Hypoxic modification in the radiation treatment for prostate cancer

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Kent Yip

Mount Vernon Cancer Centre,
Rickmansworth Road,
Northwood,
HA4 2RN

This thesis is dedicated to Clare, Amelia and Annabelle for their sacrifice, endless support, encouragement and patience.

Declaration

I, Kent Yip confirm that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Introduction

Tumour hypoxia exists among patients with prostate cancer. It is associated with resistance to radiotherapy and increased likelihood of relapse post treatment. The concurrent administration of carbogen and nicotinamide with radiotherapy has been shown to improve survival in patients with bladder cancer and selected patients with head and neck cancer, but this approach has not been attempted previously in patients with prostate cancer. Inhalation of carbogen alone can improve the oxygenation status of prostate cancer as evaluated by functional MR imaging. However, androgen deprivation therapy (ADT) causes collapse in the tumour vasculature, and patients with high risk prostate cancer routinely receive three months of androgen deprivation therapy prior to the start of their course of radical radiotherapy. The ability of carbogen administration to reverse tumour hypoxia during radiotherapy may thus be compromised.

Methods

Fifty patients with high risk prostate cancer were recruited into the single arm phase 1b/II PROCON (PROstate CarbOgen and Nicotinamide) clinical trial. They received carbogen and nicotinamide during their course of radiotherapy after they had undergone three months of neoadjuvant hormone treatment. Prevalence of urinary and gastrointestinal toxicities at two, four and twelve weeks of completing radiotherapy were recorded. PSA progression free and overall survival were calculated using the Kaplan Meier method. Twenty patients among them also underwent functional MR imaging (BOLD, diffusion weighted and dynamic contrast enhanced sequences) before and during their radiotherapy to assess the oxygenation

status of their prostate cancer in response to carbogen and the state of their vasculature.

Results

None of the patients developed grade 3 or worse acute urinary or gastrointestinal toxicity, and the side effect profile is comparable to contemporary clinical trials. Despite the antivascular effect of prior hormone treatment, as confirmed by the drop in the mean Ktrans value (18-25%) following three months of ADT, the application of carbogen remained effective in reversing tumour hypoxia as demonstrated by the mean reduction in R2* value of 5.8% following the administration of carbogen. The 5 year overall survival for the entire cohort was 92%, and the 5 year PSA progression free survival was 87%.

Conclusion

The concurrent administration of carbogen and nictotinamide in patients receiving a course of radical radiotherapy for their prostate cancer is safe, and can improve tumour hypoxia despite the antivascular effect of prior hormone treatment. The 5 year PSA progression free and overall survival rates are comparable to those reported by other contemporary trials for patients with high risk prostate cancer. Future randomised clinical trials involving the use of carbogen and nicotinamide alone, or in combination with other systemic treatments, should focus on patients with hypoxic prostate cancer.

Impact Statement

The lack of oxygen (hypoxia) in prostate cancer can adversely impact on treatment outcome. The results presented in this thesis show that asking patients with prostate cancer to breath in a mixture of oxygen and carbon dioxide (carbogen) during their course of radiotherapy is safe, and that it can improve the oxygenation level in the cancer and may increase the effectiveness of radiotherapy in some patients. A larger study, specifically targeting patients whose cancers have a low oxygenation level, is required to further evaluate this approach, alone or in combination with other therapeutic intervention.

Acknowledgement

This trial was conceived and designed by Dr Roberto Alonzi and Professor Peter Hoskin. They also wrote the protocol and secured the funding from Prostate Cancer UK (formerly Prostate Cancer Charity) for my research fellowship post and the research expense associated with this trial.

I recruited patients who received radiotherapy for their prostate cancer at Mount Vernon Cancer Centre into this study, and was supported by Ms Juliet Valentine and Ms Uma Patel, therapeutic radiographers, in reviewing these patients and collecting their clinical data. Mr James Stirling and Mr Ian Simcock, diagnostic radiographers at the Paul Strickland Scanner Centre (PSSC), conducted the functional MRI scans on these patients.

I outlined the tumours in all the functional MR images, having been taught to intepret these scans by Professor Anwar Padhani and Dr Andrew Gogbashain, consultant radiolgists at the Paul Strickland Scanner Centre. In my subsequent analyses of these imaging data, I was supported by Dr Jane Taylor, research physicist at the Paul Strickland Scanner Centre.

After I had left Mount Vernon Cancer Centre, Dr Niluja Thiruthaneeswaran and Dr Anup Vinayan, oncologists at the Mount Vernon Cancer Centre, collected up to date survival data on my behalf for all the patients in the trial.

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List of publications related to this project

Poster presentation at ASTRO 2017 (San Diego)

"Hypoxia modification during prostate radiation therapy using carbogen & nicotiniamide: toxicity results from a phase 2 study (PROCON)", Thiruthaneeswaran N., Yip K., Valentine J., Patel U., Choudhury A., Hoskin P., Alonzi R. https://www.redjournal.org/article/S0360-3016(17)32162-4/fulltext

Oral presentation at RSNA 2014 (Chicago)

"Hypoxic modification during prostate radiotherapy – an evaluation of changes in the tumour microenvironment using multiparametric MRI", Yip K., Valentine J., Sterling J., Simcock I., Taylor N., Collins D., D'Arcy J., Patel U., Gogbashian A., Hoskin P. Padhani A., Alonzi R. http://archive.rsna.org/2014/14016733.html

Poster presentation at ESTRO 2013 (Geneva)

"PROCON a phase Ib/II trial to evaluate concomitant carbogen & nicotinamide during prostate radiotherapy: early toxicity report", Valentine J., Yip K., Patel U., Hoskin P., Alonzi R. https://www.sciencedirect.com/journal/radiotherapy-and oncology/vol/106/suppl/S2?page-size=100&page=8 (PO-07-03)

Ther Adv Urol 2013 Feb; 5(1):25-34

"Carbogen gas and radiotherapy outcomes in prostate cancer", Yip K. & Alonzi R. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3547529/

1.1 Overview

Each year 1.2 million men worldwide are diagnosed with prostate cancer, resulting in 358,989 deaths [1] . Prostate cancer incidence rises with age [2]. In the UK, 75% of new cases were diagnosed in men aged 65 years or older [3], and post-mortem results have demonstrated the presence of cancer cells in the prostate in 80% of men by age 90 [4]. With an ageing population in most developed countries, the diagnosis and management of prostate cancer is likely to consume an increasing proportion of the health resource. Incidence varies among men of different ethnicities, with those of Afro-Caribbean origin having the highest incidence, and Asian origin the lowest [5].

Prostate cancer is a highly heterogeneous disease in terms of its prognosis. Gleason score, stage, and presenting PSA values are used for formal risk stratification [6]. Different therapeutic strategies are appropriate for patients of different risk group, and these include active surveillance, prostatectomy, radiotherapy (RT), or a combination of RT and surgery with or without androgen deprivation. Compared to active surveillance, early surgical or radiotherapeutic intervention delay disease progression and the development of metastases but do not confer any survival advantage [7,8] for patients with low risk localised disease. For patients whose disease has progressed during active surveillance, both surgery and radiotherapy are acceptable alternatives as neither has shown to be superior [8,9]. Patients' co-morbidities and preferred avoidance of certain side effects usually determine their choice of treatment modality. Combined approach with radiotherapy and androgen

deprivation therapy (ADT), or radiotherapy, surgery and ADT are usually indicated for patients with intermediate or high risk disease [10]

1.2 Radiotherapy for Prostate Cancer

It has been estimated that 60% of patients with newly diagnosed prostate cancer will undergo radiotherapy in the UK [11]. Most of them will receive external beam radiotherapy (EBRT), with the rest treated with brachytherapy. In EBRT photons are generated externally by linear accelerators, whereas in brachytherapy the radioactive sources of photons are actually placed inside the prostate gland itself. This can be achieved either through the permanent placement of iodine-125 (^{125}I) seeds, or the loading of iridium-192 (^{192}Ir) sources through interstitial catheters temporarily inserted into the prostate gland. Apart from the practical differences in their implementation, these two brachytherapy techniques also differ in their rate of radiation delivery. In ^{125}I seeds brachytherapy radiation is delivered at a rate of 0.01 to 0.3Gy/hour, thus it is classified as low dose rate (LDR) brachytherapy. In contrast, the dose rate achieved using ^{192}Ir is 12 to 400Gy/hour, hence this method is termed high dose rate (HDR) brachytherapy. Both LDR and HDR brachytherapy can be used as monotherapy, or as a boost before or after a course of external beam radiotherapy [12–15].

1.3 Hypoxia in tumour microenvironment

The growth and progression of cancer is vitally dependent on its supporting host tissue [16] and the development and maintenance of a functional vascular supply is one the hallmarks of cancers [17]. However, the architecture of this vasculature is chaotic and sub optimal [18], resulting in poor oxygen delivery and tumour hypoxia. As tumour outgrows its blood supply diffusion limited chronic hypoxia ensues; the cyclical temporary collapse of the immature and chaotic vasculature also leads to perfusion limited acute hypoxia.

