Clonogenic assays for three prostate cancer cell lines with varying levels of DNA repair mutations treated with LET-optimized pencil beam scanning protons and evaluation of dose rate effect

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ABSTRACT

Purpose: To investigate how varying levels of DNA repair mutations affect the LET_d sensitivity of prostate cancer cells.

Methods: The prostate cancer cell lines Du145 (low DNA repair mutation burden), PC3 (moderate mutation burden), and LNCaP (high mutation burden) were cultured and seeded in petri dishes for *in vitro* irradiation. AP/PA pencil beam scanning proton beams were used to create 2, 3, 4, and 5 keV/µm uniform LET-optimized fields with uniform dose to the bottom of the petri dish planning target volume; AP/PA 6x x-rays with uniform dose were used as a reference. Plan-specific quality assurance was performed for each field. The clonogenic assay was performed 13-16 days after irradiation to calculate surviving fractions and fit linear-quadratic cell survival curves. PC3 was also irradiated with single-layer and spread-out Bragg peak (SOBP) fields to test if LET-dependence could be confounded by a dose rate effect (i.e., high LET_d at the distal edge in SOBP fields is confounded with less overlapping layers resulting in higher average dose rates).

Results: The overall relative radiosensitivity between cells lines showed a strong correlation with increased levels of DNA repair mutations, with Du145 showing low sensitivity, PC3 moderate sensitivity, and LNCaP high sensitivity. DNA repair mutations were also correlated with LET_d sensitivity, with PC3 showing unequivocal LET-dependence and LNCaP showing statistically significant differences between x-rays and protons, both with RBEs up to 1.3; Du145 showed no LET-dependence. Statistical significance in PC3 survival was seen between both levels of LET_d and dose rates with LET_d having a larger effect. A higher dose rate resulted in a larger LET-dependent RBE and higher LET_d resulted in a higher dose-rate-dependent RBE

Conclusion: DNA repair mutations were correlated with overall radiosensitivity as the strongest effect and LET_d sensitivity as a secondary effect. Higher LET_d and increased dose rate had a mutually enhancing effect on RBEs.

