resection		
No	Ref.	–
Yes	4.25 (2.51 to 7.18)	<0.001
NCCN risk group[c]		
Very low/Low	Ref.	–
Intermediate	0.78 (0.36 to 1.72)	0.54
High/Very High	1.11 (0.46 to 2.70)	0.81
Prior prostatectomy		
No	Ref.	–
Yes	1.29 (0.83 to 2.01)	0.26
Hormone therapy		
No	Ref.	–
Yes	1.19 (0.74 to 1.92)	0.47
Inclusion of pelvic nodes in radiotherapy field		
No	Ref.	–
Yes	0.61 (0.36 to 1.03)	0.07
Inclusion of seminal vesicles in radiotherapy field		
No	Ref.	–
Yes	1.12 (0.72 to 1.73)	0.62
Brachytherapy		
None	Ref.	–
HDR	0.90 (0.40 to 2.02)	0.81
LDR	0.16 (0.02 to 1.23)	0.08
Bladder V40 Gy, percent[d]	1.02 (0.97 to 1.06)	0.48
Bladder V50 Gy, percent[d]	1.03 (0.97 to 1.08)	0.37
Bladder V60 Gy, percent[d]	1.04 (0.98 to 1.10)	0.23
Bladder V65 Gy, percent[d]	1.06 (0.99 to 1.13)	0.07
Bladder V70 Gy, percent[d]	1.13 (1.04 to 1.23)	0.004
Bladder V75 Gy, percent[d]	1.07 (0.99 to 1.16)	0.08

Abbreviations: HR, hazard ratio; CI, confidence interval; BMI, body mass index; NCCN, National Comprehensive Cancer Network; EBRT, external beam radiotherapy.

[a] Autoimmune diseases included inflammatory bowel disease (*N* = 43), rheumatoid arthritis (*N* = 29), ankylosing spondylitis (*N* = 1), systemic lupus erythematosus (*N* = 3), scleroderma (*N* = 1), and other collagen vascular disease (*N* = 13). Participants could report more than one autoimmune disease.

[b] Prior abdominal surgery included appendectomy (*N* = 182), cholecystectomy (*N* = 59), and/or others (*N* = 349). Participants could report more than one type or prior abdominal surgery.

[c] Due to small numbers, patients in the Very low (*N* = 7) and Low (*N* = 92) groups were combined, and patients in the High (*N* = 246) and Very high (*N* = 17) groups were combined.

[d] Bladder volume receiving the stated radiation dose was derived from dose-volume-histograms capturing EBRT dose and does not account for dose received from brachytherapy. Thus, the model was fit among those patients who did not receive brachytherapy and had complete dosimetry data available (*N* = 1,315). The HR is per 5% increase in the percent of the bladder receiving the stated dose.

Table 3
Hazard ratios from a multivariable grouped relative risk model of time to hematuria assessing the effect of ACEi adjusted for clinical factors. The model was stratified by enrollment site and includes 1,372 study participants with non-missing data for all variables in the model.

Characteristic	Adjusted HR (95% CI)	*P*-value
ACEi use during radiotherapy		
No	Ref.	-
Yes	0.51 (0.28 to 0.94)	0.030
Heart disease		
No	Ref.	-
Yes	0.32 (0.15 to 0.67)	0.003
Prior transurethral resection		
None	Ref.	-
TURB only	4.03 (0.96 to 17.0)	0.058
TURP only	4.05 (2.28 to 7.18)	<0.001
TURP and TURB	5.17 (1.23 to 21.8)	0.025
Pelvic radiotherapy		
No	Ref.	-
Yes	0.55 (0.32 to 0.96)	0.037
Bladder V70 Gy, percent[a]	1.13 (1.04 to 1.22)	0.004

[a] Bladder V70 Gy was derived from dose-volume-histograms capturing EBRT dose and does not account for dose received from brachytherapy. The HR is per 5 percent of the bladder receiving 70 Gy.

sistent with the lack of effect of ACEi use on rectal bleeding in the present analysis.

