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Title: A Randomised Feasibility Trial of Stereotactic Prostate Radiotherapy with or without Elective Nodal Irradiation in High-Risk Localised Prostate Cancer (SPORT Trial)

Short running title: P-SABR +/- ENI in high-risk prostate cancer

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Conflict of interest statement

The authors declare no potential conflicts of interest.

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Data availability statement for this work

Research data are stored in an institutional repository and can be shared upon request to the corresponding author.

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Abstract

Purpose

To establish the feasibility of a randomised clinical trial comparing stereotactic ablative radiotherapy (SABR) to the prostate-only (P-SABR) or to prostate plus pelvic lymph nodes (PPN-SABR) in patients with unfavourable intermediate- or high-risk localised prostate cancer and explore potential toxicity biomarkers.

Materials & Methods

Thirty adult men with at least one of the following features; clinical MRI stage T3a N0 M0, Gleason score ≥ 7 (4+3), PSA > 20 ng/mL were randomised 1:1 to P-SABR or PPN-SABR. P-SABR patients received 36.25Gy/5 fractions/29 days, PPN-SABR patients also received 25Gy/5 fractions to pelvic nodes with the final cohort receiving a boost to the dominant intraprostatic lesion of 45-50 Gy. γH2AX foci numbers, citrulline levels and circulating lymphocyte counts were quantified. Acute toxicity information (CTCAE v4.03) was collected weekly at each treatment and at six weeks and three months. Physician-reported late RTOG toxicity was recorded from 90 days to 36 months post-completion of SABR. Patient-reported quality of life (EPIC and IPSS) scores were recorded with each toxicity timepoint.

Results

The target recruitment was achieved and treatment successfully delivered in all patients.0% and 6.7% (P-SABR) and 6.7% and 20.0% (PPN-SABR) experienced acute grade ≥ 2 gastrointestinal (GI) and genitourinary (GU) toxicity respectively. At 3 years, 6.7% and 6.7% (P-SABR) and 13.3% and 33.3% (PPN-SABR) had experienced late grade ≥ 2 GI and GU toxicity respectively. One patient (PPN-SABR) had late grade 3 GU toxicity (cystitis and haematuria), no other grade ≥ 3 toxicity was observed.

33.3% and 60% (P-SABR) and 64.3% and 92.9% (PPN-SABR) experienced a minimally clinically important change (MCIC) in late EPIC bowel and urinary summary scores respectively.

γH2AX foci numbers at 1 hour post-first fraction were significantly higher in PPN-SABR arm compared to P-SABR arm (p=0.04). Patients with late grade ≥ 1 GI toxicity had significantly larger falls in circulating lymphocytes (12 weeks post-radiotherapy, p=0.01), and a trend towards higher γH2AX foci numbers (p=0.09), than patients with no late toxicity. Patients with late grade ≥ 1 bowel toxicity and late diarrhoea experienced greater falls in citrulline levels (p=0.05).

Conclusions

A randomised trial comparing P-SABR to PPN-SABR is feasible with acceptable toxicity. Correlations of γH2AX foci, lymphocyte counts and citrulline levels with irradiated volume and toxicity suggest potential as predictive biomarkers. This study has informed a multicentre UK randomised phase III clinical trial.

Keywords

Prostate; stereotactic; elective nodal irradiation; toxicity; biomarkers

Introduction

Prostate cancer is the most common cancer in men, accounting for over 1.4 million new cancer diagnoses per year (1). For patients with high-risk localised prostate cancer, standard treatment options include radical external beam radiotherapy (EBRT) to the prostate and seminal vesicles in combination with twelve to thirty-six months of hormone therapy (2, 3). Multiple studies have demonstrated that biochemical control improves with the delivery of higher radiation doses (4, 5, 6, 7, 8). Multiple strategies have been investigated over the past decade to improve biochemical outcomes and reduce toxicity, including elective pelvic nodal irradiation (ENI), hypofractionation, image-guided radiotherapy and hydrogel spacers. While the role of ENI has been controversial, it is often delivered in patients deemed to be at high risk of pelvic lymph node involvement (9, 10, 11).

There is a growing body of evidence indicating that prostate cancer is sensitive to higher radiation doses per fraction (hypofractionation) (12, 13). Reducing the number of radiotherapy treatments also has significant advantages with regard to cost, required number of hospital visits, and patient convenience (14).

Stereotactic ablative therapy (SABR) is an external beam radiotherapy technique which involves the precise delivery of a higher dose of radiation, using typically five fractions of radiotherapy (15). Given the fractional sensitivity of prostate cancer, this allows for escalation of dose delivered to the prostate relative to conventional EBRT with a view to maximising tumour control without a corresponding increase in toxicity. While SABR has been extensively studied in favourable risk localised prostate cancer there is a lack of randomised evidence demonstrating its safety and efficacy in high risk disease and a lack of studies comparing SABR to prostate with or without ENI.

The site of the dominant intraprostatic lesion (DIL), the sub-volume of prostate containing the bulk of tumour, is often the site of local recurrence following radiotherapy (16). This region, therefore, determines the clinical outcome for most patients and there is good evidence that targeting the DIL

for focal boost dose escalation can improve the therapeutic index compared to dose escalation to the whole prostate (17). Results from cohort studies show acceptable toxicity and good early PSA control following focal boosts (18, 19). While these studies used conventional fractionation, SABR has been successfully used to deliver prostate boosts alongside conventional EBRT (20, 21).

Currently, the main method of predicting side effects from radiotherapy is based on the dose received by healthy organs and whether co-morbidities are present. However, a proportion of patients will experience significant toxicity despite receiving acceptable dose to the organs at risk (OARs). A biomarker predicting toxicity before treatment or early in treatment may allow adjustment of the radiotherapy plan to reduce the risk of late toxicity.

Phosphorylated gamma-H2AX (γH2AX) and serum citrulline have been shown to change in response to radiation exposure. γH2AX is an early marker of radiation-induced DNA double stranded breakage that forms discrete quantifiable nuclear foci and is detectable even after exposure to low doses of radiation (22). Levels in lymphocytes peak 30-60 min after irradiation with subsequent rapid decay in an exponential fashion (23). γH2AX foci in circulating lymphocytes following radiotherapy have been studied in lung cancer (24, 25) and prostate cancer (26, 27) and are associated with radiation dose but correlation with toxicity is less well established.

A major limiting factor with prostate and pelvic radiotherapy is damage to the small bowel. Citrulline is an amino acid that plays a role in the urea cycle. Circulating citrulline levels are derived almost exclusively from small intestinal enterocytes and reduced levels occur in gastrointestinal disease and short bowel syndrome (28). There is emerging evidence that citrulline levels decrease during abdominal and pelvic radiotherapy in keeping with bowel side effects (29, 30), but this has not consistently been shown.

The aim of our study was to establish the feasibility of a randomised clinical trial comparing SABR to the prostate alone (P-SABR) with SABR to the prostate plus pelvic lymph nodes (PPN-SABR). In

addition to this, an exploratory analysis of the changes in γH2AX and serum citrulline levels during radiotherapy exposure was also performed. Feasibility was determined by the i) willingness of clinicians to recruit and patients to enter the study, ii) the technical feasibility of delivering this treatment and iii) acceptable toxicity; with the longer-term goal of using these data to inform a multicentre randomised controlled trial of prostate and pelvic nodal SABR.

Methods

Study design and participants

A prospective non-blinded randomised controlled feasibility study was designed and implemented at a single UK academic cancer centre. Eligible patients were men ≥ 18 years with recently diagnosed histologically confirmed National Comprehensive Cancer Network (NCCN) unfavourable intermediate- or favourable high-risk prostate adenocarcinoma with at least one of the following features; clinical MRI stage T3a N0 M0, Gleason score ≥ 7 (4+3), PSA > 20 ng/mL (31). Patients with cT3b and cT4 disease were excluded. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 and a life expectancy of five years or greater. Patients with a prostate volume > 90cc or IPSS > 19 were excluded. Participants were planned to receive twelve to thirty-six months of androgen deprivation therapy (ADT) as part of their standard treatment.

The trial was registered on XXXXX (identifier XXXXX). Ethical approval was obtained from the XXXXX Research Ethics Committee (ethics number XXXXX). All participants received written study information and provided written informed study consent. A CONSORT diagram of the trial profile is provided in Fig. 1.

