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Germline variants disrupting microRNAs predict long-term genitourinary toxicity after prostate cancer radiation

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Abstract

Background and purpose: The purpose of this study was to determine whether single nucleotide polymorphisms disrupting microRNA targets (mirSNPs) can serve as predictive biomarkers for toxicity after radiotherapy for prostate cancer and whether these may be differentially predictive depending on radiation fractionation.

Materials and methods: We identified 201 men treated with two forms of definitive radiotherapy for prostate cancer at two institutions: 108 men received conventionally-fractionated radiotherapy (CF-RT) and 93 received stereotactic body radiotherapy (SBRT). Germline DNA was evaluated for the presence of functional mirSNPs. Random forest, boosted trees and elastic net models were developed to predict late grade ≥2 GU toxicity by the RTOG scale.

Results: The crude incidence of late grade ≥2 GU toxicity was 16% after CF-RT and 15% after SBRT. An elastic net model based on 22 mirSNPs differentiated CF-RT patients at high risk (71.5%) versus low risk (7.5%) for toxicity, with an area under the curve (AUC) values of 0.76–0.81. An elastic net model based on 32 mirSNPs differentiated SBRT patients at high risk (64.7%) versus low risk (3.9%) for toxicity, with an area under the curve (AUC) values of 0.81–0.87. These models were specific to treatment type delivered. Prospective studies are warranted to further validate these results.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2021.12.040.

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Conclusion: Predictive models using germline mirSNPs have high accuracy for predicting late grade ≥2 GU toxicity after either CF-RT or SBRT, and are unique for each treatment, suggesting that germline predictors of late radiation sensitivity are fractionation-dependent. Prospective studies are warranted to further validate these results.

Keywords

Toxicity; SBRT; IMRT; Prostate; Germline; SNPs

Given the high effectiveness of definitive treatments for localized prostate cancer, quality of life following treatment is a paramount factor in patient-physician shared decision-making [1]. After definitive radiotherapy, a major quality of life limiting toxicity is late genitourinary (GU) toxicity, which could manifest as increased urinary frequency, retention, pain, and bleeding. The 5-year late grade ≥2 GU toxicity rates following modern radiotherapy ranges from 12% to 15% [2,3] with an insidious increase over time. Overall, these rates appear to be similar whether a patient is treated with conventionally-fractionated radiotherapy (CF-RT; 1.8–2.0 Gy per fraction over 39–45 treatment sessions) or stereotactic body radiotherapy (SBRT; >7 Gy per fraction over 5 or fewer sessions) [4,5]. While these data indicate that on a population level, there are no aggregate-level differences in toxicity, there may indeed be patients who exhibit fraction-dependent radiosensitivity.That is, some patients may have more toxicity after high dose per fraction versus low dose per fraction radiation.

There are considerable data suggesting that genomic factors may be important in determining clinical radiosensitivity, with several studies suggest that germline single nucleotide polymorphisms (SNPs) in multiple genes may be associated with GU toxicity after radiotherapy, however, those reported have only modest accuracy for predicting toxicity [6–8]. Emerging data suggest an important role for microRNAs (miRNAs) - small, non-coding RNA elements [9]. miRNAs are global regulators of stress response pathways, including the local and systemic response to radiation [10]. Beyond miRNAs themselves, germline single nucleotide polymorphisms disrupting their target binding or regulatory regions (termed mirSNPs) appear to be integral to determining the response to radiation [1011]. Recently, miSNPs were found to predict wound-healing toxicity after SBRT in sarcoma [12]. In order to identify and characterize whether a signature from this pool of mirSNPs could help to aid in the prediction of late GU toxicity following CF-RT versus SBRT in a fractionation-dependent manner, we performed a translational study of germline DNA from 201 prospectively treated patients.

