Determination of optimal drug dose and light dose index to achieve minimally invasive focal ablation of localized prostate cancer using WST11- Vascular Targeted Photodynamic (VTP) therapy

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Key Words: Prostate, cancer, TOOKAD, VTP, Focal therapy

Abstract

Objective

To determine the optimal drug and light dose for prostate ablation using WST11 (TOOKAD® Soluble) for Vascular Targeted Photodynamic (VTP) therapy in men with low-risk prostate cancer.

Patients and methods

Forty men received a single dose of 2, 4 or 6mg/kg WST11 activated by 200J/cm light at 753 nm. WST-11 was given as a 10 minute intravenous infusion. The light dose was delivered using cylindrical diffusing fibres within hollow plastic needles positioned in the prostate using transrectal ultrasound scan (TRUS) guidance and a brachytherapy template. Magnetic resonance imaging (MRI) was used to assess treatment effect at 7 days, with assessment of urinary function (International Prostate Symptom Score [IPSS]), sexual function (International Index of Erectile Function [IIEF]) and adverse events at 7 days, 1, 3 and 6 months. TRUS guided biopsy was performed at 6 months.

Results

Thirty-nine of 40 treated men completed follow up. The Day 7 MRI showed maximal treatment effect (95% of the planned treatment volume) in men who had a WST11 dose of 4mg/kg, light dose of 200J/cm and light density index (LDI) of >1. In the 12 men treated with these parameters, the negative biopsy rate was 10/12 (83%) at 6 months, compared to 45% (10/26) for the men who had either different drug doses (n=10), or an LDI of <1 (n=16). Transient urinary symptoms were seen in the majority of men, with no significant difference in IPSS score between baseline and 6

months. IIEF scores were not significantly different between baseline and 6 months.

Conclusion

4mg/kg TOOKAD® Soluble activated by 753nm light at a dose of 200J/cm and an LDI >1 resulted in treatment effect in 95% of the planned treatment volume and a negative biopsy rate at 6 months of 83% in a group of 12 men.

Introduction

Currently, men who are diagnosed with early prostate cancer face the dilemma of whether to undergo active surveillance or have treatment directed to the whole prostate, with surgery or radiotherapy [1, 2]. Active surveillance involves repeated biopsies and frequent prostate-specific antigen (PSA) testing, often results in anxiety, and cross-over to active therapy occurs with or without evidence of progression [3]. Whole gland treatment is associated with a known set of side-effects, that most men wish to avoid [4]. The increasing precision of modern diagnostics is permitting a more refined characterisation of risk and has introduced the relatively novel attribute of tumour location [5,6]. This transition has permitted the substitution of an anatomical target for therapy – in other words the contour of the prostate – to one that is defined by the location and burden of the tumour. The result is tissue preserving or focal therapy [7], an approach that has proved beneficial in most other solid organs.

Vascular-targeted photodynamic therapy (VTP) uses a photosensitising agent (WST11, TOOKAD® Soluble), activated by 753nm light to generate reactive oxygen species (ROS) within blood vessels. These ROS lead to vessel thrombosis and subsequent tissue necrosis around the light delivery fibre. The bacteriochlorophyll derivatives are a novel generation of light-activated vascular occluding photosensitizing agents which includes WST09

(padoporfin; palladium bacteriopheophorbide) and its water-soluble derivative WST11 (padeliporfin; palladium bacteriopheophorbide monolysotaurine). Pre-clinical studies have demonstrated their efficacy for the ablation of prostatic tissue [8]. Phase II studies demonstrated that WST09 effectively produced ablation in targeted volumes of prostate tissue. However, intraoperative hypotension occurred in association with cardiovascular effects that were thought to be linked to the solubilizing excipient Cremophor® [9]. In order to avoid these side effects, the hydrophilic derivative WST11 was developed, which is soluble in aqueous solutions, so that Cremophor® is not required [10]. Preclinical studies in several animal models have shown that treatment with WST11 leads to occlusion of the entire tumour vasculature within a few minutes of treatment, leading to tumour ablation [11, 12, 13].

The present paper reports the findings from a Phase II, prospective, multicentre, open-label, single intravenous (IV) dose study of WST11- VTP, which was designed to determine the optimal drug concentration and light dose parameters to achieve prostate ablation in men with early prostate cancer. The primary efficacy criteria were the histological assessment of a 6-month biopsy, and assessment of volume of hypoperfusion on gadolinium enhanced magnetic resonance imaging (MRI) scan at 7 days. Safety and quality of life were also assessed.

Patients and methods

Patients

Men (>18 years of age) diagnosed with low-risk prostate cancer, who were suitable for active surveillance, were invited to take part in the study. The main exclusion criteria were: Prior or current treatment for their cancer, including hormonal manipulation (excluding 5-alpha reductase inhibitors) or androgen supplements in the last 6 months, radiation therapy, chemotherapy, or trans-urethral resection of the prostate and the use of any photosensitising medication for 1 month before and 1 week after the VTP procedure. In men diagnosed by transrectal ultrasound scan (TRUS) guided biopsy, the histological entry criterion was limited to Gleason pattern 3+3. For men diagnosed by transperineal template guided biopsy, secondary Gleason pattern 4 was permitted provided that it was low burden (<3 cores positive per lobe and </=3mm maximum cancer core length). No other restrictions were placed on the number of positive cores present at baseline. The upper limit of PSA prior to consent was 10ng/ml, with a maximum clinical stage of T2b (radiological stage T2c acceptable).