A dedicated trial management group (meeting every two months) provided overall supervision of the trial on behalf of the trial sponsor and trial funder and ensured that the trial was conducted according to Good Clinical Practice guidelines. An independent Data Monitoring Committee oversaw the safety of trial participants and met regularly (minimum twice per year) to review toxicity data.

Interventions

All 30 patients in the trial received a minimum of 3 months of neoadjuvant ADT as standard of care, with the view to its continuation for a minimum of twelve months in total. Three fiducial markers

and a polyethylene glycol hydrogel spacer (SpaceOAR®; Boston Scientific, Marlborough, MA) was inserted transperineally after their initial planning CT scan. Patients were scanned in the supine position with an empty rectum and comfortably full bladder with knee rests and ankle stocks. Enemas were used for 2 days prior to and on the day of CT planning and on each day of treatment. They underwent a second planning CT scan in the same position at least seven days post-fiducial implant. All patients also underwent a post-procedure multiparametric MRI prostate for radiotherapy planning which aided target and OAR, particularly prostatic urethra, delineation. Patients treated with P-SABR received 36.25 Gy in five fractions to the prostate planning target volume (PTV) and 40 Gy in five fractions to the prostate clinical target volume (CTV) delivered once weekly over five weeks (29 days). Patients treated with PPN-SABR received the same treatment with the addition of 25 Gy in five fractions to the pelvic nodal PTV. The proximal 10-20 mm of seminal vesicles were included within the prostate PTV and the remainder of the seminal vesicles was prescribed 25 Gy in five fractions. Due to acceptable initial toxicity rates, the protocol was amended so the final cohort of 10 patients could receive a simultaneous boost to the dominant intra-prostatic lesion (DIL) of 45-50 Gy in five fractions, irrespective of their randomisation. The trial design is summarised in Fig. S1.

The prostate CTV consisted of the prostate, any extra-prostatic extension and the base (proximal 1-2 cm) of the seminal vesicles. The prostate PTV was formed using a 5 mm isotropic expansion of the prostate CTV. The pelvic lymph node planning target volume (PTVln) was formed using a vessel expansion method previously described by the PIVOTAL clinical trial protocol (32) using a 7 mm margin in all planes; where necessary, this could be reduced to 5 mm in order to prioritise OAR dose-volume constraints (DVCs). The DIL boost gross tumour volume (GTV) was defined using the diagnostic MRI T2 and diffusion weighted images. A 3 mm margin in all planes was applied to the GTV (excluding OARs) to form the boost CTV, then cropped if necessary to remain inside the prostate PTV and outside OARs, including the planning organ at risk volumes (PRV). OARs were prioritised

over DIL boost volume coverage to limit the risk of toxicity. Expected coverage of target structures are presented in Table S1.

OAR dose constraints are described in Table S2 and are similar to those used in multicentre randomised trials (33). Where necessary, radiotherapy doses and/or volumes were amended to prioritise OAR constraints. The isodose distribution of a sample plan in axial and coronal views is demonstrated in Fig. S2. Patients were treated weekly with volumetric arc therapy on a linear accelerator (Varian Truebeam, Palo Alto, USA) with CBCT image guidance before each treatment aligned to gold fiducial markers. Patients were reviewed weekly during treatment; on follow-up, they were reviewed six-weekly for three months, then three-monthly until one year and six-monthly until five years.

Endpoints

The primary aim of the study was to demonstrate the feasibility of performing a randomised trial comparing P-SABR to PPN-SABR in men with unfavourable intermediate or high-risk localised prostate cancer. Therefore, the primary endpoints were recruitment (30 patients), technical delivery of SABR, physician-reported acute gastrointestinal (GI) and genitourinary (GU) toxicity (Common Terminology Criteria for Adverse Events (CTCAE v4.03)) until 90 days post completion of SABR), and patient-reported quality of life (QOL) (Expanded prostate cancer index composite (EPIC) and international prostate symptom score (IPSS) scores until 90 days after completion of SABR). Acute toxicity information was collected weekly at each treatment and at six weeks and three months post-completion of SABR. Secondary endpoints included physician-reported late GI and GU toxicity (Radiation Therapy Oncology Group (RTOG) late toxicity scores from 90 days to 36 months post completion of SABR) and late patient-reported QOL (as assessed by EPIC and IPSS scores from 90 days to 2 years post completion of SABR). Late toxicity was measured every three months for the first year then every six months thereafter.

Exploratory biomarker endpoints included the measurement of γH2AX foci in circulating lymphocytes and serum citrulline to ascertain the temporal pattern of changes during and after treatment, to compare small volume P-SABR treatment to large volume PPN-SABR (Fig. S2) and to correlate biomarkers with acute and late GI and GU toxicity.

Blood samples for γH2AX foci in circulating lymphocytes and serum citrulline were taken at initial screening, immediately prior to and one hour after the first fraction, prior to each additional radiation exposure and at follow up visits six weeks and three months after completion of radiotherapy. Quality of life (QOL) questionnaires were completed at initial screening, prior to every radiation exposure, six weeks after completion of radiotherapy, then every three months for the first year and planned for every six months thereafter until five years after completion of radiotherapy. White cell counts, including lymphocytes were measured at baseline, end of radiotherapy and 6 and 12 weeks post-radiotherapy to monitor haematological function.

Processing of blood samples for translational analysis

Blood samples collected for biomarker analysis were transferred to the laboratories on ice within 2 hours of collection and processed immediately. Plasma for citrulline analysis was collected following centrifugation at 1000 x g for 10 minutes at 4°C. Plasma was stored in 100 µL aliquots in Eppendorf tubes® at -80°C until analysis. Mononuclear cells for γH2AX analysis were collected following Ficoll-Paque® (GE Healthcare)separation. Briefly, 2mL of whole blood was layered on top of 2 mL of Ficoll-Pague®, and this was centrifuged at 400 x g for 30 minutes at 4°C. After centrifugation, the layer containing the mononuclear cells was carefully transferred to a fresh 15 mL tube, 10mL of PBS was added to wash the cells and a further centrifugation step was carried out for 10 minutes at 200 x g and 4°C to pellet the cells. Cells were resuspended in freezing media (90% FBS (Gibco) and 10% DMSO (AppliChem)) and slowly frozen to -80°C. Cells were stored at -80°C until staining was carried out.

Citrulline analysis

Citrulline levels were determined using a Citrulline ELISA (Invitech LDN). Briefly, the plasma was diluted 1:4 in the provided buffer, and a derivatisation step was carried out using the plate included. Subsequently, the ELISA was carried out using the samples and standards. Citrulline antiserum was mixed with the samples and standards and incubated overnight at 4°C. Three washes were carried out manually before the addition of the enzyme conjugate, which was incubated for 30 minutes on a shaker at 600 rpm at room temperature. Three further washes were carried out, after which the samples were incubated in substrate for 30 minutes on a shaker at 600 rpm at room temperature before the addition of the stop solution. The absorbance was read using a multiplate reader immediately, and citrulline levels were determined relative to the standards used.

γH2AX immunofluorescent staining

Mononuclear cells were thawed and washed in PBS to remove all of the freezing media before immunofluorescence staining was carried out. Briefly, cells were cytospun onto Superfrost™ Plus slides (VWR) for 10 minutes at 500 rpm and fixed with 4% formaldehyde (Polysciences) for 10 minutes followed by three PBS washes. Permeabilization was achieved by adding PBS with 0.5% Triton X-100 (Sigma) for 10 minutes followed by three further PBS washes. Cells were then blocked for one hour using PBS with 5% FBS. γH2AX primary antibody (Millipore) was added for one hour at room temperature at a 1:2,000 dilution, followed by three washes in PBS with 0.1% TWEEN®-20 (VWR), and an anti-mouse Alexa Fluor 488 secondary antibody (Invitrogen) at 1:2,000 was added for one hour at room temperature. Cells were again washed three times with PBS with 0.1% Tween®-20, and slides were mounted using ProLong Gold with DAPI (Invitrogen). The slides were left to dry in

the dark at room temperature overnight, and then transferred to -20°C for long term storage. Slides were blinded to the researcher before counting. γH2AX foci were counted in 200 cells per sample using a Zeiss fluorescent microscope with an x63 objective.