Methods

Participants and treatment characteristics

The patient population included 108 patients receiving CF-RT on a single-arm prospective study at Oslo University Hospital [13] and 93 patients receiving SBRT on two single arm prospective studies at the University of California, Los Angeles (NCT01059513 [$n = 63$] and NCT02296229 [$n = 30$]) (Table S1–2). Patients included in this sub-study provided signed consent for collection and analysis of germline DNA for the evaluation of predictors of

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toxicity and efficacy. A minimum follow-up of six months was required for inclusion (all patients on the protocol that had available germline DNA met this criteria). This study was approved by the Ethics Committee of the Health Region South/East of Norway as well as the Institutional Review Board at the University of California, Los Angeles. Patients in the CF-RT cohort received 74 Gy in 37 fractions to the prostate and 50 Gy in 25 fractions to the pelvic lymph nodes as described previously [13]. The initial portion was delivered with intensity modulated radiotherapy techniques, with expansions on the prostate ranging from 13-15 mm and imaging guidance predominantly consisting of alignment to bony markers. Patients in the SBRT cohort received 40 Gy in five fractions to the prostate, as described previously [14]. SBRT plans were delivered by volumetric modulated arc therapy, with a planning margin of 5 mm around the prostate, reduced to 3–5 mm posteriorly. Inter- and intrafractional motion management relied on alignment to intraprostatic fiducial markers. The toxicity scale used was the RTOG scale, rather than the more contemporary Common Terminology Criteria for Adverse Events (CTCAE) scale. This scale is functionally similar to the RTOG scale with respect to significant (i.e., moderate or greater) toxicity. Toxicity was assessed q3 monthly in the first year, then q6 monthly for two years, and then annually. Late toxicity events were defined as events occurring >90 days after radiotherapy.

Analysis of germline DNA

Genomic DNA from peripheral blood mononuclear cells, whole blood, or normal tissue found in biopsy specimens was isolated using standard techniques and analyzed in a single Clinical Laboratory Improvement Amendments–certified laboratory as previously described [15]. Biomarkers were identified from a pool of miRNA-based biomarkers discovered and determined to be functional previously through sequencing and bioinformatic approaches [16]. Mutations in DNA damage repair and response genes and immune response genes, in the key gene targets of miRNAs known to be critical in the DNA damage or immune response, and in the promoters of miRNAs known to be important in these responses were prioritized. Therefore, for this analysis, panels were run using the Sequenom platform, an analysis which included approximately 300 single nucleotide polymorphisms or deletions. Each panel was run with internal controls that used Taqman Genotyping as the gold standard. To compare the genetic variation between the CF-RT and SBRT cohorts, we calculated the fixation index for each analyzed mirSNP (Fig. S1) [17].

Variable selection and model fitting

Statistical models and analyses were conducted in R (version 4.0.0). All mirSNPs with variance close to zero (nearZeroVar::caret version 6.0–84)[27] or that had an almost perfect correlation ($r^2 \geq 0.99$) were removed. Fisher's exact test was used to test the pairwise independence between each mirSNP and the outcome of experiencing a late grade ≥2 GU toxicity event as scored on the Radiation Therapy Oncology Group (RTOG) scale [18]. This scoring system was developed in 1985 and grades the severity of radiation-induced reactions from 0 to 5, with grade 2 toxicity being considered moderate. For the initial assessment of pairwise independence, the p-values were only used for model selection and thus no adjustment for multiplicity of testing was performed. Toxicities studied were restricted to GU toxicity rather than gastrointestinal (GI) toxicity since the low event rate in either cohort

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of GI toxicity precluded meaningful statistical analysis. Furthermore, we evaluated grade ≥2 toxicity, rather than grade ≥3, GU toxicity, due to limitations of such events.

Due to the unequal distribution of patients with and without toxicity, up-sampling was used (upSample::caret version 6.0-84) [19] to create balanced populations. Random forest (randomForest::randomForest version 4.6-14) [20], boosted trees (gbm::gbm version 2.1.5) [21], and elastic net (glmnet::glmnet version 3.0-2) [22] models were then generated to predict toxicity in each patient for both the CF-RT and the SBRT cohorts. In all models, SNP mutation status was included as a categorical predictor (3 categories). The p-value threshold for inclusion in the models were 0.3 except for the boosted tree model for the SBRT cohort, which had a p-value threshold of 0.15. Each model was run using various parameters, and model performance was assessed for sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), F1 score, and AUC (AUC::cvAUC version 1.1.0), using held-out samples [23]. To reduce selection bias and overfitting, these metrics were calculated using leave-one-out-cross validation (LOO-CV). Cross validation (CV), as opposed to external validation on a held-out test sample, is more appropriate for medium to low sample sizes, as reliance on a small test data-set leads to unreliable statistical inference. In this context, CV has been shown to provide a nearly unbiased estimate of the expected prediction error [24]. We considered nested CV as an alternative to simple CV and found that our results did not vary significantly. Finally, we evaluated toxicity-free survival for patients in both the CF-RT and SBRT cohorts stratified into low and high-risk groups based on mirSNP signatures [25].