Ethics statement

The study was conducted within 9 university hospital centres in Europe (France, the Netherlands and United Kingdom) and Canada.

The protocol, all protocol amendments, the proposed Patient Informed Consent Document were submitted to the following Independent Ethics Committees or Institutional Review Boards for approval. All patients gave signed informed consent to participate before any study-related activities were performed.

Study design

This was a multicentre, phase II, open-label, single IV dose escalation, 6-month, clinical trial, designed to determine the optimal treatment conditions necessary to achieve prostate cancer ablation. Patients were followed for 6 months post-treatment. MRIs of the prostate were assessed 1 week, 3

months and 6 months after the VTP procedure, and a prostate biopsy was undertaken at 6 months post-procedure.

The trial was registered at ClinicalTrials.gov (NCT00975429) and in the EudraCT register (2008-000876-26).

VTP procedure

WST11- VTP treatment comprised a single IV administration of WST11 (TOOKAD® Soluble; STEBA Biotech, L-2613 Luxembourg) at a dose of 2, 4 or 6mg/kg, using 753nm laser light at a fixed power (150mW/cm) and energy (200J/cm) delivered through transperineal interstitial optical fibres positioned in the prostate. The rate of infusion varied according to body weight since the duration of the infusion remained constant (10 minutes).

Preclinical data had shown predictable ablation volumes using 2mg/kg of WST11 with a light energy dose of 200J/cm [15]. These were the treatment parameters for the first 3 men in the study. The drug dose escalation plan comprised an increase to 4 and 6mg/kg, contingent on the effects on prior patients. The decision to escalate the dose was based on the volume of hypoperfusion (treatment effect) on the Day 7 MRI and any AEs reported to the Data Safety Monitoring Board.

The procedure was performed under general anaesthesia. The procedure has been previously described in detail [9, 10]. A transperineal template (Mick Radio-Nuclear Instruments, Inc, USA) was used to position hollow, transparent, plastic brachytherapy-like catheters (Best Medical International, Inc, USA, model 1117-20 and Medlight SA, Switzerland, model iCAT-2.0-200) into the prostate, using TRUS image guidance, in accordance with a previously devised MRI-based treatment plan. Cylindrically diffusing optical fibres (Medlight SA, Switzerland, model RD and CeramOptec GMBH, Germany, model CD) were inserted into the catheters. The number and positioning of the fibres were adapted to each patient in order to obtain zonal to sub-total destruction of the prostate areas of interest. Examples of the different treatment plans with the corresponding MRI result at Day 7 (mid-

gland axial plan after gadolinium injection) are shown in Figure 1. The decision on which plan to use was taken by a Treatment Planning Guidance Team, comprising the radiologist, who did the treatment planning based on the pre-treatment MRI, the Principal Investigator, and the consultant urologist. The Team's decision was based on the number of positive cores and the extent and location of disease (eg, unilateral or bilateral disease), but depended also on the dose escalation scheme at the time an individual patient was enrolled.

The length of diffusing fibre varied between 1 and 4cm, in 0.5cm increments; the length was chosen to allow a 5-mm safety margin between the end of the diffuser and the base, the apex and the capsule of the prostate, unless a larger safety margin was deemed necessary, such as at the start of a new drug dose group. Laser light at 753nm was delivered to the prostate, using a multichannel diode laser (V-Gen Electro Optics Ltd, Israel, model 8CH-753 Mk II). A maximum of 2 lasers with 8 channels (16 fibres in total) were used per patient. Light monitoring was done by the use of optical detector fibres (Medlight SA, Switzerland, model IP) in the prostate, rectum and urethra, connected to a continuously recording light dosimeter (V-Gen, model 8CH-PDT-DOS-1).

The single WST11 IV dose was a 10-minute infusion, conducted using automatic syringes, through a fast flow peripheral line (antecubital catheter) or a central line. This was followed by continuous illumination of the prostate gland through optical fibres for 22 minutes and 15 seconds in order to activate the WST11. Light delivery was initiated at the end of the WST11 infusion in order to coincide with the peak serum concentration of WST11. The total light dose varied according to the length and number of optical diffusing fibres. This phase was precisely monitored by continuous clinical surveillance and was stopped in the event of occurrence of a significant AE. The total duration of the whole procedure was about 2 hours (including anaesthesia, fibre placement and illumination with the laser light). At the end of the illumination, the laser was turned off and the fibres withdrawn from the prostate.

A urinary catheter was left in situ until the next morning. The patient was required to wear protective eye wear and stay in low level light for the first hours after the treatment.

Efficacy and Safety Criteria

The primary efficacy criterion was the result of a 12-core prostate biopsy, 6 months after VTP. Success was defined as a negative biopsy in the treated lobe(s).

The secondary efficacy criterion was the volume of VTP effect assessed as the volume of intraprostatic hypoperfusion on the Day 7 MRI. The MRI protocol used a pelvic phased array coil with a 1.5T MRI, although the manufacturer varied between centres.

