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Corresponding author:
Mark E. Artz; Email: markartz@alum.mit.edu

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Method for determining a Prostate Cancer LET Sensitivity Index (PCLSI) using tumour-specific DDR mutations for proton RBE

Mark E. Artz[1], Michael J. Vieceli[1], Jiyeon Park[1], Mohammad Saki[1], Yawei Zhang[1], Eric D. Brooks[2], Nancy P. Mendenhall[1], Perry B. Johnson[1] and Hardev S. Grewal[1]

[1]Department of Radiation Oncology, University of Florida College of Medicine, Jacksonville, FL, USA and [2]Premier Radiation Oncology Associates, Clearwater, FL, USA

Abstract

Purpose: This study introduces the prostate cancer linear energy transfer sensitivity index (PCLSI) as a novel method to predict relative biological effectiveness (RBE) in prostate cancer using linear energy transfer (LET) in proton therapy based on screening for DNA repair mutations.
Materials and Methods: Five prostate cancer cell lines with DNA repair mutations known to cause sensitivity to LET and DNA repair inhibitors were examined using published data. Relative Du145 LET sensitivity data were leveraged to deduce the LET equivalent of olaparib doses. The PCLSI model was built using three of the prostate cancer cell lines (LNCaP, 22Rv1 and Du145) with DNA mutation frequency from patient cohorts. The PCLSI model was compared against two established RBE models, McNamara and McMahon, for LET-optimized prostate cancer treatment plans.
Results: The PCLSI model relies on the presence of eight DNA repair mutations: AR, ATM, BRCA1, BRCA2, CDH1, ETV1, PTEN and TP53, which are most likely to predict increased LET sensitivity and RBE in proton therapy. In the LET-optimized plan, the PCLSI model indicates that prostate cancer cells with these DNA repair mutations are more sensitive to increased LET than the McNamara and McMahon RBE models, with expected RBE increases ranging from 11%–33% at 2keV/μm.
Conclusions: The PCLSI model predicts increasing RBE as a function of LET in the presence of certain genetic mutations. The integration of LET-optimized proton therapy and genetic mutation profiling could be a significant step toward the use of individualized medicine to improve outcomes using RBE escalation without the potential toxicity of physical dose escalation.

Introduction

Prostate cancer is the predominant oncological disease affecting men worldwide.[1] Despite improvements in diagnostic and therapeutic capabilities, optimization of patient-specific treatment remains challenging. In radiation oncology, the complex interplay between radiation dose delivery and cellular response is crucial. The relative biological effectiveness (RBE) in radiation therapy depends on the cellular response to radiation, influenced by factors including cellular genetics.[2]

Radiobiological studies emphasize linear energy transfer (LET) as a key determinant of cellular radiation response. LET refers to the energy deposited by ionizing radiation per unit path length.[3] Higher LET radiation, found in proton, helium and carbon beams, causes more significant DNA damage compared to low LET radiation, like X-rays or gamma rays. This increased damage translates to a greater RBE.

High LET radiation causes more double-strand breaks (DSBs), while photon-based radiation causes more single-strand breaks (SSBs).[4] DSBs rely on homologous recombination repair (HRR) pathways, whereas SSBs can use additional repair mechanisms. The reliance on HRR for high LET radiation stems from the need for high-fidelity repair mechanisms to address the complexity and cluster of DNA damage, ensuring accurate restoration of genomic integrity.[5] Poly (ADP-Ribose) polymerase inhibitors (PARPi), such as olaparib, are a class of drugs used to systemically inhibit SSB repair, directing cells toward DSB repair pathways. Cells with mutations in HRR genes, such as BRCA2,[6] are less likely to survive with PARPi and are more sensitive to high LET radiation.[7–13]

DNA repair mutations are the primary driver of prostate cancer. Mutations in the androgen receptor (AR) and BReast CAncer (BRCA2) genes play prominent roles in prostate cancer and prostate cancer progression; within metastatic prostate cancer, almost 85% of patients have AR mutations and almost 47% have BRCA2 mutations.[14,15] Almost 60% of non-metastatic patients

have AR mutations and almost 50% have a PTEN mutation.[16] This genetic domain critically interacts with radiation dosimetry. Dose escalation in prostate cancer correlates with improved 5-year prostate-specific antigen (PSA) control outcomes,[17] with DNA repair mutations providing an opportunity to escalate RBE dose. Patients with these mutations may experience higher RBE doses, especially with high LET radiation modalities such as proton therapy. High-risk prostate cancer patients are likely to benefit from RBE escalation, as they have both a need for 5-year PSA outcomes improvement[18] and harbour an increased DNA repair mutation burden.

Other RBE models, such as the McMahon and McNamara models, are informed by analysis of several cell lines and their response to LET, which are generalized models that often have a high amount of uncertainty for cells with low α/β ratios such as prostate cancer.[19,20] Although there have been several publications demonstrating that the inhibition of individual DNA repair genes increases the sensitivity of cells to LET, the relationship between these genetic mutations and LET has not been evaluated for prostate cancer with LET values in the range of proton therapy. The DNA repair mutations used in the PCLSI were chosen due to both the prevalence in prostate cancer and impact on sensitivity to radiation and LET. Using a limited number of genes allows the PCLSI model to potentially be used with genetic screening of prostate cancer biopsies and would only require sequencing of a targeted number of genes, which is less costly than more broad sequencing strategies, for example the prostate cancer genetic risk assessment tool Decipher uses 22 genes.[21] The genes used in the PCLSI model are likely a subset of the genes in the Decipher prostate cancer risk tool, as the PCLSI genes are often studied for both disease progression risk and responsiveness to therapeutics.

DNA repair mutations are not only the primary driver of progression and castration resistance but also the common mechanism of action for both PARPi and LET sensitivity in prostate cancer.[22–28] In cell studies, mutations in genes such as BRCA1, BRCA2, P53 and ATM play significant roles in sensitivity to DSB damage. In one experiment, cell lines without BRCA1 mutations exhibited an RBE of only approximately 0.98 in low LET and 1.5 in increased LET proton beams, when compared to X-rays. In contrast, cells with BRCA1 mutations showed a much larger RBE difference, 1.3 in low proton LET vs 3.5 in increased LET proton beams.[7] In cells, ataxia-telangiectasia-mutated (ATM) inhibitor produced a similar increase in RBE as BRCA1 mutations, with an RBE near 1.1 in low LET and an RBE of 3.2 with increased LET.[7] Certain lung cancer phenotypes with BRCA mutations have exhibited RBE values of 1.77 when compared against 250-kVp X-rays.[29] These studies show significant increases in RBE from increased LET in the presence of inhibited DNA repair genes.

