



Circulating Cell-Free DNA Correlates with Body Integral Dose and Radiation Modality in Prostate Cancer

Natalie A. Lockney, MD; Randal Henderson, MD; Steven G. Swarts, PhD; Zhenhuan Zhang, MD, PhD; Bingrong Zhang, DVM, MD; Jennifer Li, BS; Robert A. Zlotecki, MD, PhD; Christopher G. Morris, MS; Katherine Casey-Sawicki, MA; Paul Okunieff, MD

Department of Radiation Oncology, University of Florida College of Medicine, Gainesville and Jacksonville, FL, USA

Abstract

Purpose: The RadTox assay measures circulating cell-free DNA released in response to radiotherapy (RT)-induced tissue damage. The primary objectives for this clinical trial were to determine whether cell-free DNA numbers measured by the RadTox assay are (1) correlated with body integral dose, (2) lower with proton RT compared with photon RT, and (3) higher with larger prostate cancer RT fields.

Patients and Methods: Patients planned to receive proton or photon RT for nonmetastatic prostate cancer in the setting of an intact prostate or postprostatectomy were eligible for the trial. Plasma was collected pre-RT and at 5 additional daily collection points beginning 24 hours after the initiation of RT. Data from 54 evaluable patients were analyzed to examine any correlations among RadTox scores with body-integral dose, RT modality (photon versus proton), and RT field size (prostate or prostate bed versus whole pelvis).

Results: Body integral dose was significantly associated with the peak post-RT RadTox score ($P = .04$). Patients who received photon RT had a significant increase in peak post-RT RadTox score ($P = .04$), average post-RT RadTox score ($P = .04$), and day-2 RadTox score (all minus the pre-RT values for each patient) as compared with patients who received proton RT. Field size was not significantly associated with RadTox score.

Conclusion: RadTox is correlated with body integral dose and correctly predicts which patients receive proton versus photon RT. Data collection remains ongoing for patient-reported RT toxicity outcomes to determine whether RadTox scores are correlated with toxicity.

Keywords: circulating DNA; cell-free DNA; protons; prostate cancer; radiation dosimeter

Submitted 24 June 2020
Accepted 10 July 2020
Published 15 Sept 2020

Corresponding Author:
Paul Okunieff, MD
Department of Radiation
Oncology
College of Medicine
University of Florida
2000 SW Archer Rd
PO Box 100385
Gainesville, FL 32610-0385,
USA
Phone: +1 (352) 265-0287
pokunieff@ufl.edu

Original Article

DOI
10.14338/IJPT-20-00033.1

© Copyright
2020 The Author(s)

Distributed under
Creative Commons CC-BY

OPEN ACCESS

<http://theijpt.org>

Introduction

Although physics dosimetry is the key clinical indicator of tumor control probability and complication risk, dose decisions are based on population statistics and fail to incorporate a patient's predisposition to the benefits and risks of radiation. Radiation is often used in combination with surgery or systemic therapies (eg, chemotherapy and immunotherapy); hence, unforeseen toxicity is common. A subset of patients will experience severe radiotoxicity, but an unknown subset might tolerate a higher and more-curative dose [1]. As such, these patients, who may receive a dose specified for a more-sensitive subset of

the population, potentially receive less-effective treatment. Without an effective method to incorporate biology, physics dosimetry is an accepted limitation of radiotherapy.

Research into increasingly effective biodosimetric technologies, aimed at triage of a radiation or nuclear event, presents an opportunity for clinical implementation of a personalized toximeter, rather than solely a dosimeter. We have demonstrated in animal models that circulating cell-free DNA (cfDNA), evaluated using the DiaCarta (Richmond, California) SuperbDNA RadTox, is a measure of cell death within the radiation field [2, 3]. The measurement correlates with whole and subtotal body radiation dose. In preclinical models, it scales with toxicity when combined with other stressors (eg, wounds or simulated sepsis). The method is a patented measurement of total human DNA in the circulation with *Alu* retrotransposons as the DNA marker.

Building on those studies, we conducted a clinical trial in patients undergoing radiotherapy (RT) for prostate cancer to compare RadTox as a potential toximeter with physics dosimetry. Prostate cancer was chosen because it is commonly treated with radiation alone and has a high tumor-control rate; thus, symptoms can be assigned to radiation, rather than chemotherapy, surgery, or tumor recurrence. Our primary objectives were to determine whether plasma cfDNA results measured by the RadTox assay are (1) correlated with body integral dose, (2) lower with proton RT compared with photon RT, and (3) higher with larger prostate cancer RT fields. Our secondary objective (ie, to determine whether plasma cfDNA numbers measured early during RT course are higher in patients who experience grade 2 or higher RT toxicity) will be reported separately, once all patients have completed 1-year follow-up.

