A genome-based model for adjusting radiotherapy dose (GARD): a retrospective, cohort-based study

Jacob G Scott, Anders Berglund, Michael J Schell, Ivaylo Mihaylov, William J Fulp, Binglin Yue, Eric Welsh, Jimmy J Caudell, Kamran Ahmed, Tobin S Strom, Eric Mellon, Puja Venkat, Peter Johnstone, John Foekens, Jae Lee, Eduardo Moros, William S Dalton, Steven A Eschrich, Howard McLeod, Louis B Harrison, Javier F Torres-Roca

Summary

Background **Despite its common use in cancer treatment, radiotherapy has not yet entered the era of precision medicine, and there have been no approaches to adjust dose based on biological differences between or within tumours. We aimed to assess whether a patient-specific molecular signature of radiation sensitivity could be used to identify the optimum radiotherapy dose.**

Methods **We used the gene expression-based radiation-sensitivity index and the linear quadratic model to derive the genomic-adjusted radiation dose (GARD). A high GARD value predicts for high therapeutic effect for radiotherapy; which we postulate would relate to clinical outcome. Using data from the prospective, observational Total Cancer Care (TCC) protocol, we calculated GARD for primary tumours from 20 disease sites treated using standard radiotherapy doses for each disease type. We also used multivariable Cox modelling to assess whether GARD was independently associated with clinical outcome in five clinical cohorts: Erasmus Breast Cancer Cohort (n=263); Karolinska Breast Cancer Cohort (n=77); Moffitt Lung Cancer Cohort (n=60); Moffitt Pancreas Cancer Cohort (n=40); and The Cancer Genome Atlas Glioblastoma Patient Cohort (n=98).**

Findings **We calculated GARD for 8271 tissue samples from the TCC cohort. There was a wide range of GARD values (range 1·66–172·4) across the TCC cohort despite assignment of uniform radiotherapy doses within disease types. Median GARD values were lowest for gliomas and sarcomas and highest for cervical cancer and oropharyngeal head and neck cancer. There was a wide range of GARD values within tumour type groups. GARD independently predicted clinical outcome in breast cancer, lung cancer, glioblastoma, and pancreatic cancer. In the Erasmus Breast Cancer Cohort, 5-year distant-metastasis-free survival was longer in patients with high GARD values than in those with low GARD values (hazard ratio 2·11, 95% 1·13–3·94, p=0·018).**

Interpretation **A GARD-based clinical model could allow the individualisation of radiotherapy dose to tumour radiosensitivity and could provide a framework to design genomically-guided clinical trials in radiation oncology.**

Funding **None.**

Introduction

Radiotherapy is an efficacious and cost-effective treatment that is received by up to two-thirds of all patients with cancer in the USA. It is estimated to be responsible for 40% of all cancer cures, yet represents only 5–10% of all cancer-related health expenditures. Despite its therapeutic importance, it is under-represented in the national portfolio of clinical trials (eg, only 5·5% of US National Cancer Institute trials involve radiotherapy).[1,2]

The sequencing of the human genome has paved the way for the era of precision medicine, which aims for the right treatment to be delivered to the right patient at the right time. The US National Institutes of Health defines precision medicine as an approach to disease prevention and treatment based on individual differences in environment, genes, and lifestyle.[3] Although the genomic era has affected the delivery of chemotherapy and targeted biological agents,[4] it has yet to affect radiotherapy, the most commonly used therapeutic agent in oncology.[5] A central principle in precision medicine is that cancer therapy should be tailored to individual tumour biology.[6] Despite this, radiotherapy dose protocols are uniform (ie, one-size-fits-all), with the underlying assumption that every patient has the same opportunity to benefit from radiotherapy. However, genomic studies have shown biological heterogeneity to be a central characteristic of cancer.[7]

Previously, our group has focused on the identification of pan-tissue radiotherapy-specific biomarkers. We hypothesised that the biological networks that regulate radiosensitivity and radioresistance would be conserved across disease sites. We thus developed a gene-expression-based radiosensitivity index as a molecular estimate for cellular survival fraction at 2 Gy (SF2). We did this by training a linear regression model to predict the experimental SF2 value for 48 cancer cell lines from nine different disease sites, based on the expression of ten specific genes extracted from an interaction network developed using a systems biology approach.[8–10]

The gene-expression-based radiosensitivity index was validated in vitro and was shown to predict tumour

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Department of Radiation Oncology (J G Scott MD, J J Caudell MD, K Ahmed MD, T S Strom, MD, E Mellon MD, P Venkat MD, Prof P Johnstone MD, Prof E Moros PhD, Prof L B Harrison MD, J F Torres-Roca MD), **Department of Integrated Mathematical Oncology** (J G Scott), **Department of Integrated Bioinformatics and Biostatistics** (A Berglund PhD, Prof M J Schell PhD, W J Fulp MS, B Yue MS, E Welsh PhD, Prof J Lee PhD, S A Eschrich PhD), **DeBartolo Family Personalized Medicine Institute** (Prof W S Dalton MD, Prof H McLeod Pharm D), **and Department of Chemical Biology and Molecular Medicine** (J F Torres-Roca), **Moffitt Cancer Center and Research Institute, Tampa, FL, USA; Department of Radiation Oncology, University of Miami, Miami, FL, USA** (I Mihaylov PhD); **and Department of Medical Oncology and Cancer Genomics, Erasmus Medical Center, Rotterdam, Netherlands** (Prof J Foekens MD)

Correspondence to:
Dr Javier F Torres-Roca, Moffitt Cancer Center and Research Institute, Tampa, FL 33612, USA
javier.torresroca@moffitt.org

Research in context

Evidence before this study

We searched PubMed for articles published up to Sept 15, 2016, using the terms "precision medicine AND radiation therapy" as well as "precision medicine AND radiation therapy AND genomics". Before this study, the term precision radiation therapy generally referred to anatomic and geometric precision in the delivery of a given radiation dose to a defined tumour target, using clinical factors, such as tumour size, tumour response, and imaging features. There are several groups that have shown that there are biological differences that determine both tumour and normal tissue response to radiation, but to date no clinical strategy has been developed to integrate these biological differences into customised radiation in clinical practice.