DNA repair-inhibited cells treated with X-rays compared to X-rays+olaparib experienced significant increases in RBE, similar in scale to DNA repair-inhibited cells treated with increased LET alone. Three PTEN-deficient prostate cancer cell lines showed an RBE of 1.4 from the addition of olaparib.[30] In non-prostate cancer cell lines, two cell lines with inhibited DNA repair gene XRCC2 showed an RBE of approximately 2.0[26] and three cell lines with chemically inhibited BRCA1/2 demonstrated an RBE of 1.3–1.5.[31] These types of cellular response studies further demonstrate that cells with inhibited DSB repair experience similar increases in RBE from increased LET as with the addition of DNA repair inhibitors like PARPi in low LET radiation such as X-rays, although the RBE enhancement from LET has been demonstrated to be significantly greater than olaparib in DNA repair-inhibited cells.

Among the DNA repair inhibitors, the PARPi olaparib has been demonstrated as particularly effective radiosensitizing agent, especially in prostate cancer.[8,25,26,30–32] Given that sensitivity to DNA repair inhibitors and LET heavily depends on mutations in the same DNA repair genes, PARPi and other published data were used to build a mathematical model to identify DNA repair mutations that predict proton LET sensitivity.

This study introduces the Prostate Cancer LET Sensitivity Index (PCLSI) as a novel method to predict prostate cancer cell sensitivity to proton therapy, emphasizing the influence of genetic mutations. The interplay between DNA repair inhibitors and sensitivity to high LET radiation is of particular interest in LET-optimized proton therapy. Both hinge on the integrity of DSB repair pathways. Cells harbouring mutations in DSB repair genes manifest increased vulnerability to both PARPi and high LET radiation.[8]

The PCLSI is particularly promising when combined with LET optimization. LET-optimized proton therapy can provide higher LET to prostate cancer tissue, resulting in more DSBs per J/kg than conventional photon therapy. The impact of several genes that may influence LET sensitivity and thus proton therapy's RBE are quantified. The PCLSI model is also compared against established RBE models for single field optimization (SFO) and LET-optimized prostate cancer treatment plans.

Methods and Materials

Five prostate carcinoma cell lines with published olaparib survival data were investigated: LNCaP,[33] VCaP,[34] 22Rv1,[35] PC3[36] and Du145.[37] These cell lines are derived from a lymph node metastasis, spinal cord metastasis xenograft, primary prostate cancer xenograft, bone metastasis and brain metastasis, respectively. The LNCaP, VCaP and 22Rv1 cell lines were AR positive, while PC3 and Du145 cells were AR negative. LNCaP and VCaP are PSA positive, while PC3 and Du145 are PSA negative; 22Rv1 is negative for the PSA protein but positive for PSA mRNA.[38] The cell lines evaluated in this study were purchased from the American Type Culture Collection (ATCC).[6,39]

Olaparib ($C_{24}H_{23}FN_4O_3$, MW: 434 g/mol), a PARPi, has been studied for the treatment of adult patients with germline or somatic HRR gene-mutated metastatic castration-resistant prostate cancer (mCRPC). Olaparib's mechanism induces synthetic lethality in cancer cells, particularly those with HRR deficiencies, such as BRCA1 or BRCA2 mutations.[40] In HRR-deficient cells, olaparib-induced loss of base excision repair (BER) results in the accumulation of unrepaired SSBs. During DNA replication in S phase, these SSBs lead to replication fork collapse, ultimately generating deleterious DSBs. In cells with intact HRR, these DSBs can be effectively repaired. However, in HRR-deficient cells, such as those with BRCA mutations, these DSBs either remain unrepaired or are mended through error-prone DNA repair mechanisms (i.e., nonhomologous end joining), ultimately triggering genomic instability and apoptotic cell death.

The model also uses the published data regarding the Du145 prostate cancer cell line sensitivity to proton beam LET[39,41] and the DNA repair mutation profile included from the Catalogue Of Somatic Mutations In Cancer (COSMIC) cell line project's mutations database (Figure 1A). Du145 was used as the common calibration cell line to estimate the change in LET required to produce a similar sensitization from the available spectrum of a PARPi, olaparib, doses.[6] Du145 was chosen because it is one of the only prostate cancer cell lines with published LET sensitivity data

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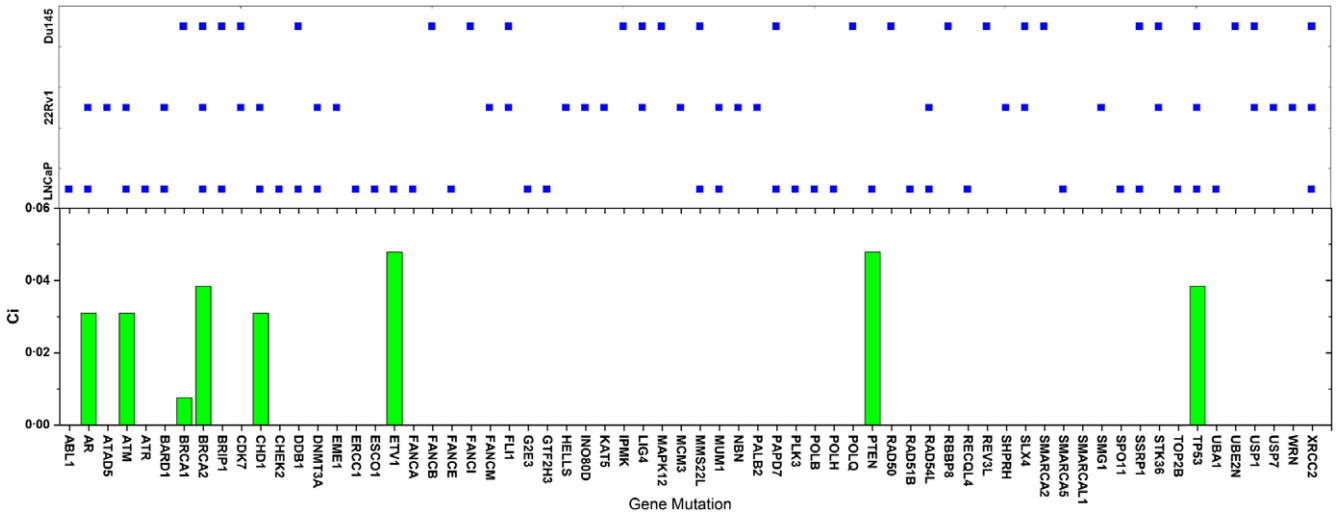


Figure 1. Presence of DNA repair mutations and resulting PCLSI coefficients. (A) Presence of DNA repair gene mutations (blue square) in each modelled cell line. A total of sixty-seven gene mutations are shown and data are taken from the COSMIC database. Resultant coefficient Ci of $(LET \times Gene_i) \cdot C_i = (RBE_{PCLSI} - 1.16)$ of different gene mutations is shown (B).

in proton therapy and it contains a relatively moderate DNA repair mutation burden compared to the other prostate cancer cell lines that were studied. Of the five cell lines included in the olaparib data, three cell lines were used to fit the PCLSI model, Du145, 22Rv1 and LNCaP. These three cell lines had mutations with published incidence frequency from genetic screening of prostate cancer patients.[14,16] PC3 and VCaP were excluded because they did not have the presence of any of the eight genes with known mutation frequencies and had no effect on the PCLSI model.

