Definitive radiotherapy improves locoregional control and survival in inoperable non–small cell lung cancer patients. However, radiation-induced toxicities (pneumonitis/esophagitis) are common dose-limiting inflammatory conditions. We therefore conducted a pathway-based analysis to identify inflammation-related single-nucleotide polymorphisms associated with radiation-induced pneumonitis or esophagitis. A total of 11,930 single-nucleotide polymorphisms were genotyped in 201 stage I–III non–small cell lung cancer patients treated with definitive radiotherapy. Validation was performed in an additional 220 non–small cell lung cancer cases. After validation, 19 single-nucleotide polymorphisms remained significant. A polygenic risk score was generated to summarize the effect from validated single-nucleotide polymorphisms. Significant improvements in discriminative ability were observed when the polygenic risk score was added into the clinical/epidemiological variable-based model. We then used 277 lymphoblastoid cell lines to assess radiation sensitivity and expression quantitative trait loci (eQTL) relationships of the identified single-nucleotide polymorphisms. Three genes (PRKCE, DDX58, and TNFSF7) were associated with radiation sensitivity. We concluded that inflammation-related genetic variants could contribute to the development of radiation-induced toxicities.

Locally advanced non–small cell lung cancer (NSCLC) is frequently treated with radiotherapy either alone or with chemotherapy. Although higher doses of radiotherapy have been associated with improved outcomes, some patients develop radiation-induced toxicity (even death) that often necessitates dose reductions that hamper the effectiveness of therapy.

Acute normal tissue toxicity (pneumonitis or esophagitis) following radiotherapy for NSCLC is primarily due to hyperinflammation of the lung or the esophagus following exposure to radiation. The development of either toxicity is multifactorial and difficult to predict. Although some clinical and dosimetric variables are associated with toxicity, these variables by themselves are not reliable predictors due to individual variation. Thus, objective biomarkers are sorely needed to minimize events and improve treatment efficacy.
positives, a validation analysis was undertaken, bringing the total population of NSCLC patients included in the current analysis to 465—the largest study of its type yet performed. To better understand the potential functional consequences of the identified toxicity-associated variants, we utilized a lymphoblastoid cell line (LCL) model system of radiation sensitivity. Our approach incorporates discovery, validation, and functional assessment of inflammation-related genetic variation as a step toward the identification of meaningful genetic markers that can be used in the clinic to guide treatment decisions for NSCLC patients receiving radiation.

RESULTS

Patient characteristics
A total of 201 NSCLC patients (109 men and 92 women) with a mean age of 65 years were included in the discovery phase.

| Variable | Discovery, n (%) | Validation, n (%) |
|----------|-----------------|------------------|
| Age, mean (SD) | 65.0 (9.5) | 62.9 (10.4) |
| Sex | | |
| Male | 109 (54%) | 122 (56%) |
| Female | 92 (46%) | 98 (44%) |
| Smoking pack-years (SD) | 51.7 (29.0) | 53.7 (28.9) |
| Clinical stage | | |
| Stage I | 18 (9%) | 26 (12%) |
| Stage II | 25 (12%) | 24 (11%) |
| Stage IIIA | 98 (49%) | 104 (47%) |
| Stage IIIB | 60 (30%) | 66 (30%) |
| Performance status | | |
| 0 | 60 (30%) | 51 (23%) |
| 1 | 96 (48%) | 99 (45%) |
| 2–4 | 17 (8%) | 30 (14%) |
| FEV1 percentage (mean, SD) | 68.2 (21.1) | 67.6 (18.8) |
| DLCO percentage (mean, SD) | 66.7 (21.6) | 62.1 (19.0) |
| Planned target volume (mean, SD) | 667.0 (440.8) | 755.0 (481.0) |
| MED (mean, SD) | 26.8 (11.6) | 30.1 (13.1) |
| MLD (mean, SD) | 15.4 (5.1) | 18.8 (9.4) |
| Radiation type | | |
| 2D | 24 (12%) | 94 (43%) |
| 3D | 30 (15%) | 102 (46%) |
| IMRT | 111 (55%) | 24 (11%) |
| Proton | 36 (18%) | NA |
| Concurrent chemoradiotherapy | 117 (58%) | 135 (61%) |
| Total | 201 | 220 |