Hypoxia in the tumour microenvironment has long been established to be associated with inferior prognosis and resistance to both chemotherapy and radiotherapy in a variety of tumour sites [19]. Compromise in repairing DNA double strand breaks under hypoxic condition can drive genetic rearrangements [20,21]. Coupled with the inherent selective pressure exerted by hypoxia, the high mutagenic rates among the heterogeneous mass of cancer cells resulting from this genomic instability will favour the development of tumour clones of a more aggressive phenotype, because changes adopted by cells to survive and thrive under hypoxic conditions are themselves crucial to the process of metastasis [22–24]. These changes include switching to anaerobic respiration for energy production [25] , increasing oxygen delivery by stimulating the growth of new blood vessels [25], and increasing cellular motility to seek out an alternative host environment [26].

Central to these changes is the upregulation of HIF-1, which mediates cellular responses to hypoxia through its impacts on a range of cellular physiological processes, such as angiogenesis, apoptosis, cellular metabolism and proliferation.

HIF-1 is a heterodimeric transcription factor composed of two subunits HIF-1α and HIF-1β. The NH_2 terminus transactivation domain (N-TAD), the COOH terminus transactivation domain (C-TAD), and the oxygen dependent degradation domain (ODDD) are the three main functional domains of HIF-1α. Whilst HIF-1β is constitutively expressed, the activity of HIF-1α is upregulated by hypoxia through two pathways: one von Hippel-Landau gene product (pVHL) dependent and the other one pVHL independent. In the presence of oxygen, HIF-1α is hydroxylated by the HIF-1 prolyl hydroxylases at its two proline residues (P^{402} & P^{564}) in the ODDD. This leads to its polyubiquination by the product of the von Hippel-Landau gene (pVHL) and recognition by proteosomes for degradation. Under hypoxic conditions, the HIF-1 prolyl hydroxylases are inactive. HIF-1α is thus no longer targeted for degradation and is able to translocate into the nucleus, dimerizes with HIF-1β to form a heterodimeric transcription factor to promote the transcription of its downstream effectors. Binding of the co-factor CBP/p300 to the C-TAD in the HIF-1α is necessary to initiate transcription, and under normoxic condition Factor Inhibiting HIF-1α (FIH-1) promotes the hydroxylation of the asparagine residue of HIF-1α (N^{803}) and blocks the interaction between CBP/p300 and C-TAD. The activity of HIF-1α is also subject to hypoxia independent controls mediated by growth factor signalling, Mdm2 and Hsp90 pathways [29]. Downstream effectors of HIF-1 include VEGF (Vascular Endothelial Growth Factor) which promotes angiogenesis; OPN (osteopontin) which regulates inflammatory response and cell migration; GLUT-1 (Glucose Transporter 1) which is involved in cellular metabolism of glucose; lysyl oxidase which regulates endothelial-mesenchymal transition [27,28]; CXCR 4 (C-X-C motif receptor 4) which has been implicated in the extravasation of tumour cells and trafficking of cancer stem cells [29]. Figure 1.1, taken from Masoud and Li [30],

provides a more comprehensive list of the downstream effectors of HIF-1 and their functions.

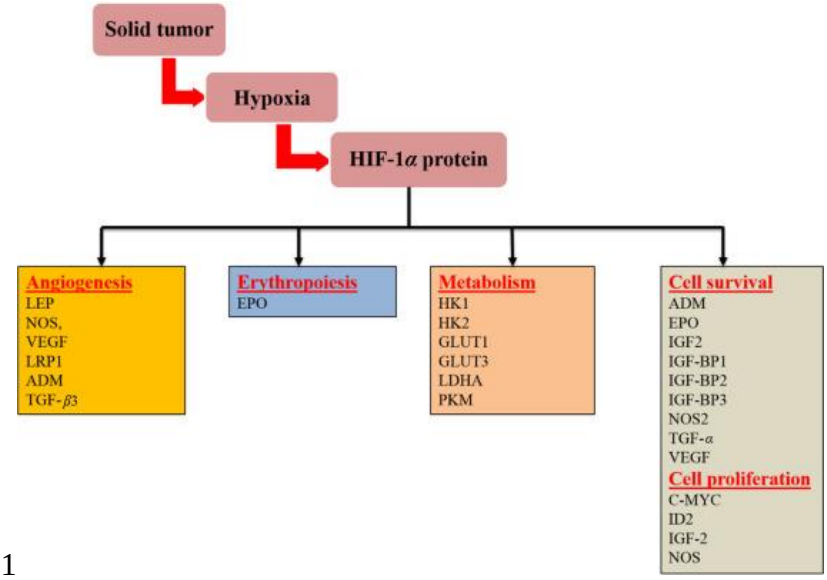


Figure 1.1

1.4 Hypoxia and radiotherapy

The oxygen fixation hypothesis has been the classical explanation for the enhancement of radiation induced cytotoxicity by oxygen [31]. However, there are other mechanisms, such as its role on cell cycle progression and its effect on tumour vasculature which may also contribute to this effect.

Electrons generated by ionising radiation through the Compton and pair production effects interact with water molecules to produce hydroxyl radicals. These cause damage to the DNA by reacting with the various hydrogen atoms of its deoxyribose backbone [32]. In the presence of oxygen, the hydroxyl radicals can form peroxyl radicals. As the resultant DNA damage from peroxyl radicals are much harder to repair compared to those from hydroxyl radicals [31], the delivery of radiotherapy in the presence of oxygen will thus more likely to result in cytotoxicity.

Radiosensitivity is cell cycle dependent. In general, cells are most sensitive to radiation during mitosis and in the G2 phase of the cell cycle, less so in G1, and least sensitive during the latter part of the S phase [33], which may be mediated by an increase in the repair of DNA double strand breaks (DSB) by the homologous recombinant (HR) repair pathway [34–36]. Hypoxia can induce cell cycle arrest in all phases, in particular at the G1/S interface, whilst re-oxygenation can promote resumption of cell cycle progression [37,38].

However, chronically hypoxic cells may potentially be more radiosensitive compared to normoxic ones on re-exposure to oxygen [37,39,40] due to the impairment of their HR mechanism under chronic hypoxia [39,41]. In theory, this impairment of HR can be further exploited for therapeutic gain through the use of an inhibitor of the poly (ADP-ribose) polymerase (PARP) pathway [42]. Oxidative stress from background cellular metabolism results in the formation of single strand breaks (SSB) and nucleotide base damage. PARP is a critical component for their repair. In vitro studies have shown that cells with defective BRCA1 & BRCA2, which are involved in the homologous recombination (HR) repair mechanism of double strand breaks, are particularly sensitive to PARP inhibition [43,44]. Suppression of PARP function prevents background endogenous SSBs from being repaired; the increased number of SSBs will cause the collapse of replication forks and generate double DSBs, which eventually result in cell death if left unrepaired due to defects in the HR-DSB repair mechanism and reliance on the error prone non homologous end joining system [45].

Experimental data by Garcia-Barros et al. [46] and Moeller & Dewhirst [47,48] lend support to the hypothesis that tumour response to radiation may be determined not

only by tumour cell phenotype but also by its microvascular radiosensitivity. The chronically hypoxic tumour micro-environment can become re-oxygenated during a course of conventionally fractionated radiotherapy. As outlined earlier, this improved oxygenation status may be advantageous to radiotherapy by resulting in more lethal RT induced DNA damage and allowing cell cycle progression to the more radiosensitive G2/M phase. However, Moeller et al have shown that this acute re-oxygenation can also lead to the generation of reactive oxygen species. In response, HIF-1 is up-regulated and promotes radio-resistance in the endothelial cells of the tumour vasculature [47]. This protection can be overcome by either blockading HIF-1, or using very high radiation dose per fraction, which can induce endothelial apoptosis by the alternative sphingomyelinase (ASMase) and ceramide pathway [49,50].

The practice of using dose greater than 2 Gray per fraction is termed hypofractionation. Moderately hypofractionated radiotherapy schedules for the treatment of prostate cancer have become the standard of care in the UK after confirmation of their equivalence to conventionally fractionated schedules in terms of toxicities and efficacy [51]. The practical and economic advantages with this approach are obvious – fewer fractions mean fewer hospital visits by the patients and less demand on machine time. In addition, prostate cancer cells are believed to have a low alpha/beta ratio and thus a better therapeutic ratio can be achieved using higher doses per fraction [52]. Improvements in radiotherapy technology in the forms of intensity modulated radiotherapy (IMRT) and image guided radiotherapy (IGRT) have enabled the boundary of dose per fraction to be pushed back further, and "extreme hypofractionation", where the whole RT course for prostate cancer can be completed in as few as 4 fractions, is the norm when stereotactic body radiotherapy is

employed [53]. This is the subject of a current phase 3 clinical trial [54]. When using such schedules, the potential radiobiological advantage of inducing tumour endothelial apoptosis, as discussed earlier, must be balanced against the disadvantage of insufficient time for re-oxygenation to take place. Both classical radiobiology in vitro studies and mathematical modelling [55] have confirmed the detrimental effect of hypoxia on hypofractionation. In fact, the use of hypoxic modifiers was shown to benefit moderately hypofractionated schedules more than conventionally fractionated ones [56], and their use have been strongly advocated in the era of extreme hypofractionation [57].