PSLSI Model Development

Relating olaparib sensitization to LET sensitization

Published α and β values for Du145 response to photon therapy were used as reference values from which Δα values were calculated by modelling sensitization by exploitation of mutations in DSB repair pathways by olaparib or LET. The linear-quadratic model (LQM) is used to describe the relationship between surviving fraction (SF) of cells and dose,[42] the LQM is shown in Equation 1. In Equation (1), the SF of a particular cell type in response to radiation dose (D) is shown where α describes lethal damage from single particles, such as DSBs. Lower values of α result in a broad shoulder for the SF curve and higher values of α resulting in straightening of the SF curve, often seen in radiation with increased LET. β describes multiple hit cell death, such as from SSBs, and the SF curve at higher doses. β does not show LET dependence in LET sensitivity studies consistent with its mechanistic meaning.[39,41,42] The change in α required to produce the relative change in SF at the published doses of olaparib was calculated using Equation 2 for a dose of 2Gy.[8,31] This sensitization model chose α because α is dependent on LET; this is also consistent with LET-dependent cell survival curves.[39,41]

$$SF(\mathrm{D}) = e^{-(\alpha * D + \beta * D^2)} \tag{1}$$

SF_1 and SF_2 are taken from the same cell line at two different LET values

$$-ln(SF_1) = \alpha_1 * D + \beta_1 * D^2$$

$$-ln(SF_2) = \alpha_2 * D + \beta_2 * D^2$$

$$\beta_1 = \beta_2 \text{ due to } \beta \text{ being LET indepdent } [39, 41, 42]$$

$$-ln(SF_1) - \alpha_1 D = -ln(SF_2) - \alpha_2 D$$

$$\alpha_2 - \alpha_1 = \Delta\alpha = \frac{ln(SF_1) - ln(SF_2)}{D} \tag{2}$$

To calculate RBE from Δα, the dose required to produce a SF of 10% was calculated using Equation 3 with an $\alpha_{ref} = 0.14$ and $\beta = 0.03$ taken from published clonogenic assay data.[41] The LQM was solved for dose using the quadratic formula with both $\alpha_{ref}+0$ (lower LET) and $\alpha_{ref}+\Delta\alpha$ (higher LET), and the ratio of these two doses provides the RBE value for radiation with increased LET and greater Δα. The Δα used in the model is shown in Table 1 and relative sensitization of the SF in Figure 2. The change in RBE was then calculated using Equation 4. The LET range that would produce the corresponding ΔRBE was calculated using the linear model of Du145 sensitivity to LET.[43] The LET increase required to produce the RBE enhancement was estimated by dividing the change in RBE by the published Du145 coefficient of LET sensitivity of 0.084.[43] Using the estimated LET range, the RBE response to LET of each cell line was estimated using least squares fit. The reference RBE of each cell line at 1.9 keV/μm was calculated using Equation 5 and added to the RBE enhancement values, and k is the individual cell line linear RBE relationship with LET. The values of LET and RBE used in fitting the PCLSI model are shown in Table 2, and the range of LET value from 2.0 to 4.5 keV/μm refers to the LET spectrum present clinical proton therapy plans.[43]

$$(\alpha_{ref} + \Delta\alpha) * D + \beta * D^2 + ln(SF_2) = 0$$

$$D = \frac{-(\alpha_{ref} + \Delta\alpha) + \sqrt{(\alpha_{ref} + \Delta\alpha)^2 - 4 * \beta * \ln(0.1)}}{2 * \beta} \tag{3}$$

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Table 1. Values of $\Delta\alpha$, ΔRBE and RBE calculated using the sensitization of cell line surviving SF treated with olaparib with Du145 LET and olaparib sensitivity used for cross-calibration. The RBE and LET equivalent values were used to model the PCLSI. k_{lsqr} is a least squares regression of the relationship between LET and RBE for each cell line. The ΔRBE values were added to the RBE_{ref} values calculated using Equation 5 for an LET of 1.9 keV/µm

	Cell lines				
	LNCaP	VCaP	22Rv1	PC3	Du145
Dose [µM]			$\Delta\alpha$		
0.0	0.000	0.000	0.000	0.000	0.000
2.5	0.299	0.07	0.247	0.112	0.042
5.0	0.446	0.07	0.327	0.118	0.053
10.0	0.525	0.105	0.422	0.186	0.112
20.0	0.525	0.099	0.299	0.178	0.264
			ΔRBE		
k_{lsqr}	0.244	0.040	0.151	0.073	0.084
ΔLET [keV/µm]	LNCaP	VCaP	22Rv1	PC3	Du145
0.00	0.000	0.000	0.000	0.000	0.000
0.94	0.643	0.134	0.521	0.220	0.079
1.19	1.008	0.134	0.711	0.233	0.100
2.62	1.212	0.207	0.947	0.380	0.220
6.67	1.212	0.194	0.643	0.364	0.560
			RBE		
LET Eq. [keV/µm]	LNCaP	VCaP	22Rv1	PC3	Du145
1.900	1.625	1.244	1.449	1.307	1.322
2.841	2.268	1.378	1.970	1.527	1.400
3.097	2.633	1.378	2.160	1.540	1.422
4.526	2.837	1.451	2.396	1.687	1.542
8.584	2.837	1.439	2.093	1.671	1.881

Abbreviations: Surviving Fraction (SF), Radiobiological Biological Effectiveness (RBE), Linear Energy Transfer (LET), Prostate Cancer LET Sensitivity Index (PCLSI).