DLCO, diffusing capacity or transfer factor of the lung for carbon monoxide; FEV1, forced expiratory volume in 1 s; IMRT, intensity-modulated radiation therapy; MED, median esophageal dose (Gy); MLD, median lung dose (Gy); NA, not available; PTV, planning target volume (cm³).

Grade 2 or higher pneumonitis or esophagitis were present in 70 (37%) and 90 (45%) patients, respectively. Thirty patients (15%) experienced both toxicities. All patients were smokers with a median smoking history of 51.7 pack-years. The majority of patients had stage IIIA (49%) or IIIB (30%) disease. Only 8% patients had a pretreatment performance score of 2 or higher. A total of 117 (58%) patients received concurrent chemoradiotherapy; the majority (55%) of these patients were treated with intensity-modulated radiation therapy, to a median dose to the lung of 15.9 Gy and a median esophageal dose of 28.4 Gy. The validation phase included 220 NSCLC patients. Of this group of patients, 77 (35%) had grade 2 or higher pneumonitis and 113 (51%) had esophagitis, with 47 (21%) patients having both pneumonitis and esophagitis. Age, sex, smoking status, clinical stage, and pretreatment performance status were comparable between the validation and discovery phases (Table 1).

Individual SNP analysis
A total of 11,930 SNPs from 904 inflammation-related genes were included in the discovery analysis. Of these, 1,208 were significantly associated with esophagitis and 1,321 with pneumonitis at \( P < 0.05 \). Genotyping data for the validation phase from a previous genome-wide association study for lung cancer risk\(^{12}\) were available for 226 SNPs for esophagitis and 234 SNPs for pneumonitis.

Esophagitis
Ten SNPs were validated as being significantly associated with esophagitis (Table 2). The most significant SNP, rs1239344, is located in the 3’UTR region of OSMR (encoding for
Table 2  Inflammation-related genetic variants associated with radiation-induced pneumonitis and esophagitis

| Chr | Gene | Location | SNP  | Model  | Discovery (OR (95% CI)a | P   | Validation (OR (95% CI)a | P   | Meta-analysis (OR (95% CI)) | P   | P-het |
|-----|------|----------|------|--------|--------------------------|-----|--------------------------|-----|----------------------------|-----|-------|
|     |      |          |      |        | OR validation (95% CI)    |     | OR meta (95% CI)         |     |                            |     |       |
| 5   | OSMR | 3′-UTR   | rs1239344 | DOM   | 2.45 (1.14–5.26)         | 0.021 | 4.15 (1.68–10.28)        | 0.002 | 3.05 (1.70–5.47)           | 1.78× 10⁻⁴ | 0.383 |
| 19  | TNFSF7 | 3′-UTR   | rs7259857 | ADD   | 0.50 (0.30–0.84)         | 0.008 | 0.50 (0.28–0.90)         | 0.021 | 0.50 (0.34–0.74)           | 4.48× 10⁻⁴ | 0.981 |
| 2   | PRKCE | Intron   | rs940052 | DOM   | 0.34 (0.16–0.75)         | 0.007 | 0.37 (0.15–0.90)         | 0.030 | 0.35 (0.19–0.64)           | 5.49× 10⁻⁴ | 0.912 |
| 13  | FGFR4 | 5′-UTR   | rs4772468 | DOM   | 2.56 (1.20–5.47)         | 0.015 | 2.76 (1.14–6.80)         | 0.027 | 2.64 (1.48–4.72)           | 1.04× 10⁻³ | 0.897 |
| 6   | TAP1 | Intron   | rs3819721 | ADD   | 2.30 (1.12–4.72)         | 0.023 | 2.55 (1.14–5.68)         | 0.022 | 2.41 (1.41–4.11)           | 1.28× 10⁻³ | 0.851 |
| 12  | CD4  | Intron   | rs2707212 | DOM   | 2.70 (1.23–5.92)         | 0.013 | 2.23 (1.04–4.79)         | 0.040 | 2.45 (1.42–4.23)           | 1.34× 10⁻³ | 0.730 |
| 19  | LILRP2 | Exonb   | rs270771 | DOM   | 0.28 (0.08–0.93)         | 0.037 | 0.10 (0.02–0.66)         | 0.017 | 0.21 (0.08–0.57)           | 2.30× 10⁻³ | 0.375 |
| 10  | IL15RA| 3′-UTR   | rs1998521 | ADD   | 1.78 (1.03–3.08)         | 0.037 | 1.82 (1.02–3.26)         | 0.043 | 1.80 (1.21–2.68)           | 3.67× 10⁻³ | 0.958 |
| 2   | TANK | 3′-UTR   | rs7309  | DOM   | 2.42 (1.06–5.55)         | 0.036 | 3.02 (1.04–8.77)         | 0.043 | 2.63 (1.37–5.06)           | 3.76× 10⁻³ | 0.750 |
| 6   | AGER | 5′-UTR   | rs204993 | ADD   | 0.56 (0.32–0.98)         | 0.043 | 0.54 (0.30–1.00)         | 0.050 | 0.55 (0.36–0.83)           | 4.82× 10⁻³ | 0.950 |