Sample Size

The sample size was pragmatically determined as sufficient to demonstrate willingness of clinicians at a single institution to approach patients, and for these patients to enter an RCT comparing P-SABR to PPN-SABR. It was felt to be sufficient to demonstrate the safety and technical feasibility of delivering this complex radiotherapy and to provide toxicity information to power a subsequent multi-centre RCT.

Randomisation

This was a non-blinded study. The randomisation schedule was generated by the XXXXX independent of the investigators. This was carried out through the provision of sealed allocation envelopes, held under lock and key by a data manager independent of the trial.

Statistical Methods

Toxicity (Kaplan Meier curves) and QOL outcomes (waterfall plots) were plotted using GraphPad Prism version 9.4.0 (GraphPad Software, San Diego, California, USA). A minimally clinically important change (MCIC) in EPIC QOL was defined as a decrease of five points for GI toxicity and six for GU toxicity as previously published (34). The purpose of this study was to demonstrate feasibility not to formally compare the treatment groups with respect to toxicity.

Citrulline levels relative to baseline levels were calculated and graphs were plotted using GraphPad Prism 9. Average γH2AX foci numbers per cell and relative lymphocyte counts were plotted and correlations were completed using Pearson correlations in GraphPad Prism 9. For both γH2AX and

citrulline analysis, T-tests were used to determine the significance of any differences between the two treatment arms, and also between patients with and without toxicity. Error bars represent the standard error of the mean, * denotes $p<0.05$, ** denotes $p<0.01$ and *** denotes $p<0.001$. Multivariate analyses (MVA) of RTOG late grade ≥ 1 bowel toxicity and patient-reported late diarrhoea by citrulline levels at second and third fractions and by treatment group were also performed. Continuous citrulline data was standardised by subtracting the mean and dividing by the standard deviation so that each data point was represented in standard deviations from the mean. Standardised citrulline and a binary indicator of group (i.e., P-SABR or PPN-SABR) where then analysed in cox regression models for late grade ≥ 1 bowel toxicity and patient-reported late diarrhoea. The analysis was performed in R 4.2.1 (35) using the *coxph* function from the *survival* package (36).

Results

Primary endpoints

Recruitment

Between October 2016 and December 2018, 51 patients were given patient information sheets for the study. Of these, 31 patients were randomized. One patient was excluded prior to commencement of SABR (due to spacer gel infiltration of the anterior rectal wall) and 15 patients in each group were included in the analysis. At the time of reporting, median follow up was 48 months (range 30-60 months). The target recruitment of 30 patients was achieved (CONSORT diagram, Fig. 1).

Patient characteristics

Demographic and tumour characteristics of included patients are summarised in Table 1. The median age at presentation was 67 years (interquartile range (IQR) 61.5-70 years). Median PSA at

initial presentation prior to commencement of ADT was 11.02 ng/mL (IQR 7.16–20.39). 83% of patients had NCCN high-risk localized prostate adenocarcinoma with 57% of patients diagnosed with clinical stage T3a N0 M0 disease.

Technical delivery

The radiotherapy plan achieved target planning target volume (PTV) dose coverage in all cases with only minor deviations from protocol observed as summarised in Table S1. Six patients in the PPN-SABR group had PTV(ln) margins reduced to 5mm to optimise bowel doses. In the final cohort of 10 patients, a radiotherapy boost of 50 Gy was achieved for one patient while eight had a boost of 45 Gy in five fractions to the DIL. One patient had tumour involving most of the prostate gland and was deemed unsuitable for delivery of a boost. All rectal dose metrics were achieved with only one patient in each arm not fulfilling a single bladder constraint. In the P-SABR group, all but one patient achieved bowel constraints. Within the PPN-SABR group, two patients exceeded the bowel V18.1Gy constraint with values of 106cc and 100.5cc rather than 100cc. The urethra was visualised for these 10 patients with only one patient not achieving the constraints as shown in Table S2.

Acute toxicity and quality of life

In total 0% (95% confidence interval (CI) 0%-20.3%) and 6.7% (95% CI 0.3%-29.8%) of patients treated with P-SABR and PPN-SABR respectively experienced acute CTCAE grade 2 GI toxicity (HR not possible, p=0.32), and 6.7% (95% CI 0.3%-29.8%) and 20.0% (95%CI 7.0%-45.2%) of those treated with P-SABR and PPN-SABR respectively experienced acute CTCAE grade 2 GU toxicity (HR 0.33, p=0.29) (Fig. 2 and Table S4). No patient experienced grade ≥ 3 acute toxicity.

33.3% (95% CI 15.2%-58.3%) and 53.3% (95% CI 30.1%-75.2%) of patients treated with P-SABR and 50% (95% CI 26.8%-73.2%) and 64.3% (95% CI 38.8%-83.7%) of those treated with PPN-SABR experienced a MCIC change in acute EPIC bowel and urinary summary scores respectively (Fig. 3 and Table S4). Mean increase in IPSS score from baseline was 7.7 (standard deviation (SD) 5.9) for the P-SABR arm and 8.3 (SD 5.1) for the PPN-SABR arm (Fig. 3). Among those who experience ≥ 1 MCIC in acute urinary summary scores, mean increase in IPSS score from baseline was 8.4 (SD 4.7) compared to 7.0 (SD 7.5) among those who experienced < 1 MCIC in the P-SABR group and 9.8 (SD 4.4) versus 4.2 (SD 3.3) in the PPN-SABR group.

Secondary endpoints

Late toxicity

In total one patient (6.7%,95% CI 0.3%-29.8%) and two patients (13.3%, 95% CI 2.4%-37.9%) in the P-SABR and PPN-SABR groups respectively experienced late RTOG grade ≥ 2 GI toxicity (HR 0.48, p=0.54) and 6.7% (95% CI 0.3%-29.8%) and 33.3% (95% CI 15.2%-58.3%) of those treated with P-SABR and PPN-SABR respectively experienced late RTOG grade ≥ 2 GU toxicity (HR 0.18, p=0.07) between 90 days and 3 years following radiotherapy (Fig. 2 and Table S4). One patient treated with PPN-SABR experienced late grade 3 GU toxicity (cystitis and haematuria). At 36 months, the point prevalence of grade ≥ 2 GI toxicity in the P-SABR group (data for 14 patients available) was 7.1% (95% CI 0.4%-31.5%) and GU toxicity was 0% (95% CI 0%-21.5%), and in the PPN-SABR group (data for 15 patients available) prevalence was 6.7% (95% CI 0.3%-29.8%) and 6.7% (95% CI 0.3%-29.8%) respectively (Fig. S3).

33.3% (95% CI 15.2%-58.3%) and 60% (95% CI 35.7%-80.2%) of patients treated with P-SABR and 64.3% (95% CI 38.8%-83.7%) and 92.9% (95% CI 68.5%-99.6%) of those treated with PPN-SABR experienced a MCIC in late EPIC bowel and urinary summary scores respectively (Fig. 3 and Table S4). Mean increase in IPSS score from baseline was 5.8 (SD 6.9) for the P-SABR arm and 5.7 (SD 6.3)

for the PPN-SABR arm (Fig. 3). Among those who experienced ≥ 1 MCIC in late urinary summary scores, mean increase in IPSS score from baseline was 8.6 (SD 7.4) compared to 1.7 (SD 3.3) among those who experienced < 1 MCIC in the P-SABR group and 5.2 (SD 6.2) versus 3.0 (SD -) in the PPN-SABR group. Additional data regarding toxicities and quality of life are contained in the supplementary material (Fig. 4 and 5).

Exploratory endpoints

γH2AX foci in peripheral blood mononuclear cells were analysed as a marker of DNA damage in order to determine if detectable DNA damage was greater in PPN-SABR patients with larger treated volumes compared to P-SABR patients who had the prostate and seminal vesicles alone treated (Fig. S2) and to determine if γH2AX could predict toxicity. γH2AX foci increased 1 hour after first SABR fraction in all patients, with significantly higher foci numbers observed with PPN-SABR compared to P-SABR (p=0.04, Fig. 4a). Lymphocyte counts were observed to be significantly decreased with PPN-SABR during (pre #5, 36.1% drop, p<0.001) and after radiotherapy (6 weeks post, 33.2% drop, p<0.001 and 12 weeks post, 38.7% drop, p<0.001) compared to P-SABR (Fig. 4b). There were significant correlations between increased γH2AX foci numbers 1 hour after fraction 1 and decreased lymphocyte counts at the end of treatment (p=0.02) and 3 months after treatment (p=0.00) (Fig. 4c). Citrulline levels were also measured; larger decreases in citrulline were observed in the PPN-SABR group during treatment (one week after treatment start, Pre #2, p=0.006 and Pre #3, p=0.01) (Fig. 4d), compared to P-SABR.