Added value of the study

In this study, we develop and validate the genomic-adjusted radiation dose (GARD), a clinical model for genomic radiation dosing that could allow the individualisation of radiotherapy dose to tumour radiosensitivity and provide a framework to design genomically-guided clinical trials in radiation oncology. To assess the usefulness of GARD in a clinical setting, we generated GARD values in primary solid tumours from 20 disease sites, tested GARD as a predictor of clinical outcome in five independent clinical datasets, and developed a GARD-based prediction model that accurately predicted the observed impact of increasing the radiotherapy dose in a clinical trial.

Implications of all the available evidence

Precision medicine encompasses all therapeutic applications for patient care. With multidisciplinary care becoming standard for most patients with cancer, it is crucial that precision medicine is expanded beyond medical oncology. GARD potentially provides a clinically actionable framework that could allow the integration of biological differences into radiotherapy dose. GARD represents, to the best of our knowledge, the first opportunity to depart from the uniform application of radiotherapy and design efficient, genomically-guided clinical trials in radiation oncology.

response to preoperative radiotherapy in patients with rectal cancer or oesophageal cancer and to predict clinical outcome in patients with breast cancer, head and neck cancer, glioblastoma, pancreatic cancer, and metastatic colorectal cancer treated with radiotherapy.[8,9,11–15] These data support the concept that clinical benefit from radiotherapy (ie, the effect radiotherapy has on clinical outcome) is non-uniform and is highest in a subpopulation of genomically-distinct patients (ie, a radiosensitive population). We use this notion to derive the concept of radiation therapeutic effect.

The linear quadratic model was first proposed by Lea[16] to describe the biological response to radiation. The linear quadratic model is used to estimate different radiation fractionation schemes with similar clinical effect, and has been successfully used in several large randomised trials in prostate cancer comparing standard fractionation with hypofractionation.[17] Integrating individual biological differences into radiotherapy protocols is a key step towards realising the promise of precision medicine. We hypothesise that the gene-expression-based radiosensitivity index, together with the linear quadratic model, in the form of the genomic-adjusted radiation dose (GARD), can serve as the basis for precision medicine in radiation oncology. We aimed to develop and validate the GARD model by generating GARD scores and modelling associations between GARD and clinical outcomes after radiotherapy.

Methods

Study design and cohort population

The Total Cancer Care (TCC) protocol is a prospective tissue collection protocol that has been active at Moffitt Cancer Center (Tampa, FL, USA) and 17 other institutions since 2006.[18] We used primary tumour samples from patients enrolled in the TCC protocol (TCC cohort) to calculate GARD scores and assess the range of GARD values within and between tumour types. We used samples from 20 disease sites from patients who received standard of care treatment at the discretion of their physician, mostly between 2010 and 2012. TCC patients consent for their samples to be collected and profiled.

We used data from five clinical cohorts (Erasmus Breast Cancer Cohort, Karolinska Breast Cancer Cohort, Moffitt Lung Cancer Cohort, Moffitt Pancreas Cancer Cohort, and The Cancer Genome Atlas Glioblastoma Patient Cohort) to assess whether GARD was associated with clinical outcomes. All cohort studies received institutional approval.

The Erasmus Breast Cancer Cohort comprises patients with T1–T3 primary tumours and no clinical evidence of lymph node metastases (N0) treated at the Erasmus Medical Center (Rotterdam, Netherlands).[11] We used data for 263 patients who received lumpectomy plus whole-breast radiotherapy with or without a boost to the tumour cavity, with total doses ranging from 40 Gy to 74 Gy, delivered at 1·8–2 Gy per fraction. The primary outcome, early metastasis, was defined as a distant recurrence in the first 5 years following completion of primary treatment. Raw gene expression data are publically available (GSE2034, GSE5327).

The Karolinska University Hospital, Radiumhemmet Cohort (Karolinska Cohort) is a prospective cohort of patients with breast cancer treated at the Karolinska University Hospital (Solna, Sweden), between Jan 1, 1994, and Dec 31, 1996.[19] The cohort included patients with T1–T3 primary tumours with or without clinical evidence of lymph node metastases (N0–N1). We used data for

77 patients who received segmentectomy or mastectomy plus radiotherapy of 50 Gy in 25 fractions delivered to the conserved breast or chest wall, with or without local nodes. No patient received a tumour cavity or chest-wall-scar boost. All patients underwent axillary dissection. Follow-up data were obtained from the Swedish Breast Cancer Registry and was supplemented with patient charts as previously described.[19] The primary endpoint was relapse-free survival (any distant, regional, or local relapse from the end of primary treatment).

The Moffitt Lung Cancer Cohort comprises archived tumours that were resected between 2000 and 2010 from patients in the TCC and Moffitt Cancer Center tissue database.[20] We used tissue samples and data for 60 patients who had pathologically confirmed, American Joint Committee on Cancer version 6, stage IIIA or IIIB non-small-cell lung cancer and underwent surgical resection and post-operative radiotherapy with a mean dose to the planning target volume of 54·8 Gy (range 43·2–70 Gy). Recurrence was assessed based upon the determination of the treating physician in clinical source documentation. Gene expression data were obtained from TCC. Investigators received written informed consent for tissue acquisition and molecular profiling and follow-up.

The Moffitt Pancreas Cancer Cohort comprises patients with pancreatic cancer from the TCC and Moffitt Cancer Centre tissue database. We used data for tissue samples available for analysis from 40 patients who underwent upfront surgical resection for pancreatic cancer between 2000 and 2011 and received radiotherapy with concurrent fluorouracil or gemcitabine chemotherapy. The median radiation dose was 50 Gy (range 43·2–54 Gy) in 180 to 200 cGy daily fractions for a median of 28 fractions (range 24–30) to the pancreatic tumour bed and regional lymphatics. Patients were excluded if they received neoadjuvant therapy. Gene expression data were obtained from TCC. Investigators received written informed consent for tissue acquisition and molecular profiling and follow-up.

We used data for 98 patients from The Cancer Genome Atlas (TCGA) Glioblastoma Patient Cohort who underwent radiotherapy and concurrent temozolomide treatment. Patient data were included for analysis if gene expression array data[21] were available with a sample that included 50% tumour or more. Patients were excluded if they received neoadjuvant treatment or had low MGMT expression. Clinical and array-based gene expression data (Affymetrix HT Human Genome U133 Array Plate Set level 2) was downloaded from TCGA.