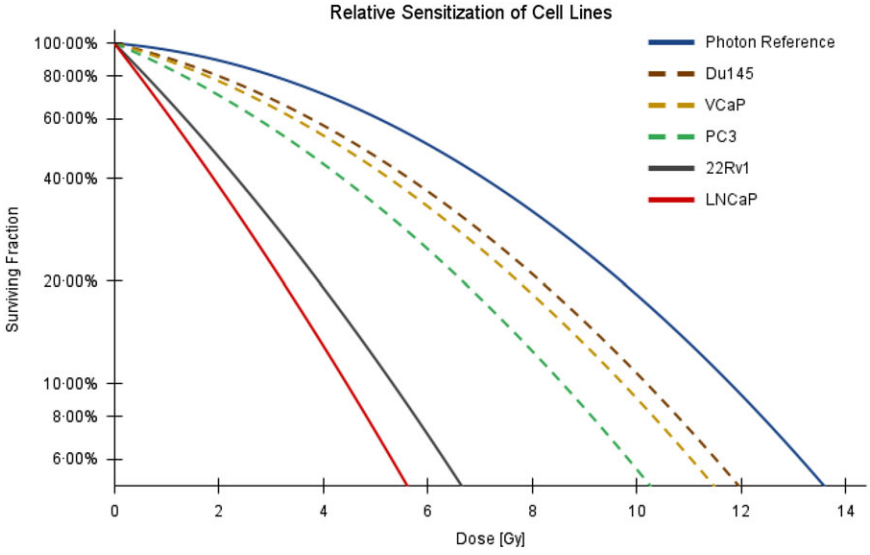


Figure 2. Predicted relative sensitization of cell lines. Surviving fraction of each prostate cancer cell line using α_{ref} and β_{ref} from published photon data (blue) and estimated $\Delta\alpha$ at 3.1 keV/µm. Du145, VCaP and PC3 (doted) showed moderate sensitization while 22Rv1 (black) and LNCaP (red) showed the most significant sensitization.

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$$RBE_{(\alpha_{ref}+\Delta\alpha)} = \frac{D_{(\alpha_{ref}+0)}}{D_{(\alpha_{ref}+\Delta\alpha)}} \quad (4)$$

$$RBE_{ref} = 1.16 + k * 1.9\ [\text{keV}/\mu\text{m}] \quad (5)$$

Establishing coefficients for DNA repair mutation sensitivity

To capture the relationship between RBE sensitivity and HRR mutations, a linear system of equations was devised. A system of equations was created such that the RBE as a function of LET of a cell line with a known DNA repair mutation profile could be calculated from its PCLSI (Equation 6). The PCLSI relies on the summation of all coefficients, C_i, from each individual gene including PCLSI model, $Gene_i$. For an individual patient, only the DNA repair mutations present in the biopsy are summed, genes with mutations are noted as present with a value of one and those not present with a zero. An example case with DNA mutation profile and PCLSI use is presented in the Clinical Case Study section. The presence of DNA repair mutations in prostate cancer cell lines was sourced from publication,[6] and the presence of each mutation is displayed in Figure 1A.

$$PCLSI = \sum (C_i \times Gene_i) \quad (6)$$

The PCLSI is a metric of LET sensitivity created by multiplying the coefficient of sensitivity caused by the presence of each genetic mutation,C_i by one if the mutation is present or zero if the mutation is not present, denoted by$Gene_i$. A system of equations was created from DNA repair mutations presented in the COSMIC database from the cell lines Du145, 22Rv1 and LNCaP. The system of equations was solved such that the coefficients of each genetic mutation best fit the RBE at each LET. The system of equations was solved with a linear least square in MATLAB to best fit the coefficients to the reference SF data.[6]

Prostate Cancer LET Sensitivity Index (PCLSI) & coefficient determination

The proposed PCLSI model utilizes the tissue-specific DNA repair mutation profile to estimate the sensitivity of RBE to LET from the SF of prostate cancer cell lines with varying DNA repair mutation profiles. The RBE multiple when compared to the reference dose expressed in Cobalt Gray Equivalent (CGE) is given in Equation 7, denoted as.RBE_{PCLSI} In Equation 7, PCLSI replaces k from Equation 5, introducing the presence of DNA repair mutations to the relationship between RBE and LET. The equivalent biological impact in CGE, RBE_{Dose} is given as RBE_{PCLSI} multiplied by $Dose_{physical}$ and is shown in Equation 8. The linear model intercept for LET = 0 was taken from the published fit of Du145 LET sensitivity.[41,43]

$$RBE_{PCLSI} = 1.16 + PCLSI \times LET \quad (7)$$

$$RBE_{Dose} = (1.16 + PCLSI \times LET)\ x\ Dose_{physical} \quad (8)$$

To calculate the coefficients of sensitivity to each genetic mutation, C_i a system of equations was evaluated using least squares to fit the coefficients, using the MATLAB[44] function *lsqr*.[45] Equation 9 shows the conversion of Equation 7, RBE_{PCLSI} into the form of $A \cdot x = b$ to be solved by MATLAB.

$$(LET \times Gene_i) \cdot C_i = (RBE_{PCLSI} - 1.16)\ of\ the\ form\ A \cdot x = b \quad (9)$$

Prostate Cancer LET Sensitivity Index (PCLSI) statistical sensitivity

The robustness and reliability of the PCLSI model were evaluated using multiple statistical sensitivity analyses. A bootstrap analysis[46] with 1000 iterations was performed to establish confidence intervals for each gene coefficient, providing insight into the stability of the gene-specific contributions to LET sensitivity. Leave-one-out cross-validation (LOOCV)[47] was implemented across all data points to assess the model's predictive performance, with root mean square error (RMSE) calculated to quantify prediction accuracy. Perturbation analysis[48] was conducted by perturbing the input data with 5% random noise over 1000 iterations to evaluate the model's stability to measurement uncertainties. The coefficient of variation and standard deviation were calculated to assess the variability in gene coefficients under these perturbations. This comprehensive sensitivity analysis framework provided quantitative measures of the model's reliability and identified the relative confidence in each gene's contribution to LET sensitivity prediction.

RBE Model Comparison

The PCLSI model was compared to two published LET-dependent RBE models. The first model is the McMahon model,[49] a linear LET-dependent model with the RBE calculated as

$$\text{RBE}_{McM} = 1 + \kappa \text{LET} \quad (10)$$

where $\kappa = 0.055$ μm/keV is a constant fit to cell survival data from non-prostate cell lines.[50,51] This fitted κ-factor is consistent with the optimized κ-factor that minimized the variability in the modelled McNamara RBE dose for α/βs ranging from 2 Gy to 10 Gy can be considered a generalized-tissue model.

