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## STROGAR – STrengthening the Reporting Of Genetic Association studies in Radiogenomics

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### Abstract

Despite publication of numerous radiogenomics studies to date, positive single nucleotide polymorphism (SNP) associations have rarely been reproduced in independent validation studies. A major reason for these inconsistencies is a high number of false positive findings because no adjustments were made for multiple comparisons. It is also possible that some validation studies were false negatives due to methodological shortcomings or a failure to reproduce relevant details of the original study. Transparent reporting is needed to ensure these flaws do not hamper progress in radiogenomics. In response to the need for improving the quality of research in the area, the Radiogenomics Consortium produced an 18-item checklist for reporting radiogenomics studies. It

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### Conflict of interest

All authors declare no conflict of interest.

is recognised that not all studies will have recorded all of the information included in the checklist. However, authors should report on all checklist items and acknowledge any missing information. Use of STROGAR guidelines will advance the field of radiogenomics by increasing the transparency and completeness of reporting.

## Keywords

Radiogenomics; Reporting guidelines; Normal tissue toxicity; Genetics

Radiogenomics is a multi-disciplinary scientific research field aiming to link human genomic variability to a cancer patient's likelihood of developing toxicity following radiotherapy [1]. Over 80 publications to date reported results of studies investigating correlations between genetic markers and radiotherapy toxicity. Although many reports published *p*-values that were nominally statistically significant, findings have proved difficult to reproduce [2–8]. One problem is that many studies carry out multiple comparisons without controlling the rate of false positive findings. As the human genome contains around 11 million single nucleotide polymorphisms (SNPs), the prior probability that a given SNP is associated with the phenotype is low. Validation of significant associations in independent cohorts is key to progress in radiogenomics, and requires greater standardisation of study designs, data collection and data analysis. This can only be achieved by adopting a common set of guidelines for reporting radiogenomics studies.

Radiogenomics shares many similarities with other genetic and observational epidemiology studies but has unique challenges that warrant a separate set of checklist items to complement the CONSORT, STROBE and STREGA guidelines [9–11]. STROBE guidelines were used to develop checklist items for reporting information on participants, methods and results [9]. The STREGA extension was drawn on for genetic epidemiology items, specifically for data sources and measurement, and statistical methods [10]. New items were added to address radiotherapy aspects. An 18-item checklist is presented for reporting radiogenomics studies (Table 1).

## Explanation and elaboration of checklist items

The following sections explain briefly the relevance of each checklist item for radiogenomics, and elaborate on the details required for manuscript reviewers and readers, referencing exemplary papers where available.

### Items 1–3 – title and abstract; introduction: background/rationale and objectives

See STROBE explanation and elaboration paper [12].

### Item 4 – methods: study design

Samples and data can be collected in observational studies (cohort, case-control, cross-sectional) or within randomised controlled trials. For recent examples of different study designs see Kerns et al. and Barnett et al. [4,13]. An earlier paper by Bentzen elaborates on the importance of improving study designs in radiation oncology [14]. Information should

be given on the research question or hypothesis being tested; primary and secondary endpoints; and statistical power and justification of patient numbers. Reporting the statistical power of the study is important so that definitive conclusions can be drawn about the strength of association between toxicity and a genetic variant that the study was powered to detect; for example SNPs with a minor allele frequency of 25% or greater and genotype relative risk of 1.5 or greater. An example description of a power calculation is in Barnett et al. [4].

#### **Item 5 – methods: patient population**

Detailed information on the process leading to participant inclusion in a study is important because participants might differ from the target population to which any findings will be applied. Such information aids readers in understanding and potentially trying to reproduce the methods in validation studies. For cohort, case-control and cross-sectional studies, the population from which the sample was selected and the method of recruitment should be described. The number of individuals at each stage of recruitment should be accounted for ideally using a flow diagram, comparable to CONSORT [11]. Depending on the type of study, this may include the numbers of patients: examined for eligibility, confirmed as eligible, included in the study, with follow-up data, genotyped, successfully genotyped and analysed. Matching may be used in radiogenomics studies to make groups directly comparable for potential confounders or effect modifiers and reduce the complexity (as in cohort studies) or ensure the similarity in the distribution of variables and potential confounders between cases and controls (in case-control studies) [15,16]. For matched studies, the matching criteria and number of exposed and unexposed patients should be given in cohort studies and for case-control studies the number of controls per case. Though replication studies may not have the same matching information available, this will at least allow for assessment of limitations and potential discrepancies in the patient population that may account for lack of replication of SNP association. For examples of how to detail information see Barnett et al. and Fig. 1 in Kerns et al. [4,13].