Procedures

We assayed tumours from adult patients enrolled in the TCC protocol on Affymetrix Hu-RSTA-2a520709 (Affymetrix; Santa Clara, CA, USA), which contains approximately 60 000 probesets representing 25 000 genes. Chips were normalised using iterative rank-order normalisation.[22] Batch effects were reduced using partial-least squares. We extracted from the TCC database normalised, debatched expression values for 13 638 samples from 60 sites of origin and the ten radiosensitivity index genes (*AR*, *c-Jun*, *STAT1*, *PKC-beta*, *RelA*, *cABL*, *SUMO1*, *PAK2*, *HDAC1*, and *IRF1*). We excluded all metastatic duplicate samples and disease sites with fewer than 25 samples. We included samples irrespective of receipt of radiotherapy.

Gene-expression-based radiosensitivity index scores were generated previously for all clinical cohorts except for the Moffitt Lung Cancer Cohort.[11] To calculate radiosensitivity index scores for this cohort, we normalised gene expression values from Affymetrix U133A CEL files using the robust multiarray average (RMA) algorithm[23] and did linear scaling to avoid negative values. We ranked each of ten genes in the algorithm based on gene expression (highest expressed gene is ranked at ten and lowest at one) and calculated the radiosensitivity index using the predetermined algorithm (equation 1):

$$\begin{aligned}\text{RSI} = {} & -0{\cdot}0098009 * AR + 0{\cdot}0128283 * cJun + 0{\cdot}0254552 * \\ & STAT1 - 0{\cdot}0017589 * PKC - \text{beta} - 0{\cdot}0038171 * \\ & RelA + 0{\cdot}1070213 * cABL - 0{\cdot}0002509 * SUMO1 - \\ & 0{\cdot}0092431 * PAK2 - 0{\cdot}0204469 * HDAC1 - \\ & 0{\cdot}0441683 * IRF1\end{aligned}$$

Statistical analysis

The linear quadratic model proposes that there are two parameters that impact on radiotherapy cytotoxicity, one that is proportional to radiotherapy dose (α) and one that is proportional to the square of the dose (β). The linear quadratic model in its simplest form is represented by (equation 2):

$$S = e^{-nd(\alpha + \beta d)}$$

Here, e is the natural logarithm, with S representing the surviving fraction after n fractions of radiation, each of dose (d). α represents the linear radiosensitivity parameter and β represents the quadratic radiosensitivity parameter. Effect (E) was calculated as (equation 3):[24]

$$E = nd(\alpha + \beta d)$$

Here effect represents the cytotoxic effect of radiotherapy in cell lines (a higher effect results in higher cytotoxicity).

We derived GARD scores using the linear quadratic model, the individual gene-expression-based radiosensitivity index, and the standard of care radiation dose and fractionation schedule for each patient (appendix p 1). The calculation for GARD is similar to biologically effective dose, except that patient-specific α is derived by substituting radiosensitivity index for survival (S) in equation 1, where dose (d) is 2 Gy, n=1, and β is a constant (0·05/Gy²).[25] A higher GARD value predicts a higher

See Online for appendix

radiation therapeutic effect. We make the assumption that radiation therapeutic effect is equivalent to clinical benefit. We calculated GARD using a script written into Excel.

For analysis of the TCC cohort, we ranked each patient's GARD from highest to lowest. We assigned radiotherapy dose and fractionation protocols for each disease type: subclinical (45 Gy in 25 fractions), microscopic (60 Gy in 30 fractions), and macroscopic disease (≥70 Gy in 35–40 fractions). We then defined three GARD levels corresponding to the proportion of patients within each radiotherapy dose group. We compared median GARD between disease sites for each assigned dose group using the Fisher's exact test. We created violin plots using MATLAB R2016a (The MathWorks, Natick, MA, USA) and the "Violin Plots for plotting multiple distributions (distributionPlot.m)" toolbox from MATLAB Central File).[26]

For each clinical cohort, we ranked GARD values from highest to lowest and grouped patients into three radiotherapy dose groups: low, intermediate, and high. We then defined three GARD levels (low, medium, and high), corresponding to the proportion of patients within each dose group. We estimated distant-metastasis-free survival in the Erasmus Breast Cancer cohort using the Kaplan-Meier method and used the log-rank test to identify differences by high versus low GARD score, dichotomised at the 75th percentile, based on previous gene-expression-based radiosensitivity index analyses.[11] We calculated biologically effective dose assuming a constant αβ ratio of 2·88 for breast cancer as previously described ($BED_{2\cdot88}$).[27,28] We assessed the correlation between GARD and $BED_{2\cdot88}$ with Spearman correlation. Proportional hazards regression analyses were used to calculate the effect of biologically effective dose on distant-metastasis-free survival. Proportional hazards regression analyses were also used for multivariate analysis of GARD as a continuous variable or dichotomous variable in oestrogen-positive patients. We compared sociodemographic and clinicopathological characteristics between patients included and excluded from the Erasmus cohort using Fisher's exact test for categorical variables, including gene-expression-based radiosensitivity index, and Wilcoxon rank-sum test for continuous variables.

For the five clinical cohorts, we used multivariable Cox proportional hazards regression to assess the association between GARD and the studied endpoint, adjusting for potential confounders and using a backward elimination model with a significant level-to-stay of 0·10. GARD cut-points for the Karolinska breast cancer (75th percentile), glioblastoma (75th percentile), and pancreatic cancer (50th percentile) cohorts were similar to the gene-expression-based radiosensitivity index cut-points used in previous analyses. We tested two cut-points for the lung cancer cohort (75th percentile and 60th percentile [which was eventually used]); Bonferroni correction was performed for multiple testing in this cohort.

Since GARD is a model combining gene-expression-based radiosensitivity index with the linear quadratic model, we compared the performance of GARD to the radiosensitivity index in the Erasmus Breast Cancer Cohort using backward elimination in a multivariable model fitted with candidate variables (hormone receptor status, T stage, age, GARD, $BED_{2\cdot88}$ and radiosensitivity index) to calculate the likelihood ratio.