ADD, additive model; Chr, chromosome; CI, confidence interval; DLCO, diffusing capacity or transfer factor of the lung for carbon monoxide; DOM, dominant model; FEV1, forced expiratory volume in 1 s; OR, odds ratio; P-het, P value for heterogeneity; UTR, untranslated region.

aAdjusted for age, sex, pack-years, clinical stage, performance status, concurrent chemoradiotherapy, radiation treatment type, FEV1 percentage, DLCO percentage, planned volume, and median dose (median lung dose for pneumonitis; median esophageal dose for esophagitis).13 In pseudogene.

oncostatin M receptor) and is located in a predicted microRNA binding site for seven microRNAs based on predictions in the PolymiRTS database.13 This SNP was associated with a greater than twofold increased risk of developing esophagitis in both the discovery (odds ratio (OR) = 2.45, 95% confidence interval (CI) = 1.14–5.26, P = 0.021) and validation (OR = 4.15, 95% CI = 1.68–10.28, P = 0.002) phases. This finding was highly significant (P = 1.78× 10⁻⁴) in the combined meta-analysis, with a 3.05-fold increase in risk of esophagitis (95% CI = 1.70–5.57).

Pneumonitis

Nine SNPs were significantly associated with pneumonitis in the validation population (Table 2). The most significant SNP, rs107711, is located in the 3′UTR region of CDK1 (encoding for cyclin-dependent kinase 1) and is predicted to create a new binding site for miR-1306-5p.13 This SNP was significantly associated with a higher risk of pneumonitis in both phases of the study under the dominant model (ORdiscovery = 2.67, 95% CI = 1.26–5.63, P = 0.010; ORvalidation = 2.33, 95% CI = 1.21–4.48, P = 0.011). In the meta-analysis, this increase remained significant (ORmeta = 2.47, 95% CI = 1.51–4.04, P = 3.08× 10⁻⁴).

Polygenetic risk score analysis

To quantitate the effect of multiple risk genotypes, polygenic risk scores (PRSs) were calculated to better assist in identifying those at highest risk for radiation-induced toxicity (Table 3).