#### **Item 6 – methods: radiation exposure**

The absorbed dose distribution across relevant normal tissues varies considerably with modern radiotherapy. This variability must be considered in radiogenomics studies, as toxicity depends on the distribution of dose in space and time (dose–volume effect and dose fractionation) [17]. Accurate dosimetry and appropriate quality assurance is needed to reduce the non-genetically related variation in toxicity so that replication studies can be compared with original reports of SNP-phenotype association. Authors should report methods for dosimetry or state if the information is not available.

If the critical normal tissue structure is known and relatively small (<1 cm in width), then it is important to report an accurate estimate of absorbed dose at that location (or mean dose to the contoured structure). Larger structures require a more extensive analysis of dose–volume relationships. In some cases, patient cohorts may be treated so uniformly that individual dosimetric parameters have little predictive value. In other cases, especially when inter-institutional data are used, or when the prescription dose varies within a cohort, variation in dose–volume parameters will cause variability in the incidence of toxicity and should be

included in any predictive model or as a covariate in multivariable analysis [18]. Dosimetric variability that is unaccounted for will reduce the ability to detect any genetic component to risk. A more detailed discussion on reporting dose–volume–toxicity studies is given by Jackson et al. [19]. If this information is available, authors should report details on how it was collected and used in the analysis. There are currently several examples of analyses that incorporate both dose and genetic risk factors [4,7,20,21]). Studies are increasingly involving multiple cohorts, and EQD2 can be calculated to quantify prescribed doses and differences in dose per fraction across studies, as illustrated in the Radiogenomics Consortium meta-analysis of TGF $\beta$ 1 studies [7].

### Item 7 – methods: phenotype(s)

The phenotype is radiotherapy toxicity, which can occur early (during or within weeks of treatment) or late (3 months to many years later). Second cancer induction is a very late toxicity [22]. The time when toxicity is assessed is important for radiogenomics because late effects can manifest many years after irradiation and can progress in severity [23]. The intensity with which follow-up information is sought and obtained influences incidence and prevalence estimates [24]. Cultural or socioeconomic differences in compliance to planned follow-up visits could potentially become confounding factors in radiogenomics studies unless they are accounted for in the analysis. Studies should specify whether toxicity was recorded at a single time point or the maximum grade from a series of follow-up assessments so that replication studies can be assessed with respect to similarity or difference in follow-up schedule. For some tumour types baseline symptoms are correlated with toxicity after radiotherapy. For example, late toxicity following radiotherapy to the prostate can be similar to the symptoms of prostate cancer, benign prostate disease and bladder disorders. Change in function from baseline may be calculated, or baseline function included as a covariate in the analysis. It is important that studies state whether baseline function and symptomology were assessed before the start of radiotherapy and, if so, provide a clear explanation of methods to account for these in the analysis.

There are multiple endpoints of toxicity both for the different tissues irradiated (e.g. skin telangiectasia, bowel obstruction or lung pneumonitis) and also within a tissue or organ (e.g. breast shrinkage, oedema, pigmentation, telangiectasia and pain) [5]. Several normal tissues may be irradiated, such as bowel, bladder and reproductive organs following radiotherapy for tumours in the pelvis. As some SNPs identified are likely to be endpoint specific, the endpoints studied should be carefully defined.

There are multiple scales for grading toxicity, e.g. the RTOG (Radiation Therapy Oncology Group)/EORTC (European Organisation for Research and Treatment of Cancer) late effects scale [25]; the LENT SOMA (Late Effects Normal Tissues: Subjective, Objective, Management and Analytic) system [26,27], now largely superseded by the NCI Common Terminology Criteria for Adverse Effects version 4.0 (CTCAEv4.0) [28]. Both physician- and patient-reported outcomes (PROs) can be obtained [24,29,30]. Authors should report on the instruments used for recording toxicity to provide readers with a clear understanding of how phenotypes were defined.