We hypothesised that there would be a threshold that separates high and low GARD subpopulations, towards which a clinician can adjust the radiotherapy dose to increase radiation therapeutic effect. Given the equation for GARD, it could be expected that an increasing proportion of patients will achieve the GARD threshold with escalating radiotherapy doses. To test this possibility, we built a model to test whether GARD could predict benefit from radiotherapy shown in a real-life cohort. We used assumptions as observed in the Erasmus Breast Cancer cohort (eg, GARD threshold 75th percentile, radiosensitivity index distribution, hazard ratio [HR] between high GARD and low GARD of 2·11) to develop a formula for the potential effect on distant-metastasis-free survival of radiotherapy dose escalation in breast cancer predicted by the GARD-based model (equation 4)

$$\frac{a * HR + (-a) * 1}{b * HR + (1-b) * 1}$$

In this equation, a and b are the estimated proportions of patients to achieve the GARD threshold dose level at the radiotherapy doses being compared (range 50–76 Gy *vs* 50 Gy reference dose). We assume an improvement in distant-metastasis-free survival in the high GARD population similar to that observed in the Erasmus cohort (HR=2·11). We use the GARD equation to calculate the distribution of GARD values at 66 Gy (a) and 50 Gy (b), assuming that the radiosensitivity index distribution of a random breast cancer population is the same as that observed in the Erasmus Breast Cancer Cohort. We then estimated the percentage of patients that would have reached the GARD threshold value at each radiotherapy dose. We chose to compare 50 Gy and 66 Gy doses to simulate a completed clinical trial in patients with early stage breast cancer (T 1–2, N 0–1) breast cancer (EORTC 22881-10882). EORTC 22881-10882 randomly assigned 5318 patients to postoperative whole-breast radiotherapy of 50 Gy, with a 16 Gy boost (n=2661) or without a boost dose (n=2657).[29] We generated HRs comparing distant-metastasis-free survival after radiotherapy as calculated in our GARD-based model and compared it with the published results of EORTC 22881-10882. The difference in outcome between high-GARD and low-GARD populations used for this modelling experiment was derived from the multivariable analysis of the Erasmus cohort.

All tests were two-sided with a significance level of 0·05. We used SAS version 9.3 for all analyses.

Role of the funding source

There was no funder for this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

GARD values for the 8271 tissue samples in the TCC cohort ranged from 1·66–172·4 (figure 1, appendix pp 2–3). 2517 (30%) samples were assigned the 45 Gy dose level, 4877 (59%) were assigned the 60 Gy dose level, and 877 (11%) were assigned the 70 Gy or more dose level. Using these proportions, we derived three GARD levels by percentile: low (0 to 30·40), middle (30·41 to 89·40), and high (89·41 to 100). There was a wide range of GARD values within each uniform radiotherapy dose group (figure 1). Patients assigned a dose of 45 Gy had GARD values from 3·03 to 56·34. Patients assigned to 60 Gy had GARD values from 1·66 to 122·38. Patients assigned to 70 Gy or more had GARD values from 9·73 to 172·4.

By taking gene-expression-based radiosensitivity index into account, GARD scores show that a higher radiotherapy dose does not always result in a higher radiotherapeutic effect across a population. For example, if GARD was exclusively related to radiotherapy dose it would be expected that patients assigned to 45 Gy would be in the low GARD group. However, although 1456 (58%) of 2517 patients assigned to 45 Gy were in the low GARD group, 1037 (41%) of 4877 patients were in the middle GARD group (figure 1). Moreover, 558 (21%) of 4877 patients in the middle GARD group and 38 (4%) of 877 patients in the high GARD group were assigned to 45 Gy (figure 1). Thus highly radiosensitive patients (those with a low radiosensitivity index) assigned to 45 Gy had GARD values that were similar to some patients assigned to higher radiotherapy doses (60 or 70 Gy) but who were less radiosensitive. Similarly, although most patients assigned 70 Gy were in the high GARD group, 1023 (11%) of 4877 patients in the middle GARD group were assigned 70 Gy (figure 1).

For cancers usually treated with 70 Gy radiotherapy, patients with cervical cancer and oropharyngeal head and neck cancer had the highest median GARD values (figure 2A). Median GARD was higher in patients with oropharyngeal head and neck cancer (39·71) than in those with non-oropharyngeal head and neck cancer (32·56; p=0·0417), which is consistent with the superior clinical outcomes after radiotherapy in patients with oropharyngeal cancer.[30] In the group of disease sites usually treated with 60 Gy, glioma (median GARD 16·55) and sarcoma (17·94) had lower GARD compared with all other disease sites at this dose level (p<0·0001; figure 2B). GARD also shows that the radiotherapy therapeutic effect at 60 Gy is larger in non-melanoma skin cancer (median GARD 25·80) than in melanoma (21·17; p=0·01144). Oesophageal cancer and rectal cancer are usually treated with preoperative radiotherapy; oesophageal cancer had a higher GARD than did rectal cancer (p=0·00032; figure 2C). Gastric and pancreatic cancer are both commonly treated with postoperative radiotherapy; GARD identified a higher predicted radiation therapeutic effect for gastric cancer than for pancreatic adenocarcinoma (p=0·00171; figure 2C).

Patients included in our analysis of the Erasmus Breast Cancer Cohort did not have significantly different sociodemographic and clinicopathological characteristics compared with those we excluded (appendix pp 1–2). Establishing generalisability for the Erasmus Breast Cancer Cohort population, we show the radiosensitivity index distribution between patients in this cohort and those in the TCC cohort did not significantly differ (appendix p 11). GARD values were widely distributed in patients in this cohort (median 27·22, range 4·01–104·25; figure 3A). GARD by actual radiotherapy dose group is in the appendix (p 4). Patients who had a GARD at or above the 75th percentile for this cohort (≥38·9; high GARD) had longer distant-metastasis-free survival compared with those with GARD below the 75th percentile (low