The mean PRS for esophagitis was similar for both the discovery population (6.10; range: 2.44–9.34) and the validation population (6.12; range: 2.42–9.34). There was a consistent association with increased risk of developing esophagitis with per score increase in the PRS (ORdiscovery = 3.73, 95% CI = 2.42–5.75, P = 2.72× 10⁻⁹; ORvalidation = 3.03, 95% CI = 2.03–4.53, P = 6.38× 10⁻⁸; ORmeta = 3.33, 95% CI = 2.48–4.48, P = 1.11× 10⁻¹⁵). A similar effect was observed for pneumonitis, with a mean PRS of 5.20 (range: 1.61–10.19) in the discovery population and 5.07 (range: 0.88–9.34) in the validation population. The PRS was positively associated with a trend of significantly increased risk of pneumonitis (ORdiscovery = 1.97, 95% CI = 1.54–2.52, P = 8.29× 10⁻³; ORvalidation = 1.84, 95% CI = 1.45–2.32, P = 3.62× 10⁻²; ORmeta = 1.90, 95% CI = 1.60–2.25, P = 1.58× 10⁻¹³).

We then tested the ability of the identified genetic variants to enhance the prediction of radiation-induced toxicity in a subset of the population with complete clinical and genotyping information. A strong improvement of discrimination ability was observed for esophagitis when adding identified loci into...
the risk model. In the baseline model created with the clinical and epidemiological variables included in the main effect analysis, the area under the curve (AUC) for the receiver operating curve (ROC) was 0.799. With the inclusion of the PRS, there was a significant shift in the AUC to 0.936. Bootstrap resampling confirmed the significant increase in the AUC (ΔAUC = 0.137, 95% CI = 0.111–0.236; Figure 1a). A shift in the AUC was also observed for pneumonitis. The AUC for the baseline model was at 0.755, and with the addition of the PGs into the baseline model the AUC increased to 0.794. This improvement in the prediction discrimination when adding genetic markers was shown to be significant following 1,000 bootstrap resamplings (ΔAUC = 0.039, 95% CI = 0.001–0.123; Figure 1b).

Functional correlation with radiosensitivity of significant SNPs
Following imputation, 4,786 additional SNPs were identified in the 18 candidate regions harboring the 19 validated SNPs. Of these, 135 (116 imputed and 19 genotyped) were significantly associated with radiation-induced toxicities in the combined discovery and validation population. We selected these SNPs to assess for functional correlation with radiation sensitivity via the LCL model system that incorporates baseline host gene expression and cytotoxicity following radiation treatment. A total of 45 SNPs in three genes (PRKCE, DDX58, and TNFSF7) were found to be significantly associated with radiation response, which is more than the 5.83 that would be expected by chance alone (P = 1.21 × 10⁻²⁷). PRKCE and TNFSF7 SNPs also showed eQTL relationships (Supplementary Table S1 online).

PRKCE is located on chromosome 2 and encodes for protein kinase C, epsilon. The genotyped variant, rs940052, is located in an intron and was associated with significantly decreased risk for esophagitis (OR<sub>meta</sub> = 0.34, 95% CI = 0.19–0.62, P = 4.03 × 10⁻⁴). Although this SNP was not correlated with radiosensitivity, 40 imputed SNPs within the region surrounding PRKCE were found to be significantly associated with not only risk of esophagitis but also radiation response and cis-regulation of PRKCE. For example, an imputed SNP (rs11125035) located 3′ to rs940052, also in an intronic region, was associated with significantly decreased risk of esophagitis (OR<sub>meta</sub> = 0.34, 95% CI = 0.19–0.62, P = 4.03 × 10⁻⁴). This same variant was also borderline significantly associated with radiation response in terms of both AUC (P = 0.145, P = 0.019) and GI50 (P = 0.123, P = 0.046). Moreover, this SNP showed a borderline significant cis-eQTL relationship with DDX58 expression (r = 0.115, P = 0.058).