Results

Median follow-up periods were 8.5 years (standard deviation 2.6 years) in the CF-RT group and 3.2 years (standard deviation 0.5 years). Notably, all patients treated with CF-RT identified as Caucasian, while 19% of the patients in the SBRT cohort did not identify as Caucasian. Seventeen of the 108 patients in the CF-RT cohort (16%) and 14 patients in the SBRT cohort (15%) experienced late grade ≥2 GU toxicity. Associations between late toxicity and mirSNPs for the CF-RT and SBRT cohorts are shown in Tables 1 and 2. Twenty-two mirSNPs (involving 19 genes) in the CF-RT cohort and 32 mirSNPs (involving 30 genes) in the SBRT cohorts met the initial p-value threshold of 0.3 for inclusion in machine learning models. In the CF-RT cohort, 10 of the mirSNPs (45%) were in genes associated with the immune response (accounting for eight genes), and seven mirSNPs (32%) were in genes associated with DNA repair (accounting for five genes). In the SBRT cohort, 15 mirSNPs (47%) were in genes associated with the immune response (accounting for 14 genes), and 6 (19%) were in genes associated with DNA repair. Given the imbalances in race, we evaluated fixation indices of the mirSNPs analyzed in this study, only two mirSNPs had fixation indices >0.2, with only one having an index >0.25. The top six mirSNPs sorted by fixation index were IL8_rs4073 (0.887), BMP2_rs1980499 (0.249), IL6_rs1800797 (0.172),IL6_rs1800795 (0.162), TNNT2_rs3729843 (0.160), CD274_rs2297136 (0.128) (Supplement Fig. S1).

Bar charts showing the proportions of patients within each cohort who had toxicity, stratified by mirSNP-based risk classification, are shown in Fig. 1. CV-based estimates

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of performance metrics for random forest, elastic net, and boosted trees models trained to predict toxicity after CF-RT or SBRT are shown in Table 3. The elastic net model for toxicity after CF-RT performed best, with an AUC of 0.81 and an F1 score of 0.71. The random forest and boosted trees models still had good performance with AUC ranges of 0.76–0.78 and F1 ranges of 0.54–0.65. For the elastic net model, the negative predictive value (NPV) was 94%, with a specificity of 97%. For predicting toxicity after SBRT the elastic net model also performed the best, with an AUC of 0.87 and an F1 score of 0.71. Once more, the other models still had good performance, with AUCs of 0.81 for both and F1 scores of 0.53–0.63. The NPV of the elastic net model for SBRT was 0.96, and the specificity was 0.92. Any given mirSNP had a relatively low impact on the overall model F1 scores in isolation, with changes in F1 score after removal of any mirSNP ranging from −0.05 to +0.18 (Fig. S2).

To investigate the uniqueness of the final models for predicting toxicity to CF-RT versus SBRT, we applied the identified signatures of toxicity for each, to the other, i.e., we evaluated the mirSNP signature for CF-RT toxicity to the SBRT cohort, and vice versa. We found that these models did not predict toxicity to the alternative treatment course (Table S2). All models had AUC values <0.55 and F1 scores of 0.25 or less. The PPVs in these models ranged between 0.17–0.25, and the NPVs were all >82%. A bar graph showing the stratification of each cohort into high-risk and low-risk of toxicity subgroups based on this reversed classification scheme is shown in Fig. S3.

Finally, we evaluated toxicity-free survival curves for patients in the CF-RT and SBRT cohorts based on both mirSNP signatures (Fig. 2). Among patients receiving CF-RT, patients predicted to be at high risk of toxicity based on the CF-RT-derived mirSNP signature had significantly shorter toxicity-free survival when compared against men predicted to have a lower risk of toxicity ($p < 0.0001$). Similarly, among patients who received SBRT, patients predicted to be at higher toxicity based on the SBRT-derived mirSNP signature had a significantly shorter toxicity-free survival ($p < 0.0001$) than men predicted to have a lower risk of toxicity. If stratified by the SBRT-derived mirSNP signature, however, no significant differences were seen between toxicity-free survival in patients receiving CF-RT ($p = 0.25$). An analogous result was seen if the SBRT cohort was stratified by the CF-RT mirSNP signature ($p = 0.43$). These findings again support the unique application of each signature to the specific fractionation schema, CF or SBRT.