1.5 Androgen deprivation therapy and tumour hypoxia

The importance of androgen in the development of prostate cancer was established by Huggins and Hodges more than 50 years ago [58,59]. The transcriptional activations of many genes responsible for cellular differentiation, proliferation and apoptosis are driven by the androgen receptor (AR) pathway [60]. In addition, cross talks exist between AR and other known oncogenic factors such as EGF, IGF, TGF-β, VEGF, FGF and MAPK [61,62]. Androgen deprivation therapy (ADT) is the established first line treatment for patients with metastatic prostate cancer; it is also used in the neoadjuvant, concomitant and adjuvant setting for those with non metastatic prostate cancer undergoing EBRT [63–69]. For patients with locally advanced or high risk prostate cancer longer duration of ADT from 2 to 3 years is better than shorter duration of 4-6 months [70,71]. However, whilst patients with intermediate risk prostate cancer also benefit from neoadjuvant and concurrent ADT [72], treatment with ADT beyond the completion of RT may confer no additional benefit [73]. The benefits of ADT in conjunction with radiotherapy for patients with

co-mordities may also be limited [67]. In contrast to EBRT, the benefit of ADT for patients undergoing brachytherapy is less certain, particularly among the elderly [74,75]. ADT, radiotherapy and hypoxia interact with each other in numerous ways. These include the induction of androgen hypersensitivity by hypoxia [76], the enhancement of radiation induced apoptosis by ADT [77], and the anti-angiogenic effect of ADT [77] which can be both beneficial and detrimental to RT efficacy.

Classical teaching states that radiation induced cytotoxicity is primarily mediated by mitotic cell death. However, the role of apoptosis may have been under-estimated [78,79]. The lack of concordance of the results of clinical studies relating the level of apoptosis and treatment outcomes in a variety of human tumours led Hendry and West to conclude that the relative contribution of apoptotic and mitotic cell death in determining the radio-sensitivity and response of tumours might be tumour type and stage dependent [80]. In the era of molecularly targeted therapy, Meyn et al [81] argued that restoration of prompt primary apoptosis directly induced by RT (as opposed to apoptosis secondary to mitotic catastrophe) by novel therapeutic agents might improve tumour response to radiation. In vitro and clinical evidence exist to support an association between BCL-2, an anti-apoptosis protein, and response to irradiation by prostate cancer cells. The use of agents that specifically target BCL-2, such as HA14-1 [82], methyl jasmonate [83] and antisense oligonucleotide [84] , concomitantly with RT all reduced clonogenic survival following irradiation in animal studies. Expression of BCL-2 has been found to be associated with inferior outcome following radiotherapy in numerous reports [85–89]. If apoptosis is involved in mediating radio-sensitivity in prostate cancer, androgen blockade may potentiate the effect of irradiation by negating androgen mediated inhibition of apoptosis [90] . This interaction between irradiation and androgen to determine the

apoptotic fate may involve the c-Jun NH_2 terminal kinase pathway [91–93] which inactivates BCL-2 via its phosphorylation [94,95]. Furthermore, hypoxia may provide the pressure to select for prostate cancer or endothelial cells with increased BCL-2 expression and resistance to apoptosis [96,97].

Hypoxia can up-regulate the androgen receptor pathway, and thus induce the cancer cells to become hypersensitive to androgen, through both ligand dependent [98,99] and ligand independent mechanisms [100]. Whilst ADT may help overcome this hypersensitivity, it can potentially worsen tumour hypoxia due to its effect on the tumour vasculature. Studies using animal models have provided very strong evidence that androgen withdrawal leads to a reduction in tumour blood supply, possibly via the promotion of endothelial cell apoptosis [101–106]. Results from non invasive imaging studies using DCE-MRI in patients with prostate cancer before and after they have received androgen deprivation therapy are supportive of these findings [107–109] . However, the impact of this vascular degeneration on the oxygen status of the tumour environment is less clear. In one animal model study, the HIF 1α protein level was significantly elevated following castration; this would be suggestive of an increase in hypoxia [110]. In another study, Ming et al [111] showed a decrease in tumour oxygenation level by 45% in a LNCaP xenografted tumour model within 24 hours of commencement of bicalutamide. Alonzi et al. confirmed this finding non-invasively by evaluating the tumour oxygen status of prostate cancer patients before and after three months of goserelin treatment using ISW and dynamic MRI [107]. In contrast, Milosevic et al [112], using direct micro-electrodes measurements, showed that pO_2 increased after at least 3 months of treatment with the anti-androgen bicalutamide. With electrode measurements, only a small proportion of the cancer can be sampled, and the position of the needle in relation to the tumour location is

never known with certainty, hence samples may have been taken from benign tissues rather than from the tumours. Also the introduction of electrodes into the prostate might have resulted in a transient artefactual effects, eg, a transient increase in blood flow due to the inflammatory effect caused by the invasive procedure. These may explain the conflicting results obtained by the latter group. We shall return to this discrepancy in more detail in chapter 4.

1.6 Hypoxia and prognosis in prostate cancer – clinical data

Hypoxia has been demonstrated in prostate tumours by immunohistochemical studies, direct measurement and imaging. Carnell et al [113] carried out immunohistochemical analyses on whole prostate specimens from 37 patients who had undergone radical prostatectomy for their localised prostate cancer. Prior to surgery, patients were intravenously given pimonidazole, which was used as an extrinsic marker of tumour hypoxia. Antibodies raised against pimonidazole were used to identify regions of hypoxia in the prostate specimen. Pimonidazole binding was present in the prostate carcinomas of 34 out of the 37 patients. Correlation between strong pimonidazole staining and Gleason score was demonstrated.

Immunohistochemical studies carried out by Vergis *et al.* on 308 patients who had either undergone radical prostatectomy or radiotherapy demonstrated that those whose tumours had an increased expression of HIF-1α and / or VEGF were more likely to experience biochemical relapse following their initial local treatment [114]. Osteopontin expression was predictive of outcome following surgery but not radiotherapy per se. However, further analysis suggested that patients with high osteopontin expression in their tumours, hence those with more hypoxic tumours,

were more likely to benefit from escalation in radiotherapy dose [115]. The prognostic value of hypoxic markers HIF-1α and osteopontin to PSA progression free survival has been confirmed in a separate cohort [116]. They showed that patients with high osteopontin expression were more likely to respond to dose escalation via brachytherapy boost. In addition to OPN and HIF 1α, GLUT-1 expression was also found to be of prognostic value; in contrast to OPN expression, those with positive GLUT 1 expression did not benefit from dose escalation, whilst those with negative GLUT-1 expression did. It was hypothesised that those with GLUT-1 positive disease had such severely hypoxic tumour that dose escalation alone was insufficient to overcome treatment resistance. GLUT-1 [117], together with other hypoxic markers such as VEGF [118,119] and factor inhibiting HIF-1 [120] have been demonstrated to predict treatment outcome in other patient cohorts.

The pO_2 of the tumour microenvironment can be measured directly. Movsas *et al.*[121] inserted Eppendorf pO_2 micro-electrodes into the prostate of 12 patients during surgery or brachytherapy. Muscle readings were used as an internal control. They found that pO_2 measurements from the pathologically involved portions of the prostate to be significantly lower than those from normal muscle or benign regions of the prostate. Using direct Eppendorf electrode measurements, Parker *et al.* showed that localised prostate cancers were often markedly hypoxic and that significant heterogeneity in oxygenation level was present within the prostate tumours of individual patients as well as between patients with similar tumours [122].

Having established the existence of hypoxia in prostate cancer, Movsas *et al*. went on to show that a low prostate cancer : normal tissue pO_2 ratio was predictive of PSA progression free survival following brachytherapy treatment [123]. In a different

cohort of 247 patients with localised prostate cancer, Milosevic et al [124] inserted trans-rectal needle-electrodes under ultra-sound scan guidance into the prostate of these patients prior to their RT. 40 to 80 individual oxygen readings were taken from each patient along 2 to 4 linear measurement tracks through regions of prostate most likely to contain tumour based on previously established radiological, clinical and histological information. The percentage of pO_2 readings of less than 10mmHg (HP_{10}) was used to assess the degree of tumour hypoxia in each patient. They found HP_{10} to be predictive of early biochemical relapse, and suggested that patients with hypoxic tumours were more likely than those with well oxygenated tumours to develop biochemical failure within the first 48 months of completion of treatment. However, caution must be exercised when interpreting results from electrode studies. As highlighted by Stewart et al [125], the insertion of the electrodes themselves can disturb tumour blood flow and hence influence oxygen level. This concern is supported by the experimental findings by van den Berg et al [126], who assessed the tumour oxygenation and blood perfusion of subcutaneously implanted rhabdomyosarcoma tumours in rats following the placement of HDR catheters into the tumour. They found both the tumour perfusion and oxygenation levels to be marked reduced 1 hour after catheter implantation. Moreover, the Eppendorf technique only measured the mean oxygen level in the tract rather than the overall oxygen status of the tumour. Furthermore, in the report by Movsas, the type of anaesthesia used had an impact on the oxygen level readings [121].