Adverse Events (AEs) were recorded and laboratory evaluations were performed periodically during the 6-month follow-up period; AEs were coded using the Medical Dictionary of Regulatory Activities (MedDRA), version 12.0. The patients also underwent 12-lead electrocardiograms (ECGs) at baseline, during the VTP procedure and peri-operatively. Toxicities were graded according to the National Cancer Institute's Common Terminology Criteria for AEs (CTCAE), version 3.0. The relationship of the AEs was assessed with respect both to the study treatment and to the technical procedure of the VTP. Urinary and erectile function prior to VTP, and at 1, 3 and 6 months post−treatment, were assessed using the International Prostate Symptom Score (IPSS) and International Index of Erectile Function (IIEF-5) quality of life questionnaires, respectively.

Statistical Methods

Descriptive statistics (n, mean, standard deviation (SD), median, minimum and maximum) were calculated for quantitative variables; frequency count by category was given for qualitative variables. Confidence intervals were given where appropriate. If not otherwise stated, all statistical tests were 2-sided and at the 5% level of significance, and provided 95% confidence.

The only statistical inference performed was the 1-sided binomial exact test for the proportion of patients with negative biopsy assessment at Month 6. This study served to generate hypotheses on the primary efficacy objective for a future phase III clinical study. A significant difference between the proportion under the null hypothesis and the alternative of at least 25% was seen as clinically relevant.

Results

Forty-two men were enrolled in the study with 4 excluded from the final efficacy analysis (Figure 2). The baseline characteristics of the 42 enrolled men are shown in Table 1. Two patients underwent anaesthesia for the procedure, but did not receive the drug or light dose: one patient due to a rectal abnormality which precluded satisfactory ultrasound probe placement, and another due to ECG changes suspicious for ischaemia which were seen after the start of the anaesthesia. Two additional patients underwent the full VTP procedure, but could not be evaluated for efficacy: one man had the incorrect lobe treated and the other was staged as T3a at Month 6, which on central radiologist review was confirmed as being present and missed prior to VTP. The patient in whom the wrong lobe was treated was retreated in the correct lobe after the Month 6 biopsy.

Of the remaining 38 men, 3 men had significant protocol deviations (laser malfunction at the time of treatment, n=2) and administration of an ultrasound contrast agent at the time of the procedure (n=1).

Three patients were treated with WST11 at a dose of 2mg/kg, and 2 patients received the 6mg/kg dose. In the 3 patients treated at the lowest dose, little effect was seen on the Day 7 MRI scans and therefore this dose was deemed to be sub-optimal and no further patients were enrolled at 2mg/kg. The 6mg/kg dose was deemed not suitable for smaller glands, as the treatment effect with 3 fibres appeared to be difficult to maintain within the boundaries of the prostate gland. The 6mg/kg was not evaluated further in this study, but

was evaluated in study PCM203, where it did not result in larger volumes of effect than 4mg/kg. .

Thus, the majority of patients (n=33) were dosed at 4mg/kg. All patients who received the WST11 dose were treated with a light energy of 200J/cm, except for 2 patients who received an energy dose of 350J/cm due to a laser malfunction, and were excluded from the final efficacy analysis.

In total, 28 men had a drug dose of 4mg/kg, a light dose of 200J/cm and a minimum of 3 light fibres. An exploratory analysis of these men showed that the light dose per volume of prostate was an important determinant of both treatment effect on contrast enhanced MRI at 1 week, and of 6-month biopsy result. The light density index (LDI) was defined as:

$$LDI = \frac{\sum lengths\ of\ illuminated\ fibres\ (cm)}{volume\ of\ targeted\ prostate\ (mL)}$$

In the 4mg/kg group, 12 men had an LDI of $\geq$1, and 16 men an LDI of <1 (Figure 2).

Efficacy

6-month biopsy

One patient declined the 6-month biopsy, so 37 biopsies were available for the analysis of the primary efficacy criterion and 38 MRI scans were available for the secondary efficacy criterion.

Of those men who could be evaluated (n=37), 20 had a negative 6-month biopsy (53%), while of those men in the optimal dose-energy group (n=28), 15 had a negative 6-month biopsy (54%) (Table 2). However, when the assessment was limited to men with the optimal treatment conditions (4mg/kg, 200J/cm, LDI >1), the percentage having a negative biopsy increased to 83% (10/12 men).

Histopathological analysis of the 6-month biopsies showed that fibrosis was the most frequent histopathological finding after VTP. There were no foci of prostate cancer found within areas of fibrosis.

Gadolinium-enhanced MRI at 1 week

The prostate volume was seen to increase between baseline and 7 days from a mean of 50.7mL to 63.9mL across the 38 men who underwent Day 7 MRI. This swelling reduced over time, and, combined with fibrosis showed a reduction to a mean volume of 41.8mL at 3 months (n=36), and 40mL at 6 months (n=33).

The volume of treatment effect was defined as the volume of hypoperfusion at Day 7 divided by the mean of the intended treatment volume at baseline and at 7 days.

In the 38 evaluable men at Day 7, the mean percentage treatment effect (treatment volume/whole prostate volume) was 38%. In men in the 4mg/kg group (n=33), the mean treatment effect was 42%. The majority of these men had had treatment in one lobe only.