Methods

Study Design and Patient Selection

This institutional review board–approved (IRB201601961) clinical protocol was funded by a University of Florida (UF) subcontract through a phase I Small Business Innovation Research (N44CA180036, Alexandria, Virginia) awarded to DiaCarta, Inc (Richmond, California). The study was conducted at the UF Department of Radiation Oncology (Gainesville, Florida) and the UF Health Proton Therapy Institute (Jacksonville, Florida). Eligible patients met the following inclusion criteria: diagnosis of adenocarcinoma of the prostate planned for RT with protons or photons, clinical stage I to III tumors, 18 years or older, and an Eastern Cooperative Oncology Group score 0 or 1. Although eligible patients had not yet started definitive treatment with chemotherapy or radiation, androgen-deprivation therapy and prostatectomy were allowable. Exclusion criteria included metastatic disease, history of prior rectal or other pelvic malignancy, active inflammatory bowel disease, prior pelvic RT for any reason, and psychiatric or addictive disorders or other conditions that would preclude the patient from meeting study requirements. All enrolled patients provided written, informed consent.

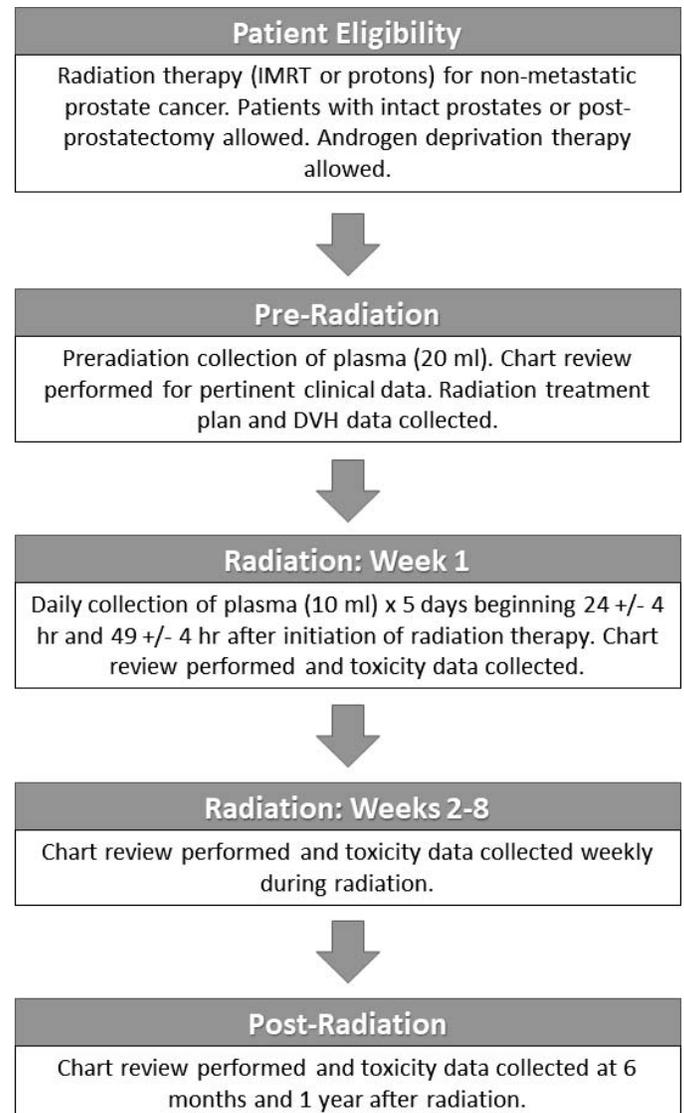
As outlined in **Figure 1**, venous blood was collected in 10-mL BD Vacutainer plastic blood collection tubes with K₂ ethylenediaminetetraacetic acid (Hemogard Closure, Greiner Bio-One, Frickenhausen, Germany) according to the following schedule: (1) in the week preceding the initiation of radiotherapy (20 mL), and (2) daily for 5 on-treatment days beginning 24 ± 4 hours after the initiation of radiotherapy (10 mL each). Radiation-treatment plan details were volumetrically recorded. Patient-reported toxicity outcomes were recorded before treatment, weekly during treatment, and after treatment at 6 months and 1 year.

Radiation Treatment

Proton therapy and intensity-modulated RT (IMRT) with photons were allowed for the primary treatment of prostate cancer or for treatment in the postprostatectomy setting. **Table 1** summarizes the allowed radiation-treatment dose and fractionation schedules according to standards of care.

Proton therapy or IMRT could also be used in the postprostatectomy setting as postoperative treatment for patients with risk factors that indicate a high risk of locoregional recurrence or for salvage treatment for patients with documented pelvic recurrence or rising prostate-specific antigen (PSA) in the absence of distant metastasis. Proton therapy and IMRT to the prostate bed could be treated with 1.8 GyRBE or Gy per day to a total of 64.8 to 70.2 GyRBE or Gy, with possible localized boost of gross recurrence to 73.8 GyRBE or Gy total. Patients considered to be at significant risk of lymph node involvement with cancer could be treated with IMRT to the prostate bed and pelvic lymph nodes at 1.8 Gy/d to a total of 45 Gy, followed by a proton therapy or IMRT boost to the prostate bed of 1.8 GyRBE or Gy per day to a total of 64.8 to 70.2 GyRBE or Gy, with possible localized boost of an area of gross recurrence to 73.8 GyRBE or Gy total.

Figure 1. Summary of clinical study design. Abbreviation: IMRT, intensity-modulated radiation therapy; DVH, dose-volume histogram.