γH2AX foci numbers (Fig. 5a), lymphocyte counts (Fig. 5c) and citrulline levels (Fig. 5e) were then compared in patients with CTCAE v4.03 grade 0 and patients with grade ≥ 1 acute GI toxicity, no significant differences were observed. However, there were trends towards higher γH2AX foci numbers (p=0.09), and larger decreases in citrulline (p=0.05) (Fig. 5b and 5d) and significantly

greater decreases in lymphocyte counts (12 weeks post-radiotherapy, p=0.01) in patients with late grade ≥ 1 GI toxicity compared to patients with late grade 0 toxicity (Fig. 5f).

At 36 months, 33% of patients with relative citrulline levels ≤ median at second fraction and 40% with relative citrulline levels ≤ median at third fraction had experienced late bowel toxicity versus 20% with relative citrulline levels > median at second fraction and 13% with relative citrulline levels > median at third fraction (HR=1.69, p=0.45 and HR=3.10, p=0.13 respectively) (Fig. S6a, S6b). When stratified by treatment group, 33% of patients in the PPN-SABR group had experienced late bowel toxicity versus 20% in the P-SABR group (HR=1.79. p=0.40) (Fig. S6c).

At 36 months, 57% of patients with relative citrulline levels ≤ median had experienced one MCIC for late bowel toxicity versus 40% of patients with relative citrulline levels > median (HR=1.66, p=0.32) (Fig. S7a). 64% of patients in the PPN-SABR group had experienced one MCIC for late bowel toxicity compared to 33% in the P-SABR group (HR=2.28, p=0.11) (Fig. S7b). 86% of patients with relative citrulline levels ≤ median had experienced one MCIC for late diarrhoea compared to 60% of patients with relative citrulline levels > the median (HR=2.04, p=0.06) (Fig. S7c). Seventy-nine percent of patients in the PPN-SABR group had experienced one MCIC for late bowel toxicity compared to 67% in the P-SABR group (HR=1.33, p=0.47) (Fig. S7d).

MVA accounting for treatment group was performed. MVA of late grade ≥ 1 bowel toxicity by citrulline levels at second fraction and third fractions demonstrated a 65% (HR=0.35, p=0.07) decrease and a 42% (HR=0.58, p=0.30) in the relative hazard per standard deviation in citrulline respectively. MVA of one MCIC for patient-reported late diarrhoea by citrulline levels at second and third fractions demonstrated a 51% (HR=0.49, p=0.42) decrease and a 39% decrease (HR=0.61, p=0.43) in the relative hazard per standard deviation in citrulline respectively (Table S5).

Discussion

Our study demonstrates the feasibility of the addition of ENI to P-SABR in the treatment of patients with unfavourable intermediate- and high-risk prostate adenocarcinoma. We demonstrate that recruitment to this study was acceptable to patients and clinicians, that it was technically feasible to deliver this radiotherapy and that the treatment was well tolerated.

To date, the evidence for SABR with ENI for higher-risk prostate cancer has been limited to single-arm, single institution studies delivering a variety of doses. Forty gray in five weekly fractions to the prostate with 25 Gy in five fractions ENI has been used in a study by Alayed et al. (37) and in the SATURN trial (38), with no grade ≥ 3 toxicities in either trial. However, the FASTR trial (39) of 40 Gy to the prostate and 25 Gy to pelvic lymph nodes in five weekly fractions was terminated early after higher than anticipated late toxicities among the initial 16 patients enrolled. The subsequent FASTR 2 trial (40) which used a lower dose to the prostate of 35 Gy and smaller PTV margin (4 mm versus 5 mm) without ENI was better tolerated with four (14.8%) acute grade 2 GU toxicities and one (3.7%) acute grade 2 GI toxicity, five (17.9%) and five (21.7%) late grade 2 GU toxicities at 6 months and one year, and no late grade ≥ 2 GI or any grade ≥ 3 toxicities. Hannan et al. (41) studied dose-escalated SABR of up to 47.5 Gy to the prostate, 55 Gy to the DIL and 25 Gy to the pelvic lymph nodes in 5 weekly fractions with late grade ≥ 2 GU and GI toxicities of 20% and 7% respectively and late ≥ 3 GU and GI toxicities of only 2% and 0% respectively. A recent retrospective study by Murthy et al. (42) compared P-SABR and PPN-SABR in 220 cases with a median dose of 36.25 Gy to the prostate ± 25 Gy in five fractions over two weeks reported significantly higher rates of acute grade 2 GI toxicity and late grade 2 GU toxicity in the PPN-SABR arm compared to the P-SABR arm (29.4% vs 14.7%, p=0.01 and 45.6% vs 25.0%, p=0.00 respectively). Rates of late grade 3 toxicity were low in the trial with 2.5% GU and 1% GI (42). In our study, both physician-reported and patient-reported acute GI and GU toxicity and decline in QOL were greater with PPN-SABR compared to P-SABR arm in our study, although toxicity rates in both were acceptable with no acute grade ≥ 3 toxicity, cumulative three-year late toxicity rates of 6.7% and 6.7% (P-SABR) and 13.3% and 33.3% (PPN-SABR) for grade

≥ 2 GI and GU toxicity respectively, and only one late grade ≥ 3 toxicity (GU), comparable with previously published trials (37, 38, 41, 42). Point toxicity rates at three years were lower than cumulative rates for both GI and GU late toxicities in the PPN-SABR group demonstrating recovery from treatment-induced toxicities. This further suggests long term tolerability of SABR with ENI. While mean increase IPSS scores from baseline were similar between groups, mean increases were greater among those who experienced ≥ 1 MCIC in acute and late urinary summary scores than those who did not for both groups.Among men with high risk localised prostate cancer there was no improvement in overall survival demonstrated with ENI in the RTOG 77-06 (43), RTOG 94-13 (11) and GETUG-01 (44) trials. However, all these studies were carried out in the pre-IMRT era (and the pre-CT era in the case of RTOG 77-06) and there remains considerable debate as to the role of ENI for men with high-risk disease, particularly given that several studies have demonstrated improvements in biochemical control with the addition of ENI to prostate radiotherapy (45, 46). The recent POP-RT trial (47) demonstrated improved biochemical failure-free survival and disease-free survival with ENI and prostate radiotherapy compared to prostate-only radiotherapy in high-risk prostate cancer. At median follow up of 44.5 months, cumulative late grade ≥ 2 toxicity in the prostate-only radiotherapy group was 3.8% and 7.5% for GI and GU toxicities respectively and in the ENI plus prostate radiotherapy group was 6.5% and 17.7% respectively (48). Results from the ongoing PIVOTAL boost (32) and RTOG-0924 (49) trials comparing EBRT to the prostate with or without ENI will provide additional data in this space. No randomised studies comparing P-SABR to PPN-SABR have been performed to date. While the low number of patients who received a focal radiotherapy boost prevents statistical comparison, it is reassuring that toxicity rates did not appear to be significantly increased compared to those patients who did not receive a boost and suggests that a boost is well tolerated in this patient cohort.

To our knowledge, this is the first randomised study to examine γH2AX and citrulline in a randomised trial of patients treated with different radiotherapy volumes, small P-SABR volumes

compared to large PPN-SABR volumes. The presence of double-stranded DNA breaks and subsequent cell death is indicated by the significant correlations between increased γH2AX foci numbers one hour after the first fraction and decreased lymphocyte counts at the end of treatment and three months after treatment (Fig. 4c) (22). In the PPN-SABR arm in our study, there were greater decreases in citrulline (Fig. 4d), indicating the presence of small bowel damage secondary to pelvic radiotherapy. The findings are consistent with those of a study by Onal et al. of 53 patients receiving pelvic irradiation for a variety of cancers, in which citrulline levels were found to decrease at the end of radiotherapy (30). The trends towards higher γH2AX foci numbers and greater decreases in citrulline (Fig. 5d, 5f, 6, S4), and significantly greater decreases in lymphocyte counts in patients with late grade 1 GI toxicity and diarrhoea compared to patients with no toxicity (Fig. 5e) suggest their potential as predictive biomarkers although larger studies are required. MVA of serum citrulline suggests it may add more information to predict the risk of late bowel toxicity than treatment group alone.