In an exploratory analysis of the treated lobe only, the 12 men of the 4mg/kg group with an LDI $\geq$1 had a mean treatment volume effect (treatment effect/lobe volume) of 95% versus 59% ($p<0.01$) in those with an LDI <1. “

The correlation between the LDI and the volume of effect is shown in Figure 3.

Extraprostatic treatment effect (hypoperfusion outside of the prostate) was observed in 18 of 38 men; in all cases this resolved on follow-up imaging, and was not associated with any clinically evident damage to adjacent organs, fistula or incontinence.

Adverse Events (AEs)

34 patients (81%) reported a total of 131 AEs. All AEs were mild or moderate in intensity, with the exception of a melanoma in one of the subjects, which occurred after a number of months. This was considered severe but unrelated to the either the study drug or the procedure.

28/42 patients (66.7%) experienced at least 1 AE related to the technical procedure and 14 patients (33.3%) experienced at least 1 AE related to the study drug. The most common AEs related to the technical procedure were coded to the Reproductive System and Breast Disorders system organ class (SOC) (haematospermia, n=5; erectile dysfunction, n=4; and perineal pain, n=4) and Renal and Urinary Disorders SOC (dysuria, n=9; hematuria, n=8; and transient urinary retention, n=2). Due to the small number of patients treated at 2 and 6 mg/kg, no analysis can be made on the difference in the level of side effects observed between the treatment doses.

Five patients experienced SAEs of which 3 were related to the technical procedure. Two patients had large extraprostatic necrosis caused by an excessive light dose due to a calibration error; corrective measures were taken immediately to ensure it did not reoccur and both patients recovered without sequelae. One patient had pelvic pain which prolonged hospitalisation by 1 day and was due to the technical procedure. The 2 other SAEs (deep vein thrombosis at 4 months post-procedure and dysuria secondary to a herpetic eruption at 3 days post-procedure) were considered unlikely to be related to the treatment.

Of the 40 patients who received the complete VTP procedure, none withdrew due to AEs.

An analysis of the paper ECG recordings was performed by CardiaBase, an ECG core lab. The results indicated a marked prolongation of the Fridericia-corrected QT (QTcF) interval after the induction of anaesthesia. However, as no further prolongation of the QTcF interval was observed after the injection of WST11, the earlier QTcF changes were deemed due to anaesthetic drugs.

There were some minor changes in the biochemical parameters including transient elevation of hepatic enzymes in 12 men in the immediate postoperative period. This had no clinical consequences, and resolved without specific treatment. The elevation in hepatic enzymes was deemed by the investigator related to anaesthesia, as it is not unusual to observe such an increase after a general anaesthetic. No long-term consequences were expected as these elevations were always mild (mostly <2 x upper limit of normal) and transient.

The safety data of the 40 patients have been reviewed in detail by the Data Safety Monitoring Board. No significant safety issue was identified and the general tolerability of the procedure was considered good.

Quality of Life Questionnaires

Questionnaires were evaluable for 34 patients at baseline and Month 6.

IPSS questionnaire

For all 34 evaluable men, the mean IPSS score for questions 1-7 decreased from 7.3 at baseline, to 6.6 (10.1% mean change) at Month 1, 5.4 (-31.7%) at Month 3 and 5.1 (-17.3%) at Month 6. Of the 29 evaluable men in the optimal treatment group (4mg/kg), mean IPSS score for questions 1-7 decreased from 6.3 at baseline, to 5.6 (15.2% mean change) at Month 1, 3.7 (-36.7%) at Month 3, and 3.8 (-18.1%) at Month 6.

For the quality of life domain of the IPSS score (Question 'How would you feel if your urinary symptoms stayed the same for the rest of your life?'), where a higher score indicates greater unhappiness with symptoms, there was a significant fall in the QoL score from 2.1 to 1.3 from baseline to Month 6 ($p<0.013$). The results were comparable between all patients and those receiving the optimal treatment.

IIEF-5 Questionnaire

For all 34 evaluable men, the mean baseline score was 17.7, falling to 13.8 at Month 1, then rising to 16.5 at Month 3, and 16.6 at Month 6. There was no

statistical difference between baseline and Month 3 or Month 6. Of the 29 evaluable men in the optimal (4mg/kg) treatment group, the mean baseline score was 18.0, falling to 13.3 at Month 1, then rising to 16.4 at Month 3, and 17.0 at Month 6. Of the 25 patients who had a baseline score of over 15, 4 patients (27%) had an IIEF decrease of more than 10 points at Month 6. Over the 6-month period of the study, 4 patients developed de novo erectile dysfunction as defined by a decrease of the IIEF score of 10 points or more.

Discussion

TOOKAD® Soluble VTP exhibits many of the desired attributes of a minimally invasive, well-tolerated procedure that can be targeted to a pre-determined volume of prostate tissue and administered in an ambulatory setting as a one-off procedure. This study has contributed to defining the optimal treatment conditions to achieve predictable ablation volumes within the prostate and so provide a method by which clinicians can selectively ablate target volumes of tissue.