DISCUSSION
Inflammation is believed to be the most important cellular process contributing to the etiology of esophagitis and pneumonitis. In this study, we applied a targeted, systematic approach to assess the association of inflammation-related SNPs with radiation-induced toxicity. We focused on Caucasian NSCLC patients to maintain a homogeneous population, and the effect of population substructure is minimal for both pneumonitis and esophagitis (data not shown). To minimize potential false-positive findings, our study design included a validation step with analysis of additional samples and also incorporated functional genomic analyses to provide a potential biological basis for the observed associations.

Among the variants identified in the analysis of pneumonitis or esophagitis, several loci showed functional significance. The intronic SNP rs11795343 in DDX58, associated with increased pneumonitis risk, was significantly associated with radiation responses and host gene expression in the LCLs. DDX58 encodes a DEAD box protein involved in host immune response.16 The homolog of DDX58 in a pig model system has been shown to play a role in infectious disease.17 Schneider et al. found that the

Table 3 Polygenetic risk score (PRS) for radiation-induced toxicity

| Toxicity | Discovery | Validation | Meta-analysis |
|----------|-----------|------------|---------------|
|          | Event n (%) | No event n (%) | OR (95% CI)<sup>a</sup> | P | Event n (%) | No event n (%) | OR (95% CI)<sup>a</sup> | P | OR (95% CI) | P | P-het |
| Esophagitis (10 SNPs) | 90 (44.8%) | 111 (55.2%) | 3.73 (2.42–5.75) | 2.72 × 10⁻⁹ | 113 (51.8%) | 105 (48.2%) | 3.03 (2.03–4.53) | 6.38 × 10⁻⁸ | 3.33 (2.48–4.48) | 1.11 × 10⁻¹⁵ | 0.493 |
| Pneumonitis (9 SNPs) | 70 (37.0%) | 119 (63.0%) | 1.97 (1.54–2.52) | 8.29 × 10⁻⁸ | 77 (38.9%) | 121 (61.1%) | 1.84 (1.45–2.32) | 3.62 × 10⁻⁷ | 1.90 (1.60–2.25) | 1.58 × 10⁻¹³ | 0.693 |

<sup>a</sup>Adjusted for age, sex, pack-years, clinical stage, performance status, concurrent chemoradiotherapy, radiation treatment type, FEV1 percentage, DLCO percentage, planned volume, and median dose (median lung dose for pneumonitis; median esophageal dose for esophagitis).
expression of genes involved in oxidative stress and viral infection response, including DDX58, was increased in airway epithelial cells from patients with chronic obstructive pulmonary disease. This variant is not located in known The Encyclopedia of DNA Elements (ENCODE) regulatory elements, suggesting that a currently unknown element is present in this region or that this variant is in linkage disequilibrium with a yet undiscovered causal SNP that mediates DDX58 function.

Variants in PRKCE and TNFSF7 resulted in decreased risk of esophagitis and were also found to be correlated with radiation response and host gene expression in the LCLs. Protein kinase C epsilon (PRKCE) is a member of the protein kinase C family that can phosphorylate a number of protein targets and that participates in a diverse array of cellular processes. It is also known to promote NSCLC growth and enhance lung cancer cell survival through suppression of apoptosis. Studies have found that PRKCE is associated with radiation-induced cellular changes. In our study, rs940052 and 35 imputed SNPs in PRKCE were significantly associated with esophagitis, radiation response, and host gene expression. It is likely that this SNP could result in altered expression of PRKCE, therefore affecting response to radiation toxicity in the surrounding normal cells. The third significant variant was located within TNFSF7 encoding for a tumor necrosis factor (TNF) ligand family member that contributes to T-cell proliferation and activation. TNFSF7 (also known as CD70) is upregulated by radiation exposure, which results in T-cell activation. Our results for the first time suggest a potential important role of this gene in the development of esophagitis and radiosensitivity.