### Item 8 – methods: genotyping strategy and quality control

Authors should report the steps taken to ensure the high quality of genotyping data. For example, authors should report processes to prevent sample mix-up, such as ‘barcoding’ with a set of highly polymorphic SNPs that are present on the genotyping platform and genotyped separately to compare with array results. To ensure readers are confident that results are not biased by sample mix-up, authors should report whether duplicate samples were assayed in multiple experiments and concordant results obtained. Authors should also report whether pair-wise comparisons were performed to check for cryptic relatedness (i.e. unknown kinship). It is important to report samples excluded from analyses on the basis of question of identity or low call rate, as substantial differences in call rates between cases and controls can lead to spurious results. For transparency, authors should report the numbers of patients in whom genotyping was attempted and was successful.

Details should be given on the genotyping or sequencing platform chosen. If discordant findings are reported between studies, it is helpful to know whether one study used a platform with a higher call and lower error rate than the other, or whether one study ran all cases and controls in separate batches, which could contribute to differences in results. For the same reasons, authors should report how the data were cleaned: whether poorly performing samples were removed, and whether poorly performing or monomorphic and rare SNPs were filtered out. Some useful data checks that can inform the reader of the quality of data and potential sources of bias include the following: (i) checks for batch or study centre effects or for unusual patterns of missing data, including marked differences in the call rates between the cases and controls; (ii) a Hardy–Weinberg equilibrium (HWE) check to determine whether deviations from HWE are systematic from inbreeding, population stratification or subject selection as opposed to being limited to a discrete number of SNPs and possibly an indication of phenotype association; and (iii) check of SNP association distribution with the log quantile–quantile (QQ) *p*-value plot [31].

It is also important that authors report methods used for handling missing data. Authors should report if, and how, they investigated whether missingness is systematic between cases and controls. A few missing genotypes should not introduce bias; however, for multipoint analyses, many individuals might be missing one or a few genotypes, which could be a compounding effect. If data imputation is used to address missingness, this must be stated and the approach used reported.

### Item 9 – methods: data analysis and statistical methods

The analysis of data from radiogenomics studies is dominated by three major issues: (1) high dimensionality of the data set, (2) confounding factors and, for late toxicity, (3) censoring.

**High dimensionality data sets in radiogenomics**—Three main factors may contribute to the high dimensionality of radiogenomics data sets: (i) the scoring of a larger number of toxicity items often evaluated repeatedly over time; (ii) the availability of a large number of dose–volume parameters for each individual in the study population; and (iii) the often very large set of SNPs considered in each study. In a study addressing the impact of

just seven SNPs upon two different normal tissue endpoints (corresponding to 14 individual tests), the probability of at least one being significant by chance – even under the null hypothesis – is >50% assuming the conventional 5% significance level at each comparison. Thus, authors should interpret their results in the context of the experiment wide false positive rate, study-specific Q–Q plots, and/or genome-wide significance level rather than simply report the *p*-values for the individual SNPs. Another approach is to use independent validation data sets for testing the statistical significance of the top findings of the discovery study sample. Along similar lines, when reporting multiple-comparisons corrected data in radiogenomics, it is important to provide details on dose–volume measures included in multivariable models. A classical dose–volume histogram (DVH) contains a very large number of points and thus could represent a large number of possible ‘dosimetric’ variables tested. The potential for false-positive associations is due to the number of not only SNPs tested, but also the DVH parameters used.

**Confounding in radiogenomics**—A *confounding factor* or *confounder* is a variable that correlates (positively or negatively) with both the dependent variable (radiotherapy toxicity) and the independent variable (genotype) thus causing a spurious relationship between the two. A factor affecting the risk of radiotherapy toxicity is not a true confounder, unless it is also associated with genotype, typically through ancestry. Population stratification results in differences in allele frequencies between cases and controls because of systematic differences in ancestry rather than association of genes with disease. This is a significant confounder in all genetic association studies, and can make comparisons across studies with differing ancestry difficult. Radiogenomics studies can involve multi-ethnic cohorts because of the difficulty in obtaining large sample sizes with detailed clinical data from ethnically uniform populations. Authors should report methods used for assessing and correcting for population stratification, and corrected and non-corrected data should be compared.