More specifically, biopsy data, post-treatment MRI outputs and analysis of the safety data indicate that 4mg/kg WST11, 200J/cm energy and an LDI $\geq$1 are probably optimal treatment conditions for prostate ablation using the VTP procedure. In addition the Day-7 MRI result proved to be a good predictor of the 6-month biopsy result, with a mean 95% treatment effect on Day-7 MRI correlating with an 83% negative biopsy rate in men receiving the optimal treatment parameters, compared to a mean treatment effect of 58% and a negative biopsy rate of 31% in the 16 men who received 4mg/kg WST11 with an LDI of <1. This correlation is supported by both preclinical data [14] and those from the post-radiotherapy prostate [9].

In terms of tolerability and safety, WST11 fared well. No cases of hypotension were reported, which had been a concern with the previous formulation WST09. All adverse events following treatment were mild or moderate, apart

from 1 severe case (neoplasm) in the 4mg/kg group, which was considered unrelated to the study medication or procedure. There were 5 SAEs, all in the 4mg/kg group. None were considered related to the study drug, but 3 of the 5 were considered to be related to the study procedure: 2 necrosis events caused by excessive illumination due to device malfunction and 1 of pelvic pain, which was considered serious as it prolonged hospitalisation by 1 day.

Additional unexpected benefits were also recorded. The cohort exhibited a fall in IPSS score that was accompanied by a slight improvement in the quality of life. Erectile function was difficult to assess given that many of the subjects recorded poor or absent baseline function. Overall there was mild deterioration.

These results need to be considered in the light of some inevitable methodological limitations. First, the number of patients was small as this was an early phase II trial. As it was a dose escalation study, the results of doses other than 4mg/kg were confined to very small samples. A follow-on study (PCM 203) was designed to explore the use of 4mg/kg WST11 and an LDI of 1 in a larger study population. Following completion of PCM 203 a European multicenter phase III study randomizing men to WST11 VTP or active surveillance was set up and has now completed recruitment and randomization of 400 men.

Second, due to the limited sensitivity of TRUS biopsy, a negative biopsy at 6 months post-VTP procedure does not necessarily confirm the absence of tumour. In the context of low-risk prostate cancer on standard TRUS biopsy, 1 in 4 men may have no cancer found on repeat TRUS, although 1 in 4 may be upgraded or upstaged [15]. We considered the use of transperineal template guided biopsy both prior to and following VTP, but decided that the requirement for 3 general anaesthetics in the 6-month study would be too great a burden for the patient. Men who had a template biopsy at baseline (5/41, 12%) were those enrolled in centres with a high volume of men having template biopsy.

Third, this study had a short follow-up of 6 months, and longer term histological results and patient-reported outcomes are going to be needed in order to establish oncological efficacy.

The attributes of the VTP procedure are very different to existing focal therapy platforms that rely principally on generating extremes of temperature in order to induce coagulative necrosis. These energy sources require an iterative and responsive mode of delivery that is surgeon-driven and is challenging to teach and to learn [16]. WST11 VTP can be planned in advance and the execution is limited to the placement of needles within a sector of the prostate under ultrasound control - a skill that is widely held and easily taught. This, together with the opportunity to be amenable to quality control is a welcome attribute.

This study has permitted a standardisation of the procedure, which is an essential step in the evaluation of any health technologies. It has permitted the design and subsequent approval of a Phase III study in Europe (EUDRACT registration number: 2008-000876-26).

Conclusions

This study has shown that the optimal drug dose, light dose and light density index for prostate ablation using TOOKAD® Soluble are 4mg/kg WST11 activated by 753nm light at a dose of 200J/cm and a light density index of $\geq$1. These parameters applied in a group of 12 men resulted in treatment effect in 95% of the planned treatment volume and a negative biopsy rate at 6 months of 83%.

Further work to assess this within a European phase III randomized controlled trial comparing WST11 VTP to active surveillance is ongoing, with recruitment of more than 400 men completed.

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Conflicts of Interest

Dr. BARRET reports other from Intuitive Surgical, outside the submitted work;
Dr. Scherz reports grants, personal fees and non-financial support from null, during the conduct of the study; non-financial support from null, outside the submitted work; In addition, Dr. Scherz has a patent null issued, and a patent null licensed.

References

1. Cooperberg MR, Broering JM, Carroll PR. Time trends and local variation in primary treatment of localized prostate cancer. J Clin Oncol. 2010 28(7):1117-23.
2. Cooperberg MR, Broering JM, Kantoff PW, Carroll PR. Contemporary trends in low risk prostate cancer: risk assessment and treatment. J Urol. 2007 178(3 Pt 2): S14-9.
3. Klotz L. Active surveillance for prostate cancer: a review. Curr Urol Rep. 2010 11(3):165-71.
4. Parker WR, Montgomery JS, Wood DP, Jr. Quality of life outcomes following treatment for localized prostate cancer: is there a clear winner? Curr Opin Urol. 2009 19(3):303-8.
5. Kirkham APS, Emberton M, and Allen C. "How Good Is MRI at Detecting and Characterising Cancer Within the Prostate?" *European Urology* 50, no. 6 (2006): doi:10.1016/j.eururo.2006.06.025.
6. Moore, CM, Robertson NL, Arsanious N et al. "Image-Guided Prostate Biopsy Using Magnetic Resonance Imaging-Derived Targets: A Systematic Review." *European Urology* (2012) doi:10.1016/j.eururo.2012.06.004. .
7. Valerio M, Ahmed HU, Emberton M et al, "The Role of Focal Therapy in the Management of Localised Prostate Cancer: A Systematic Review." *European Urology* (2013)doi:10.1016/j.eururo.2013.05.048.
8. Huang Z, Chen Q, Luck D, et al. Studies of a vascular-acting photosensitizer, Pd-bacteriopheophorbide (TOOKAD), in normal canine prostate and spontaneous canine prostate cancer. Lasers Surg Med. 2005 36(5):390-7.
9. Trachtenberg J, Weersink RA, Davidson SR, et al. Vascular-targeted photodynamic therapy (padoporfin, WST09) for recurrent prostate cancer after failure of external beam radiotherapy: a study of escalating light doses. BJU Int. 2008 102(5):556-62.
10. Azzouzi AR, Barret E, Moore CM et al. "TOOKAD(®) Soluble Vascular-targeted Photodynamic (VTP) Therapy: Determination of