Overall, the majority of the 17 genes implicated in risk of acute radiation-induced toxicity are involved in a set of key cellular processes related to inflammation—this includes the nuclear factor κB, mitogen-activated protein kinase/c-Jun N-terminal kinase, and Janus kinase/signal transducer and activator of transcription pathways. All these pathways have extensive support for their role in inflammation. More relevant to the current study, there is evidence that they play a role in response to radiation (reviewed by Dent and Valerie).
the observed associations of these genes with the risk of developing pneumonitis and esophagitis.

Increasingly, the focus in genetic association studies has moved from single-variant effects to the combined effect of multiple variants on risk for complex diseases and traits. Previous studies have demonstrated the benefits of using polygenic risk scores for risk estimates of body mass index, prostate and breast cancer, and bladder cancer. Similarly, in this study we tested the effect of accumulated genetic information and potential for improved risk stratification to identify those at high risk of radiation-induced toxicities. Two PRSs were developed based on the validated loci for pneumonitis and esophagitis. These scores were able to identify those at high risk in both the discovery and validation populations with relatively high effect sizes. Rather than consider the effect from each SNP individually, the generated PRSs summarized the information from all preselected genetic loci and more accurately represent the genetic risk of each patient. Therefore, as shown in the prediction models developed, this approach of assessing the cumulative effect across a panel of genetic variants holds more power to accurately predict toxicity risk, increasing the potential for clinical application.

Previous studies have developed prediction models based on clinical and dosimetric variables to identify patients at high risk for developing radiation-induced toxicities, with varied accuracy. In the current study, we demonstrated the ability of genetic information to improve prediction discriminative accuracy. The AUCs for the models that incorporated genetic information were within the range that could have the potential for clinical translation. The esophagitis model with the PRS information reached a predictive power of >93%, making it potentially a strong tool to better stratify patients into different risk groups and to help minimize toxicity. Further efforts will be needed to validate and calibrate these models in external populations.

In conclusion, our study provides strong evidence that genetic variation in inflammation-related pathways has a significant effect on the risk of developing radiation-induced acute normal tissue toxicities in lung cancer patients. Functional analysis from the LCL model system provides additional evidence supporting our findings and information on the potential mechanism underlying our identified associations. Although not definitive evidence regarding a biological effect underlying the observed associations, the functional assessment in the LCL model system provides additional evidence in support of the validity of the genetic loci being associated with radiation toxicity in NSCLC patients and presents potential mechanistic links for these events that are worthy of further analysis. Our hope is that these findings can guide future, in-depth investigation, including the in vitro experiments proposed here, regarding these mechanisms. Together, the validated genetic loci and the constructed PRS could have great power to be used in the clinic to guide personalized decisions regarding optimal radiation dosages for NSCLC patients who receive primary radiation therapy.

**METHODS**

**Patient population and data collection.** Patients were recruited as part of an ongoing lung cancer case–control study at the University of Texas MD Anderson Cancer Center initiated in 1995. All patients were Caucasian, histologically confirmed stage I–III NSCLC patients who were treated with definitive radiotherapy or concurrent chemoradiation therapy. The dose and fraction were prescribed according to tumor stage, size, and site; surgery status was also considered, and patients generally received 55–70 Gy at 1.8–2 Gy per fraction with or without chemotherapy. We included only patients with a radiation total dosage ≥45 Gy in order to focus only on those who received definitive radiation treatment. We excluded any patient treated with stereotactic or hypofractionated radiotherapy (defined as >3 Gy per fraction). During an in-person interview, demographic and epidemiologic variables were collected. Clinical and treatment information (pretreatment performance status, treatment regimens, and radiation dosimetric variables) was abstracted from medical records. Grade 2 or higher pneumonitis and esophagitis were scored based on the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0) guidelines. All patients signed a written informed consent prior to enrollment. This study was approved by the University of Texas MD Anderson Cancer Center institutional review board.