1.7 Pre-clinical studies of hypoxia modification during radiotherapy

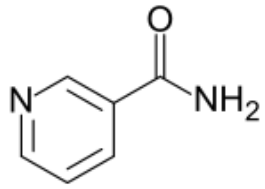
Inhalation of hyperbaric oxygen (> 1 atmospheric pressure) during radiotherapy provides the most direct means to raise oxygen partial pressure (pO_2) in the blood. However, the practical difficulties in the simultaneous delivery of hyperbaric oxygen

and radiotherapy in clinical trials, together with the increase in normal tissue side effects [127,128], have led researchers to favour alternative means to improve tumour oxygen supply.

Carbogen is a normobaric gas mixture of carbon dioxide and oxygen, which is usually administered at one of two concentrations (95% O_2 with 5% CO_2 or 98% O_2 with 2% CO_2). Carbogen has been demonstrated to improve the oxygenation of both experimental and human tumours. However, there is evidence to suggest that, at least in some tumours, enhanced blood flow may also contribute to its action. This gas mixture increases intravascular oxygen availability resulting in greater oxygen uptake by tumours. Carbogen also transiently opens non-functional blood vessels, likely to be a result of the CO_2 component of the gas mixture. This further increases oxygen delivery to regions of perfusion-limited hypoxia and also causes an increased leakage of molecules from the plasma to the extracellular space. Extracellular tumour pH has been shown to decline in response to carbogen gas breathing, in particular if the tumours are large and hypoxic [129].

The rationale for the use of a high oxygen-content gas to improve tumour oxygenation is that the resulting increase in arterial pO_2 will enhance the diffusion of oxygen into the tissue. The addition of 2% or 5% CO_2 was originally proposed to counteract any vasoconstriction induced by pure oxygen breathing. A study in a murine tumour model was performed to determine how the CO_2 content of the inspired gas influences radiosensitivity. Gas mixtures containing 0, 1, 2.5 and 10% CO_2, balanced with oxygen, were compared with 5% CO_2 + 95% O_2. Measurements of tumour oxygenation and perfusion were also made during the breathing of each

gas. The results showed that the level of radiosensitisation achieved is dependent on both the CO_2 content of the inspired gas and the duration of gas breathing. No radiosensitisation was evident following inhalation of 90% O_2 + 10% CO_2. All other gases elicited radiosensitisation. However, that achieved with 100% O_2 disappeared at the extended pre-irradiation breathing time of 45 min. Changes in oxygenation, as measured by pO_2 electrodes, did indicate improved oxygenation status following the inhalation of the gases. However, the time course and extent of the changes did not mirror accurately the changes in radiosensitization. All the gases with a CO_2 content of 2.5% or greater induced a 10-20% reduction in microregional blood flow. This study implies that the decreased radiosensitisation seen at extended breathing times of 100% oxygen is unrelated to blood flow changes. The fact that radiosensitisation is seen with extended breathing times of gases containing 2.5% & 5% CO_2, despite blood flow decreases, is indicative of other overriding physiological changes, perhaps related to oxygen utilization. The studies overall indicate that, at least in the tumour investigated, radiosensitisation is maintained if the CO_2 content of the inspired gas is reduced from 5% to 2.5 or even 1% [130]. There is both pre-clinical and clinical evidence to show that carbogen gas breathing can improve tumour oxygenation in prostate cancer [131]. This translational research in murine xenografts and humans showed a mean reduction in tumour hypoxia of 6.4% ($p = 0.003$) for DU145 xenografts and 5.8% ($p = 0.007$) for PC3 xenografts. 64% of the human tumours showed an increase in tumour oxygenation during carbogen inhalation with a mean improvement of 21.6% ($p = 0.0005$).



Nicotinamide (chemical structure as above), also known as niacinamide and nicotinic acid amide, is the amide of nicotinic acid (vitamin B3 / niacin). Nicotinamide is a water-soluble vitamin and is part of the vitamin B group. Nicotinic acid, also known as niacin, is converted to nicotinamide in vivo, and, though the two are identical in their vitamin functions, nicotinamide does not have the same pharmacologic and toxic effects of niacin, which occur incidental to niacin's conversion. Thus nicotinamide does not reduce cholesterol or cause flushing. In cells, nicotinamide is incorporated into nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). NAD+ and NADP+ are coenzymes in a wide variety of enzymatic oxidation-reduction reactions. In terms of hypoxia modification, nicotinamide appears to reduce impaired tumour blood flow and thus affects the proportion of acutely hypoxic cells. A major component of its activity is the improvement of tumour oxygenation resulting from a reduction in microregional ischaemia. Nicotinamide is known to reduce arterial blood pressure in rodents, suggesting a vascular component in its mechanism of action. A direct effect on supplying blood vessels probably contributes to the oxygenating action of nicotinamide in tumours. Although the precise mechanism remains obscure, studies on murine tumours suggest that nicotinamide, at least at high doses, reduces the occurrence of transient decreases in microregional perfusion [132,133].

Both carbogen and nicotinamide have radiosensitizing properties. When used together in animal models they produce enhancement ratios of 1.9 with conventionally fractionated radiotherapy and 2.8 with accelerated radiation schedules [134–136].

1.8 The concept of synthetic lethality as applied to hypoxia

As discussed earlier, chronic hypoxia may render prostate cancer cells more radiosensitive by decreasing the synthesis of homologous recombination proteins (HR) [39,41]. Chan et al. [137] showed in an ex-vivo clonogenic assay that HR deficient hypoxic cells pre-treated with ABT-888, a PARP inhibitor [138], 24 hours prior to being irradiated, had lower surviving fraction compared to the control. The 24 hour gap between the final dose of ABT-888 and 5 Gray irradiation ensured that the ABT-888 had been washed out completely from the system and thus any synergistic effects observed would be the result of prior SSBs formation induced by ABT-888. Using a lung cancer pre-clinical model, Jiang et al showed that the radiosensitizing effect of Olaparib, a commercially available PARP inhibitor, was only observed in hypoxic tumours associated with down regulation of the homologous recombinant protein Rad51, but not in normoxic tumours. Moreover, this synergistic effect with radiation was mediated by increased unrepaired DNA double strand break following treatment with Olaparib [139].

The results from three early phase clinical trials testing an oral PARP inhibitor appear to substantiate this concept of synthetic lethality whereby cells defective in their HR mechanism are particularly sensitive to PARP inhibition. In the one trial which was open to patients regardless of their BRCA status, durable objective antitumor activity

was observed only in those with BRCA 1 or BRCA 2 mutations [140]; in two subsequent trials which specifically targeted those with the above mutations, objective response or stable disease were seen in 70% of ovarian [141] and 80% [142] of breast cancer patients – quite an impressive figure in a heavily pre-treated population. In a subsequent phase 3 study in which patients with advanced or metastatic ovarian cancer who harboured either a BRCA 1 or BRCA 2 mutation, the use of maintenance Olaparib resulted in an improvement in the 3 year progression free survival from 27% to 60% [143]. Prostate cancer patients with BRCA2 loss showed a 100% response rate to Olaparib in a phase 2 clinical trial [144], and have improved radiological progression free survival when they receive Olaparib compared to enzalutamide or abiraterone after development of castrate resistant cancer in the soon to be presented randomised phase III trial PROfound. [145]. Three early phase clinical trials are currently underway investigating the combination of Olaparib with radiation – two in GBM (NCT02229656 & ISRCTN52658296), and one in head and neck cancer (NCT02229656). It will be interesting to note if hypoxic markers may predict any potential synergism.

1.9 Clinical Studies of hypoxic modification in other tumour sites

A meta-analysis of hypoxia modification during radiotherapy has supported the beneficial impact of combining standard radiotherapy with different methods of hypoxic modification [56]. 10,108 patients treated in 86 randomised trials were identified. The odds ratios for the outcome of loco-regional control and survival were 0.77 (95% CI 0.71-0.86) & 0.87 (95% CI 0.80-0.95) respectively in favour of the interventional group.

The various hypoxia modification strategies tested in early phase clinical trials have been comprehensively reviewed by Oronsky et al [146]. However, only two of them have been incorporated into routine standard clinical practice. They are the use of nitro-imidazole compounds and carbogen with nicotinamide.

Nitroimidazole compounds such as misonidazole, pimonidazole and nimorazole, undergo reductive activation under hypoxic conditions. When they enter a viable cell they undergo a single electron reduction to form a potentially reactive species. In the presence of normal oxygen level they are immediately reoxidised. In hypoxic tissue the low oxygen concentration is not able to effectively compete to reoxidise them; further reductions thus take place, culminating in the association of the reduced nitroimidazole with various intracellular components. These reduced nitroimidazoles can mimick oxygen and render DNA damage induced by radiation more difficult to repair. In the diagnostic setting, they are used to identify areas of hypoxia in immuno-histochemical analyses and nuclear medicine imaging [147]. Therapeutically, the concurrent administration of nimorazole during radiotherapy in the treatment of patients with pharyngeal and supra-glottic laryngeal carcinoma has become the standard in Denmark following the encouraging results of the pivotal DAHANCA 5 trial. In this phase III randomised controlled trial, the use of nimorazole improved the 5 year local regional control rate from 33% to 49% [148]. Agents which are selectively cytotoxic against hypoxic cells are very attractive in principle. Tirapazamine is the most developed drug in this class. Despite its early promise, it has not been shown to augment the effect of radiation in a randomised phase III trial for patients with advanced head and neck cancer [149].