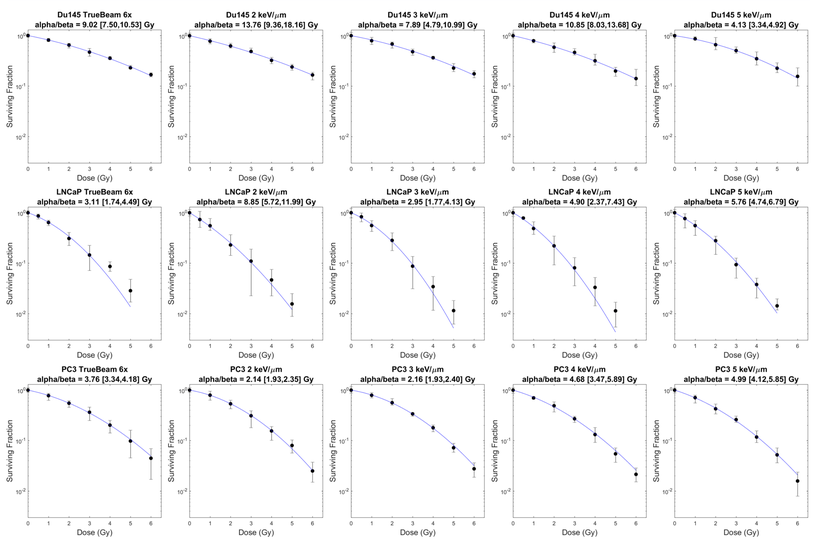
INTRODUCTION

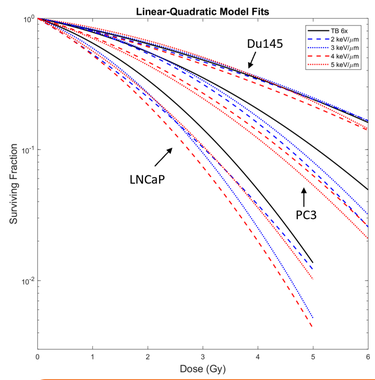
LET_d optimization in proton therapy has potential to improve the 5-year freedom from biochemical progression through RBE-dose-escalation.[1] LET-dependent RBE for the prostate cancer cell line Du145 has been previously investigated,[2,3] although this represents only one cell line out of a limitless number of patient-specific variants. **DNA repair mutations, such as in the homologous recombination repair (HRR) mechanism for double strand DNA breaks, may play a large role in predicting prostate cancer LET_d sensitivity.** Several studies have shown that non-prostate cancer cell lines with HRR mutations are selectively sensitive to high LET_d protons compared to x-rays.[4-8] In pharmacology, the poly (ADP-ribose) polymerase (PARP) inhibitor Olaparib (which by forcing single strand DNA breaks to be repaired by double strand break mechanisms has a mutual cell killing mechanism as radiation) has been shown to be more effective in prostate cancer cells with more DNA repair mutations.[9] In our recent publication, we calibrated this Olaparib sensitivity data[9] to published Du145 LET_d sensitivity data[2] and created a prostate cancer LET_d sensitivity index (PCLSI) model with a linear coefficient determined by cell-line-specific gene weighting and inclusion.[10] The PCLSI model predicts increased LET-dependent RBEs and lower survival for cell lines with higher DNA repair mutation burdens when treated with proton therapy. **In this scheme, tumor biopsies could be genetically screened to identify which patients may be good candidates for LET_d optimization, with up to 60% of patients having DNA repair mutations.[11,12]**

To the best of our knowledge, this is the first study to perform the clonogenic assay with LET-optimized fields, which has the unique advantage of precisely knowing a robust value of the delivered LET_d compared to single-layer or SOBP fields. However, there is potential for a confounding dose rate effect. Single-layer fields deposit dose at a point with a few overlapping spots in a short time and have a high dose rate. SOBP fields have a higher dose rate distally with fewer overlapping layers decreasing the average dose rate than at proximal depths, with regions of high dose rate also corresponding to higher LET_d. LET-optimized fields require at least two overlapping beams which substantially decreases the average dose rate. A recent study showed correlation between optical toxicities and dose rate but not with LET_d.[13]

The purpose of this study is to irradiate prostate cancer cell lines with varying levels of DNA repair mutations with LET-optimized PBS protons to demonstrate correlation of DNA repair mutations with LET_d sensitivity. A secondary aim is to investigate if LET-dependent RBE depends on the dose rate.

LET-Optimized





Du145	2 keV/µm	3 keV/µm	4 keV/µm	5 keV/µm
TB 6x	0.95863	0.62645	0.0089467	0.16861
2 keV/µm		0.68334	0.012532	0.18137
3 keV/µm			0.0076499	0.11389
4 keV/µm				0.34006

LNCaP	2 keV/µm	3 keV/µm	4 keV/µm	5 keV/µm
TB 6x	0.00010429	3.5793×10^{-9}	4.008×10^{-9}	6.9915×10^{-7}
2 keV/µm		0.16399	0.13461	0.8631
3 keV/µm			0.88294	0.12438
4 keV/µm				0.10059

PC3	2 keV/µm	3 keV/µm	4 keV/µm	5 keV/µm
TB 6x	0.0019166	0.0055282	7.9457×10^{-7}	6.4605×10^{-10}
2 keV/µm		0.40908	0.068239	0.00074636
3 keV/µm			0.0016437	1.9788×10^{-6}
4 keV/µm				0.065787

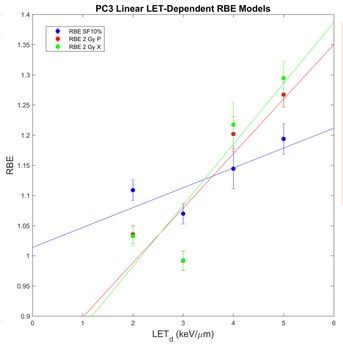
Top: **LET-optimized cell survival results** for Du145 (top row), LNCaP (middle row), and PC3 (bottom row) (error bars are 68.2% confidence intervals).
Bottom: **Linear-quadratic model fits** (left) and **p-values** (right). Green: statistically significant at Bonferroni-Holm-corrected significance level. Red: Non-significant.