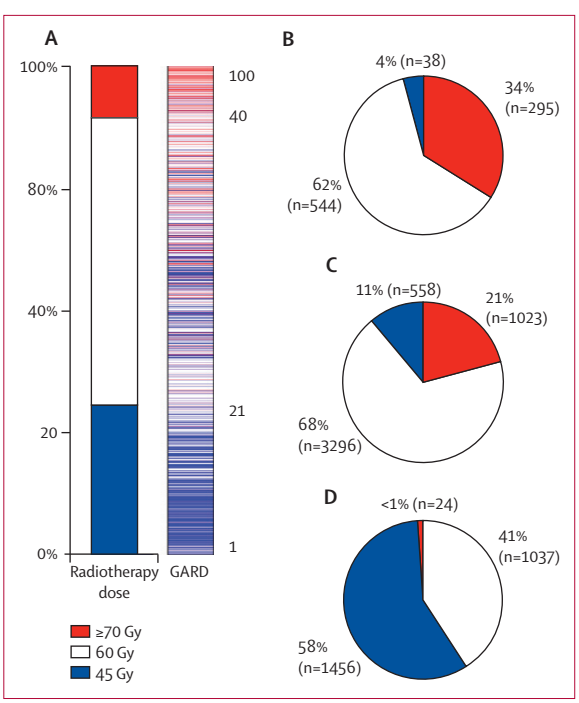
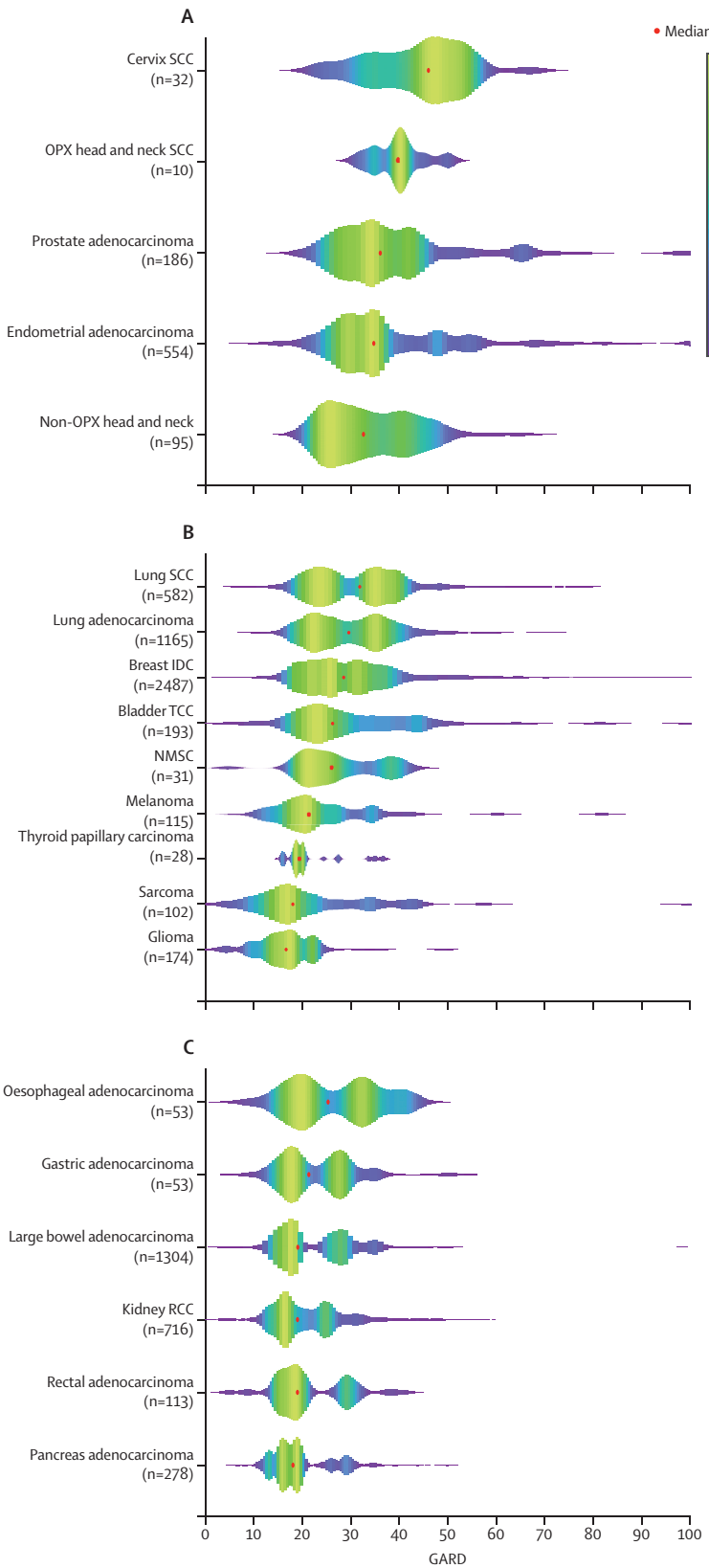


Figure 1: **A framework for genomic-adjusted radiation dose (GARD)**
(A) The left plot shows the proportion of patients in each radiotherapy dose group. On the right plot, GARD values for each individual patient are presented ranked from the highest to lowest value; each line represents an individual patient; colour relates to dose assigned. Nine patients in the cohort had a GARD higher than 100; these patients were assigned a GARD of 100. Pie charts show dose assignments for patients in GARD score groups: (B) low (0–30·4 percentile); (C) middle (30·41–89·4 percentile); and (D) high (89·41–100 percentile). GARD=genomic-adjusted radiation dose.



GARD; figure 3B). There was a weak but significant correlation between GARD and $BED_{2·88}$ (R=0·25, p<0·0001), with an increase in GARD generally associated with an increase in $BED_{2·88}$. In univariable analysis, $BED_{2·88}$ did not predict distant-metastasis-free survival (p=0·12; appendix p 5). In multivariable analyses, GARD was the only independent predictor of distant-metastasis-free survival in this cohort; hormone receptor status, T stage, age, and surgery type were not independently associated with distant-metastasis-free survival (tables 1, 2). GARD was also an independent variable predicting clinical outcome in oestrogen-receptor-positive patients in the Erasmus breast cancer cohort (appendix pp 8–9). In multivariable analysis, GARD was significantly associated with relapse-free survival in the Karolinska Breast Cancer Cohort, local control in the Moffitt Lung Cancer Cohort, and overall survival in the TCGA Glioblastoma Cohort and the Moffitt Pancreas Cancer Cohort (table 2).