Discussion

In this post hoc translational study of 201 patients enrolled on two prospective protocols, we developed models that could reliably predict late GU ≥2 toxicity after either CF-RT or SBRT based on germline mirSNPs. These models all had AUC values of >0.75 (>0.8 for all models developed in the context of SBRT), with the elastic net model for toxicity after CF-RT and SBRT having AUCs of 0.81 and 0.87, respectively; both had F1 scores of 0.71. The values indicate a high accuracy for predicting toxicity. Notably, models that predicted for toxicity after CF-RT did not predict toxicity for patients treated with SBRT, and vice versa. Overall, these data strongly suggest that variants in germline DNA can predict for significant GU

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toxicity after definitive radiotherapy, and that these germline signatures appear to predict for toxicity dependent on fractionation schema.

There are several limitations to this study. First, this constitutes a post-hoc analysis of subset of prospectively treated patients and is therefore retrospective by nature. While toxicity was scored prospectively, selection biases may have affected which patients contributed to the analysis, however, none had biomarker testing before this study was completed. Second, patients in the CF-RT cohort had a significantly larger area of tissue radiation, both by virtue of the pelvic nodal coverage and due to larger planning volumes around the prostate target. However, this difference alone would not account for the poor performance of models trained on the SBRT cohort in the CF-RT cohort, and modern pelvic nodal radiotherapy does not appear to be associated with increased toxicity [26]. Third, there may be other underlying differences between patients treated with CF-RT and SBRT that could explain the apparent uniqueness of the predictive models to each population. For instance, all patients treated with CF-RT identified as Caucasian, while 19% of the patients in the SBRT cohort did not identify as Caucasian. Race has been previously identified as a potential predictor for toxicity following radiotherapy, and any difference in toxicity based on race might also explain the difference in model performance [27]. However, when evaluating the fixation indices of the mirSNPs analyzed in this study, only two mirSNPs had fixation indices >0.2, with only one having an index >0.25. Fourth, patient-reported outcomes, rather than physician-scored toxicity, would ultimately be the most appropriate metric for whether a toxicity is “severe” or not [28–30], and such patient-reported outcomes were not available for this study. Fifth, other important factors, such as smoking status, dosimetry, medical comorbidities such as diabetes, could have influenced the development of late grade ≥2 GU toxicity, and we did not have sufficient information to comprehensively incorporate these important features into our models. Regardless of these potential limitations, our findings are strong and appear to be in line with the data generated by others that there are important components in the germ-line DNA that can predict radiotoxicity. Additionally, there was no manner to account for interobserver variability in toxicity assessment. Further, we optimized our analysis using the binary event of “toxicity” vs. ‘no toxicity” as we felt that endpoint was more important to patients than the toxicity-free interval. While our time-to-toxicity analyses confirm that our predictive signatures also are associated with toxicity-free survival, our training and validation processes were not designed with that endpoint. Finally, follow-up time was unequal with far greater follow-up in the CF-RT cohort. As more toxicity events occur in medium-term follow-up in the SBRT cohort, our results could be impacted.

Kerns *et al.* recently reported an individual patient data meta-analysis of six genome-wide association studies who underwent RT with a mix of different radiotherapy schedules (including patients who received brachytherapy and post-prostatectomy radiation) [7]. In a multivariable model that included SNPs using a genome-wide association study approach found to be associated with time to toxicity, the SNP rs17599026 was found to afford a statistically significant 37% increased risk of grade ≥1 urinary frequency. However, inclusion of SNP data into a predictive model for the risk of increased urinary frequency led to a modest increase in the concordance index from 0.56 without inclusion of SNP data to 0.57 with inclusion of the SNP data. Lee *et al.* assessed the performance of a random forest

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model for predicting changes in patient-reported GU symptoms after brachytherapy with or without external beam radiotherapy [8]. For the endpoint of weak stream, the predictive model had an AUC of 0.70. Most SNPs included in their model were in genes associated with neurogenesis and ion transport, which are associated with the development of lower urinary tract symptoms regardless of radiation [31].

In contrast, the present study has identified highly predictive models of functional variants that predict late GU toxicity in a fractionation-dependent fashion without the incorporation of baseline clinical characteristics and instead utilizing machine-learning approaches trained exclusively on germline mirSNPs. These models predicted for a broader endpoint of general, significant (i.e., grade ≥2) GU toxicity, over simply a weak urinary stream. Moreover, the mirSNPs identified in our model are largely related to immune response and DNA repair, pathways known to be directly related to the radiation response and thus are logical candidates for late toxicity.