DNA Repair Mutations showed dependence on:

- **Overall Radiosensitivity**: Du145 survived higher than PC3 which survived higher than LNCaP
- **LET_d Sensitivity**: Du145 showed no LET-dependence, PC3 showed statistically significant differences between x-rays, low LET_d, and high LET_d, and LNCaP showed statistically significant differences between protons and x-rays (the LNCaP cell line is inherently more variable)

RESULTS

		α (Gy^{-1})	β (Gy^{-2})	α/β (Gy)	$RBE_{SF10\%}$	RBE_{2GyP}	RBE_{2GyX}
Du145	TB 6x	0.18 ± 0.01	0.02 ± 0.00	9.02 ± 1.52	-	-	-
	2 keV/µm	0.21 ± 0.01	0.02 ± 0.00	13.76 ± 4.40	-	1.06 ± 0.04	1.06 ± 0.04
	3 keV/µm	0.17 ± 0.02	0.02 ± 0.01	7.89 ± 3.10	-	0.96 ± 0.05	0.96 ± 0.05
	4 keV/µm	0.21 ± 0.01	0.02 ± 0.00	10.85 ± 2.83	-	1.10 ± 0.03	1.10 ± 0.04
	5 keV/µm	0.13 ± 0.01	0.03 ± 0.00	4.13 ± 0.79	-	0.89 ± 0.03	0.91 ± 0.03
LNCaP	TB 6x	0.33 ± 0.06	0.11 ± 0.03	3.11 ± 1.38	-	-	-
	2 keV/µm	0.56 ± 0.03	0.06 ± 0.02	8.85 ± 3.13	1.11 ± 0.07	1.19 ± 0.05	1.23 ± 0.05
	3 keV/µm	0.39 ± 0.06	0.13 ± 0.03	2.95 ± 1.18	1.14 ± 0.08	1.15 ± 0.06	1.15 ± 0.06
	4 keV/µm	0.54 ± 0.07	0.11 ± 0.04	4.90 ± 2.53	1.23 ± 0.09	1.27 ± 0.07	1.31 ± 0.07
	5 keV/µm	0.49 ± 0.02	0.09 ± 0.01	5.76 ± 1.02	1.10 ± 0.06	1.15 ± 0.05	1.17 ± 0.05
PC3	TB 6x	0.19 ± 0.01	0.05 ± 0.00	3.76 ± 0.41	-	-	-
	2 keV/µm	0.16 ± 0.01	0.08 ± 0.00	2.14 ± 0.21	1.11 ± 0.02	1.04 ± 0.02	1.03 ± 0.02
	3 keV/µm	0.15 ± 0.01	0.07 ± 0.00	2.16 ± 0.24	1.07 ± 0.02	0.99 ± 0.02	0.99 ± 0.02
	4 keV/µm	0.27 ± 0.03	0.06 ± 0.01	4.68 ± 1.12	1.14 ± 0.03	1.20 ± 0.03	1.22 ± 0.04
	5 keV/µm	0.29 ± 0.02	0.06 ± 0.01	4.99 ± 0.87	1.19 ± 0.03	1.27 ± 0.02	1.29 ± 0.03



Left: **α and β fitting parameters** and **RBEs** (with 68.2% confidence intervals)
Right: PC3 **LET-dependent model fits** (equations below)

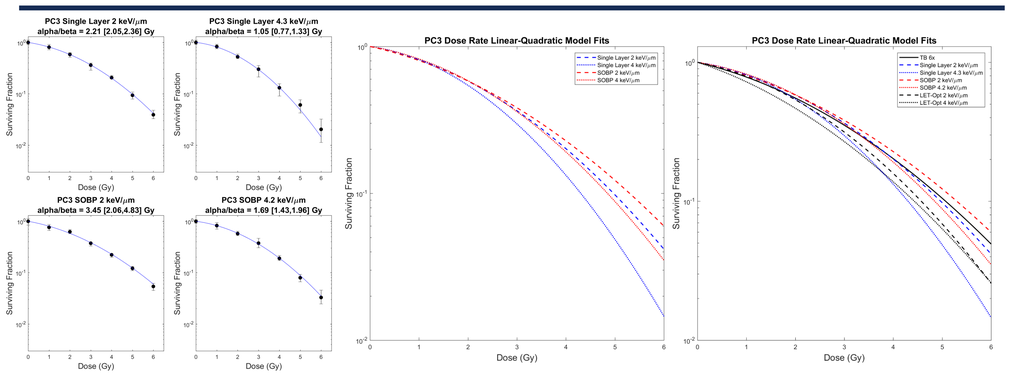
- Du145 RBEs were near 1
- LNCaP had RBEs up to 1.31 but did not correlate with LET_d
  - Larger variability inherent to LNCaP cell line
- PC3 had RBEs up to 1.29 that increased with LET_d