In the multivariate model to compare GARD with the radiosensitivity index, the radiosensitivity index was the last variable eliminated, yielding a final model including GARD and $BED_{2·88}$, with an overall likelihood ratio χ^2 value of 10·45 (p=0·0054). Nine patients were excluded from this analysis because of missing oestrogen and progesterone receptor status. When these patients were included back, as is analytically preferable, the model had an overall likelihood ratio χ^2 value of 12·07 (p=0·0024). Notably, the corresponding model excluding GARD with only radiosensitivity index and $BED_{2·88}$ had a likelihood ratio χ^2 value of 6·95 (p=0·0310). The gain of 5·12 points (ie, 12·07 subtract 6·95) is substantial, demonstrating that the GARD and $BED_{2·88}$ model is much better than the gene-expression-based radiosensitivity index.

In figure 4, we identify a subset of 23% (78 of 344) of patients (radiosensitivity index 0·18–0·35) that could achieve the GARD threshold (GARD ≥38·9) if they received radiotherapy doses of 45–75 Gy. Using the observed distribution of high versus low GARD subpopulations in the Erasmus Breast Cancer Cohort at each delivered radiotherapy dose to estimate the potential benefit of GARD, we estimate that uniform and unselected radiotherapy dose escalation would result in an overall slight improvement in distant-metastasis-free survival (figure 4B, C). The predicted improvements, while substantial, would not be noticed in an unselected randomised trial. For example, our model estimates that dose escalation from 50 Gy to 66 Gy would result in an

Figure 2: **GARD score distribution and density within 70 Gy (A), 60 Gy (B), and 45 Gy (C) dose levels, by disease site**
The red dot represents the median GARD value for each disease site at assigned dose levels. Colours in the plot correlate with the sample density. GARD=genomic-adjusted radiation dose. SCC=squamous cell carcinoma. OPX=oropharyngeal. IDC=invasive ductal carcinoma. TCC=transitional cell carcinoma. NMSC=non-melanoma skin cancer. RCC=renal cell carcinoma.

increased distant-metastasis-free survival (HR 0·92). A trial with 80% power to detect this difference without genomic guidance would require 14489 patients (appendix p 9). By contrast, a GARD-based approach predicts that the benefit of dose escalation from 50 Gy to 66 Gy would require 230 patients if using only a subgroup of patients with gene-expression-based radiosensitivity index scores lower than 0·31 (appendix p 10).

We compared the potential benefit calculated by GARD for dose escalation to 66 Gy with the results of the randomised EORTC 22881–10882 trial of 5318 patients with breast cancer.[29] In this trial, after a median follow-up of 17·2 years, dose escalation resulted in a lower local (ipsilateral) recurrence (HR 0·65, 99% CI 0·52–0·81; p>0·0001) and no difference in distant metastasis (1·06, 0·92–1·24; p=0·29). The estimated distant-metastasis-free survival for dose escalation to 66 Gy calculated by GARD (HR of 0·92) was within the lower 99% CI of the HR reported by EORTC 22881–10882 for this endpoint.[30] The estimated HR for distant-metastasis-free survival is a quarter of the observed effect on local recurrence (HR 0·65), consistent with the 4:1 relationship between local recurrence and breast cancer death observed in the EBCTCG meta-analysis,[31] although it should be noted that more recent analyses of radiotherapy benefit do not show the same ratio.[32]

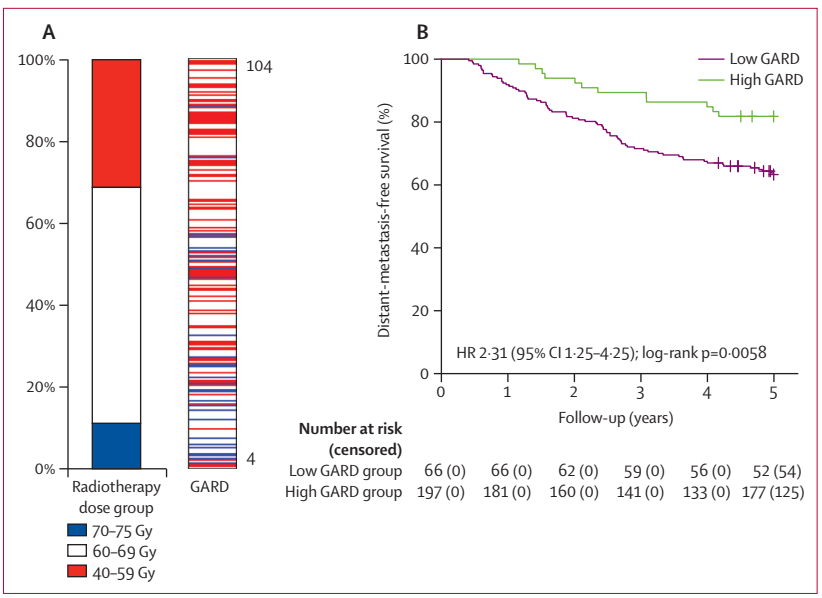


***Figure* 3: GARD and distant-metastasis-free survival in the Erasmus Breast Cancer Cohort**
(A) GARD values for each individual patient are presented ranked from the highest to lowest value; each line represents an individual patient and colour relates to radiotherapy dose received. The number of patients in each group and the GARD ranges are online (appendix p 4). (B) Kaplan-Meier plot for distant-metastasis-free survival comparing patients with high GARD (≥38·9; the 75th percentile) with patients with low GARD (<38·9). HR is from univariable analysis. If no event occurred, then cases were censored at 5 years GARD=genomic-adjusted radiation dose. HR=hazard ratio.