Optimal Treatment Conditions and Assessment of Effects in Patients with Localised Prostate Cancer." *BJU international* 112, no. 6 (2013): doi:10.1111/bju.12265.

11. Fleshker S, Preise D, Kalchenko V, Scherz A, Salomon Y. Prompt assessment of WST11-VTP outcome using luciferase transfected tumors enables second treatment and increase in overall therapeutic rate. Photochem Photobiol. 2008 84(5):1231-7.
12. Brandis A, Mazor O, Neumark E, Rosenbach-Belkin V, Salomon Y, Scherz A. Novel water-soluble bacteriochlorophyll derivatives for vascular-targeted photodynamic therapy: Synthesis, solubility, phototoxicity and the effect of serum proteins. Photochem Photobiol. 2005 81(4):983-93.
13. Ashur I, Goldschmidt R, Pinkas I, et al. Photocatalytic generation of oxygen radicals by the water-soluble bacteriochlorophyll derivative WST11, noncovalently bound to serum albumin. J Phys Chem. 2009 113(28):8027-37.
14. Huang Z, Haider MA, Kraft S, et al. Magnetic Resonance Imaging correlated with the histopathological effect of Pd-Bacteriopheophorbide (TOOKAD) Photodynamic therapy on the normal canine prostate gland. Lasers Surg Med. 2006 38(7):672–81.
15. Berglund RK, Masterson T, Vora KC, Eggener SE, Eastham JA, Guillonneau BD. Pathological upgrading and upstaging with immediate repeat biopsy in patients eligible for active surveillance. J Urol. 2008 180(5):1964–7; discussion 1967-8.
16. Dickinson L, Hu Y, Ahmed HU, Allen C, Kirkham AP, Emberton, Barratt D. Image-directed, Tissue-preserving Focal Therapy of Prostate Cancer: A Feasibility Study of a Novel Deformable Magnetic Resonance-ultrasound (MR-US) Registration System. BJUI (2013)doi:10.1111/bju.12223.

Figure Legends

Figure 1 Schematic Diagram of Treatment Plans and Corresponding Day 7 MRI Results

The 3 treatment plans for focal therapy of low-risk, early stage prostate cancer by WST-mediated VTP therapy, which were considered by the Treatment Planning Guidance Committee, are displayed. The possible different fibre configurations used are illustrated.

The red circles indicate the possible location of the fibres; the blue circle indicates the position of the urethra in the prostate gland.

The corresponding magnetic resonance imagining (MRI) scans, taken on Day 7, are displayed underneath.

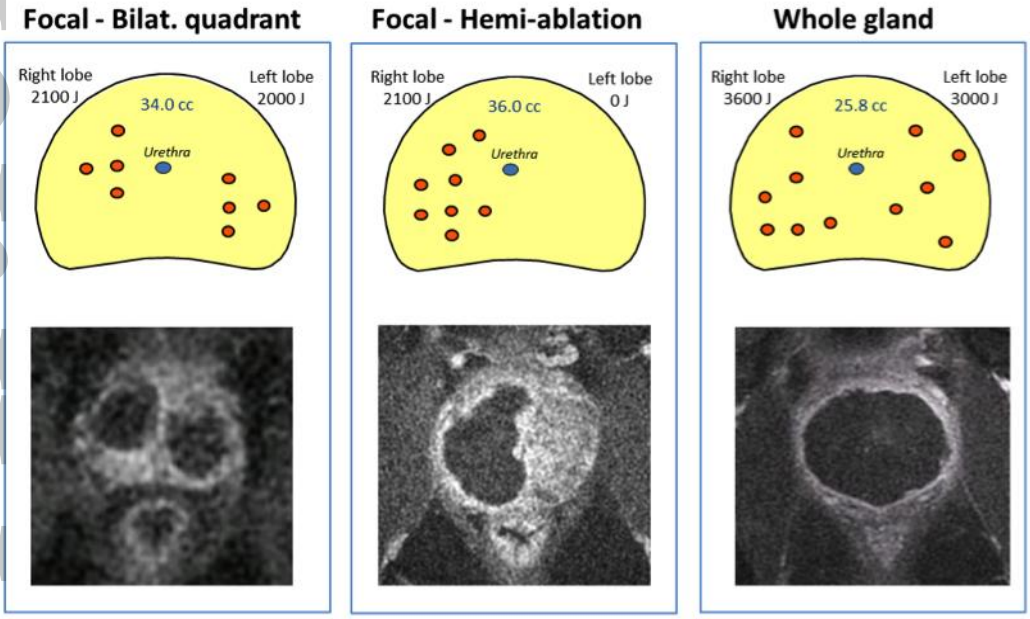
Figure 2 Flow Chart Showing Participants in the Study