Downloaded from <http://meridian.allenpress.com/ijpt/article-pdf/7/2/21/2660372/2331-5180-7-2-21.pdf> by guest on 18 January 2021

Dose-volume histogram (DVH) data were generated for the first 4 fractions of RT to account for the initial phase of treatment because the RadTox assay was performed before RT and on days 1 to 5 of RT before any boost or before cone-down phases of RT. In addition, DVH data for the plan summary, encompassing the initial phase of treatment and any boost phases of treatment, were also recorded.

cfDNA Measurement Using the RadTox Assay

Blood specimens were kept at room temperature for no more than 1 hour before plasma isolation. If plasma was not isolated within 1 hour, then the specimens were stored on ice or at 4°C. To isolate plasma, blood specimens were centrifuged at 2000g for 20 minutes at 20°C. After centrifugation, the plasma in each tube was removed without disturbing the white buffy coat layer above the packed red cells. Plasma was transferred into 1-mL microcentrifuge tubes and frozen at -80°C within 4 hours of collection.

Plasma specimens were then shipped in dry ice to DiaCarta, where the RadTox assay was performed to measure DNA concentration (nanograms of DNA per milliliter of plasma).

Statistical Analysis

JMP Pro (version 13.0.0, SAS Institute, Cary, North Carolina) was used for all statistical analysis. An independent *t* test provided estimates of statistical significance between categorical explanatory variables and a series of continuous metrics related to RadTox.

Table 1. Allowed radiation treatment dose and fractionation schedules.

Patient group	Radiotherapy	Dose per fraction	Total dose
Intact prostate			
Prostate + proximal SV alone	Photon IMRT	1.8– 2.0 Gy	78–79.2 Gy
	Proton	2.0 GyRBE	78 GyRBE
		2.5 GyRBE	70 - 72.5 GyRBE (hypofractionated regimen)
Prostate + proximal SV + PLN	Photon IMRT	2 Gy	46 Gy, followed by prostate + proximal SV boost
Prostate + proximal SV boost	Photon IMRT	1.8–2.0 Gy	32–34.2 Gy (total dose to prostate + SV = 78–79.2 Gy)
	Proton	2 GyRBE	32 GyRBE (total dose to prostate + SV = 78 Gy)
Postprostatectomy			
Prostate bed alone	Photon IMRT	1.8 Gy	64.8–70.2 Gy ± boost to gross recurrence
	Proton	1.8 GyRBE	64.8–70.2 GyRBE ± boost to gross recurrence
Prostate bed + PLN	Photon IMRT	1.8 Gy	45 Gy, followed by prostate bed boost
Prostate bed boost	Photon IMRT	1.8 Gy	19.8–25.2 Gy (total dose to prostate bed, 64.8–70.2 Gy)
	Proton	1.8 GyRBE	19.8–25.2 GyRBE (total dose to prostate bed, 64.8–70.2 GyRBE)
Gross recurrence boost	Photon IMRT	1.8 Gy	3.6–9 Gy (total dose to gross recurrence, 73.8 Gy)
	Proton	1.8 GyRBE	3.6–9 GyRBE (total dose to gross recurrence, 73.8 GyRBE)

Abbreviations: SV, seminal vesicles; IMRT, intensity-modulated radiation therapy; GyRBE, gray relative biologic effectiveness; PLN, pelvic lymph node.

Results

Patient Characteristics and Radiation Treatment

Between October 2016 and March 2017, 70 patients signed consent for the study; 54 patients (77%) were evaluable. A total of 16 of the 70 patients either withdrew (6 of the 16 patients no longer willing to undergo blood draws) or became ineligible because of the absence of radiation start date (8 of 16 patients had insurance or travel issues and were treated elsewhere), development of metastatic disease (1 patient), or hospitalization (1 patient). **Table 2** outlines patient characteristics. Fifty-one of the 54 patients (94%) underwent all 6 blood draws, and 3 patients (6%) missed only 1 blood draw. The median patient age at time of RT was 69.8 years (range, 52.3–85.2 years). A total of 42 patients (78%) received proton RT alone, whereas 12 patients (22%) received either IMRT alone or IMRT followed by a proton boost. Because proton boosts did not occur during the sample collection period, those patients were assigned to the IMRT group. The median prescribed RT dose for all patients was 78 Gy (range, 66.6–78 Gy).

RadTox Levels

The median pre-RT RadTox score was 34.8 ng/mL (range, 11.6–130.6 ng/mL). The median of the maximum (peak) of the 5 daily post-RT RadTox scores was 63.1 ng/mL (range, 21.9–252.7 ng/mL), and the median of the average of the 5 daily post-RT RadTox scores was 49.8 ng/mL (range, 19.9–180.7 ng/mL).

As shown in **Figure 2**, 3 patterns of change in RadTox scores were seen among the patients. Many patients (18 patients, 33%) had little change in their RadTox score during the first week of RT. These patients have preliminarily been termed the “low” group, which is defined by a peak/pre RadTox ratio (peak post-RT RadTox level/pre-RT RadTox level) of < 1.5. Most patients (32 patients, 59%) had a slow rise in their RadTox level; this “intermediate” group had a peak/pre RadTox ratio of 1.5- to 3-fold. A few patients (4 patients, 7%) had larger increases in their peak/pre RadTox ratio of > 3-fold and are tentatively considered the “high” group.