	Hazard ratio (95% CI)	p value
GARD score <75th percentile *vs* GARD score (>75th percentile)	2·11 (1·13–3·94)	0·018
Hormone receptor status*		
ER+ and PR– or ER–and PR+ *vs* ER+ and PR+	1·28 (0·76–2·17)	0·35
ER– and PR– *vs* ER+ and PR+	0·98 (0·57–1·68)	0·93
T stage 2, 3, or 4 *vs* T stage 1	1·36 (0·86–2·16)	0·18
Age (years)		
41–55 *vs* <40 years	0·78 (0·42–1·43)	0·41
56–70 *vs* <40 years	0·84 (0·44–1·59)	0·59
71–83 *vs* <40 years	0·37 (0·12–1·13)	0·08
Lumpectomy *vs* surgery (mastectomy)	1·97 (0·88–4·43)	0·10

GARD=genomic-adjusted radiation dose. ER=oestrogen receptor. PR=progesterone receptor. *Nine patients did not have hormone receptor status and were excluded.

***Table* 1: Multivariable analysis of the Erasmus Breast Cancer Cohort for distant-metastasis-free survival**

Discussion

We show that GARD varies widely within populations and tumour types, and is associated with outcomes in five clinical cohorts and in a model comparing predictions to real-world results. Several threads of evidence support the clinical value of GARD. GARD is largely based on gene-expression-based radiosensitivity index and the linear quadratic model, both of which have extensive clinical validation. Gene-expression-based radiosensitivity index has been validated as a predictor of outcome in multiple datasets of radiotherapy-treated patients and the linear quadratic model has been used as the basis for dose and fractionation in clinical radiation oncology.[8,9,11–15,27,33] GARD ranges are consistent with the clinical heterogeneity of radiotherapy therapeutic benefit. For example, the higher GARD scores for glioma versus sarcoma, oropharyngeal versus non-oropharyngeal head and neck cancer, oesophageal cancer versus rectal cancer, non-melanoma skin cancer versus melanoma, and gastric cancer versus pancreatic cancer reflect better radiotherapy outcomes for these disease types in clinical studies.[30,34–40] We also show the clinical validity of GARD in patients with breast cancer who did not receive chemotherapy or hormonal therapy, thus limiting confounding factors. Additionally, there was substantial variation in the radiation doses delivered to patients in this cohort, which allowed radiotherapy dose to affect GARD. GARD has been developed to enable adjustment of radiotherapy dose to match an individual tumour's radiosensitivity, with higher GARD values predicting a higher therapeutic effect from radiotherapy. Therefore, it is reasonable to test the clinical validity of GARD by testing whether patients with higher GARD values have better clinical outcomes.

Our analyses show that GARD is an independent predictor of a radiotherapy-specific outcome and outperforms both gene-expression-based radiosensitivity

	Median follow-up (months)	Events	Radiotherapy dose range (Gy)	GARD range	Endpoint	HR from multivariable analysis (95% CI)	p value	Adjustment factors
Erasmus Breast Cancer Cohort (n=263)	60	23	40–74	4·01–104·25	Distant-metastasis-free survival*	2·11 (1·1–3·9)	0·018	Oestrogen and progesterone receptor status, age, surgery (*vs* lumpectomy), and T-stage
Karolinska Breast Cancer Cohort (n=77)	87	19	50	8–60	Relapse-free survival†	7·4 (1·4–138)	0·014	Hormonal therapy, chemotherapy, and oestrogen and progesterone receptor status
Moffitt Lung Cancer Cohort (n=60)	37	23	45–70	15–125	Local control‡	3·4 (1·3–9·1)	0·016	Surgery, stage, histology, lymphovascular invasion
TCGA Glioblastoma Cohort (n=98)	11	76	12·6–97·0	0·4–46·0	Overall survival§	1·9 (1·1–3·3)	0·019	Age, performance status
Moffitt Pancreas Cancer Cohort (n=40)	68	27	45–54	16–40	Overall survival§	2·6 (1·1–6·0)	0·029	CA 19·9, margin lymph nodes

TCGA=The Cancer Genome Atlas. *Primary endpoint defined as distant recurrence in the first 5 years following completion of primary treatment. †Primary endpoint defined as any relapse distant, regional, or local from the end of primary treatment. ‡Defined in this study as time from surgical resection to cancer recurrence within the irradiated field. If no event occurred, then cases were censored at the date of last clinical evaluation. Cases in which more than 4 months elapsed without a clinical evaluation were censored at the date of antecedent clinical evaluation.[22] §Defined in this study as the interval from surgery to date of death.

***Table 2*: Clinical cohort description and multivariate analysis for the effect of GARD on selected endpoints**

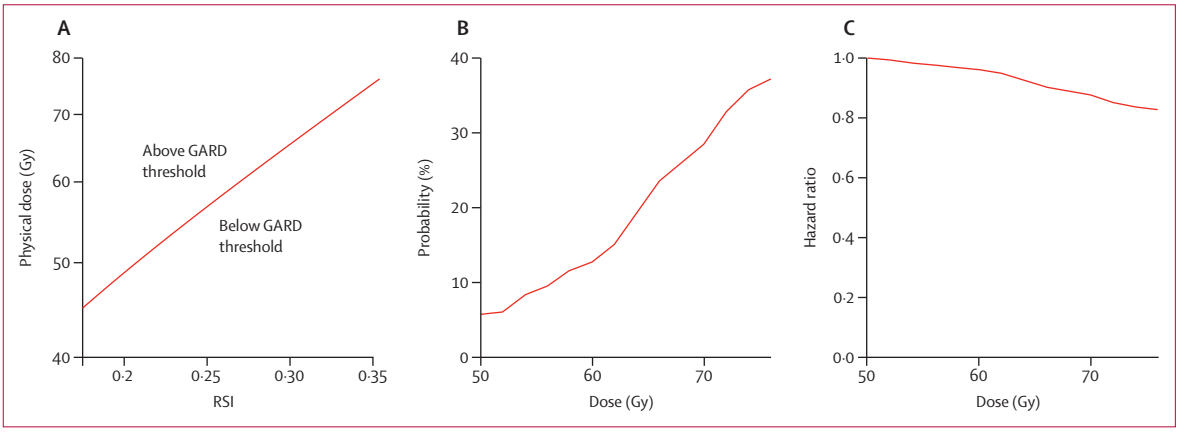


***Figure 4*: A model for genomic-informed radiation dose in breast cancer**
(A) The red line shows the physical radiotherapy dose required to meet the GARD threshold (GARD≥38·9) with increasing RSI. This curve is based on the radiotherapy effect calculated for distant metastasis (not local control) using the Erasmus Breast Cancer Cohort. (B) The probability of patients reaching the GARD threshold (GARD≥38·9) in an unselected population as a function of radiotherapy dose. (C) Estimates of the potential therapeutic effect (HR for high versus low GARD subpopulations) of increased radiotherapy dose (the reference dose is 50 Gy). GARD=genomic-adjusted radiation dose. RSI=radiosensitivity index. HR=hazard ratio.