An example is erectile dysfunction after brachytherapy for prostate cancer where a recent study showed that African American race/ethnicity is significantly associated with increasing log-odds for better erectile function even after adjustment for pre-treatment sexual health-related quality of life score and age [32]. This would mean that any genotype with significantly higher (or lower) prevalence in African Americans than Whites could show a spurious association with erectile dysfunction in a mixed patient sample of African Americans and Whites. In other words, race/ethnicity is a confounder for this endpoint. The link between ancestry and radiotherapy toxicity could also be due to variations in life-style factors; smoking for example lowers the risk of radiation pneumonitis [33], and the prevalence of smoking varies considerably in the United States according to race/ethnicity [34], again making smoking a potential confounder in radiogenomics studies.

Technique-dependent variation in radiation dose distribution – with a resulting effect on radiotherapy toxicity – and/or in time– dose-fractionation schedules could become a confounding factor especially in multi-centre radiogenomics studies where demographics vary between centres or where the use of a specific technique was related to race/ancestry, perhaps through socioeconomic status. An example is a SEER-MEDICARE based study showing that the use of intensity-modulated radiotherapy for head and neck cancer from 2000 through 2005 ranged from 11.3% of cases in Kentucky to 40.4% of cases in Hawaii,



creating a possible association with genotypes due to differences in ancestry in the two populations [35].

Any report of a radiogenomics study should carefully consider possible confounding factors and describe attempts at adjusting for these in the data analysis.

**Censoring and the analysis of late effects**—(Right-) censoring occurs when an endpoint requires prolonged observation of a patient. An example is skin telangiectasia, which can appear 10 years or more following irradiation [36]. For a patient without telangiectasia 5 years after radiotherapy, it is conceivable that the patient will never develop telangiectasia or, alternatively, the time to development of telangiectasia exceeds 5 years, i.e. the observation is *censored*. Special statistical methods are required to adjust for censoring and should be reported, see for example Bentzen et al. [37].

In some matched case control studies, controls (i.e. patients who had not reached the endpoint when last seen) are only included if they have a prescribed minimum follow-up (e.g. 5 years). This creates an asymmetry between cases and controls, as it appears unreasonable to disregard events that occur early after radiotherapy, i.e. in this case before 5 years. The problem in a radiogenomics context is that 5-year survivors are likely to have more favourable prognostic factors compared to the whole population of patients, e.g., having less advanced cancer or less likely to develop intercurrent disease, for example related to smoking. This again can lead to issues with confounding (see Section “Confounding in radiogenomics”).

#### **Item 10 – results: patient characteristics**

Description of patient characteristics and their exposures helps readers to assess the generalisability of the study findings. Information about potential confounders and effect modifiers, including whether and how they were measured and accounted for in the analysis (described in Methods, Item 9) influences judgments about study validity and relevance of findings. Authors should provide the description for the overall patient sample as well as for subgroups, such as patients presenting with events. Continuous variables can be summarised using the mean and standard deviation, or the median and inter-quartile range. Ordinal and categorical variables should be presented as frequency distributions.

Genotype distribution of patients with and without the event of interest can be compared for potential effect modifiers, such as age, body mass index, and co-morbidity (e.g. diabetes, collagen vascular disease). Information on the amount of missing data for relevant parameters (in tables or figures) should be provided for assessment of potential bias or generalisation of results. This also applies to the extent of loss to follow-up. Duration and extent of follow-up for the available outcome data can be provided as a summary with either the median or mean follow-up time, where appropriate as well as the minimum and maximum follow-up times.

#### **Item 11 – results: phenotype(s)**

As in any epidemiologic study, details of the numbers of cases (and controls if used) with quantitative outcomes such as the mean, median, and range should be given and how these

have been obtained. However, some aspects of phenotype reporting are specific to radiation oncology. For example, there is under-reporting in clinical trials of toxicity deemed to be less important or not requiring surgical correction [38]. Some radiogenomics studies include only relatively high-grade toxicity in analyses (i.e. cases and controls), since it is sometimes difficult to capture low grade toxicity. It may be clinically relevant to account for the full spectrum of toxicity in the study population. If data are missing then the way in which this is handled should be reported (*linked with Item 9*).