Strengths and limitations

A strength of our study is the control arm of P-SABR, allowing direct comparison with PPN-SABR. In addition, blood samples were blinded to researchers prior to biomarker analysis, which eliminated measurement bias when assessing the response of γH2AX foci and serum citrulline to radiotherapy. Additionally, all patients underwent MRI following spacer/fiducial placement which aided target and OAR delineation.

Limitations include lack of blinding during the radiotherapy planning and delivery process and during clinician assessment of toxicity. Blinding of these endpoints, however, was not felt to be practical within clinical practice. Median follow-up in the study was relatively short at 48 months, which may have resulted in some later toxicities not being identified at the time of analysis; this is, however,

longer than the reported median follow-up in the SATURN trial (25.7 months) and in the study by Hannan et al. (18 months). All patients in this trial are planned for five years of follow-up in total.

Future research

This trial has been expanded to incorporate an additional ten patients treated on alternate days instead of weekly and is currently recruiting. This change in fractionation schedule aims to reduce the overall treatment duration for patients. Results from this study have informed a larger (n=536) multicentre, randomised phase III trial comparing P-SABR and PPN-SABR which commenced in 2022 and is powered for both toxicity and efficacy (NCT05613023).

Conclusions

This study demonstrates that a randomised trial comparing P-SABR to PPN-SABR is both feasible and acceptable to patients and clinicians. Overall toxicity rates and patient-reported QOL scores remain comparable to published data. Further investigation of γH2AX foci in circulating lymphocytes and serum citrulline levels as potential biomarkers for radiation normal tissue damage is warranted. This study has informed a multicentre phase III randomised controlled trial.

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Figure 1. CONSORT diagram of patients in the trial.

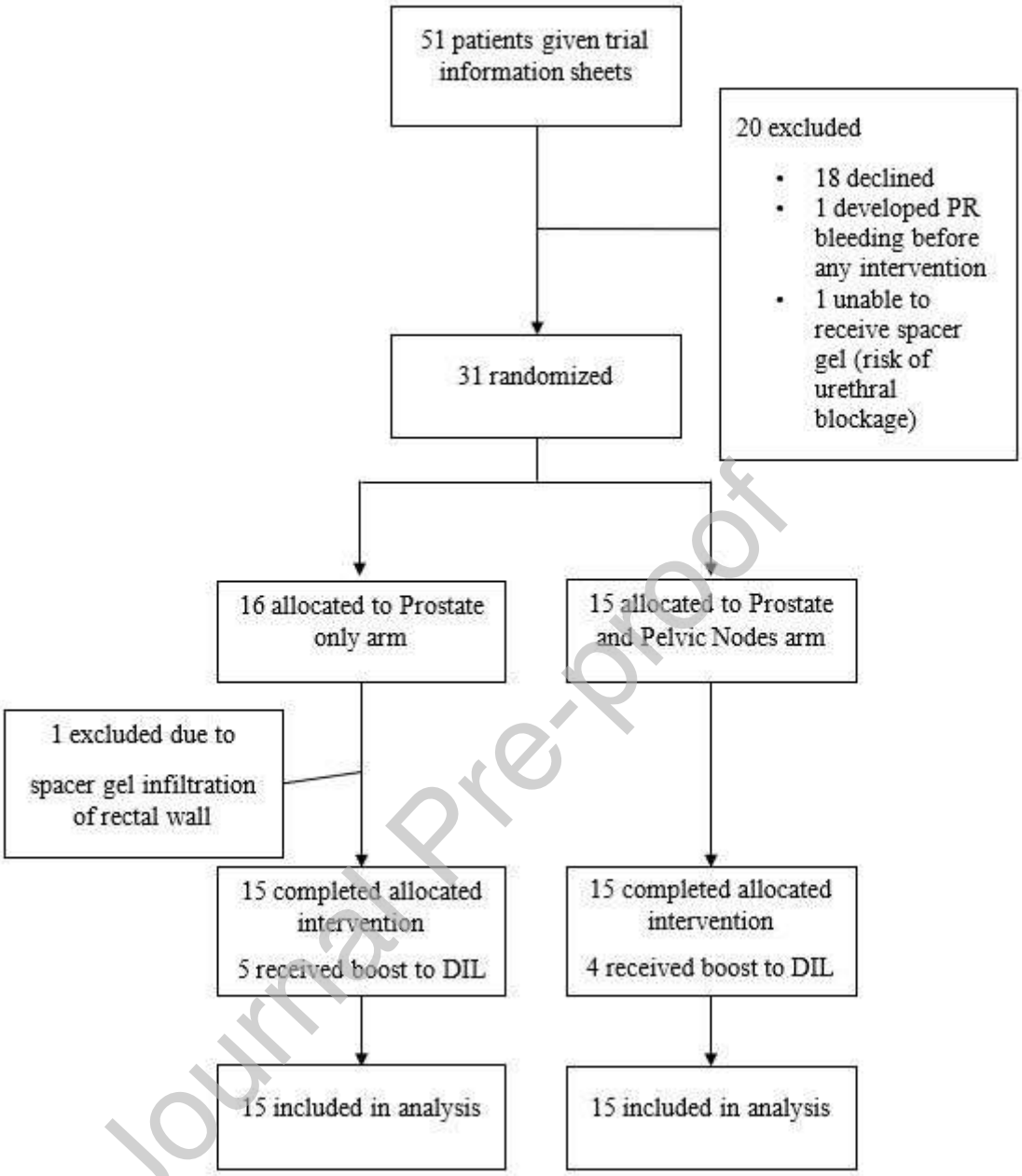
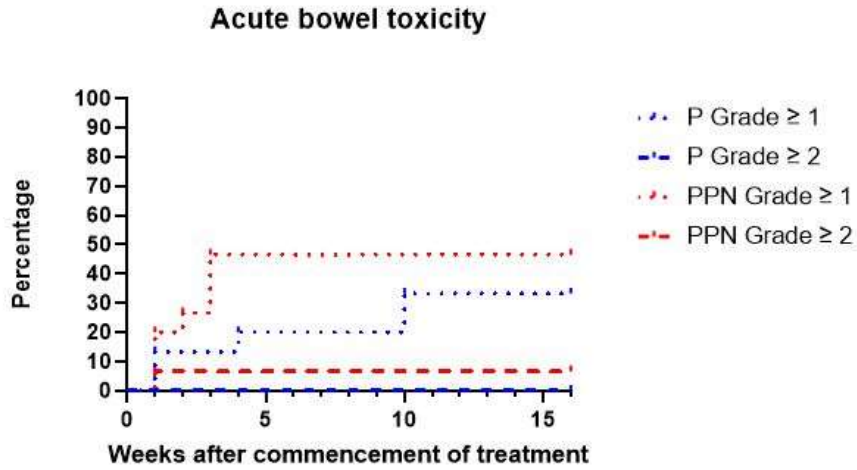
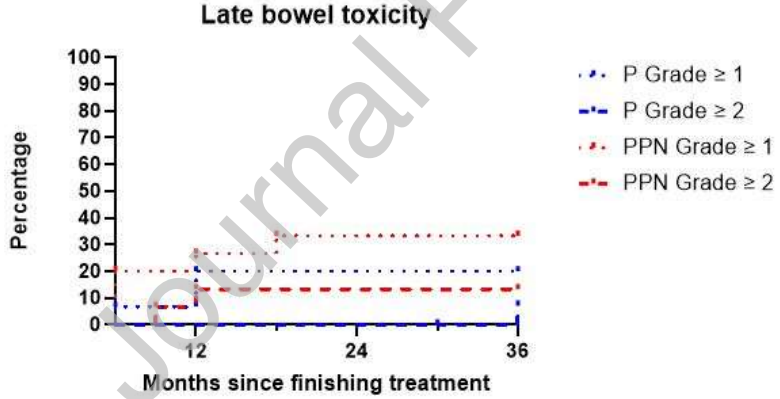


Figure 2. Kaplan-Meier curves demonstrating percentage of clinician-reported (a) acute bowel, (b) late bowel, (c) acute urinary and (d) late urinary toxicity.