index and $BED_{2\cdot88}$ in patients in the Erasmus Breast Cancer cohort. Furthermore, GARD was an independent predictor of clinical outcome in four additional independent cohorts of patients with breast cancer, glioblastoma, lung cancer, and pancreatic cancer. In both breast cancer cohorts, we used endpoints chosen by the original investigators. Although local control is the classic endpoint used to measure radiotherapy effectiveness, and radiosensitivity index has been previously shown to predict local recurrence in breast cancer, distant-metastasis-free survival has emerged as an appropriate endpoint for radiotherapy-based interventions[30,41,42] thus making distant-metastasis-free survival and relapse-free survival relevant endpoints for an radiotherapy-focused analysis.[42] The glioblastoma dataset was obtained from TCGA and overall survival was the outcome reported. Since radiotherapy has been shown to impact overall survival in glioblastoma, we think this is a relevant endpoint for GARD in this disease.[43] Data for both the pancreas and lung cancer cohorts were obtained from our institutional database. We chose to report overall survival for the pancreas cancer cohort since there are data that, although controversial, support the notion of post-operative radiotherapy having an effect on overall survival in this disease.[44] Finally, we chose local control for the lung cancer cohort since this is a classic endpoint used for the most direct clinical effect for radiotherapy.

This work has several important implications. First, we identify genomically-distinct populations that derive differential benefit from radiotherapy. Furthermore, we

provide a method by which to customise radiation dose to match the radiosensitivity of an individual patient's tumour with existing technology. We provide, to the best of our knowledge, the first framework to design genomically-stratified, radiotherapy-based trials using specifically defined genomic subpopulations. Thus, a key potential utility of this work is the use of GARD to test genomic-based radiation dosing to improve clinical outcomes. Genomic-based clinical trial design could greatly improve the efficiency of clinical trials in radiation oncology and lead to a reduction in both the number of patients required to test a hypothesis and the time to complete the trial, both of which should lead to substantial cost-savings. We emphasise that GARD is not a predictive assay or biomarker for clinical outcome but rather a model to adapt the prescribed radiation dose to match individual tumour radiosensitivity. It is not trained to predict clinical outcome, but was developed as a novel genomic radiotherapy prescription framework.

Since we propose GARD as an approach for individualised radiotherapy dose to match tumour radiosensitivity, a reasonable question is what is the clinical opportunity for patient-specific dose optimisation? Radiotherapy doses in clinical practice today have been empirically optimised, resulting in reasonable disease control and toxicity. However, our findings suggest that current uniform radiotherapy dose protocols can be further optimised with tumour-specific genomic data. GARD could provide a scientific framework to adjust radiotherapy doses that have already shown to be safe, both in terms of increasing tumour control (increasing dose to more resistant tumours) and decreasing complication risks (lowering the dose to more sensitive tumours), although this concept requires further validation. Finally, it should be emphasised that GARD only accounts for tumour radiosensitivity and that additional biological tumour parameters (ie, proliferation, hypoxia, and DNA repair) as well as patient parameters (ie, normal tissue toxicity) would further improve the ability of clinicians to optimise radiotherapy dose. Normal tissue toxicity is one parameter of central importance and approaches to better estimate individual risk are in development.[45] However, in their absence, a reasonable approach for GARD-based radiation dose optimisation could involve isotoxic dose escalation.[46]

Although our modelling framework is simple and based on classically accepted principles, we have made several assumptions to complete our analyses. Specifically, we assume that the risk of recurrence and gene-expression-based radiosensitivity index distribution in the Erasmus cohort are similar to a normal lymph node-negative breast cancer population. This assumption seems reasonable in light of the observation that the gene-expression-based radiosensitivity index distribution between the Erasmus Breast Cancer cohort and the TCC are not significantly different. Furthermore, it is possible that since patients in the Erasmus Breast Cancer cohort were not treated with systemic hormonal or chemotherapy, our model is overestimating the benefit of radiotherapy. We have also made the assumption that the quadratic component of radiation response, β, is constant. Because we do not attempt to model different ranges of fractional (daily) dose, this assumption should not qualitatively affect our conclusions. Additionally, we acknowledge that our model does not address normal tissue radiosensitivity, which could further allow the personalisation of radiation dose. Finally, while we use radiotherapy as a backbone for our analyses, the calculation of GARD could use other measures of radiosensitivity or be expanded to include other biological parameters including hypoxia, DNA repair, proliferation, and the immune system.

In conclusion, a central requirement for precision medicine in radiation oncology is the ability to inform radiation dose parameters to match individual tumour biology, thus delivering the right radiation dose for the right patient. GARD provides, to the best of our knowledge, the first opportunity to genomically inform radiation dose and our findings suggest it is a feasible approach to precision radiation oncology.

Contributors

JGS, LBH, and JFT-R designed the study. WSD and JF collected data. AB, MJS, IM, WJF, BY, EW, JJC, KA, TJS, EM, PV, PJ, JF, JL, SAE, HLM, and JFT-R contributed to data analysis. JGS, AB, MJS, IM, WJF, BY, EW, JL, SAE, and JFT-R designed the figures. All authors contributed to data interpretation and writing,

Declaration of interests

JGS and JFT-R are named inventors in a patent pending for systems for providing personalised radiation therapy. SAE and JFT-R are named inventors in patent number 8,660,801, patent number 8,665,598 and patent number 7,879,545 are related to radiosensitivity index. PJ reports receipt of personal fees from Novocure. SAE is a cofounder of Cvergenx and serves on the board and as an officer for the company. He holds stock and stock options in the Cvergenx. HLM serves on the board of directors for Cancer Genetics and on an advisory board for Kew Corporation. JFT-R reports stock in Cvergenx and has a patent issued for radiation sensitivity index with royalties paid to Cvergenx, and a patent pending for GARD.

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