In humans, tolerance to carbogen can be a problem with patients feeling flushed and breathless during inhalation. These symptoms are considerably reduced if 2% CO_2 is used instead of 5% CO_2. A study comparing tumour oxygenation during inhalation of hyperoxic gas containing either 2% or 5% CO_2 has been performed [150]. Tumour pO_2 was measured in 16 patients using the Eppendorf pO_2 histograph. After breathing gas containing either 5% or 2% CO_2 an increase in median pO_2 was measured in every tumour, the frequency of low pO_2 values (less than or equal to 10 mmHg) fell from 47% to 29% in the 5% group and from 55% to 17% in the 2% group. This confirms that breathing 2% CO_2 and 98% O_2 is well tolerated and effective in increasing tumour oxygenation.

Overall, nicotinamide rarely causes side effects, and is considered generally safe as a food additive, and as a component in cosmetics and medication. In the UK, nicotinamide can be purchased over-the-counter from many high-street retailers and pharmacies for use as a nutritional supplement. The British National Formulary lists no drug interactions for nicotinamide. The most commonly encountered side effects were significant nausea and vomiting due to the nicotinamide resulting in reduced patient compliance. The common conclusion is that a dose of 80mg/kg or higher is not feasible and future studies should proceed with a starting dose of 60mg/kg.

Results from a number of phase 2 trials in the use of carbogen and nicotinamide (CON) have been promising [151]. Two phase 3 trials have been reported. In the first trial 333 patients with locally advanced bladder cancer (T2, T3 or T4a) were recruited in the UK [152]. They were randomised to receiving either radiotherapy alone (RT) or radiotherapy plus CON. The radiotherapy regimes were identical in both arms (55 Gray in 20 fractions or 64 Gray in 32 fractions). The primary end point was local

control as assessed by cystoscopic examination at 6 months, and secondary end points were overall survival, local relapse free survival and urinary and rectal toxicities. The median follow up times were 57 months and 60 months respectively for the control and combination arms. The 6 months cystoscopic control rates were 81% for the RT + CON group and 76% for the RT alone arm (p=0.3). The 3 year overall survival rates were 59% for the combination arm and 46% for the RT alone arm (p=0.04), thus representing a 13% absolute survival benefit in favour of the use of CON with RT. Late toxicities were similar in both groups.

A second trial conducted in the Netherlands involving 345 patients with locally advanced laryngeal cancer demonstrated a significant advantage for CON in terms of regional control rate amongst patients with hypoxic tumours as defined by pre-treatment high pimonidazole staining [153]. In this group of patients, there was a 40% absolute difference in their 5 year regional control rate – 100% for the RT + CON group vs 60% for the RT alone group (p=0.01). For patients with low pimonidazole staining there was no significant difference – the 5 year regional control rates were 90% (RT alone) and 94% (RT + CON).

1.10 Introducing multi-parametric magnetic resonance imaging

Analogous to a compass needle which aligns itself with the Earth's magnetic field and points to the north, in a strong magnetic field, protons in water act like tiny dipole magnets and align with the field. However, some of these protons can exist in a higher energy state and align AGAINST the field, like a southward pointing needle. When a radiofrequency pulse is applied in the direction perpendicular to the magnetic field, this "extra" energy from the radiofrequency pulse is absorbed by the protons,

resulting in more of them being in the higher energy state. When the pulse is switched off, the excess energy will be given off as these protons revert to their original lower energy state through their precessions. Their precessions around a receiver coil will in turn induce a current in the coil and generate a signal. These k space signals received are converted through Fourier Transform to generate clinical MR images.

MRI forms part of the routine workup for patients diagnosed with prostate cancer. It is used to evaluate the local extent of the cancer and involvement of pelvic lymph nodes, both of which determine the final staging of the cancer and may influence the duration of adjuvant androgen deprivation therapy following radical treatment with radiotherapy. Prostate cancer appears as hypodense lesions in T2 weighted images. Other additional MR imaging sequences such as MR spectroscopy, diffusion weighted sequences (dw-MRI), dynamic contrast enhanced T1 weighted sequences (DCE-MRI) and blood oxygen level dependent sequences (BOLD) can be obtained and their inclusion form the basis of multi-parametric MR imaging.

1.11 Diffusion weighted MR imaging

Random Brownian motions of free protons in water form the basis of diffusion weighted MR imaging. If all the water molecules in the object being scanned are fixed in their positions, applying a 90 degrees radiofrequency pulse followed in rapid succession by a 180 degrees refocusing pulse should not generate any net signal as their effects would have cancelled each other. However, if the water molecules are free to move, their positions will have changed very slightly in the time interval between the applications of the two pulses. The second 180 degree refocusing pulse

will fail to completely reverse the effect of the first 90 degree pulse, resulting in a net signal. These signals can be used to construct apparent diffusion coefficients (ADC) maps. The less cellular the micro-environment, the freer the water molecules can move and the higher the ADC of the tissue. The ADC value can be calculated by the following equation:

ADC = $\frac{\log \frac{S_0}{S_1}}{(b1-b0)}$ where the b values reflect the strength and timing of the gradients used to generate diffusion weight images. The higher the b value, the stronger the diffusion effect.

52 patients with biopsy confirmed prostate cancer who had undergone radical prostatectomy and been investigated pre-operatively with T2 weighted and diffusion weighted MR sequences were included in the study by Lim et al [154]. They showed that the addition of ADC data to T2 weighted images alone improved the diagnostic sensitivity from 67-75% to 78 - 88%, and specificity from 77-79% to 88-89%. Woodbridge et al. [155] found a negative correlation between the ADC values of the prostate tumours obtained from dw-MRI and their corresponding Gleason score among 57 patients; patients with Gleason 7 and 8 tumours had a lower mean ADC value for their disease compared to those with Gleason 6 tumours. Moreover, the ADC values were also negatively correlated with the percentage of tumour on core biopsies. On the basis of these findings, they suggested that dw-MRI might help to radiologically differentiate among patients with low, intermediate and high risk disease. This was supported by the finding from another research group, who reported a lower mean ADC tumour value in patients with Gleason 3+4 disease compared to those with Gleason 3+3 disease[156].

An increase in the ADC value of prostate tumour after a course of radiotherapy has been consistently reported (Table 1.1). This increase was observed as early as six weeks from the start of radiotherapy [157], and may continue to a year after the end of treatment [158]. Two out of the 46 patients in Song's cohort were found to have local failure and required salvage treatment with HIFU; their tumour ADC did not increase on their first post RT dw-MRI scan [159]. Pasquier et al reported that one patient out of their cohort of twelve experienced biochemical relapse three years after their completion of radiotherapy; this was the same patient whose tumour ADC decreased one year from the end of his course of radiotherapy [160]. Liu et al. demonstrated a statistically significant difference in the post radiotherapy tumour ADC values obtained 5 to 17 weeks from the end of treatment between the thirteen patients who had developed biochemical or clinical failure and the other sixty five who had remained progression free after three years [161].

1.12 Blood Oxygen Level Dependent MR Imaging (BOLD-MRI)

Oxyhaemoglobin is diamagnetic, whilst deoxyhaemoglobin is paramagnetic. The difference in their magnetic properties are exploited in Blood Oxygen Level Dependent MR imaging (BOLD-MRI). The paramagnetic nature of deoxyhaemoglobin allows it to act as an intrinsic contrast. Similar to gadolinium containing extrinsic contrast, the presence of deoxyhaemoglobin induces T2 relaxation in the tissue where it accumulates, resulting in shortened T2* time, and increased R2* value (R2*=1/T2*). The amount of deoxyhaemoglobin in the tissue at any given time will be dependent on both the hypoxic state of the tissue and its blood flow.

Sensitivity and specificity of BOLD MRI in detecting hypoxia in patients with prostate cancer has been assessed by comparing imaging results against pimonidazole staining in the corresponding prostate tissue [162]. Twenty patients who were due to undergo radical prostatectomy had pre-operative BOLD MR scans carried out. In addition, pimonidazole was administered intravenously 12-18 hours prior to their surgery. The MRI slide which contained the largest dimension of prostate tumour was identified and matched to its corresponding histopathological mounted slide of the prostatectomy specimen. A 5mm x 5mm grid was then overlaid on both of them, and each square in the grid was dichotomized as either having high or low pimonidazole staining (hypoxia versus normoxia) and high or low R2* value. BOLD MRI had a sensitivity of 88% in detecting hypoxia which was further improved with the addition of information on relative blood volume (rBV).