**SNP selection and genotyping.** Inflammation-related gene definitions and SNP identification have been described previously. Briefly, inflammation-related genes were defined based on a predefined inflammation panel and refined through database exploration (T1DBase (http://www.t1dbase.org); University of Cambridge, Cambridge, UK). Tagging SNPs within a 10-kb flanking region of each candidate gene region were identified using the Tagger pairwise method with an r2 threshold of 0.8 and minor allele frequency ≥0.05 based on genotyping data from the Caucasian population of the International HapMap Project. The final SNP list was used to build a customized Infinium II iSelect Custom Genotyping BeadChip (Illumina, San Diego, CA). Genomic DNA was extracted from peripheral blood using the QIAamp DNA extraction kit (Qiagen, Valencia, CA). SNP genotyping was performed following the standard Infinium II assay protocol. Only SNPs that had genotype data from ≥95% of all samples and samples with genotype data from ≥95% of all SNPs were included in the final data report. The genotyping for the validation phase was performed using Illumina 300k BeadChips following the same quality control criteria as in the discovery phase. We defined any association with a P value less than 0.05 as statistically significant.

**Cell-line-based assays.** Detailed information regarding the cell-line-based radiosensitivity assays has been previously described. Briefly, 277 Epstein–Barr virus-transformed LCLs (from 93 African Americans, 89 Caucasian Americans, and 95 Chinese Americans) from unrelated healthy subjects were purchased from the Coriell Cell Repository. Total RNA was extracted, and basal gene expression was measured using Affymetrix U113 Plus 2.0 GeneChips (Santa Clara, CA). Cell cultures were exposed to 137cesium γ-rays, and radiation response was measured in triplicate for each dosage. Cell proliferation was measured via MTS assays (CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay; Promega, Madison, WI), and the GI50 was determined. DNA was extracted and SNPs were genotyped using Illumina 550K and 510S SNP arrays. The quality control procedure was similar to that described above—only SNPs with a call rate > 95% and a minor allele frequency > 5% were kept in the analysis; any SNPs that were not in Hardy–Weinberg equilibrium were removed. We used a cutoff value of P < 0.001.

**Statistical analysis.** Multivariate logistic regression was used to assess the effect of individual SNPs on the risk of developing toxicity under the additive and dominant models of inheritance. Analysis of toxicity risk included adjustments for age, sex, clinical stage, smoking pack-years, performance status, concurrent chemoradiotherapy, radiation treatment type, pretreatment lung function (diffusing capacity or transfer factor of the lung for carbon monoxide and forced expiratory volume in 1 second), planning target volume, and median dose...
(median lung dose for pneumonitis; median esophageal dose for esophagitis). Meta-analysis was used to summarize effect based on the fixed-effect model. Imputation for each candidate gene region was performed using IMPUTE2 with info score ≥ 0.9 based on 1000 Genomes Project data. Polygenetic risk score calculations were based on the weighted sum of the risk alleles from all validated loci, where the weight was determined by the estimation of each SNP with its association with risk for toxicities (pneumonitis or esophagitis). The improvement of the discriminative ability of genetic information added to the baseline clinical-variable-based model was determined by comparing the AUC for each ROC. Bootstrap results based on 1,000 resamplings were used to confirm the significance of improvement in AUC when adding genetic information. eQTL and radiation response analyses were as previously described using Pearson correlations with the expression and radiation response phenotypes adjusted for race, sex, and principal components.13

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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AUTHOR CONTRIBUTIONS X.P., L.W., J.Y.C., M.A.T.H., Y.Y., N.N., G.D.J., R.K., J.A.R., R.M.W., and X.W. performed the research. X.P., L.W., J.Y.C., M.A.T.H., Y.Y., N.N., G.D.J., R.K., J.A.R., R.M.W., and X.W. designed the research. X.P., L.W., J.Y.C., M.A.T.H., Y.Y., C.L., H.D.S., N.N., G.D.J., R.K., J.A.R., R.M.W., and X.W. performed the research. X.P., L.W., M.A.T.H., Y.Y., N.N., G.D.J., R.M.W., and X.W. analyzed the data.

CONFLICT OF INTEREST The authors declared no conflict of interest.

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