The second model is the McNamara model,[20] a phenomenological LET-dependent model based on the linear-quadratic cell survival model with the RBE calculated as

$$\text{RBE}_{McN} = \frac{1}{2D_p}\left(\sqrt{\left(\frac{\alpha}{\beta}\right)_x^2 + 4D_p\left(\frac{\alpha}{\beta}\right)_x RBE_{max} + 4D_p^2 RBE_{min}^2} - \left(\frac{\alpha}{\beta}\right)_x\right) \quad (11)$$

where D_p is the proton dose per fraction, $(\alpha/\beta)_x$ is for X-rays, and RBE_{max} and RBE_{min} are calculated as

$$RBE_{max} = 0.99064 + \frac{0.35605}{(\alpha/\beta)_x}\text{LET}_d \quad (12)$$

$$RBE_{min} = 1.1012 - 0.0038703\sqrt{(\alpha/\beta)_x}\text{LET}_d \quad (13)$$

and are the asymptotic values of RBE_{McN} as $D_p \to 0$ and, $D_p \to \infty$, respectively. The McNamara model is α/β-specific and depends on the dose per fraction. It was fit to 287 α/β data points at various values of LET, of which only two data points at one value of LET are prostate cancer (Du145). Notably, the McNamara model under-predicted the Du145 RBE in multiple studies.

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Clinical Example using PCLSI

SFO and LET-optimized prostate Pencil Beam Scanning (PBS) proton therapy plans were created to compare the PCLSI model to the McMahon and McNamara models (approved by an IRB). Example tumour volumes were taken from a high-risk prostate cancer patient, stage 3 Gleason 9 PSA 7.2 with no prior irradiation. The patient received proton therapy to the prostate, seminal vessels and pelvic lymph nodes. The dose to the planning target volume (PTV) prostate/seminal vesicles (PSV) was prescribed at 46 Gy (RBE = 1.1) in 23 fractions and 32 Gy (RBE = 1.1) in 16 fractions in sequential two-phase treatment with pelvic nodes irradiated in the first phase. A Monte Carlo dose engine (RayStation 12B Research Non-Clinical, RaySearch Laboratories, Stockholm) was used to robustly optimize the dose to target volumes. The dose and LET were optimized with 50000 ions/spot and 0.5% statistical uncertainty for the final dose/LET calculation. Python scripts were written to apply the RBE models to the plan based on the voxelwise physical dose and LET. The $(\alpha/\beta)_x$s used in the McNamara model were 1.5 Gy for the prostate/PTV PSV,[43] 5 Gy for the bladder,[43] and 5.4 Gy for the rectum,[43] often used in clinical trials and other publications. Dose-volume histograms (DVHs) were used to compare the RBE doses between models. Using the published prevalence of prostate cancer DNA repair mutations,[14,15] particularly AR (84.9%) and BRCA2 (46.9%), a PCLSI expected value of 0.165 was used in the applied RBE model.

To quantify how the PCLSI model may predict improved dosimetry over the McMahon or McNamara models, we define the target to organ-at-risk (OAR) dose ratios (TODRs) for the bladder and rectum as

$$\mathrm{TODR}_{bladder} = \frac{\mathrm{D95}_{PTV\ PSV}}{\mathrm{D20}_{bladder}} \tag{14}$$

and

$$\mathrm{TODR}_{rectum} = \frac{\mathrm{D95}_{PTV\ PSV}}{\mathrm{D10}_{rectum}} \tag{15}$$

respectively. The TODRs represent how much dose can be given to the PTV PSV D95 region per unit dose in the bladder D20 or rectum D10 regions and were based on the clinical dose goals that at least 95% of the PTV PSV should receive 100% of the prescribed dose, and no more than 20% of the bladder or 10% of the rectum should receive more than 70 Gy (RBE = 1.1). The TODRs were calculated for the McMahon, McNamara and PCLSI models for both SFO and LET-optimized plans; because the PCLSI model is only applicable to the prostate, the McNamara model was used for the OARs since it is α/β specific. The TODR serves as an analog of the therapeutic ratio, where increases in the TODR are likely to result in an improved therapeutic ratio and benefit patient outcomes. A higher TODR value indicates a better ratio between tumour coverage and dose to normal tissue.

Results

Mutations in the DNA repair genes, AR, ATM, BRCA1, BRCA2, CDH1, ETV1, PTEN and TP53, had positive coefficients (Figure 1B) relating LET to RBE. Each coefficient in the PCLSI represents the individual contribution to RBE from the presence of a DNA repair mutation in a specific gene. The total PCLSI is used to describe the patient-specific tumour cell response to LET as shown in Equation 7 and 8. These DNA repair mutations were chosen to model the PCLSI from the available mutations, Figure 1A, because these mutations had published prevalence in prostate cancer patient populations[14–16] and were known to contribute significantly to LET sensitivity in proton therapy.[7,30,52] The increase in LET sensitivity appears to be a cumulative effect where the greater the number of mutations in DNA repair genes within a cell line, the greater the sensitivity to LET. Certain DNA repair mutations were found to have greater impact than others as shown in Equation 16.

$$\begin{aligned} PCLSI = {} & (0.0309 \cdot \boldsymbol{AR}) + (0.0309 \cdot \boldsymbol{ATM}) + (0.0075 \cdot \boldsymbol{BRCA1}) \\ & + (0.0384 \cdot \boldsymbol{BRCA2}) + (0.0309 \cdot \boldsymbol{CDH1}) \\ & + (0.0478 \cdot \boldsymbol{ETV1}) + (0.0478 \cdot \boldsymbol{PTEN}) \\ & + (0.0384 \cdot \boldsymbol{TP53}) \end{aligned} \tag{16}$$

ETV1 and PTEN mutations had the largest coefficients of 0.0478, demonstrating that cells with these mutations have the greatest reduction in surviving fraction as LET increases. The next largest coefficient was for mutations in the DNA repair genes BRCA2 and TP53, with values of 0.0384. BRCA1 mutations had the smallest coefficient of any of the mutations identified, with a value of only 0.0075.

Given the DNA repair mutation profile present in each of these prostate cancer cell lines, LNCaP had the largest total PSLSI score of 0.265. As shown in Table 2, this indicates that LNCaP cells are likely to have the greatest sensitivity to LET and LET-optimized proton therapy. At an LET of 2 KeV/μm, LNCaP would experience an RBE of 1.69, and at an LET of 4 KeV/μm, it is indicated to experience an RBE of 2.22. Cell lines that have relatively high PCLSI scores, above average reference values such as 0.055,[19] are likely to experience RBE values well above the 1.1 typically used to scale the physical dose of proton therapy treatment plans. These data reveal the distinct sensitivity of these cell lines to varying LETs, underscoring the importance of adapting patient-specific radiation treatment. The PCLSI calculated from the frequency of DNA repair mutations in non-metastatic prostate cancer patients[14] was 0.055, the same value published from averaging LET sensitivity across multiple cell lines.[19]

Figure 3 shows the RBE dose (top) and LET (bottom) for the SFO (left) and LET-optimized (right) plans. The SFO dose field to the PTV PSV is more uniform than the LET-optimized field. The average LET in the PTV PSV beam set, however, is 4.4 keV/μm in the LET-optimized plan and only 2.2 keV/μm in the SFO plan; meanwhile, the change in OAR LET is modest. This results in the LET-optimized plan having higher TODRs than the SFO plan (Figure 4). It is also evident that the choice of RBE model affects the TODRs. In both the SFO and LET-optimized plans, the TODRs for both the bladder and rectum were improved using the PCLSI model. Additionally, the per cent improvement in TODRs between the McNamara and PCLSI models was 26.1% for both the bladder and rectum in the SFO plan and 36.1% in the LET-optimized plan. The improvement in TODR illustrates the increased divergence of tumour mutation-specific models, such as PCLSI, from general RBE models and the importance of developing patient- and tumour-specific models when using LET optimization.