It is important for many endpoints that baseline function is reported in radiogenomics studies since the aim is to identify genetic variants associated with toxicity *specifically attributable* to radiotherapy (see Item 7). If available and relevant, summary statistics for baseline function should be reported. Other co-morbid conditions, previous treatments such as surgery, and obstetric history can give rise to patient symptoms which should not be attributed to radiotherapy (*linked with Item 7*). This applies to treatment sites such as the pelvis where pre-existing bladder and bowel symptoms are common, as are co-morbid conditions in an ageing population. For example, pre-treatment sexual potency correlates strongly with post-treatment sexual potency [32]. Given this strong association, it would be important to know whether (and how) pre-treatment sexual potency is accounted for in studies of genetic predictors, so that comparisons in SNP effect size(s) could be drawn between studies.

### **Items 12 and 13 – results: genotypes (12) and primary associations (13)**

Little et al. discuss reporting of genotype and primary association results in the STREGA guidelines [10]. We defer to their explanation for the main aspects of these checklist items. Two points are particularly relevant to radiogenomics.

First, radiogenomics association results should be reported in the context of clinical exposures (radiation dose, volume and type; see Item 6) and effect modifiers (e.g. use of chemotherapy, smoking history). Investigators should report whether they sought associations between radiation exposures and toxicity. Similarly, it is important to report whether results are adjusted or controlled for co-morbidity and surgical procedures, which may cause non-radiation related manifestation of the phenotype. This in turn, increases the variance of the dependent variable thus increasing the risk of false negative findings in a study of a given size. For example the use of clinical photographs immediately after surgery and before radiotherapy for breast cancer in the United Kingdom START trials enabled assessment of breast shrinkage due to radiotherapy [39,40]. However, much of the clinically assessed breast in duration and shrinkage at 2 years is due to surgery rather than radiotherapy [41]. In the case of significant associations, it should be reported whether genotype–phenotype association results presented are adjusted for significant non-genetic factors.

Second, as highlighted above for Items 8 and 9, the large sample size required in radiogenomics studies makes it difficult to obtain sufficiently large cohorts from ethnically uniform populations, and so cohorts are often multi-ethnic. Previous candidate gene studies largely ignored ethnicity in genotype–phenotype associations, and this undoubtedly has contributed at least in part to their lack of reproducibility. Therefore it is important that



investigators report whether ethnicity was controlled for in reporting of genotype–phenotype associations and downstream predictive modelling. If there is evidence of association, ethnicity-adjusted genotype–phenotype results should be reported.

#### **Item 14 – results: secondary analyses**

In the STROBE Explanation & Elaboration paper, Vandembroucke et al. discuss the reporting of secondary analyses, e.g., analyses of additional endpoints, sub-groups, interactions and sensitivity [12]. The problems associated with carrying out multiple analyses are particularly relevant with the phenotype of radiotherapy toxicity, where multiple toxicities are often studied in a single cohort. Due to the danger of chance findings, un-planned secondary analyses must be reported as such.

#### **Item 16 – discussion: limitations**

Given the numerous study design, endpoint and sample size challenges described above, authors should report on the limitations of their studies. It is unlikely that a study will have information on all of the potential confounders and modifiers that could influence association between genotype and toxicity phenotypes. Similarly, few studies will have complete follow-up for toxicity at regular intervals on every patient. Authors should report on the variables unavailable for the study and discuss how omitting these variables from analysis might affect their results.

In any genetic association study, the sample size and population affect the type of genetic factors identified. A smaller study will be likely to miss variants associated with very modest effect sizes or variants that have very low minor allele frequency. A study carried out in a Northern European population could potentially miss variants that are prevalent in Asian or African populations. Authors should report on the limitations of their findings with respect to whether there are likely to be clinically relevant variants yet to be identified in larger studies, with different genotyping coverage, and/or in ethnically different populations.

#### **Item 17 – discussion: interpretation**

Most radiogenomics studies aim (1) to establish predictors of treatment response and/or (2) to explore the mechanisms underlying radiation effects. If prediction is the primary purpose of the study, the discussion should, if possible, provide an estimation of the clinical utility of a test based on the reported genetic association. If exploration of biological mechanisms is the primary aim, the interpretation should include possible mechanistic implications of the findings including the possibility that the investigated SNP/s may be in linkage disequilibrium with other SNP/s and therefore may not be the causative variants. Although some common genetic alterations may affect radiosensitivity across tissue types/endpoints, others may only be relevant for individual endpoints. This distinction should also be considered in the interpretation of the results.