Importantly, a strength of this study is the homogeneity of radiation delivery in patients within each cohort, as all patients were enrolled on prospective clinical trials with strict planning constraints and all patients in each cohort received the same dose and fractionation scheme. The high NPVs of the predictive models suggest that decisions based off these models are poised to have a significant impact: if a patient's pre-test risk of late GU ≥2 toxicity is roughly 15–20% but his biomarker panel does not predict for toxicity, his chance of toxicity is dramatically reduced. Given the importance of post-treatment quality of life in patients with clinically localized prostate cancer as well as the cost of managing post-radiation complications [32,33], this is a meaningful benefit. These findings, as well as their impact on patient decision-making, warrant validation in a prospective clinical trial, which is currently underway.

Conclusions

Models based on germline mirSNPs primarily in regulatory regions of miRNA binding in multiple genes have negative predictive values exceeding 90% for late grade ≥2 GU toxicity following either CF-RT or SBRT. Models are unique for each treatment modality, as shown by our finding that models predicting toxicity after CF-RT do not predict toxicity after SBRT, and vice versa. These findings underscore that clinically relevant radiosensitivity can be strongly explained by simultaneously evaluating functional, miRNA variants in multiple genes, and that this radiosensitivity appears to be dependent on the fractionation regimen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Conflict of interest disclosures

Dr. Kishan reported receiving personal fees from ViewRay, Inc, Varian Medical Systems, Inc, and Janssen Pharmaceuticals outside the submitted work, as well as research funding from ViewRay. Dr. Weidhaas is a co-founder of MiraDx, a molecular diagnostics company that owns IP related to microRNA binding site variants, some of which were studied in this paper.

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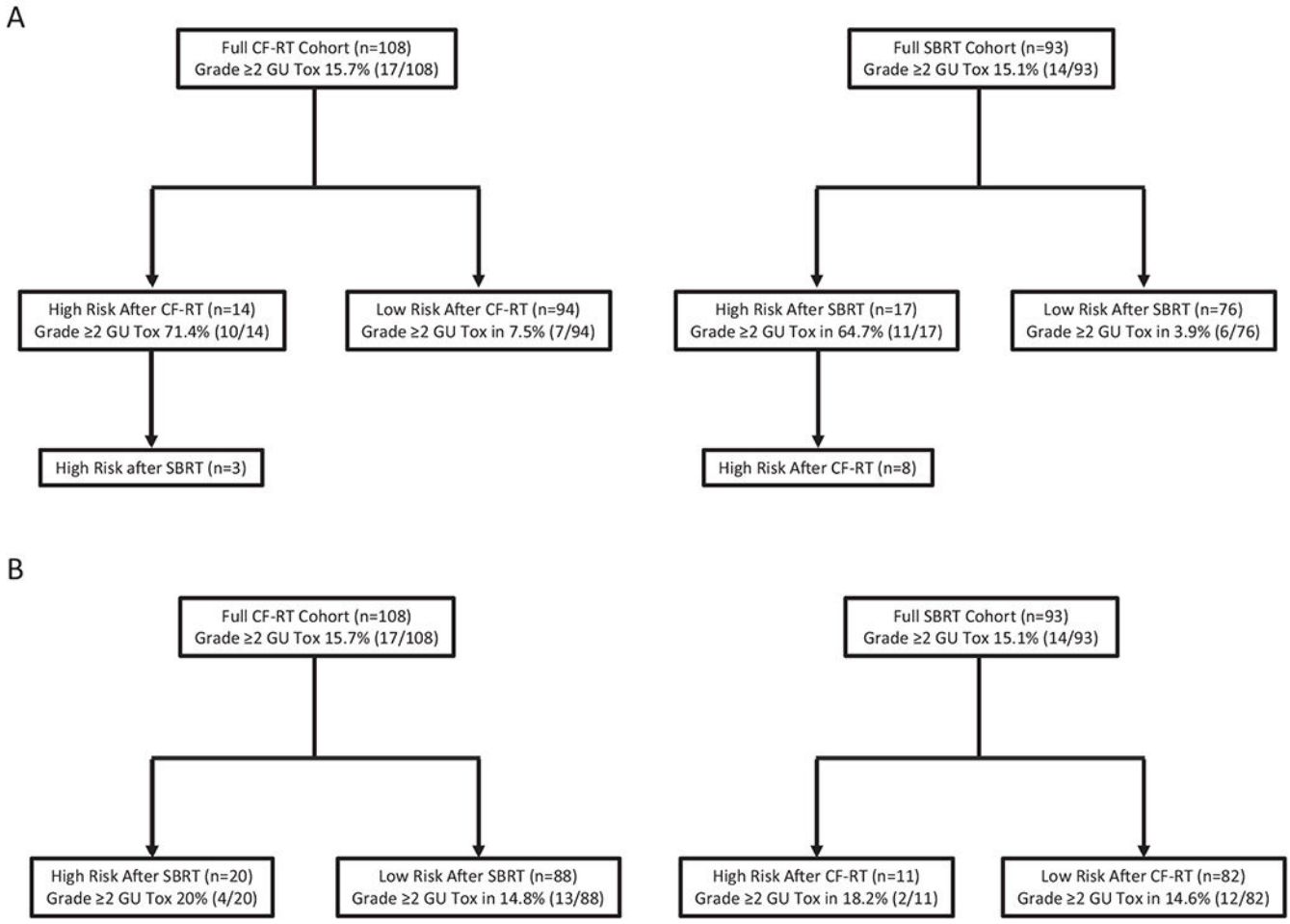