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Table 2. RBE values calculated using PCLSI. RBE value calculated using resultant coefficient at various LETs for the DNA repair mutations present in each cell line. Expected values were calculated using the PCLSI and published DNA repair mutation frequencies in prostate cancer patients[14]

Cell line/Model	LNCaP	22Rv1	Du145	McNamara	McMahon	Expected Average Met	Expected Average nonMet
PCLSI total	0.265	0.169	0.084	NA	0.055	0.165	0.055
LET (keV/μm)							
2.00	1.69	1.50	1.33	1.19	1.11	1.49	1.27
2.25	1.76	1.54	1.35	1.21	1.12	1.53	1.28
2.50	1.82	1.58	1.37	1.22	1.14	1.57	1.30
2.75	1.89	1.62	1.39	1.24	1.15	1.61	1.31
3.00	1.96	1.67	1.41	1.25	1.17	1.66	1.33
3.25	2.02	1.71	1.43	1.26	1.18	1.70	1.34
3.50	2.09	1.75	1.45	1.28	1.19	1.74	1.35
3.75	2.15	1.79	1.48	1.29	1.21	1.78	1.37
4.00	2.22	1.84	1.50	1.30	1.22	1.82	1.38
4.25	2.29	1.88	1.52	1.32	1.23	1.86	1.39
4.50	2.35	1.92	1.54	1.33	1.25	1.90	1.41

Abbreviations: Radiobiological Biological Effectiveness (RBE), Linear Energy Transfer (LET), Prostate Cancer LET Sensitivity Index (PCLSI), Met: Metastatic, nonMet: non-Metastatic.

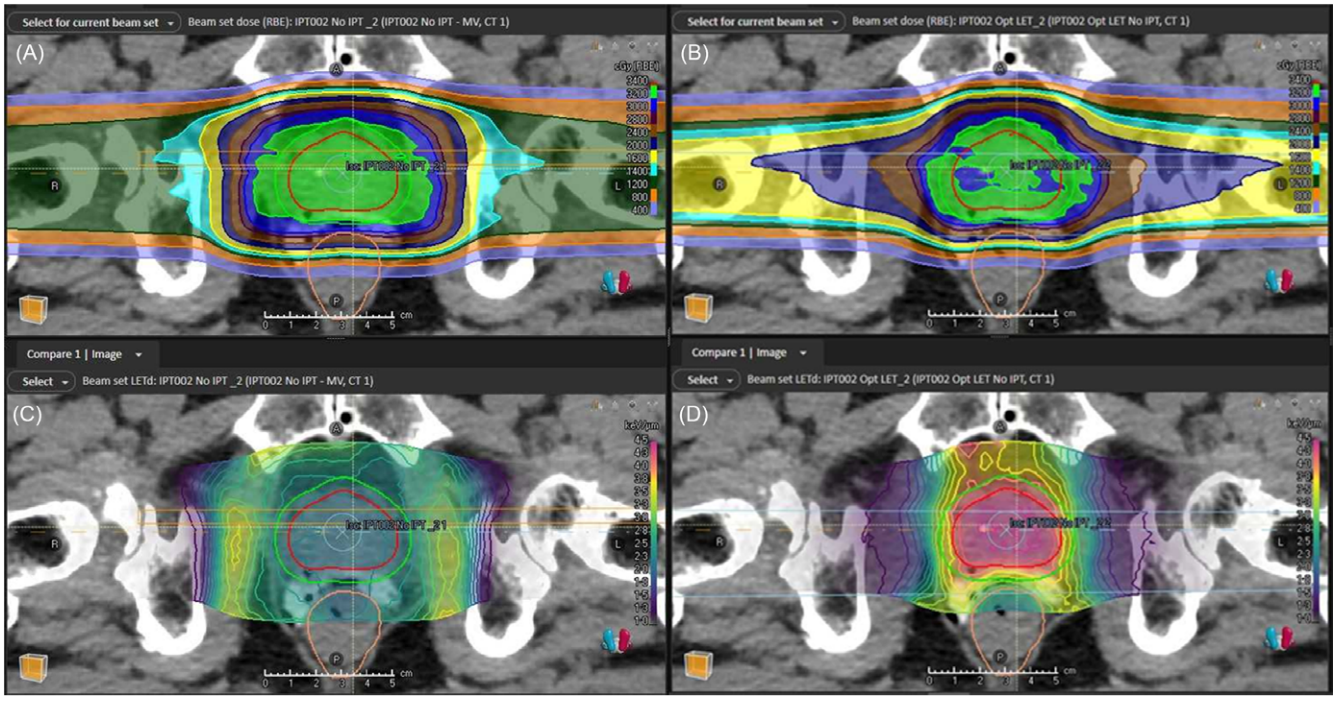


Figure 3. LET and RBE comparison between SFO and LET-optimized two-field prostate cancer proton plans. RBE = 1.1 dose (A), LET-optimized dose (B), SFO LET (C) and LET-optimized LET (D) shown from the boost phase of a PBS high-risk proton prostate cancer plan. The prostate is contoured in red, the PTV PSV in green and rectum in brown. The mean LET in the prostate is 2.2 keV/μm in the SFO plan and 4.4 keV/μm in the LET-optimized plan.

PCLSI model's statistical performance and reliability evaluation

The mean coefficients from the PCLSI model returned from bootstrapping, LOOCV and perturbation analysis align with those reported in Equation 16, with ETV1 and PTEN showing the strongest influence (0.0532, 0.0481, 0.0478) and BRCA1 showing the weakest (0.0027, 0.0067, 0.0075). This alignment helps validate the model's basic implementation.