The flow chart summarises the participants in the study and the numbers of patients that fell into the different treatment groups, based on WST11 dose (2, 4, or 6mg/kg) and light energy (200J/cm) administered and the number of interstitial optical fibres through which the infusion was delivered.
Four patient populations were defined: 1) Efficacy Evaluable Population included all patients who received the complete VTP procedure, excluding major protocol deviations identified during a blinded review before database lock; 2) Optimal Dose-Energy Population (ODEP) included all patients who received a 4mg/kg WST11 dose, 200J/cm energy dose, with at least 3 fibres and no major protocol deviations; 3) Optimal Treatment Conditions Population included patients from the ODEP that had an LDI equal or superior to the optimal LDI found during the exploratory analysis; and d) Safety Population included all patients who went through any part of the VTP procedure.
LDI = Light Density Index; VTP = Vascular-Targeted Photodynamic

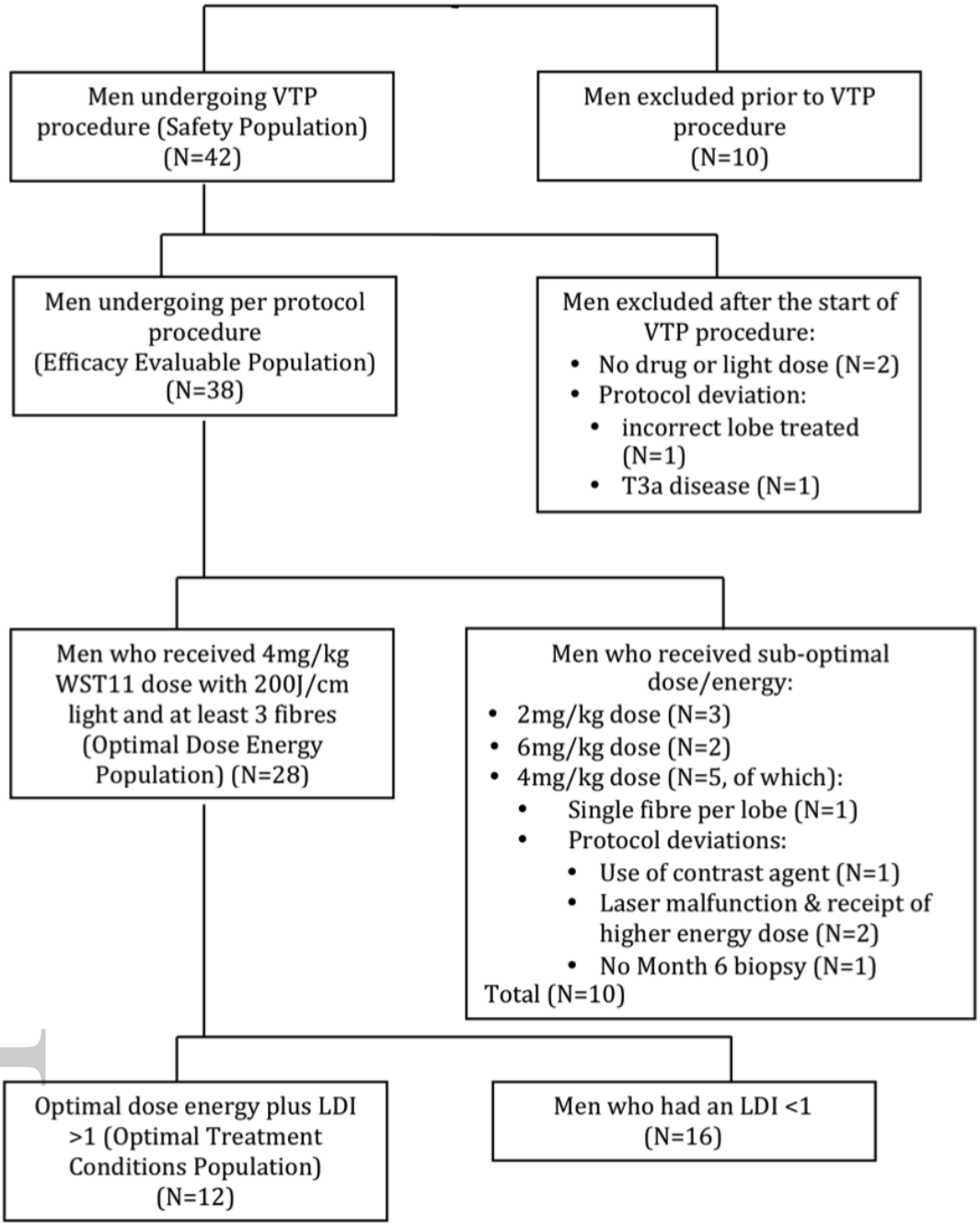
Figure 3 Correlation Between LDI Delivered and Necrosis Volume on Day 7

A positive correlation can be seen between the volume of necrosis measured on the Day 7 magnetic resonance images and the Light Density Index (LDI) delivered.