Two patients had high-baseline RadTox measurements (approximately 60 ng/mL), and 1 patient had an unusually large post-RT measurement (approximately 120–160 ng/mL) (**Figure 3**). **Table 3** shows a sample analysis of whether nuclear bone scans may be a confounder for RadTox scores. Among the 54 patients, 20 (37%) underwent standard or positron emission tomography bone scans. The median delay between the bone scan and radiation was 28 days (range, 11–46 days). Among these patients, the mean time to scan was approximately 30 days. There was no clear relationship between nuclear scans and the level of pretreatment RadTox.

Table 2. Patient and tumor characteristics, N = 54.

Characteristic	Median (range)	No. (%)
Age at RT, y	69.8 (52.3–85.2)	
Prostate cancer risk category		
Low		5 (9.3)
Intermediate		28 (51.9)
High		21 (38.9)
Pre-RT PSA, ng/mL	6.6 (0.03–66.8)	
Gleason score		
6		8 (14.8)
7		29 (53.7)
8+		17 (31.5)
Concurrent ADT during RT		
Yes		19 (35.2)
No		35 (64.8)
Surgical status		
Intact prostate		48 (88.9)
Postprostatectomy		6 (11.1)
Treatment fields		
Prostate or prostate bed ± SV		44 (81.5)
Addition of PLN		10 (18.5)
RT modality		
Proton		42 (77.8)
Photon		12 (22.2)
Prescribed total RT dose, GyRBE	78.0 (66.6–78.0)	

Abbreviations: RT, radiation therapy; PSA, prostate-specific antigen; ADT, androgen deprivation therapy; SV, seminal vesicles; PLN, pelvic lymph nodes; GyRBE, gray relative biologic effectiveness.

Correlation between RadTox Scores and DVH Parameters

We calculated body integral dose to see whether higher dose was correlated with RadTox levels. To represent the initial phase of RT, when RadTox levels were collected, body integral dose from only the first 4 fractions of RT was used. Proton therapy is delivered in a single lateral field daily, thereby allowing for 2 full cycles. As shown in **Figure 4**, the peak post-RT RadTox score (minus the baseline pre-RT RadTox score) was significantly associated with body integral dose delivered by day 4 of RT with a mean RadTox level of 36.0 ng/mL for patients with body integral dose < 20 Gy × L and 25.1 ng/mL for patients with body

Figure 2. Comparison of RadTox (nanograms of DNA per milliliter of plasma) concentration patterns as a function of first 5 dose fractions of radiotherapy (RT) using representative patients: patients 12, 4, and 36. Low group defined as those with a peak/pre-RadTox ratio (peak post-radiotherapy RadTox level/pre-RT RadTox level) of < 1.5; intermediate group defined as peak/pre-RadTox ratio of 1.5 to 3; high group defined as peak/pre-RadTox ratio > 3.

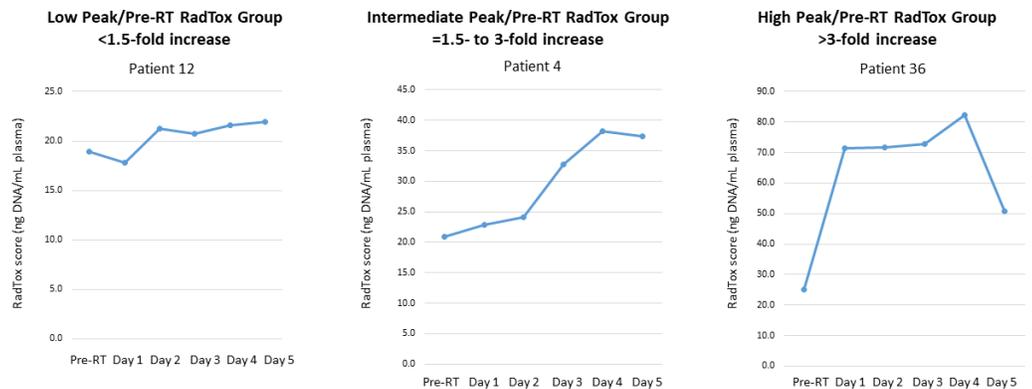
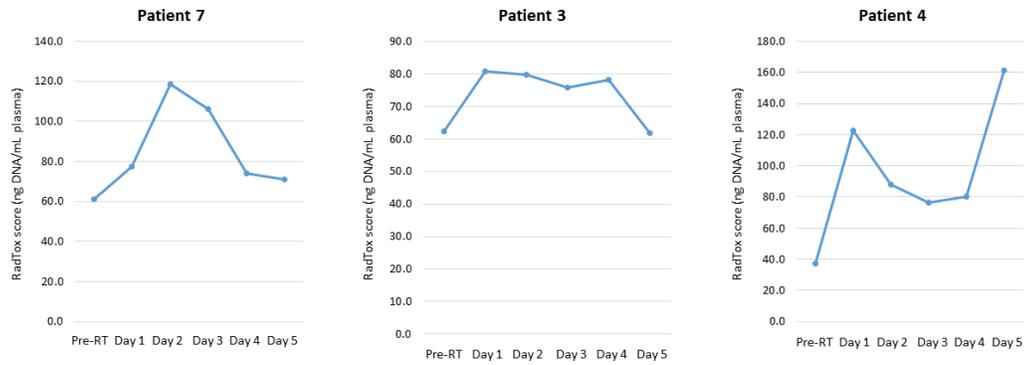