The bootstrap analysis, conducted over 1000 iterations, reveals several important insights about the model's stability. The mean value of the bootstrapped coefficients increased from 6-23%,

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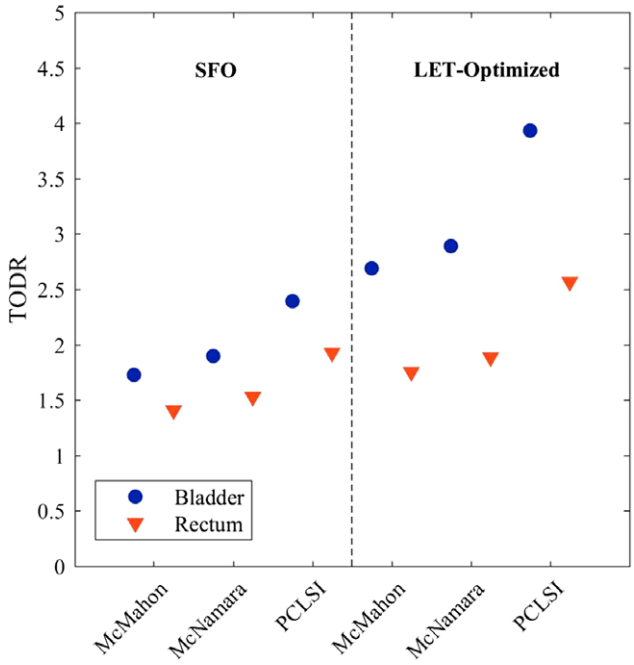


Figure 4. Bladder and rectum TODRs. Bladder and rectum target to oar dose ratios (TODRs) for both SFO and LET-optimized plans.

except for BRCA1, which reduced from 0.00748 to 0.00271. The 90% confidence intervals show that most coefficients maintain their positive influence consistently. The upper bounds demonstrate that ETV1 and PTEN could have coefficients as large as 0.1331, substantially higher than their nominal value of 0.0478. BRCA2 and TP53 emerge as the most statistically robust genes with a notably narrow confidence interval, 0.0341 to 0.0497, indicating high confidence in its effect. AR. ATM and CDH1 have a confidence interval of 0.0182 to 0.0648 indicating moderate uncertainty.

The LOOCV results, with an RMSE of 0.798, indicate moderate prediction error. The mean LOOCV coefficients are within 4% of the original PCLSI coefficients, except for BRCA1 which was 10% lower. Both LOOCV and parameter sensitivity analyses show consistent results, supporting BRCA2 and TP53 as having a reliable and well-defined contribution to LET sensitivity. The perturbation analysis shows stability, with a mean standard deviation (MSD) of 0.0043 and a coefficient of variation (CV) of 0.161. This MSD indicates that when the input data are perturbed, the resulting coefficients typically deviate by about 0.004 units from their mean values, a CV of approximately 0.16 indicates that the typical variation in coefficients represents about 16% of their mean values. In our perturbation analysis, these metrics were calculated from 1000 iterations of adding random noise, providing a robust statistical basis for assessing model stability. The relatively low standard deviation and moderate coefficient of variation suggest that while the model does show some sensitivity to input perturbations, it maintains reasonable stability in its coefficient estimates. These metrics provide quantitative evidence for the reliability of the PCLSI model's predictions in the presence of measurement uncertainties.

Discussion

The present study introduced the PCLSI model to predict the sensitivity of prostate cancer cells to proton therapy, focusing on the influence of tumour-specific genetic mutations. The role of genetic mutations in modulating radiation sensitivity is well acknowledged.[29] Our analysis highlighted several genes with varying coefficients that may play a role in LET sensitivity.

Among the identified genes, AR mutations had a coefficient of 0.0309, significant when compared to the reference of 0.055.[19] The AR gene has been implicated in the expression of many other DNA repair genes and is a cofactor of PARP1,[53] an important enzyme for detecting DNA strand breaks.[54] PARP1 serves as one of the first mechanisms to detect DNA damage and is also used in the selection of the subsequent DNA repair pathway. The success of PARP1 in the detection of DNA damage plays a significant role in the quality and efficiency of the resulting DNA Damage Repair (DDR).[54] Given that AR receptor activity governs the gene expression of several key processes in DNA damage repair, it's not surprising that AR mutations were shown to be strongly correlated with sensitivity to LET and DSBs.

E-cadherin, encoded by CDH1, also showed a coefficient of 0.0309. CDH1 acts as a tumour suppressor gene, playing a role in AR-dependent transcriptional regulation and recruiting HRR proteins for DNA DSB repairs. Mutations in CDH1 can result in hypersensitivity to ionizing radiation.[55]

The ATM gene plays a key role in DNA damage repair by encoding the PI3K-related serine/threonine kinase.[56] ATM loss hinders the DNA repair process either by germline or somatic mutations. It acts as a key signal transducer in DSB repair by sensing and cellular response. ATM mutation is also strongly associated with higher Gleason grades.[57] With a coefficient of 0.0309, even though ATM mutations have the third highest correlation with LET sensitivity along with AR and CDH1, of the genes identified in this study, it is still a significant value compared to the reference value of 0.055.

BRCA1 and BRCA2 are crucial to HRR and play a significant role in the repair of DSB DNA damage.[58,59] BCRA mutations have been shown to increase the risk of developing breast and ovarian cancers. HRR is one of the most common DDR mutations in prostate cancer, and BRCA2 is one of the most common mutations in DDR genes of prostate cancer cells.[60] BRCA1 was found to have a coefficient of 0.0075 and BRCA2 a coefficient of 0.0384. The BRCA2 was found to be of similar scale of the reference value of 0.055.[19]

ETV1 and PTEN have similar coefficients both with a value of 0.0478. Mutations in PTEN are frequently found in genomic analysis of post-prostatectomy and tumour biopsy samples.[61] Similar to ATM, PTEN also regulates PI3K. Loss of the PTEN impacts many pathways that lead to tumorigenesis, particularly in prostate cancer.[62] ETV1 plays a role in AR signaling and mutations can lead to overexpression and higher androgen metabolism and when combined with PTEN mutations lead to formation of prostate cancer in mice.[63] ETV1 was associated with more aggressive disease and poorer outcomes.[64]

TP53 mutations are one of the most common genetic mutations in cancer with TP53 mutations being reported in 50% of cancer cases. TP53 activates many cellular responses to stress including DNA repair and cell cycle arrest.[65] TP53 was found to play a significant role in the total PCLSI with a coefficient of 0.0384. When compared to the McNamara RBE model, the PCLSI predicts that tumour cells with the identified DNA repair mutations are significantly more sensitive to increased LET, especially in LET-optimized proton therapy. This indicates an opportunity to widen the therapeutic ratio in prostate cancer because this LET sensitivity is only present in the

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tumour cells and not in the surrounding healthy tissue. For prostate cancer cells with highly sensitizing mutations, like LNCaP, the RBE can be significantly higher than indicated in more generalized RBE models.