The red line is the regression line, and the blue lines are the confidence intervals of the regression line



bju_12816_f1.png



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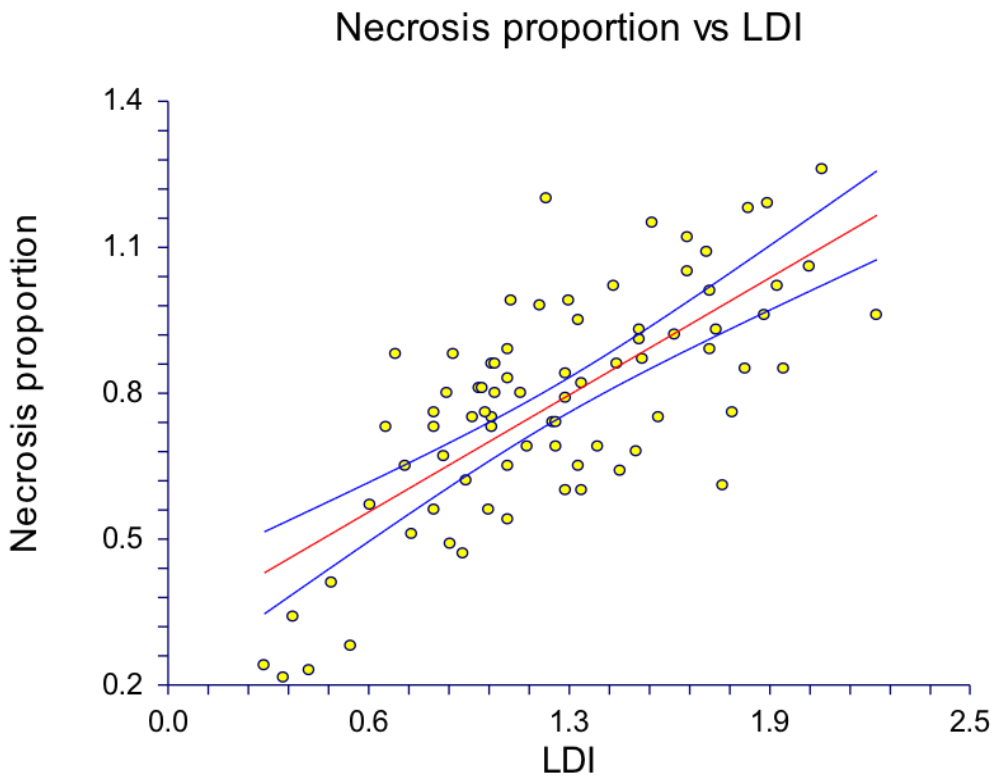

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Table 1 Patient Demographics and Other Baseline Characteristics (Safety Population)

	WST11 Dose Group			
	Mean ± SD			
	2 mg/kg	**4 mg/kg**	**6 mg/kg**	**All doses**
	N=3	**N=37**	**N=2**	**N=42**
Age at enrolment (years)	63.5 ± 5.5	63.7 ± 5.5	63.4 ± 0.3	63.9 ± 5.3
Transrectal biopsy	(n=1)	(n=34) [1]	(n=2)	(n=37)
Gleason score on TRUS biopsy	3 + 3 (n = 1)	3 + 3 (n = 34)	3 + 3 = 2	3 + 3 (n = 37)
Total no. cores analysed	12	12.8 ± 2.8	13.0 ± 1.4	12.8 ± 2.7
Number of positive cores	3	2.3 ± 1.1	1.0 ± 0.0	2.2 ± 1.1
Transperineal biopsy	(n=2)	(n=3)	(n=0)	(n=5)
Gleason score on transperineal biopsy	3 + 3 (n = 2)	3 + 3 (n = 2) 3 + 4 (n = 1)	N/A	3 + 3 (n = 4) 3 + 4 (n = 1)
Total no. cores analysed	85.5 ± 16.3	58.7 ± 18.0	-	69.4 ± 21.1
Number of positive cores	1 ± 0.0	3 ± 1.7	-	2.2 ± 1.6
Clinical staging, n (%)				
T_1	2	34	2	38 (90.5)
T_2	1	3	0	4 (9.5)

Table 2 Biopsy Results at Month 6 According to Analysis Group (All Doses Combined)

Analysis Group	No. Patients	No. (%) of Patients with Positive Biopsies	No. (%) of Patients with Negative Biopsies	p value (compared to 0.4*)
Men who received the complete VTP procedure without a major protocol deviation	38 [1]	17 (45)	20 (53)	-
Patients who had the complete VTP procedure at 4mg/kg with at least 3 fibres and no major protocol deviations	28	13 (46.4)	15 (53.6)	0.10
Men who had 4mg/kg but LDI <1	16	11 (69)	5 (31.3)	
Men who had optimal treatment conditions (4mg/kg, 200J/cm, LDI>1).	12	2 (16.7)	10 (83.3)	0.003

* the p value assess the probability of the observed results happening by chance if the null hypothesis (that the treatment has no effect) were true. The proportion of men who would have a negative confirmatory biopsy in the absence of treatment was taken as 40%, to reflect the proportions of men on active surveillance having a negative second biopsy after an initial positive biopsy