Chopra et al. [163] compared the BOLD MRI readings in men with prostate cancer against their corresponding tumour oxygenation level measured directly. Nine men who were due for placement of pre-radiotherapy intra-prostatic fiducial markers had polarographic needle electrode placed into their prostate under ultra sound guidance during the same session. 20-25 partial pressure of oxygen measurements were acquired along a linear track at 0.7mm interval between two read out points. Altogether four sets of measurements were acquired in separate needle tracks in the same region for each patient. The median partial pressure of oxygen (pO_2) values, and the percentage of measurement points with pO_2 less than 5mmHg (the hypoxic fraction) were calculated. Nine patients were recruited into this study and the data from eight patient were used for analysis. A positive correlation was found between the R2* of the tumour and the hypoxic fraction in the tumour (Spearman rank

correlation r of 0.76, p=0.02) and a trend towards negative correlation was observed between R2* and the median partial pressure of oxygen (r = -0.66, p=0.07). These findings would support the utility of BOLD MRI in detecting intra-tumoral hypoxia.

1.13 Dynamic contrast enhanced MR imaging (DCE-MRI)

The presence of gadolinium based contrast induces a shortening in the T1 time of the tissue where it accumulates. Because of the increased vascularity of tumour compared to normal tissues, contrast accumulates preferentially in tumours. Tumours are thus better visualised in contrast enhanced MR scans. This technique is developed further in dynamic contrast enhanced MR imaging to study the vascular aspect of the tumour. A bolus of gadolinium based contrast agent is administered and multiple images of the area of interest are captured at rapid succession at a series of time points. The T1 values of the tumour tissue varies with the changes in the local concentration of contrast as it diffuses from the vessels into the extra-cellular extra-vascular space (EES). Based on these temporal changes, mathematical modelling can be applied to calculate the parameters of interest which reflect the underlying vascular nature of the tumour microenvironment. These include Ve (volume of EES per unit of volume tissue), Vp (fractional blood plasma volume), and most importantly Ktrans (volume transfer constant between plasma and EES). As its name implies, Ktrans reflects the rate at which contrast diffuses from the blood vessels to the EES. This is dependent on both the permeability of the tumour vessels (their "leakiness") and the blood flow to the tumour. In tissues with a "leaky" vasculature, like tumours, the main determinant of Ktrans will be their blood flow. In the commonly applied two compartment pharmacokinetic model, the relationships

between Ve, vp, Ktrans, and concentration at time t of contrast in the plasma [Cp(t)], tissue [Ct(t)] and EES [Ce(t)] are described by the following differential equation:

$$Ve\frac{dCe(t)}{dt} = Ktrans \times \lfloor Cp(t) - Ce(t) \rfloor$$

The solution to this differential equation, as stated by Tofts (http://www.paul-tofts-phd.org.uk/DCE-MRI_siemens.pdf), is

$$Ct(t) = vpCp(t) + Ktrans \int_0^t Cp(\tau)e^{-kep\,(t-\tau)}d\tau$$

In routine diagnostic MR scans, prostate cancer appears as a hypo-dense lesion in T2 weighted MR images. Meta-analysis based on fourteen separate studies involving 484 patients by [164] showed that the Ktrans value is consistently higher for prostate cancer in both the peripheral zone and central gland compared to non-cancerous tissue. A statistically significant difference in the 75th percentile value of ktrans between patients with low and high grade prostate cancer has been reported [165]. The addition of DCE-MRI sequences to routine T2 weighted images was shown to improve the diagnostic specificity (from 37% to 88%) and positive predictive value (from 50% to 75%) compared to T2 weight MRI alone, but at the expense of reducing sensitivity (from 94% to 73%) and negative predictive value (from 89% to 75%) [166]. Similar reduction in diagnostic sensitivity with this approach was demonstrated by Delongchamps; however, this was only the case for lesions in the transitional zone but not for those in the peripheral zone [167]. In contrast, Kitajima showed an improvement in the sensitivity, specificity, positive and negative predictive value with the combined application of DCE-MRI and T2w MRI. Similar

degree of improvement was also seen with the combined application of dw-MRI and T2w MRI. The best result was obtained when all three sequences were used together [168]. In the PROMIS trial, in which multiparametric MRI was found to be superior to TRUS biopsy in diagnosing patients with clinically significant prostate cancer when evaluated against the reference test of template prostate mapping biopsy, all three sequences were incorporated [169]. They went on to suggest that the use of multiparametric MRI to triage men with clinically suspected prostate cancer might lead to an increase in detecting clinically significant cancers and a reduction in the diagnosis of clinically insignificant cancer. This in turn might allow more men to avoid having to undergo TRUS biopsies. The inclusion of both dw-MRI and DCE-MRI sequences when performing multiparametric MRI for patients with prostate cancer was recommended by a UK consensus group [170]. Similar recommendation was made in the second edition of the Prostate Imaging – Reporting and Data System (PI-RADS^{TM}) [171], though more diagnostic weight was put on information obtained from dw-MRI sequences; the main role for DCE-MRI was when diagnosis on the basis of dw-MRI was equivocal, when evaluation of dw-MRI in part or all of the prostate was technically compromised, or when multiple lesions were present in the same patient, in which case the largest DCE-positive lesion would be considered the index lesion. In 99 patients with raised PSA but previous negative standard biopsy procedure, the information provided by multiparametric MRI to guide perineal biopsy resulted in the confirmation of a cancer diagnosis in 67% of them, and over a third of these patients harboured high grade prostate cancer [172]. Both the National Institute of Clinical Excellence (NICE) and European Association of Urology recommend multiparametric MRI as the first line investigation for people with suspected clinically localised prostate cancer [173,174].

The anti-vascular effect of androgen deprivation therapy on prostate cancer has been demonstrated by the use of dynamic contrast enhanced MRI scans in multiple studies. Alonzi et al. showed a drop in the mean values of tumour blood flow, blood volume and vascular permeability among twenty patients who had undergone three months of ADT. A drop in the Ktrans value was observed in 68% of the patients, and the mean Ktrans change for all patients was a 53% reduction compared to their baseline value [175]. Barrett et al. confirmed these findings; the Ktrans fell in 30 of their cohort of 36 patients after three months of hormone treatment, and the mean drop was 56% [108]. Hotker et al. [176] likewise demonstrated a fall in the median Ktrans value in the tumours of thirty men by 48%, from 0.24/min to 0.1/min following their hormone treatment.

Low RN et al. [177] recruited 87 patients with localised prostate cancer who were due to undergo treatment with stereotactic body radiotherapy to take part in their study, in which patients underwent MRI with DCE sequence before their treatment and at the following points from the end of their treatment: two, six, twelve and twenty four months. Their radiotherapy fractionation schedules were either 38Gy/4# to the prostate only or 41.4Gy/23# to the whole pelvis followed by a 21Gy/2# boost to their prostate. When compared to the baseline, the average tumour Ktrans decreased by 40% at two months post radiotherapy, 75% at six months, 82% at twelve months and 87% at twenty four months. This was associated with a reduction in the size and enhancement of the tumours, a reduction in the size of the prostate and a steady reduction in the serum PSA level.

After external beam radiotherapy, the prostate demonstrates diffuse low signal intensity [178] on T2 weighted MR images. As recurrent cancer itself also appears as

a low signal lesion on T2, the similarity between benign tissue and active cancer can make it challenging to diagnose disease recurrence. DCE-MRI scans are particularly suited to detect recurrence, as contrast enhancement of post radiation fibrosis is slow and low, whilst recurrent active cancer will likely be highly vascular resulting in fast contrast uptake.

1.14 Aims of this project

When this project was conceived in 2008, the reported five year biochemical progression free survival rate for patients with high risk prostate cancer ranged from 40% to 69%, with boost dose to the prostate either by external beam radiotherapy or brachytherapy being associated with better survival rate [179–182]. We hypothesised that the use of carbogen and nicotinamide (CON) concurrently with a course of radical radiotherapy to the prostate would result in further improvement to the biochemical progression free survival to this cohort of patients. Whilst this combination was shown to be well tolerated among patients with bladder cancer [152], this approach had never been tested in patients receiving radiotherapy for their prostate cancer. Although the normal tissues at risk are similar for patients receiving radiotherapy for bladder and prostate cancers, those with prostate cancer would receive a higher dose of radiation, albeit to smaller volumes. We wanted to confirm that this approach would be safe in patients with prostate cancer. Furthermore, we hypothesised that the administration of CON would result in improved tumour oxygenation, despite the anti-vascular effect of androgen deprivation therapy, as evaluated by BOLD MRI scans. The conduct and results from the single arm PROCON trial form the basis of this thesis to address the hypotheses raised.

Table 1.1 Changes in ADC of tumour in response to a course of radiotherapy				
No of patients	RT schedule	Time of post RT MRI (Strength)	% change in ADC	Reference
11	78Gy/39#	Week 6 of RT. (1.5T)	14% increase	[157]
25	66 to 74Gy in 2Gy/#. Median Dose 70Gy	1 to 5 months from end of RT; mean – 1.5 months. (1.5T)	88% increase	[183]
37	78Gy/39# or 60Gy/20#	3 months from end of RT. (3T)	37% increase (78Gy/39#) and 55% increase (60Gy/20#)	[158]
46	66 to 74Gy (2Gy/#). Median dose 70Gy	1 to 5 months from end of RT; median – 3.5 months. (3T)	60% increase	[184]

2.1 Objectives of the PROCON trial

PROCON was a single arm phase 1b/II trial conducted at Mount Vernon Cancer Centre (Eudract number: 2010-021886-63) in which patients with high risk prostate cancer received carbogen and nicotinamide (CON) given concomitantly with their course of prostatic radiotherapy. It was opened in December 2011 and closed in September 2013 after recruitment had been completed. 50 patients were recruited in total over 21 months. It was conducted in agreement with the Declaration of Helsinki and the relevant UK laws and regulation. Ethical approval was granted by the South Central Oxford C Research Ethics Committee on 25-May-2011 (11/SC/0064; IRAS ID – 57169).