Findings should also be discussed in the context of other studies addressing the impact of the same SNP/s. If possible, a formal meta-analysis of the new and previous results could be considered as part of the discussion. In addition, methods to reveal potential publication bias (e.g. a funnel plot) should be considered whenever relevant.

### Item 18 – discussion: generalisation and clinical utility

Authors should discuss the clinical utility of identifying a population with higher/lower risk of developing toxicity in relation to the effect sizes found. For example, radio resistant patients might be offered a higher dose with modern techniques or radiotherapy in combination with systemic therapies with the aim of improving local control. In some cases it may be considered to avoid radiotherapy completely in individuals with a high risk of developing toxicity, provided that an effective alternative exists. For example, for prostate cancer, surgery could be offered instead of radiotherapy if a high risk of rectal bleeding or urinary discomfort is reliably identified by a test before starting treatment. Alternately, active surveillance could be considered for cancers with a very low risk of progression. In cases where therapeutic alternatives to radiotherapy are not available, a high risk of toxicity could lead to the patient being considered for new radiotherapy techniques like protons. This part of the discussion is therefore important to consider the translation of biological results to the clinic in terms of implementation and utility. If possible, specificity and sensitivity of a potential test should be discussed.

### Conclusions

Although numerous radiogenomics studies have been published, positive SNP associations have rarely been reproduced in independent validation studies. The inconsistent findings might in part be due to a high number of false positive findings because adjustments for multiple comparisons were not made. The inconsistent findings may also be due, in part, to underpowered discovery studies. It is, however, also possible that some validation studies have been false negatives due to methodological shortcomings or a failure to reproduce relevant details of the original study. Without complete and transparent reporting, these flaws will continue to hamper progress in radiogenomics. The guidelines outlined in this paper aim to correct this shortcoming. Like other reporting guidelines, STROGAR is intended as a guideline only rather than a prescription for study design and conduct – though investigators may find the guideline of some help when designing a prospective study protocol. It is hoped that the STROGAR guidelines will help researchers improve their design and reporting of new radiogenomics studies, interpret published research, and facilitate the discovery of SNPs that are genuinely associated with radiotherapy toxicity.

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**Table 1**

STROGAR – 18-item checklist for reporting radiogenomics studies.