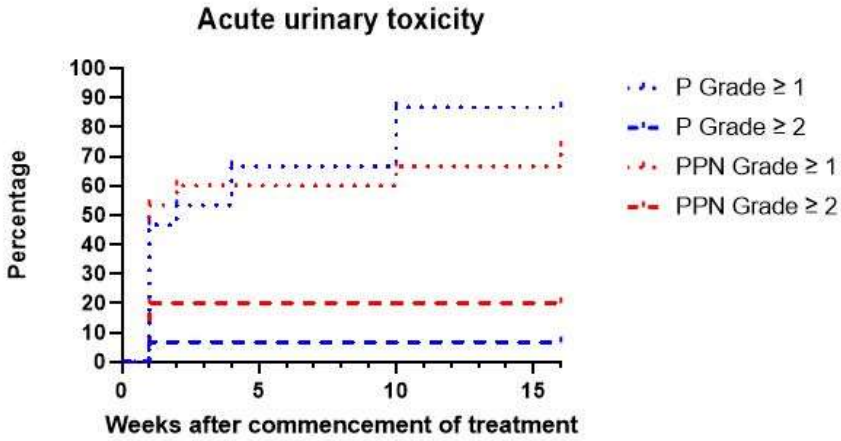
Abbreviations: P: Prostate-only radiotherapy, PPN: Prostate and pelvic nodal irradiation.



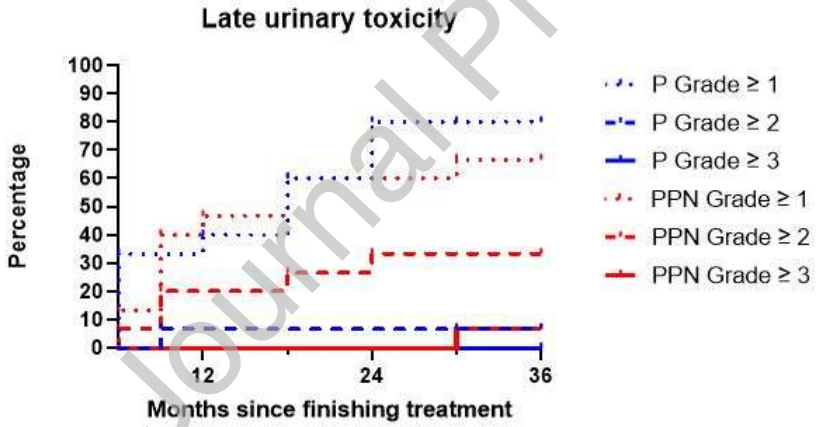
				Number at risk at each timepoint				
Group	**Censored**	**Observed**	**Total**	**2 weeks**	**3 weeks**	**4 weeks**	**10 weeks**	**16 weeks**
P Grade ≥ 1	10	5	15	13	13	13	12	10
P Grade ≥ 2	15	0	15	15	15	15	15	15
PPN Grade ≥ 1	8	7	15	12	11	8	8	8
PPN Grade ≥ 2	14	1	15	14	14	14	14	14



				Number at risk at each timepoint				
Group	**Censored**	**Observed**	**Total**	**12 months**	**18 months**	**24 months**	**30 months**	**36 months**
P Grade ≥ 1	12	3	15	14	12	12	12	12
P Grade ≥ 2	14	1	15	15	15	15	15	14
PPN Grade ≥ 1	10	5	15	12	11	10	10	10
PPN Grade ≥ 2	13	2	15	14	13	13	13	13

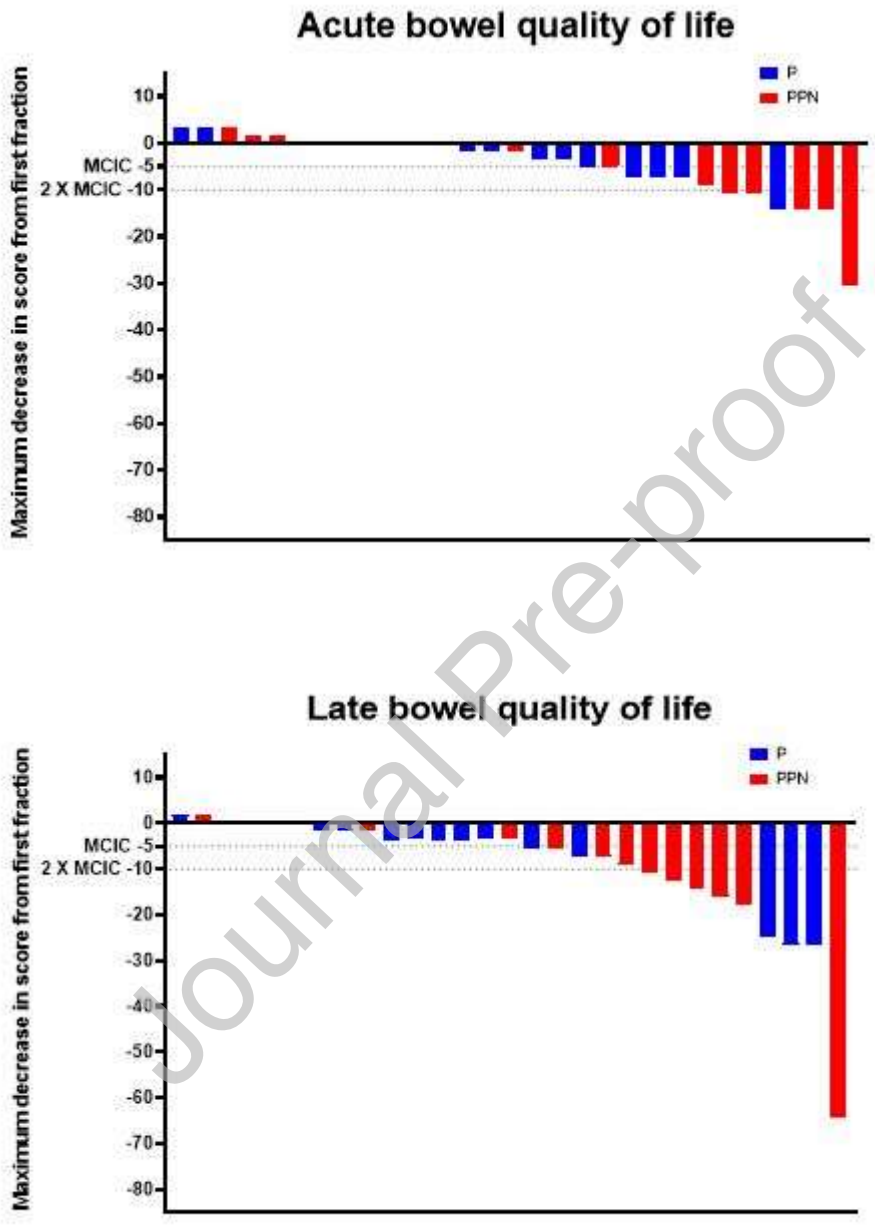


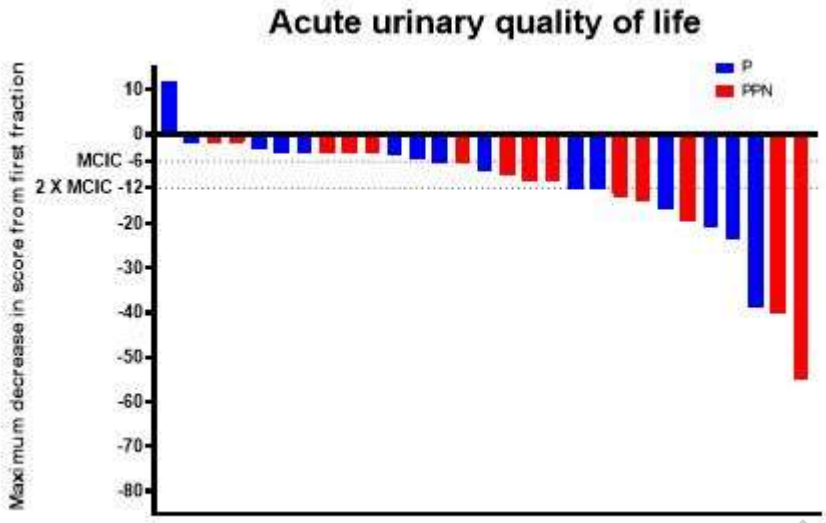
				Number at risk at each timepoint				
Group	**Censored**	**Observed**	**Total**	**2 weeks**	**3 weeks**	**4 weeks**	**10 weeks**	**16 weeks**
P Grade ≥ 1	2	13	15	8	7	7	5	2
P Grade ≥ 2	14	1	15	14	14	14	14	14
PPN Grade ≥ 1	4	11	15	7	6	6	6	5
PPN Grade ≥ 2	12	3	15	12	12	12	12	12