The objective of the phase 1b part of the study was to evaluate the safety of combining CON with a course of radiotherapy 74Gy/37# to patients with high risk prostate cancer. As outlined in the next chapter, the pre specified safety requirements were met hence the trial proceeded to phase 2. This part of the study had a clinical and an imaging component. The primary objective of the clinical component was to evaluate the efficacy of this therapeutic approach. The secondary objective was to establish the incidence of its associated acute side effects. For the imaging component, the main objective was to assess the change in tumour hypoxia in response to CON as evaluated by BOLD-MR imaging. Changes in other functional MR parameters such as Ktrans and ADC were also evaluated.

2.2 Patients'eligibility

Patients were eligible if they were older than 18 years of age with a life expectancy of more than 5 years based on their other co-morbidities, had untreated, histologically proven high risk prostate cancer as per the D'Amico criteria (any of PSA > 20ng/ml, Gleason ≥ 8, T3 disease on MRI) [6], and for whom radical radiotherapy were considered appropriate. Patients who had T4 disease, or metastatic pelvic nodal involvement on their MRI scan, or metastatic disease on bone scan, or a PSA value greater than 50ng/ml were excluded.

2.3 Treatment

All patients received bicalutamide 50mg od once daily for a total of twenty eight days. They received their first subcutanoues injection of goserelin 10.8mg as a subcutaneous depot after fourteen days of bicalutamide. Goserelin injections were then given three monthly for a total of three years.

Radiotherapy began after three months of androgen deprivation therapy. Fiducial markers were placed in their prostate before patients underwent their planning CT scans, during which contrast was given. The primary clinical target volume (CTVp) included the prostate and the seminal vesicles. The CTVp was expanded by 5mm posteriorly and 10mm in all directions to form PTVp, and the prescribed dose schedule to PTVp was 74Gy/37# over seven weeks. Pelvic nodes below the bifurcation of common iliac vessels, including the internal iliacs, obturator, presacral and external iliac nodes formed the nodal clinical target volume (CTVn). They were delineated according to the guidelines by Taylor et al [185]. The CTVn would be

expanded by 5mm in all directions to form PTVn, and the prescribed dose schedule to PTVn was 60Gy/37#. Rectum, bladder and bowel were outlined as the organs at risk, and the following normal tissue dose constraints were applied during radiotherapy planning.

	Dose(%)	Total dose (74Gy)	Max Vol(%)
Rectum	68%	50Gy	60%
	81%	60Gy	50%
	88%	65Gy	30%
	95%	70Gy	15%
	100%	74Gy	3%
	102%	75.5Gy	0%
Bladder	68%	50Gy	50%
	81%	60Gy	25%
	95%	70Gy	5%
Small Bowel	61%	45Gy	78ml
	68%	50Gy	17ml
	74%	55Gy	14ml
	81%	60Gy	0.5ml
	88%	65Gy	0ml
Sigmoid	61%	45Gy	78ml
	68%	50Gy	17ml
	74%	55Gy	14ml
	81%	60Gy	0.5ml
	88%	65Gy	0ml

Radiotherapy was delivered by static field IMRT. Planar kV images were taken before each fraction of treatment, and the locations of the fiducial markers in the

prostate on the daily pre-treatment kV images were compared to those on the digitally reconstructed radiographs from their planning scans to verify patients' set up on the treatment couch. Throughout the course of treatment, patients took oral nicotinamide tablets (Nicobion®, Mawdsleys Clinical Services, Doncaster, UK) at the dose of 60mg/kg five days per week each morning before attending for radiotherapy. The total dose for each patient was capped at 4g per day, but adjustment for those who became nauseous was permitted as set out below:

CTCAE grade of nausea	Management
≤ grade 1	Administer anti-emetic – no change in nicotinamide dose
Grade 2	Administer anti-emetic – reduce nicotinamide dose to 40mg/kg
≥ grade 3	Administer anti-emetic. Hold nicotinamide until < grade 2 then resume at 40mg/kg. Patients requiring a delay of more than 1 week should discontinue nicotinamide for the remainder of the study period.

During the delivery of radiotherapy, carbogen gas was administered at a flow rate of 10ml/min inside the LINAC room using either a facemask with airtight seal or mouth piece with nasal clip to form a closed breathing system (photo 1). Patients breathed carbogen gas for 10 minutes before and during each fraction of radiotherapy. Those who took part in the MR translational component of this study would breath carbogen gas for around 15 minutes during each MRI scan (photo 2). Some patients might experience a sensation of breathlessness and claustrophobia when breathing carbogen. This would usually be resolved with reassurance and practice, however if persistent and debilitating the patient would continue his treatment without carbogen

breathing. Other anti-cancer or investigational therapies were not permitted from the time of recruitment until the completion of the course of PROCON radiotherapy.

Photo 1 – a healthy volunteer demonstrating the breathing of carbogen via a tight fitting face mask to form a closed breathing system.

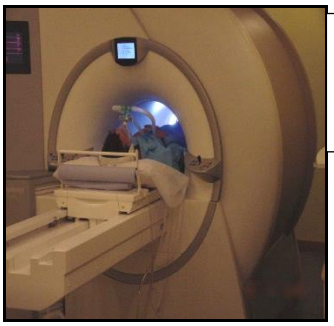


Photo 2 – a healthy volunteer demonstrating the breathing of carbogen during his MRI scan.

2.4 Clinical Assessment

All patients were reviewed at baseline within fourteen days of trial entry, and at two, four, twelve and twenty six weeks after they had completed their course of radiotherapy. Thereafter they would be followed up at six monthly intervals for a total of five years. Each assessment involved a full physical examination and history related to their gastrointestinal and urinary symptoms; these were graded according to the Common Terminology Criteria for Adverse Events (version 4.0). Their PSA value would also be checked. Except for the first four post treatment appointments, the following assessments were performed:

- International Prostate Symptom Score (IPSS)
- Functional Assessment of Cancer Therapy – Prostate (FACT-P) quality of life measurement

- IIEF-5 sexual health questionnaire

2.5 Endpoints

The primary endpoint of the phase 1b part of this trial was the incidence of grade 3 or worse abdominal or pelvic pain, gastrointestinal (GI), or genitourinary (GU) side effect, assessed in accordance to the CTCAE version 4, within four and twelve weeks from the end of their course of radiotherapy.

The primary endpoint for the phase 2 part of the study was the PSA progression free survival rate. PSA progression/biochemical relapse was in accordance to the RTOG-ASTRO 'Phoenix' criteria: PSA nadir +2ng/ml, with PSA progression free survival measured from the first day of androgen deprivation to the date of the PSA blood sample that exceeded the nadir +2ng/ml threshold. Patients who were free from biochemical relapse or lost to follow up were censored at the time when their last PSA measurement was taken. Patients who have died but had been free from biochemical relapse at their last PSA measured before their death were also censored at the time when their last PSA measurement was taken.

The secondary endpoints were the incidence of urinary and gastrointestinal toxicities of all grades, the rate of relapse free survival, and the rate of overall survival. Patients were judged to have had a relapse if they developed either a biochemical relapse as defined above, or radiological relapse as confirmed by bone, CT or MRI scans. Radiological investigations were arranged in clinically indicated symptomatic patients, but not routinely in asymptomatic patients.

In the imaging component of this study, the primary endpoint was the proportion of patients in whom a drop in the R2* value of the tumour was observed in response to the administration of carbogen. Changes in their Ktrans and ADC over the course of treatment, compared to their baseline values, would also be evaluated.

2.6 Sample size calculation and statistical considerations

For the purpose of sample size calculation, our null hypothesis was that the five year PSA progression free survival rate would be less than 40%. The alternate hypothesis was that the five year PSA progression free survival rate would be greater than 60%. 40% was the 5 year biochemical progression free survival rate for patients with high risk prostate cancer reported in one of the largest retrospective cohort who had received treatment with external beam radiotherapy to a median dose of 76Gy [179], and was comparable to the outcome of patients with high risk prostate cancer who had received 64Gy/32# of external beam radiotherapy in the UK RT01 trial [180]. Further investigation with the CON and radiotherapy combination would not be justified should the outcome of our cohort fail to match such a low bar set. With the 2 sided type 1 error rate at 5% and one sided type 2 error rate at 20%, the minimal sample size required was estimated to be 48 using a one sample test of proportion. A target of 50 patients was set to take into account potential loss of patients to follow up. 20 of them would also take part in the MRI translational part of the study.

Baseline characteristics have been summarised using descriptive statistics. PSA progression free survival and overall survival for all patients have been determined using the Kaplan-Meier method. Survival plots have been presented. Safety profile, as evaluated by CTCAEv4, were analysed and presented in terms of prevalence

across dose levels. International Prostate Symptom Score have been presented as an increase / decrease from baseline using descriptive statistics (mean, standard deviation & range).