Figure 3. RadTox (nanograms of DNA per milliliter of plasma) concentration patterns in patients with high baseline or large postradiation response as a function of the first 5 doses. Two patients had unusually high baselines (patients 7 and 3) and 1 patient had an unusually high peak (patient 4). Further analysis is necessary to identify the mechanism of these unusual results and whether they predict for poor outcomes of are potential confounders.



integral dose $\geq 20 \text{ Gy} \times \text{L}$ ($P = 0.035$). However, single RadTox levels measured on individual days of RT (days 1, 2, 3, 4, or 5) were not significantly associated with body integral dose as a binary variable ($< 20 \text{ Gy} \times \text{L}$ versus $\geq 20 \text{ Gy} \times \text{L}$).

As patients were eligible to receive different dose-per-fraction regimens, we also examined whether RadTox levels were significantly associated with dose per fraction prescribed (1.8–2.0 Gy conventional fractionation versus 2.5 Gy hypofractionation). No significant difference was found ($P > .05$).

Correlation between RadTox Levels and RT Modality

RadTox measurements were compared between patients receiving photon IMRT versus proton therapy for prostate cancer. Patients who received IMRT had a significantly increased peak post-RT RadTox score (minus the pre-RT score) of 49.4 ng/mL versus the proton group score of 25.8 ng/mL ($P = .039$) (**Figure 5A**). The average post-RT RadTox score (minus the pre-RT score) was 26.0 ng/mL for the IMRT group versus 12.8 ng/mL for the proton group ($P = .040$) (**Figure 5B**). RadTox levels measured during RT were also analyzed as a ratio to pretreatment RadTox values to account for the potential of individuals having different cfDNA clearance rates (**Figure 5C**). The difference in RadTox ratios between patients receiving IMRT and those receiving proton therapy was not statistically significant ($P = .06$ – $.08$).

Based on our previous research into the clearance kinetics of cfDNA measured in total-body irradiated nonhuman primates, we predicted that the peak in plasma cfDNA would occur approximately 48 hours after the first dose of RT. We also analyzed the RadTox data looking only at the day 2 (48 hours after the first dose of RT) RadTox measurements (minus the pre-RT values for each patient). As shown in **Figure 5D**, the average increase in RadTox by day 2 was $32.2 \pm 11.8 \text{ ng/mL}$ in the IMRT group and $14.8 \pm 4.0 \text{ ng/mL}$ in the proton group ($P = .02$).

Correlation between RadTox Scores and RT Field Size

There was no significant association found between RT field size (prostate or prostatectomy bed alone with or without seminal vesicles versus the addition of pelvic lymph nodes) and RadTox score, although there was a trend for the day 2 RadTox score ($P = .050$).

Table 3. Mean RadTox concentrations (nanograms of DNA per milliliter of plasma) in patients receiving nuclear bone scans compared with patients without scans.

RadTox score	Patients with bone scan	Patients without bone scan
Average	47.1 ± 5.8	39.1 ± 4.4
Median	45.1	31.2
Range	12.8–109.4	11.6–130.6

Figure 4. Analysis of body integral dose calculated after the first 4 fractions of radiotherapy (RT). The peak post-RT RadTox score (minus the baseline pre-RT RadTox score) was significantly associated with body integral dose delivered by day 4 of RT with mean RadTox level of 36.0 ng/mL for patients with body integral dose of < 20 Gy × L and 25.1 ng/mL for patients with body integral dose ≥ 20 Gy × L ($P = .04$).