Fig. 1.

Bar Chart of Toxicity Percentage Based on Risk-Stratification. The percentage of patients experiencing grade ≥2 genitourinary toxicity in the conventional radiotherapy (CF-RT) and stereotactic body radiotherapy (SBRT) cohorts based on single nucleotide polymorphisms disrupting microRNA targets (mirSNPs).

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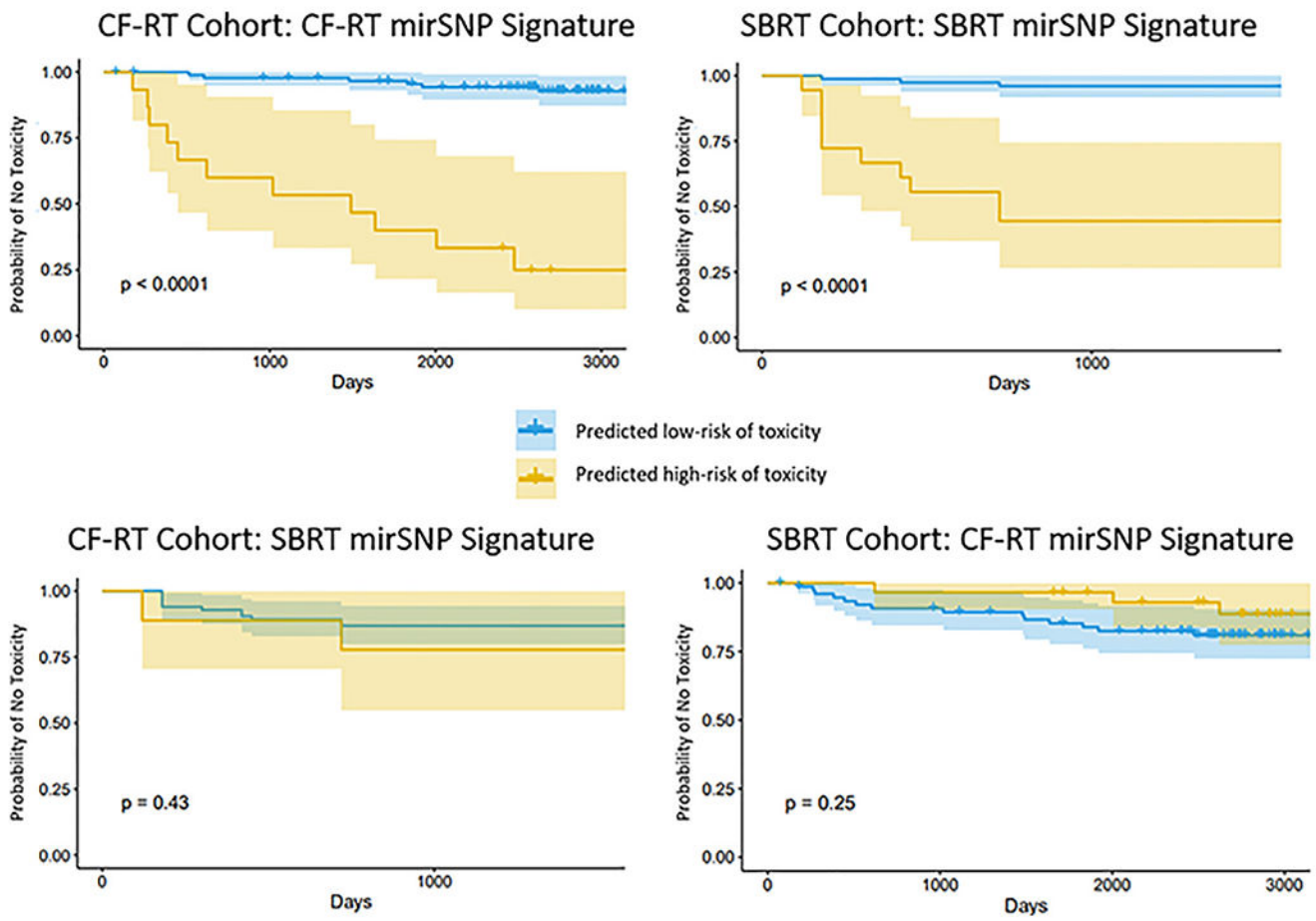