	Item number	Recommendation
<i>Title and abstract</i>		
Title and abstract	1	Include the primary outcome(s) and type of study (whether GWAS or gene-specific); provide an informative summary of the study including study design, whether discovery or validation, sample size, main endpoints, and major results.
<i>Introduction</i>		
Background/rationale	2	Note if the study is a GWAS or a candidate gene/SNP study and, if candidate gene study, rationale for choice of genes/SNPs; give a general description of the study setting.
Objectives	3	Define the primary/main outcome(s) of interest; describe the overall/long-term goal of the study; note if it is a discovery, validation, or multi-stage study. Use terminology and definitions from National Cancer Institute biomarker study guidelines [42], where applicable.
<i>Methods</i>		
Study design	4	Specify the study design (case-control, cohort); whether data were collected under a controlled trial setting; whether data were collected retrospectively or prospectively. Report power and sample size considerations.
Patient population	5	Specify the source(s) of the patients and, if multiple sources, whether they are pooled or treated as separate cohorts; define inclusion/exclusion criteria; report whether co-morbidities and medications were assessed by self-report or medical records; define methods/system used for tumour staging; describe the larger patient population from which the study sample was drawn; define how major changes in treatment protocol were handled in the analysis.
Radiation exposure	6	Specify details of radiation treatment parameters including: organ(s)-at-risk, dose–time–fractionation; dose–rate, target volume selection [ex: breast + boost], dose to critical substructures, dose–volume metric used, the type of treatment and treatment setting, radiation modality [ex: external beam vs. brachytherapy], whether single or combined treatment modalities were used, whether primary treatment or salvage therapy, imaging & planning details, ICRU recommendations followed and note relaxation of criteria, note any changes in dose or treatment protocol over the time course of enrolment and whether there were any interruptions in treatment.
Phenotype(s)	7	Specify how intra-patient or pre-treatment assessment was made and whether it is accounted for in defining phenotype(s); note whether patient reported outcomes or physician-assessed outcomes are being used to define phenotype(s); note which toxicity scoring system was used (if using a common/standard system); define the grading scales used and whether the phenotype(s) is/are defined as continuous, dichotomous or categorical; describe frequency of follow-up scheduling and diagnostic intensity; define the post-treatment timeframe for assessment of toxicity outcomes; describe whether outcome(s) is/are based on a single time point or the maximum/worst time point out of a series of follow-up assessments; note if/how competing risks were handled (such as non-radiation related manifestation of the phenotype); note any medical intervention that may influence study outcome(s).
Genotyping strategy and QC	8	Specify DNA source and isolation methods; note the methods/platform used for genotyping; specify whether genotyping was done in one stage or multiple stages; note whether genotyping was done in more than one lab or batch, and if so, how batch effects were handled; describe methods for genotype calling and cite the algorithm used; note whether genotype calling was done for the whole study sample together or in batches; describe quality control (QC) methods including concordance between duplicates, control samples, and checks for cryptic relatedness; describe methods for assessing population structure; describe SNP/CNP filtering methods including filtering on per-sample call rate, per-SNP call rate, minor allele frequency and Hardy–Weinberg equilibrium; note whether imputation was used and, if so, describe methods.
Data analysis and statistical methods	9	Define the statistical methods and models used for association testing; cite the software and settings used; describe how censoring was handled; define model selection methods used for multivariable models; describe whether all samples are analysed together or sequentially if the study involves multiple cohorts; for multi-stage studies, define methods for selecting variants to follow up in subsequent stages; describe how missing data were handled; if multiple cohorts were included, describe data harmonisation methods; note whether gene–gene interaction or gene–environment interaction was investigated; describe methods used to adjust for population structure; describe methods used to correct for multiple comparisons and/or control for risk of false-positive findings.
<i>Results</i>		
Patient characteristics	10	Report number of individuals at each stage of the study (e.g. numbers examined for eligibility, numbers confirmed eligible, included in study, completed follow-up, successfully genotyped and analysed). Give reasons for nonparticipation at each stage. Give description of the included patient sample regarding demographic (e.g. age at start of therapy, sex, race/ethnicity) and clinical

	Item number	Recommendation
		characteristics (e.g. site and stage of primary tumour, chemotherapy, hormone therapy), details of radiation exposure, where appropriate (e.g. type, dose, boost) and potential confounders and effect modifiers (e.g. life-style related factors, co-morbidities, and medications), including missing data; report length of follow-up and number of events and number of patients at risk at various follow-up times e.g. yearly. It is recommended to include a flow diagram of patients included/excluded from the study, as proposed by the CONSORT statement
Phenotype(s)	11	Report baseline function (if relevant); report numbers of responders and non-responders for dichotomous outcomes, descriptive statistics for quantitative outcome(s), or distributions for categorical outcomes.
Genotypes	12	Report call rates; numbers of samples and numbers of SNPs excluded on the basis of QC filters; if imputation was used, note which variants are imputed and which are genotyped directly; report genetically determined racial/ethnic groups or other population clusters; report genomic inflation factor as well as corrected genomic inflation factor after controlling for population structure.
Primary associations	13	For each SNP/CNP, report: common identifier (such as dbSNP rs number), minor allele identity and frequency, phenotype by genotype category, effect size (with 95% confidence interval) and <i>p</i> -value; genetic inheritance model(s) used; for multivariable analyses, report unadjusted and adjusted estimate and note which covariates were included in the model(s).
Secondary analyses	14	Report sub-group analyses and/or secondary outcomes of interest.
<i>Discussion</i>		
Key results	15	Summarise key results in the context of the study objectives given in the Introduction.
Limitations	16	Discuss limitations of the study in the context of bias (noting both direction and size), confounding, sample size and power, and representativeness of study population.
Interpretation	17	Provide an overall interpretation of the findings in the context of previous clinical studies, genetic association studies, and biological studies of radiation response.
Generalisability and clinical utility	18	Comment on the potential clinical utility of the findings in the context of the patient populations to which the results may apply.