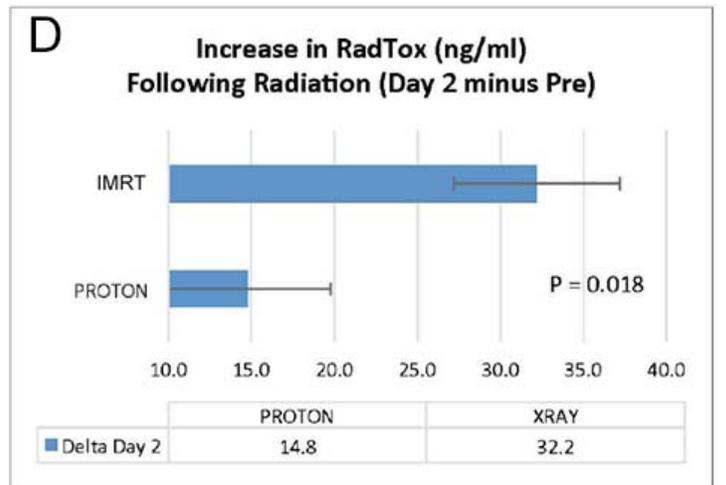
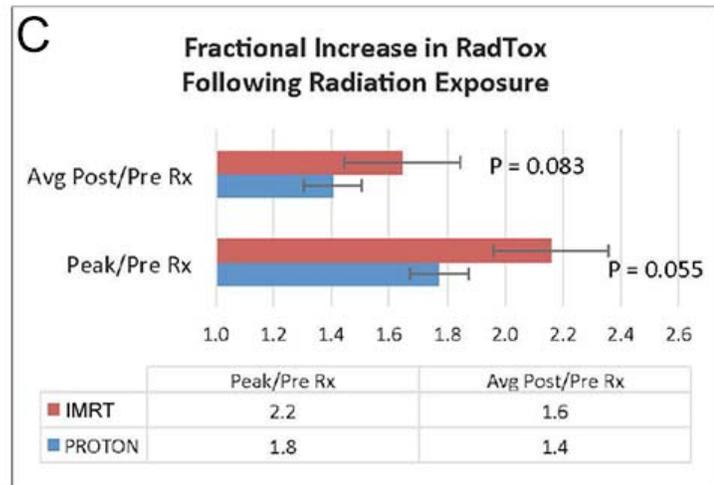
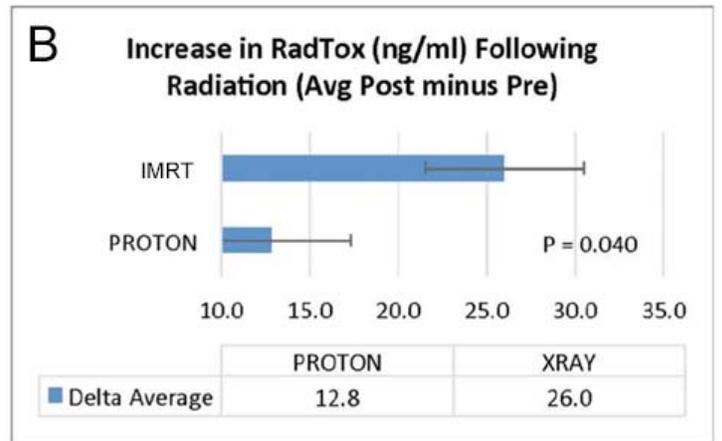
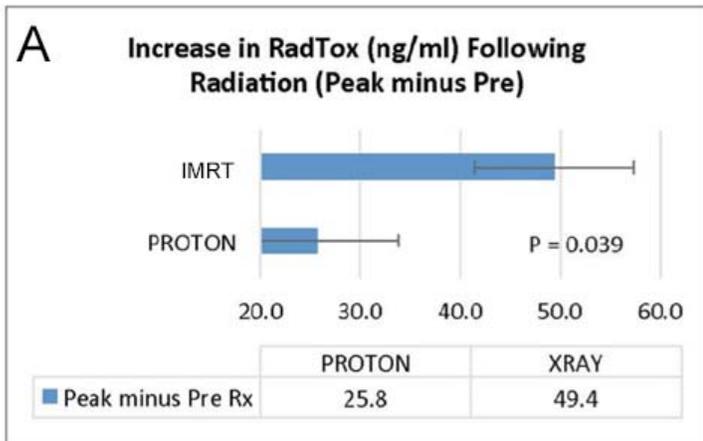
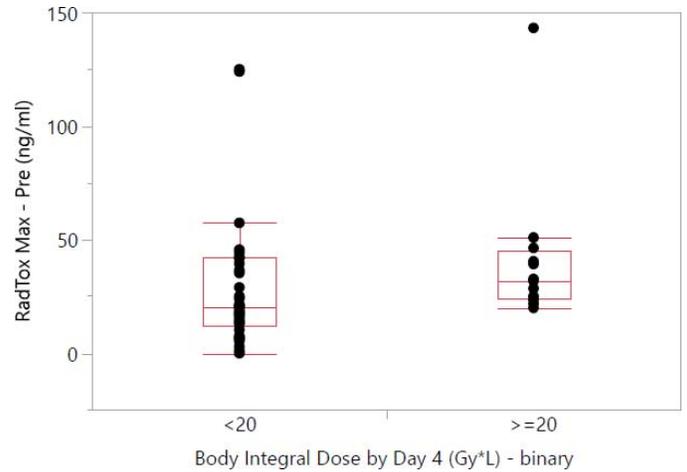


Figure 5. Comparisons of RadTox levels (nanograms of DNA per milliliter of plasma) measured in patients receiving photon intensity-modulated radiotherapy (RT) (IMRT, x-ray) versus proton RT. (A) Peak levels of post-RT RadTox (minus pre-RT levels) during the first 7 days after patients started RT were significantly higher in the x-ray group. (B) Similarly, average levels (average of the 5 daily post-RT RadTox levels minus pre-RT levels) were significantly higher in the x-ray group. (C) RadTox levels expressed as the ratios of the average post-RT/pre-RT and peak post-RT/pre-RT were not significantly different for patients who received x-rays versus protons. (D) The day 2 post-RT RadTox levels (minus pre-RT levels) in patients who received x-ray were significantly higher than those in patients who received proton therapy.