Fig. 2.

Toxicity-Free Survival Curves. Curves depict the predicted toxicity over time for men predicted to have a low or high risk of toxicity by the indicated mirSNP signature. *p*-values derived from the log-rank test.

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Table 1

Associations between late grade ≥2 genitourinary toxicity and single nucleotide polymorphisms disrupting microRNA targets (mirSNPs) in patients receiving conventionally fractionated radiotherapy.

mirSNP	No Mutation (% Tox)	Mutation (% Tox)	*p*-value	Notes
CD6_rs76677607	9.9	47.1	0.001	CD6 (immune response)
CD274_rs2297136	0.0	21.2	0.005	PDL1 (immune response)
BRCA2_rs7334543	27.5	8.8	0.014	BRCA2 (DNA repair)
NBN_rs1805794	6.2	23.3	0.017	Nibrin (DNA repair)
IL1.B_rs4848306	3.2	20.8	0.022	IL1B (immune response)
CD274_rs4742098	21.7	5.1	0.027	PDL1 (immune response)
ERCC4_rs4781562	7.7	23.2	0.035	ERCC4 (DNA repair)
MSH2_rs2303428	11.8	30.4	0.048	MSH2 (DNA repair)
rs17599026	11.9	29.2	0.056	Located on 5q31.2, previously associated with increased urinary toxicity after CF-RT
XRCC3_rs861539	7.0	21.5	0.058	XRCC3 (DNA repair)
IL2RA_rs11256497	8.0	22.4	0.062	IL2RA (immune response)
LIG4_rs3093772	19.5	3.8	0.067	DNA ligase 4 (DNA repair)
IL17D_rs7787	6.1	20.0	0.087	Homologous to IL17 (immune response)
IL6_rs1800795	4.0	19.3	0.113	IL6 (immune response)
CD274_rs4143815	20.6	8.9	0.115	PDL1 (immune response)
UNGC.96.TGFB2_NA	14.1	33.3	0.149	Secreted ligand of the TGF-beta superfamily of proteins
miR.34b.c. promoter_rs4938723	22.0	11.9	0.183	miR-34 miRNAs are mediators in the p53 pathway
MDM2_rs769412	14.3	30.0	0.191	E3 ubiquitin-protein ligase mediating ubiquitination of p53/TP53
rs3024505	18.7	9.1	0.261	Associated with inflammatory bowel disease
CETN2_rs8230	14.1	25.0	0.275	Centrin 2, possibly required for the proper duplication and segregation of the centrosome.
BRCA2_rs15869	11.3	20.0	0.292	BRCA2 (DNA repair)
IL10_rs1800872	12.1	20.0	0.298	IL10 (immune response)

mirSNP, single nucleotide polymorphisms disrupting microRNA targets; % Tox, percentage with late grade ≥2 genitourinary toxicity.