Hypertension did not affect hematuria nor did a PRS for high blood pressure. Thus, the protective effect of ACEi on the bladder appears to be distinct from hypertension. This is consistent with findings in a rat model that ACEi prevented radiation nephropathy whereas a calcium channel blocker [29] or a diuretic [30] did not, despite all reducing blood pressure. Some preclinical studies found a protective effect of angiotensin receptor blockers as well as ACEi, while others [23] did not. Evidence from clinical studies is scarce and inconsistent, and often only grouped medication data were available [23]. Given the complexity of the RAS, it is not surprising that perturbation at different points results in differing effects on tissue response to radiotherapy. Use of ACEi increases renin, angiotensin I, and angiotensin (1–7) with downstream effects on bradykinin and prostaglandins, and reduces angiotensin II [7]. Mechanistic studies are needed to understand whether the radioprotective effect of ACEi involves any of these substrates, or a mechanism separate from receptor interaction. Similarly, mechanistic studies of RAS inhibitors will provide additional information on shared mechanism(s) by different bladder toxicity symptoms. In our clinical analysis, ACEi showed a weaker, non-statistically significant protective effect on other bladder toxicity endpoints suggest there may be partial, but incomplete overlap in the molecular mechanism(s) underlying these various symptoms.

Strengths of our study include the multi-site, international patient population and prospective, longitudinal collection of PROs and medication use. The cumulative probability of patient-reported gross hematuria was similar in our study to others [31,32], though reported toxicity rates vary by treatment modality, length of follow-up, and method of assessment (PRO vs. observer assigned, type of questionnaire or grading schema). We evaluated for the first time the effect of hypertension via Mendelian randomization in a large patient population with longitudinal radiation toxicity assessments. Limitations include the non-randomized design; lack of participants who stopped ACEi use after radiotherapy precluding assessment of whether long-term use is required for radioprotection; and lack of a non-RT comparison group.

Though these findings require further evaluation in a randomized clinical trial, our study has important clinical implications given the availability of multiple classes of medications that act on the RAS. Functional studies in cell and animal models are needed to understand how the RAS is acting to protect the bladder.

Conflict of interest statement

The authors declare not conflicts of interest related to this work.

Acknowledgements

Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under K07 CA187546 (PI: S. Kerns) and UG1CA189961 (PI: Morrow); pilot funding from the University of Rochester Wilmot Cancer Institute; and Research & Academic IT, Clinical and Translational Science Institute grant support (Grant # UL1 TR002001 from the National Institutes of Health). RAPPER (PI: West) is supported by Cancer Research UK grants C1094/A18504 and C147/A25254; Drs. West and Choudhury are supported by NIHR Manchester Biomedical Research Centre funding. RADIOGEN research is supported by Spanish Instituto de Salud Carlos III (ISCIII) funding, an initiative of the Spanish Ministry of Economy and Innovation partially supported by European Regional Development FEDER Funds (INT15/00070, INT16/00154, INT17/00133, INT20/00071; PI19/01424; PI16/00046; PI13/02030; PI10/00164), and through the Autonomous Government of Galicia (Consolidation and structuring program: IN607B) given to A.Vega. Dr Tim Rattay is currently an NIHR Clinical Lecturer (CL 2017-11-002). He was previously funded by a National Institute of Health Research (NIHR) Doctoral Research Fellowship (DRF 2014-07-079). This publication presents independent research funded by the NIHR. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. The REQUITE study (PI: C. West) received funding from the European Union's 7th Framework Programme for research, technological development and demonstration under grant agreement no. 601826. We thank all patients who participated in the Radiogenomics Consortium Cohorts, the REQUITE study and all REQUITE staff involved at the following hospitals: Belgium: Ghent University Hospital, Ghent and KU Leuven, Leuven; France: ICM Montpellier, and CHU Nîmes; Germany: Zentrum für Strahlentherapie Freiburg (P. Stegmaier); ViDia Christliche Kliniken Karlsruhe (J. Claßen); Klinikum der Stadt Ludwigshafen gGmbH (T. Schnabel); Universitätsmedizin Mannheim; DKFZ also thanks Anusha Müller and Irmgard Helmbold; Italy: Fondazione IRCCS Istituto Nazionale dei Tumori, Milano and Candiolo Cancer Istitute – IRCCS, Candiolo; Spain: Complexo Hospitalario Universitario de Santiago, Santiago; UK: University Hospitals Leicester, Leicester and Manchester Biomedical Research Center, Manchester; USA: Mount Sinai Hospi-

Table 4
Association of blood pressure polygenic risk score with hypertension, time to hematuria, and time to rectal bleeding in GWAS cohorts. Effect sizes and 95% confidence intervals (CI) are per standard deviation of the polygenic risk score. Each model is adjusted for cohort.

	Hypertension (*N* = 5,288)		Time to hematuria (*N* = 5,126)		Time to rectal bleeding (*N* = 4,592)	
	OR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Blood pressure Polygenic risk score	1.38 (1.31 to 1.46)	<0.001	0.96 (0.87 to 1.06)	0.41	1.03 (0.95 to 1.11)	0.51

tal, New York. We thank Rebecca Elliott for project management of RAPPER and REQUITE.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2022.01.014.

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