Discussion

Dose distribution is the primary tool used by physicians to predict a safe treatment plan. Physicians employ radiation doses, field sizes, and organ coverage to achieve a satisfactory level of tumor control with tolerable early and late toxicity. Once treatment has started, however, there is rarely a modification, and the physician has no method of predicting which patients will be harmed by treatment until late in the therapy or after completion of the therapy. We reasoned that measurement of total tissue damage that occurs during the first week of radiotherapy could provide an individualized estimate of risk. We chose to test patients with prostate cancer because toxicity scoring is well established, and the treatment regimens have similar dosimetry with surprisingly different toxicity profiles. Thus, it can be postulated that patients who experience toxicity represent a radiosensitive population. The primary aim of this first-in-human study was to determine whether RadTox measurements during the first week of radiotherapy correlated with the current standard of physics dosimetry.

The present study was funded by the National Cancer Institute (Bethesda, Maryland) in response to a request for proposals aimed at using biodosimetry methods developed for homeland security in the cancer population. The technology for this application was also funded by a Biomedical Advanced Research and Development Authority (Washington, DC) contract (HHSO100201000003C). Our previous work showed a dose-response for whole-body radiation exposure in several animal species and in humans undergoing abdominal irradiation. In mice, we have also shown that the weight-adjusted levels of cfDNA correspond with the volume of the body exposed with partial body exposures and that strains of mice that are more radiation sensitive have differing levels of cfDNA [1, 4, 5]. Thus, we postulate that cfDNA may be a “biotoximeter.” Internationally, many groups have been developing radiation biodosimetric assays for whole-body radiation exposure. Among the most promising include circulating proteins (salivary gland amylase [4, 5]), chromosomal rearrangements [6, 7], cytokines [8], messenger RNA [9], or microRNA [10]. Some have been evaluated in patients with bone marrow transplantations or in victims of radiation exposure accidents [11, 12]. Although some of these bioassays have a clear mechanism, such as salivary gland exposure, panels often have an unknown mechanism to explain the proprietary calculations of the panel components. Few of those panels have been tested in animals with partial body exposure or in humans undergoing radiotherapy. However, there are some promising studies in patients with cancer, including the Radiotherapy Assessments during Intervention and Treatment (RADIANT trial, NCT03133286).

Our results show that cfDNA measured during the first week of radiotherapy successfully identified the patient group undergoing proton therapy. Similarly, cfDNA correlated significantly with the integral dose. However, it did not correlate with many of the usual bowel and bladder dose constraints. Because the mechanism of cfDNA is related to total cells killed by radiation during that period of time, cfDNA may provide additional biologic information beyond the dose distribution. This possibility is supported by animal studies. In mice, major surgery or trauma (splenectomy or 7-mm-diameter skin punch) does not greatly affect cfDNA nor does lipopolysaccharide injection to emulate sepsis. However, combined radiation (4 Gy), normally easily tolerated by mice, results in synergistic interactions with these concurrent toxicity amplifiers [4, 5, 7]. In the present study, 7% (4 of 54) of the patients had unusually high and sustained spikes of cfDNA during radiotherapy, a number similar to the rate of toxicity in patients with prostate cancer. Notably, 33% (18 of 54) of the patients had rather small changes in cfDNA, suggesting that they might be more radiation tolerant.

There are several limitations to this study; some of which, we will test in future studies. Most obviously, cfDNA is related to total cell death and is not specific to either the healthy tissue or the tumor. Hence, the mix of tumor-cell killing and normal tissue damage that comprise cfDNA is unknown. Organ-specific and tumor-specific markers could provide a better alternative if they were available, economical, easily performed, and quantitative. As previously mentioned, drugs, chemotherapy, and intercurrent disease are associated with different radiation tolerances. If cfDNA integrates known and unknown biologic causes of toxicity, demonstrating known patient subgroups that have higher cfDNA will be critical. In the present study, patients with autoimmune disease, chemotherapy, or previous cancer treatment (other than prostatectomy) were excluded. Thus, to evaluate cfDNA as a biomarker for those circumstances will require studies of cancers that require chemotherapy or which include high-risk populations. Prostate cancer was chosen for this study because the homogeneity of patients allows a small and easily interpretable study.

We did not observe a significant difference between the fraction sizes, which ranged from 1.8 to 2.5 Gy, or between patients receiving pelvic nodal compared with prostate or prostate bed-only radiation treatment. The study was agnostic to common treatment regimens for prostate cancer because all have similar acute- and late-toxicity profiles [13]. It is uncommon for large fraction sizes to be chosen with larger fields or postprostatectomy. Thus, physician judgment might account for that observation. Notably, the patient numbers in each fractionation subgroup were insufficient to satisfactorily test that assumption.

We did see a difference in the secondary aim for modality. Proton therapy, although achieving similar dosimetry to the IMRT optimization criteria for organs at risk, has a markedly better integral dose (our primary aim). We expected this modality-specific result because cfDNA measures total cell damage, which is related to the body integral dose. This study was not powered or designed to suggest a better modality or fractionation schedule, and none should be assumed.

A tool to predict patients who are high (or low) responders may help physicians identify those who would benefit from mitigative interventions (eg, fractionation dose changes, early 3-day recovery weekends, or preemptive medications). It may also provide confidence for dose escalation or help guide tolerable doses in cases of reirradiation. For patients undergoing stereotactic ablative radiation therapy or stereotactic body radiation therapy, the physician may be able to more confidently estimate tumor cell kill. The potential uses of cfDNA will require future investigation.