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Table 2

Associations between late grade ≥2 genitourinary toxicity and single nucleotide polymorphisms disrupting microRNA targets (mirSNPs) in patients receiving stereotactic body radiotherapy.

mirSNP	No Mutation (% Tox)	Mutation (% Tox)	*p*-value	Notes
BMP2_rs1979855	6.9	28.6	0.007	BMP2, secreted ligand of the TGF-beta superfamily of proteins
rs1893217	8.8	32.0	0.010	Associated with autoimmune diseases
BRCA2_rs15869	9.4	27.6	0.031	BRCA2 (DNA repair)
ABL1_rs11991	20.6	3.3	0.032	ABL oncogene kinase
ERCC1_rs11615	29.2	10.1	0.043	ERCC1 (DNA repair)
RAD23A_rs8240	18.7	0.0	0.064	RAD23 (DNA repair)
BATF3_rs6695772	23.7	9.1	0.076	BATF3 (immune response)
FOXP3_rs2232365	20.8	7.5	0.088	FOXP3 (immune response)
IL19_rs1798	10.6	25.9	0.106	IL19 (immune response)
LIN28A_rs9438623	17.7	0.0	0.118	LIN-28 family RNA-binding protein that acts as a posttranscriptional regulator of genes involved in developmental timing and self-renewal in embryonic stem cells
IL18R1_rs11465660	12.0	27.8	0.136	IL18 (immune response)
IL2RB_rs228942	20.4	7.7	0.141	IL24 (immune response)
CD274_rs1411262	9.8	21.4	0.150	PDL1 (immune response)
BMP2_rs3178250	9.1	20.4	0.155	BMP2, secreted ligand of the TGF-beta superfamily of proteins
SPI1_rs2071304	20.9	10.0	0.159	encodes an ETS-domain transcription factor that activates gene expression during myeloid and B-lymphoid cell development (immune response)
IL19_rs2243158	17.3	0.0	0.202	IL10 family (immune response)
rs3024505	17.3	0.0	0.202	Associated with inflammatory bowel disease
TRL4_rs4986790	17.3	0.0	0.202	Toll-like receptor 4 (immune response)
VEGFA_rs41282644	17.1	0.0	0.206	VEGFA (angiogenesis)
P2RX7_rs3751143	19.0	6.7	0.213	P2RX7, a ligand-gated ion channel
ERCC1_rs3212948	22.6	11.3	0.218	ERCC1 (DNA repair)
FANCC_rs9673	13.8	33.3	0.221	FANCC (DNA repair)
CD274_rs2297136	8.3	19.3	0.234	PDL1 (immune response)
IL6_rs2069840	8.3	19.3	0.234	IL6 (immune response)
CD274_rs2282055	10.9	21.1	0.240	PDL1 (immune response)
CD274_rs822339	10.9	21.1	0.240	PDL1 (immune response)

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mirSNP	No Mutation (% Tox)	Mutation (% Tox)	*p*-value	Notes
HAMP_rs10421768	19.2	9.8	0.252	HAMP, involved in the maintenance of iron homeostasis
HAMP_rs1882694	20.0	11.3	0.260	HAMP, involved in the maintenance of iron homeostasis
ATM_rs1800057	14.0	28.6	0.283	ATM (DNA repair)
ILRAP_rs79383051..UNGC.41.IL1RAP.	14.0	38.6	0.283	ILRAP (immune response)
KIT_rs17084733	17.6	5.3	0.286	KIT, an oncogene
rs17599026	17.3	5.6	0.29	Located on 5q31.2, previously associated with increased urinary toxicity after conventionally fractionated radiotherapy

SNP, single nucleotide polymorphism; % Tox, percentage with late grade ≥2 genitourinary toxicity.

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Table 3

Performance of predictive models for late grade ≥2 genitourinary toxicity after conventionally-fractionated radiotherapy or stereotactic body radiotherapy.

	Sensitivity	Specificity	PPV	NPV	F1 Score	AUC
Prediction of Toxicity after CF-RT						
Random Forest	0.77	0.80	0.42	0.95	0.54	0.78
Elastic Net	0.59	0.96	0.71	0.93	0.65	0.76
Boosted Trees	0.65	0.97	0.79	0.94	0.71	0.81
Prediction of Toxicity after SBRT						
Random Forest	0.86	0.76	0.39	0.97	0.53	0.81
Elastic Net	0.79	0.92	0.65	0.96	0.71	0.87
Boosted Trees	0.71	0.90	0.56	0.95	0.63	0.81

AUC, area under the curve; -RT, conventionally fractionated RT; NPV, negative predictive value; PPV, positive predictive value; SBRT, stereotactic body radiotherapy.

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