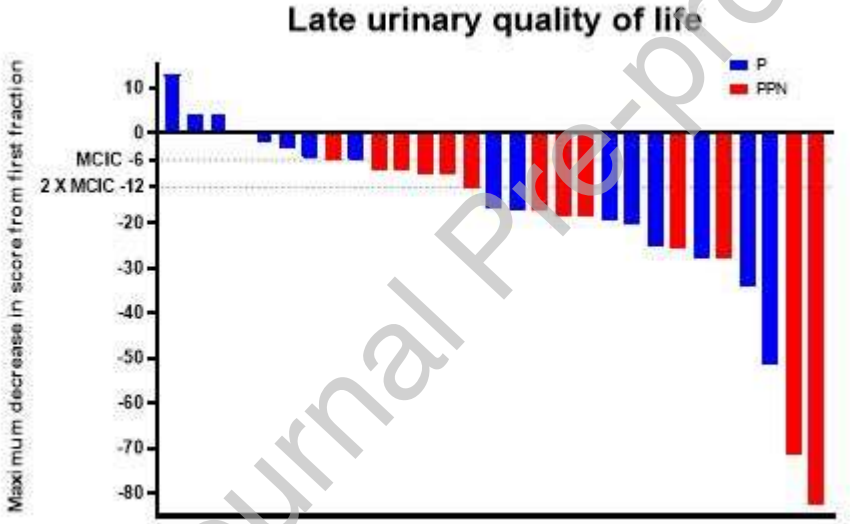


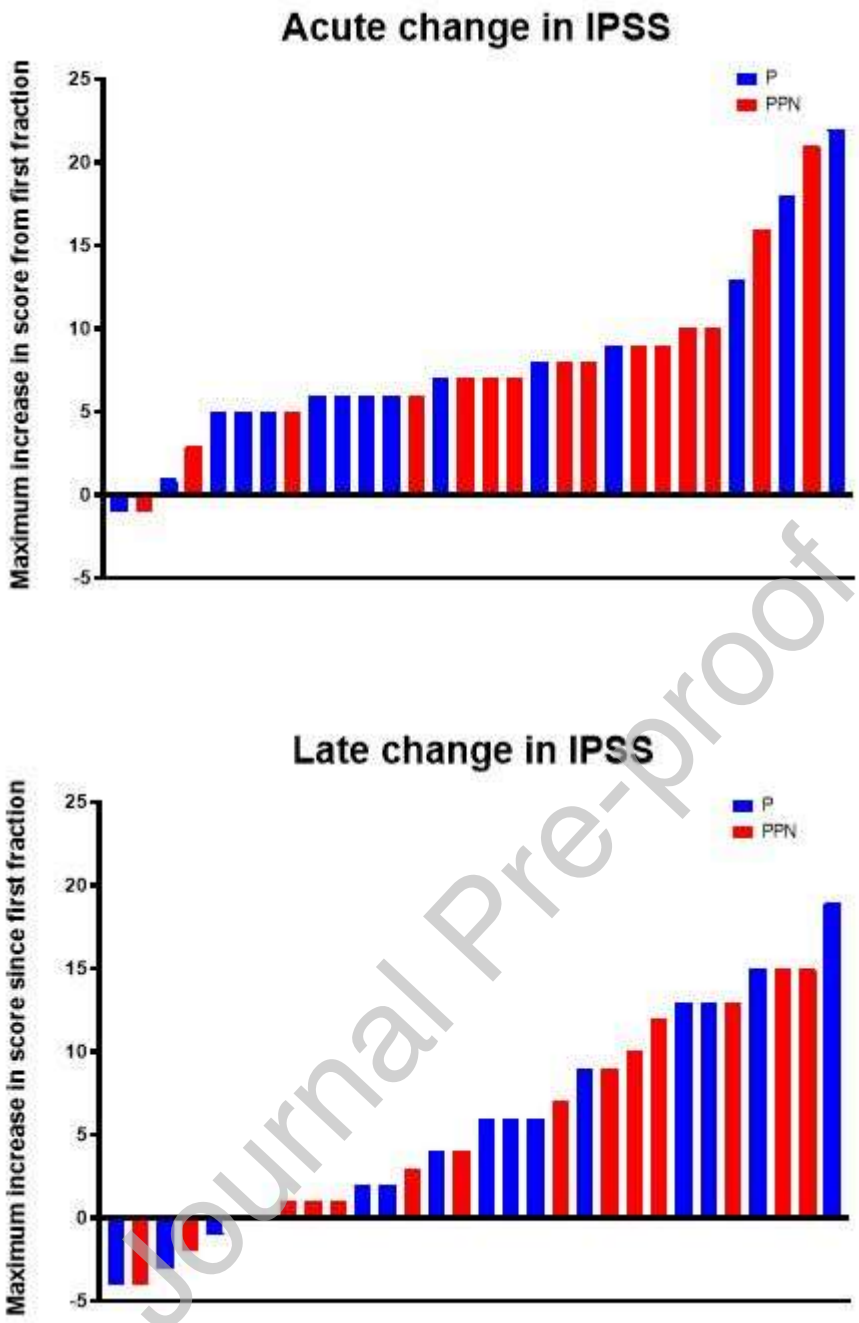
				Number at risk at each timepoint				
Group	**Censored**	**Observed**	**Total**	**12 months**	**18 months**	**24 months**	**30 months**	**36 months**
P Grade ≥ 1	3	12	15	10	9	6	3	2
P Grade ≥ 2	14	1	15	14	14	14	14	13
P Grade ≥ 3	15	0	15	15	15	15	15	14
PPN Grade ≥ 1	5	10	15	9	8	6	6	5
PPN Grade ≥ 2	10	5	15	12	12	11	10	10
PPN Grade ≥ 3	14	1	15	15	15	15	15	14

Figure 3. Maximum decrease in patient-reported EPIC summary score for (a) acute bowel, (b) late bowel, (c) acute urinary and (d) late urinary quality of life and maximum increase in IPSS summary score for acute (e) and late (f) urinary tract symptoms.





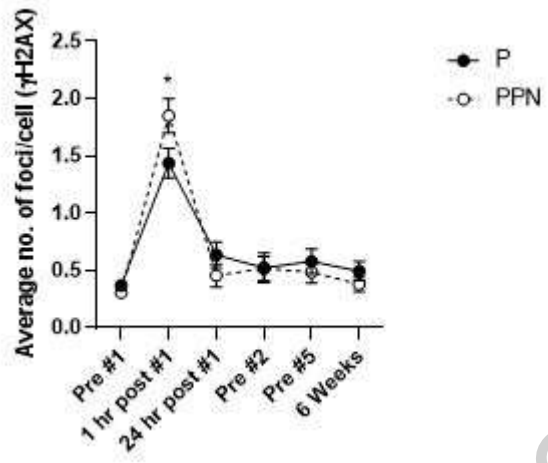


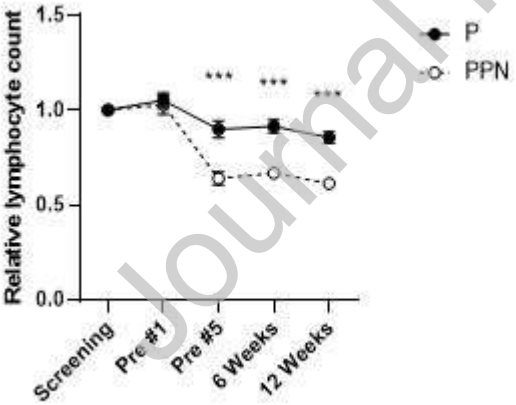


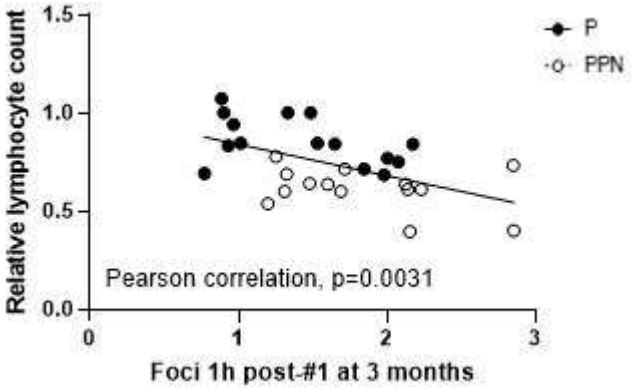
Abbreviations: EPIC: Expanded Prostate Cancer Index Composite questionnaire, IPSS: International Prostate Symptom Score, MCIC: Minimally clinically important change, P: Prostate-only radiotherapy, PPN: Prostate and pelvic nodal irradiation.