In conclusion, cfDNA can be measured during the first week of radiation. It rapidly increases after radiation with patient-specific patterns. The level of cfDNA correlates with integral dose and is higher in patients treated with IMRT compared with patients treated with protons, consistent with the improved integral dose. A subset of patients show unusually high levels of cell damage during the early stages of radiotherapy, potentially indicating that biologic factors may predispose those patients to radiotoxicity. Questions remain as to how much of the cfDNA is from tumor compared with healthy tissue and whether cfDNA will provide useful information when combined with intercurrent disease or chemotherapy. However, cfDNA appears to be a promising tool that could help physicians adjust treatment parameters early in a course of radiation treatment.

ADDITIONAL INFORMATION AND DECLARATIONS

Conflicts of Interest: Paul Okunieff, MD, is the founder of, and a stockholder in, DiaCarta, Inc. As such, he may benefit financially as a result of the outcomes of the research reported herein. In line with his UF-approved and managed conflict of interest plan and the institutional review board–approved clinical protocol, Dr Okunieff performed study activities but had no interactions with study subjects. The authors have no other relevant conflicts of interest to disclose.

Acknowledgments: The authors would like to thank Christopher Stich and the University of Florida Department of Radiation Oncology Research Office for their assistance with formatting and submitting this manuscript.

Funding: This work was supported by a phase I Small Business Innovation Research Contract from the National Cancer Institute at the National Institutes of Health (DiaCarta, N44CA180036).

Ethical Approval: All patient data were collected under internal review board–approved protocol.

References

1. Marks LB, Yorke ED, Jackson A, Ten Haken RK, Constine LS, Eisbruch A, Bentzen SM, Nam J, Deasy JO. Use of normal tissue complication probability models in the clinic. *Int J Radiat Oncol Biol Phys*. 2010;76(suppl):S10–9.
2. Zhang L, Zhang M, Yang S, Cao Y, Bingrong Zhang S, Yin L, Tian Y, Ma Y, Zhang A, Okunieff P. A new biodosimetric method: branched DNA-based quantitative detection of B1 DNA in mouse plasma. *Br J Radiol*. 2010;83:694–701.
3. Zhang L, Zhang M, Zhang B, Cao Y, Yang S, Yin L, Tian Y, Zhang K, Swarts S, Okunieff P. Radiation-induced elevation of plasma DNA in mice is associated with genomic background. *Adv Exp Med Biol*. 2012;737:147–53.
4. Dubray B, Girinski T, Thames HD, Becciolini A, Porciani S, Hennequin C, Socie G, Bonnay M, Cosset JM. Post-irradiation hyperamylasemia as a biological dosimeter. *Radiother Oncol*. 1992;24:21–6.
5. Becciolini A, Porciani S, Lanini A, Balzi M, Faraoni P. Proposal for biochemical dosimeter for prolonged space flights. *Phys Med*. 2001;17(suppl 1):185–6.
6. Lucas JN, Awa A, Straume T, Poggensee M, Kodama Y, Nakano M, Ohtaki K, Weier HU, Pinkel D, Gray J, Littlefield G. Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation. *Int J Radiat Biol*. 1992;62:53–63.
7. Tucker JD. Low-dose ionizing radiation and chromosome translocations: a review of the major considerations for human biological dosimetry. *Mutat Res*. 2008;659:211–20.
8. Partridge MA, Chai Y, Zhou H, Hei TK. High-throughput antibody-based assays to identify and quantify radiation-responsive protein biomarkers. *Int J Radiat Biol*. 2010;86:321–8.
9. Li SE, Guo F, Wang P, Han L, Guo Y, Wang XA, Li J, Lyu YM. X-ray-induced expression changes of TNFSF4 gene in human peripheral blood. *Biomed Environ Sci*. 2014;27:729–32.

10. Jacob NK, Cooley JV, Yee TN, Jacob J, Alder H, Wickramasinghe P, Maclean KH, Chakravarti A. Identification of sensitive serum microRNA biomarkers for radiation biodosimetry. *PLoS One*. 2013;8:e57603.
11. Balog RP, Bacher R, Chang P, Greenstein M, Jammalamadaka S, Javitz H, Knox SJ, Lee S, Lin H, Shaler T, Shura L, Stein P, Todd K, Cooper DE. Development of a biodosimeter for radiation triage using novel blood protein biomarker panels in humans and non-human primates. *Int J Radiat Biol*. 2020;96:22–34.
12. Lindholm C, Romm H, Stephan G, Schmid E, Moquet J, Edwards A. Intercomparison of translocation and dicentric frequencies between laboratories in a follow-up of the radiological accident in Estonia. *Int J Radiat Biol*. 2002;78:883–90.
13. Carvalho IT, Baccaglioni W, Claros OR, Chen FK, Kayano PP, Lemos GC, Weltman E, Kuban DA, Carneiro A. Genitourinary and gastrointestinal toxicity among patients with localized prostate cancer treated with conventional versus moderately hypofractionated radiation therapy: systematic review and meta-analysis. *Acta Oncol*. 2018;57:1003–10.