$$RBE_{SF10\%} = 0.033LET_d + 1.014$$

$$RBE_{2GyP} = 0.091LET_d + 0.807$$

$$RBE_{2GyX} = 0.101LET_d + 0.780$$

Dose Rate



	α (Gy^{-1})	β (Gy^{-2})	α/β (Gy)
SL 2 keV/µm	0.14 ± 0.01	0.06 ± 0.00	2.21 ± 0.16
SL 4.3 keV/µm	0.10 ± 0.02	0.10 ± 0.01	1.05 ± 0.28
SOBP 2 keV/µm	0.17 ± 0.03	0.05 ± 0.01	3.45 ± 1.39
SOBP 4.2 keV/µm	0.12 ± 0.01	0.07 ± 0.00	1.69 ± 0.26

	Dose Rate ↓	Dose Rate ↓	$RBE_{SF10\%}$ (LET_d)	RBE Enhancement (Dose Rate)
LET_d →	SL 4.3 keV/µm	SL 2 keV/µm	1.16 ± 0.02	1.06 ± 0.05
LET_d →	SOBP 4.2 keV/µm	SOBP 2 keV/µm	1.09 ± 0.05	
$RBE_{SF10\%}$ (Dose Rate)	1.13 ± 0.03	1.07 ± 0.05		
RBE Enhancement (LET_d)	1.06 ± 0.05			

PC3 Dose Rate	SL 2 keV/µm	SL 4.3 keV/µm	SOBP 2 keV/µm	SOBP 4.2 keV/µm
TB 6x	0.98288	1.1113×10^{-7}	0.0037166	0.11633
SL 2 keV/µm		2.52×10^{-12}	4.7747×10^{-6}	0.010897
SL 4.3 keV/µm				1.3003×10^{-7}
SOBP 2 keV/µm		4.3738×10^{-21}		7.4381×10^{-11}
2 keV/µm	0.00011481		9.5548×10^{-11}	
4 keV/µm		0.74374		2.7712×10^{-6}

Top left: **Single-layer and SOBP cell survival results**; **Linear-quadratic model fits** without (top middle) and with (top right) comparison to TB 6x and LET-optimized fields; Middle left: **α and β fitting parameters;** Middle right: **LET-dependent and dose-rate-dependent RBEs and RBE enhancements**; Bottom left: **P-values**. (Error bars are 68.2% confidence intervals).

- **Statistically significant differences in cell survival** between:
  - **LET_d** (lower survival with higher LET_d)
  - **Dose rate** (lower survival with higher dose rate)
- **Higher dose rate** (single-layer) had **higher LET-dependent RBEs**
- **Higher LET_d** had **higher dose-rate-dependent RBEs**

METHODS

Prostate Cancer Cell Lines

Du145 (Low mutation burden)
PC3 (Intermediate mutation burden)
LNCaP (High mutation burden)

Mutation burden determined from reference Olaparib sensitivity data and PCLSI score

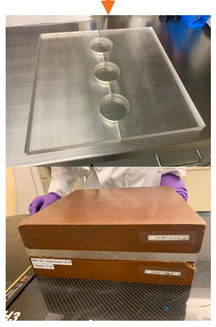
Cell Culture

ATCC recommended media + 10% FBS + 1% L-glutamine
37 °C and 5% CO_2 in humidified incubator

	AR	ATM	BRCA1	BRCA2	CDH1	ETV1	PTEN	TP53	PCLSI Score
Gene Coefficient	0.0309	0.0309	0.0075	0.0384	0.0309	0.0478	0.0478	0.0384	
Du145			X	X				X	0.084
LNCaP	X	X		X	X	X	X	X	0.265
PC3							X	X	0.086*

*PC3 may have had a higher PCLSI score if its Olaparib sensitivity data was included in the model fit