Figure 4. Lymphocyte DNA damage increases more and citrulline levels decrease more in PPN treated patients. a) Average numbers of γH2AX foci in lymphocytes of patient treated to the

prostate only (P) or prostate and pelvic nodes (PPN) before, during and after treatment. b) Lymphocyte counts for patients in P-SABR and PPN-SABR groups from before treatment to 3 months after treatment. c) Correlations between increased foci numbers 1h post fraction 1 and decreased lymphocyte counts pre fraction 5 and 3 months after treatment (Black = P, Grey =PPN). d) Relative citrulline levels for patients in P-SABR and PPN-SABR groups before, during and after treatment.







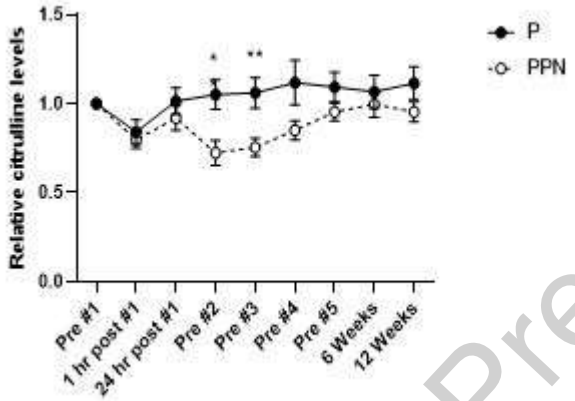
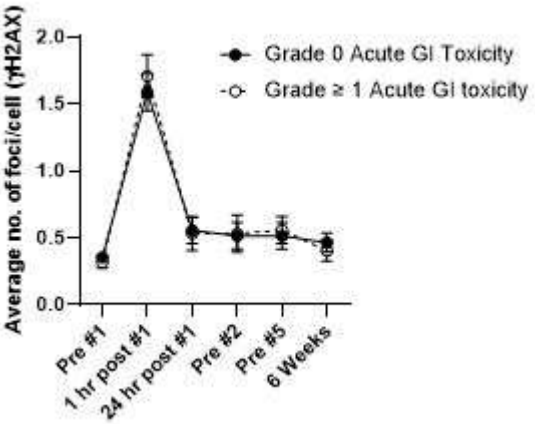
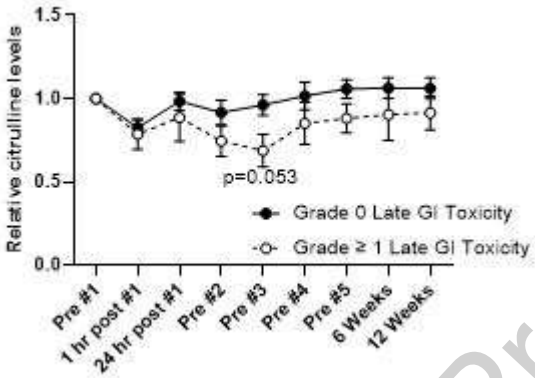
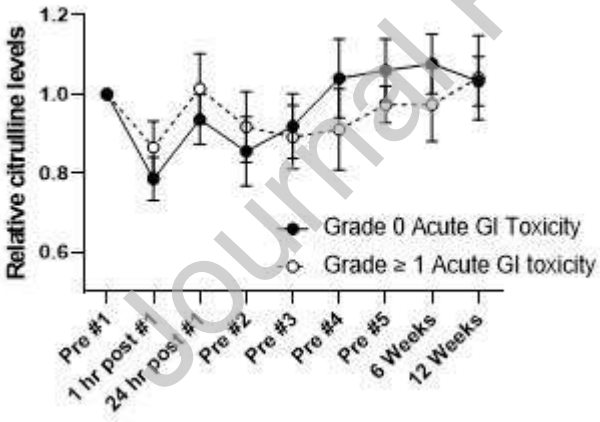
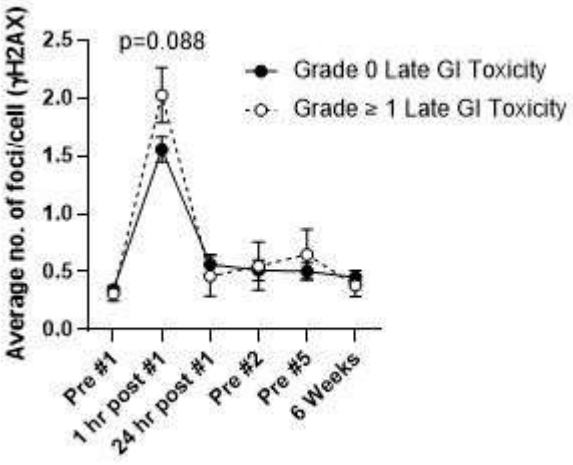


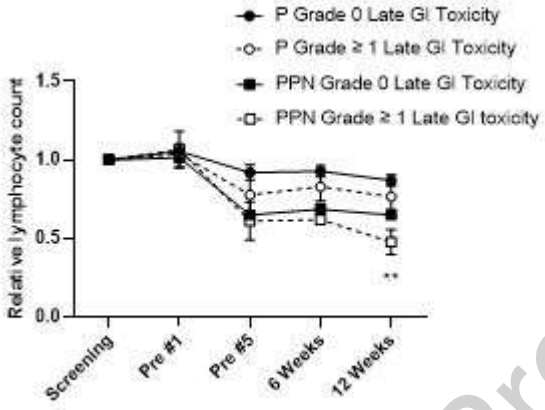
Figure 5. Increases in lymphocyte DNA damage and decreases in citrulline may predict late but not acute GI toxicity. a) Average numbers of γH2AX foci in circulating lymphocytes of patients with grade 0 or grade 1 acute GI toxicity. b) Average numbers of γH2AX foci in circulating lymphocytes of patients with grade 0 or grade 1 late GI toxicity. c) Relative citrulline levels in patients with grade 0 or grade 1 acute GI toxicity. d) Relative citrulline levels in patients with grade 0 or grade 1 late GI toxicity. e) Lymphocyte counts in patients with grade 0 or grade 1 acute GI toxicity. f) Lymphocyte counts in patients with grade 0 or grade 1 late GI toxicity.











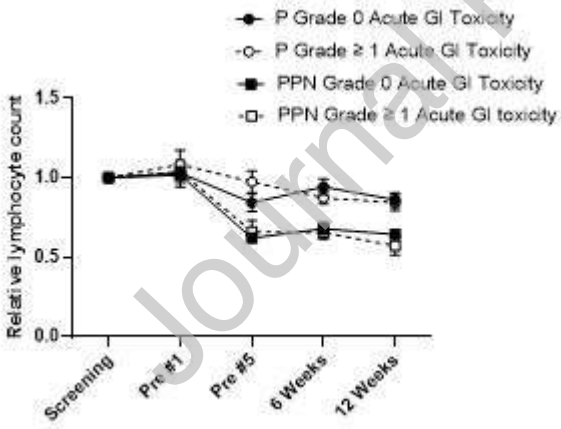


Table 1. Patient and tumour characteristics.

		All (n=30)	No ENI (n=15)	ENI (n=15)
Age	Median	67	69	64
	IQR	61.5-70	65-73	59-69
iPSA	Median	11.02	11.1	10.94
	IQR	7.16-20.39	7.52-21.56	6.67-20
Gleason	**7**	24	12	12
	8	3	2	1
	9	2	0	2
	10	1	1	0
T stage	**T2**	13	7	6
	T3a	17	8	9
Risk group	**Unfavourable intermediate**	5	3	2
	High	25	12	13

Abbreviations: ENI: Elective nodal irradiation, IQR : Interquartile range.