Genetic screening of cancer biopsies from prostate cancer patients may enable patient-specific medicine by allowing RBE dose prescriptions that deliver the optimal amount of physical dose to treat each individual patient. Dose escalation has been demonstrated to provide higher PSA relapse-free survival in prostate cancer when comparing treatments above and below 81 Gy.[66] LET optimization and tumour-specific sensitivity models can be used to improve patient outcomes by allowing dose escalation with higher RBE in prostate cancer cells without increasing the RBE dose in the surrounding healthy tissue.

Proton therapy has been shown to have high efficacy in 5- and 7-year outcomes without ADT.[67] This high-quality outcome may be provided by the high frequency of DSB DNA repair mutations in prostate cancer resulting in their increased sensitivity to even SFO proton therapy treatments, which do not benefit from LET optimization. LET optimization and genetic profiling of DNA repair mutations may allow the identification of a majority of prostate cancer patients who can achieve high-quality outcomes when using proton therapy potentially without the toxicity associated with ADT hormone therapy. The PCLSI could be formed as a standalone test for the genetic screening or incorporated into the data from existing genetic screening from products like Decipher to determine if a particular patient would benefit from increased LET in proton therapy compared to other treatment modalities such as conventional photon therapy.

The increased RBE experienced by tumours in patients with DNA repair mutations present in their tumour biopsies could allow for improved 5-year Freedom From Biochemical Progression (FFBP) similar to that experienced in physical dose escalation. In high-risk patients with photon therapy, 5yr FFBP has shown improvement from 50% at 66Gy RBE to 70% 5yr FFBP at 86Gy RBE,[68] a relationship of approximately 0.1% improvement in 5yr FFBP per Gy. Given the relationship of increasing 5yr FFBP with dose escalation in high-risk prostate cancer patients, the PCLSI predicts LET-optimized proton therapy could achieve 5yr FFBP above 80% with 96Gy RBE, 72Gy CGE, using 1.33 RBE at 3 keV/μm and taken from the PCLSI of 0.055 for non-metastatic patients shown in Table 2. This is significant because it would allow improvements in outcomes from RBE escalation above what has been achieved with photon therapy while potentially mitigating some of the toxicity risk present in physical dose escalation.

Although the PCLSI model provides insight into the role of genetic mutations and LET sensitivity, there may be some inherent limitations to this study. First, the PCLSI model relies on the existing in vitro literature data. While this model provides an approximation for the sensitivity resulting from increased LET, it might not account for all the potential biological effects in vivo that are not modulated by DNA repair mutations. Second, the PCLSI equation is linear, implying that each mutation is additive and independent of other mutations. However, in tumours, there may be interactions between mutations that can result in nonlinear effects. Third, the resultant coefficients of each of the DNA repair mutations are important. These coefficients are derived from the least square fitting. This might be appropriate if the relation between gene mutations and LET sensitivities is indeed linear, but this assumption needs to be explored in more detail across cell lines with various DNA repair mutation profiles at different LETs and doses. Although the model was not limited to positive coefficients, it is important to note that each of the genetic mutations modelled in this work resulted in positive coefficients.

Treatment plans that make use of tumour DNA repair mutation profiles, biological dose, and LET optimization have a significant chance of improving patient outcomes by increasing RBE in the tumour without affecting surrounding healthy tissue, as demonstrated by the PCLSI and improvements in biological dose distribution in this study. Preliminary clinical studies may validate the PCLSI and role of DNA repair mutations by sequencing tumour biopsies and comparing the variation in biological response of factors such as PSA score and 5yr FFBP. These treatment plans could maintain the historical physical dose but utilize LET optimization to improve the LET distribution between the tumour and surrounding tissue when compared to current SFO planning techniques.

Conclusion

This study introduced the Prostate Cancer LET Sensitivity Index (PCLSI) model, describing the influence of specific DNA repair genetic mutations on prostate cancer sensitivity to proton LET. By incorporating tumour DNA repair mutation profiles, biological dose and LET optimization, treatment plans have significant potential to improve patient outcomes by increasing RBE in the tumour without affecting surrounding healthy tissue. The PCLSI model, when compared against established RBE models, demonstrates the potential of genetic profiling of individual patient tumour biopsies in optimizing proton therapy. The integration of LET-optimized proton therapy with genetic mutation profiling could enable patient-specific RBE prescriptions, thus optimizing therapeutic outcomes while minimizing risks to normal tissue. Preliminary clinical studies may further validate the PCLSI and the role of DNA repair mutations, potentially leading to more personalized and effective proton therapy treatments for prostate cancer patients. This approach represents a significant step towards individualized medicine in radiation oncology, promising to enhance the therapeutic ratio in prostate cancer treatment.

Highlights

- Genetic screening of prostate cancer biopsies for DNA repair mutations, such as AR and BRCA2, may help identify patients that could experience a large benefit from LET-optimized proton therapy.
- The PCLSI predicts increased RBE in prostate cancer cells with a greater number of DNA repair mutations.
- The PCLSI-based RBE model predicts certain prostate cancer cells could have RBE values significantly higher than in models averaged across multiple cell lines, such as RBE = 1.1, McMahon and McNamara.
- TODR, an analog of therapeutic ratio, increased significantly when calculated using a PCLSI based on the prevalence of genetic mutations in prostate cancer patient samples.

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Competing interests. Prostate Cancer LET Sensitivity Index (PCLSI) is patent pending to Mark Artz. All remaining authors report no conflicts of interest.

Ethical approval. This study was reviewed and approved by the Institutional Review Board of the University of Florida (UFPTI 2212-PRX41, 1 December 2022).

Data sharing statement. The authors agree to share anonymized data upon reasonable request by researchers.

CRediT Statement. ***Mark Artz*** – Conceptualization, Methodology, Software, Validation, Formal Analysis; Investigation, Resources, Data Curation, Writing –

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Original Draft; Writing – Review and Editing, Visualization, Supervision, Project Administration

Michael Vieceli – Validation, Formal Analysis, Writing – Original Draft, Writing – Review and Editing

Mohammad Saki – Writing – Original Draft, Writing – Review and Editing

Jiyeon Park – Writing – Original Draft, Writing – Review and Editing

Yawei Zhang – Writing – Review and Editing

Eric Brooks – Writing – Review and Editing

Nancy Mendenhall – Resources, Writing – Review and Editing

Perry Johnson – Resources, Supervision, Project Administration, Writing – Review and Editing

Hardey Grewal – Validation, Formal Analysis, Writing – Original Draft, Writing – Review and Editing

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