Petri Dish Phantom Fabricated

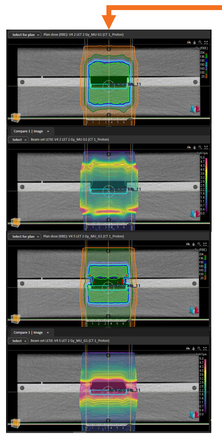


CT Simulation

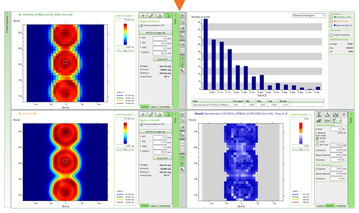
Water Equivalent Thicknesses (WET) measured
CT densities overwritten with WET-calculated densities

LET-Optimized PBS Proton Plans (RayStation)
- 2, 3, 4, 5 keV/µm plans (2 and 5 shown)
- AP/PA

6x Reference X-ray Plan
- AP/PA



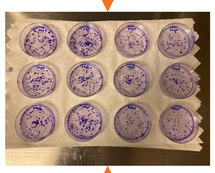
Plan-Specific QA with Gamma Analysis



Irradiation
Protons: IBA ProteusPlus
X-rays: Varian TrueBeam

Clonogenic Assay and Linear Quadratic Model Fits

$$SF = e^{-\alpha D - \beta D^2}$$



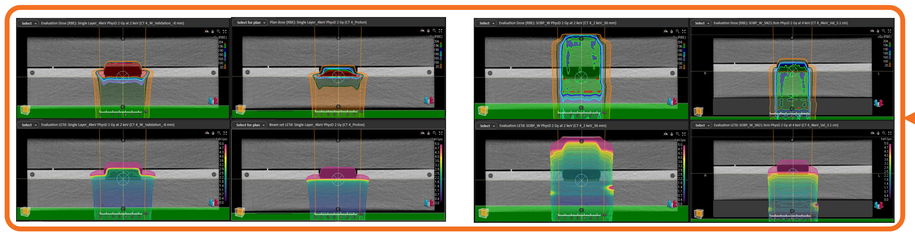
Dose Rate Proton Plans

Definitions
- Maximum Instantaneous Dose Rate (MIDR): Spot dose divided by spot dwell time
- Delivery Time Dose Rate (DTDR): Field dose divided by layer delivery time

Single-Layer Plans
- 1 energy layer (135.8 MeV)
- 2 keV/µm (10.0 Gy/s MIDR, 0.50 Gy/s DTDR)
- 4.3 keV/µm (16.0 Gy/s MIDR, 0.68 Gy/s DTDR)

SOBP Plans
- 2 keV/µm (0.02 Gy/s DTDR)
  - 13 energy layers
- 4.2 keV/µm (0.10 Gy/s DTDR)
  - 3 energy layers

LET-Optimized Plans
- Dose rate not calculated due to overlapping beams greatly diminishing average dose rate



Data Analysis

Analysis of Covariance (ANCOVA)
- Test surviving fraction data at all doses for statistical significance between two experiments
- Bonferroni-Holm correction for multiple comparisons

RBE Calculation

$$RBE = \frac{\sqrt{\alpha_x^2 + 4\beta_x D_p\left(\alpha_p + \beta_p D_p\right)} - \alpha_x}{2\beta_x D_p}$$

RBE Endpoints
- 10% surviving fraction ($RBE_{SF10\%}$)
- Surviving fraction where D_p = 2 Gy (RBE_{2GyP})
- Surviving fraction where D_x = 2 Gy (RBE_{2GyX})

CONCLUSION

DNA repair mutations in prostate cancer cells correlated with:
- **Overall Radiosensitivity**
- **LET_d Sensitivity**

Implications
- Patients with high DNA repair mutation burdens could be more radiosensitive to x-rays than low mutation burdened patients are to high LET_d protons
- High LET_d may be effective in patients with high mutation burdens
- High LET_d may result in little to no benefit in patients with low mutation burdens

Clinical potential
- Genetically screen tumor biopsies to identify which patients may benefit from LET_d optimization
- Stratify patients in clinical trial by DNA repair mutation burden so LET_d insensitive patient outcomes do not obscure true differences in LET_d sensitive patient outcomes

Dose Rate
- LET_d and dose rate had a mutually enhancing effect on RBE
  - LET-dependent RBEs were larger than dose-rate-dependent RBEs
- Proton beam dose rates should be standardized to minimize confounding dose rate effect
  - Two-beam single-field optimized (SFO) or LET-optimized may have most clinically relevant dose rate

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