Comparison between paired samples was conducted using the Wilcoxon signed rank test. The relationship between functional MRI parameters and PSA progression free survival was analysed using the Cox Proportional Hazards Regression model. The relationship between functional MRI parameters and response to carbogen in terms of improvement of the tumour hypoxic state was analysed using the logistic regression model. Correlation between two parameters was evaluated using the Spearman rank correlation test. A Spearman correlation coefficient of less than 0.3 was considered negligible [186]. All tests were two-sided and significance was defined as a p-value of ≤ 0.05. All statistical analyses were carried out using the R Project for Statistical Computing software (https://www.r-project.org), Microsoft Excel and MedCalc (https://www.medcalc.org/).

2.7 Adverse event reporting

All known toxicities from each component of the treatment are listed below and were exempted from being recorded as adverse events. All grades are in accordance with CTCAE version 4.

- Radiotherapy: All grade 1/2 gastrointestinal and genitourinary side effects as listed on CTCAE version 4
- Goserelin: hot flushes and sweating, sexual dysfunction, gynaecomastia, hypersensitivity reactions (rashes, pruritus, asthma, and rarely anaphylaxis), injection site reactions, headache, visual disturbances, dizziness, arthralgia and possibly

myalgia, hair loss, peripheral oedema, gastro-intestinal disturbances, weight changes, sleep disorders, and mood changes.

- Bicalutamide: nausea, diarrhoea, cholestasis, jaundice; asthenia, weight gain; gynaecomastia, breast tenderness, hot flushes, impotence, decreased libido; anaemia; alopecia, dry skin, hirsutism, pruritus, vomiting, abdominal pain, dyspepsia, interstitial lung disease, pulmonary fibrosis, depression, haematuria, thrombocytopenia, hypersensitivity reactions including angioneurotic oedema and urticaria, cardiovascular disorders (including angina, heart failure, and arrhythmias), and hepatic failure
- Carbogen: transient sensation of dypsnoea
- Nicotinamide: allergic reactions, grade 1/2 nausea, vomiting, diarrhoea

All other toxicities not listed above would have been considered as adverse drug reactions, reported to the chief investigator and passed onto the MHRA as indicated.

2.8 Timing for the MR scans in the imaging component of this study

All scans were carried out in the 3T Trio MRI scanner (Siemens Medical Solutions, Malvern, PA) by use of external phased array pelvic coils at the following time points:

Day 1 – baseline, prior to the use of androgen deprivation therapy (ADT)

Day 2 – first scan after three months of ADT

Day 3 – second scan after 3 months of ADT, done within one week of D2

Day 4 – after 5# of radiotherapy

Day 5 – after 15# of radiotherapy

Day 6 – after 37# of radiotherapy

2.9 MR images acquisition & region of interest definition

For T1 weighted dynamic contrast enhanced imaging, volume interpolated breath hold excitation gradient echo (VIBE) dynamic sequences were acquired (TE, 1.42 milliseconds; TR, 4.72 milliseconds; flip angles, 3° & 21°; 18 slices of 3.6mm thickness; 40 time points over a period of 6.49 minutes). A bolus of 0.1 mmol/kg of gadobutrol (Gadavist, Bayer-Schering, Burgess Hill, UK) contrast was administered at 4ml/s using a power injector, followed by a 20mL bolus of normal saline at the same rate.

For Blood Oxygen Level Dependent MR Imaging (BOLD-MRI), five spoiled gradient recalled echo (FLASH) images were acquired for twelve slices of 3.5mm thickness (TE, 4.76ms, 14.3ms, 23.8ms, 33.4ms, 61.9ms; TR, 100ms; flip angle 25°; field of view [FOV] 260mm; 192 x 192 matrix), from which relaxivity (R2*) maps were calculated.

For Diffusion Weighted MRI scans, images were acquired using the echo planar imaging technique (TR, 3500ms; TE, 74ms, FOV 260 x 260mm; matrix, 160 x 120; b values , 0, 1100, 1500 s/mm^2; 20 slices of 3.6mm thickness), from which the values for ADC were calculated and ADC map constructed.

Tumour regions of interest were defined by use of a combination of T2-weighted and contrast enhanced T1 weighted images. In general, an irregular mass of low signal intensity in the peripheral zone seen on the T2 weighted images was considered to represent tumour.

2.10 MRI data analysis

For dynamic contrast enhanced MR images, voxel based calculations were performed by using the customised software analysis package Magnetic Resonance Image Workbench (CRUK and EPSRC Cancer Imaging Centre, Royal Marsden NHS Foundation Trust, Sutton, UK). Signal intensity enhancement on the T1 weighted DCE-MRI images was assessed quantitatively by use of the pharmacokinetic model of Tofts. The cosine model is used for arterial input function [187]. The volume transfer constant of the contrast agent (Ktrans) as a percentage of unit volume of tissue was calculated for every scan. Data from all slices were combined to produce a single global tumour median value.

To calculate R2*, voxel based calculations were performed by using the customised software analysis package Diffusion View (CRUK and EPSRC Cancer Imaging Centre, Royal Marsden NHS Foundation Trust, Sutton, UK). Signal changes on BOLD-MRI were used to calculate the intrinsic T2* relaxivity rate R2*. The R2* values were calculated voxel by voxel from a straight line fit plot of InS_t against TE by least square approach, of which the gradient is –R2*.

To evaluate diffusion weighted images, voxel based calculations were performed by using the customised software analysis package DiffusionView (CRUK and EPSRC Cancer Imaging Centre, Royal Marsden NHS Foundation Trust, Sutton, UK). ADC maps were generated by calculating the ADC value in each pixel of each slice.

3.1 Patients' characteristics

PROCON was a single arm phase 1b/II trial designed to test the safety and efficacy of combining carbogen and nicotinamide (CON) treatment with radiotherapy in patients with high risk prostate cancer. Between December 2011 and September 2013 fifty patients were recruited. Twenty of them agreed to take part in the imaging component of this study, the results of which will be covered in the next chapter. The rates of patients' recruitment is as shown in table 3.1. The primary end point for the phase 1b part of the trial was the incidence of grade 3 gastrointestinal or genitourinary toxicities within 3 months of completion of radiotherapy. The primary end point for the phase 2 part was the five year PSA progression free survival rate.

The characteristics of the fifty patients who completed their treatment under the trial protocol are as summarised in table 3.2. Their median age was 70.4 years (range: 52-81). As per trial entry requirement, all of them had high risk disease according to the D'Amico criteria [6]. Thirty eight patients (76%) had MRI T stage of 3a or 3b. Fifteen patients (30%) had PSA greater than 20 at presentation. Twenty patients (40%) had a Gleason score of 8 or 9. Four patients had three high risk factors, fifteen patients had two high risk factors, and thirty one patients had one high risk factor.

3.2 Acute treatment related toxicities and compliance to trial treatment protocol

Our first three patients recruited in December 2011 had all completed their course of radiotherapy by the beginning of March 2012. None of them had developed any

grade 3 or worse genitourinary or gastrointestinal toxicity within four weeks from the end of their treatment, hence as stipulated in the trial protocol, three more patients were recruited in April 2012. The last patient in the second cohort completed his radiotherapy in June 2012. None of the first six patients had developed any grade 3 or worse genitourinary or gastrointestinal toxicity within three months from the end of their treatment, therefore the trial moved onto phase II in October 2012. Recruitment was completed in September 2013 after forty four more patients took part.

One patient (No 18) was admitted to hospital with grade 3 jaundice which self resolved. This was thought to be related to his nicotinamide and registered as a serious adverse event. His nicotinamide was stopped after 35 fractions of radiotherapy. No other grade 3 toxicities was seen. The prevalence of patients with grade 1 and grade 2 genitourinary or lower gastrointestinal toxicities during this period are summarised in table 3.3. In terms of gastrointestinal toxicities, the prevalence of grade 1 and grade 2 toxicities peaked at two weeks after completion of radiotherapy, and patients settled to their pre-treatment state twelve weeks afterwards. Recovery from genitourinary side effects appeared to take longer; their prevalence also peaked at week two but remained higher than baseline at week twelve.

In terms of evaluating their obstructive urinary symptoms, patients were classified into three groups according to their IPSS scores: 0-7 (mild symptoms), 8-19 (moderate symptoms) and 20 or more (severe symptoms). Their IPSS scores peaked at week 2. Table 3.4 summarises the median IPSS scores at each time point, and the p-value of Wilcoxon signed rank test conducted. The prevalence of patients with different severity of obstructive urinary symptoms at each time point (baseline, 2, 4,

and 12 weeks from the end of radiotherapy) are shown in figure 3.1. These differences are statistically significant, with a p value of 0.001905 (Chi-square test).

During the course of radiotherapy, twenty five patients (50%) developed nausea - six patients (12%) had grade 1 nausea, seven (14%) grade 2, and twelve (24%) grade 3. All of them were prescribed anti-emetics. The dose of nicotinamide was reduced to 40mg/kg in eleven of them, and nine of them had a break from taking nicotinamide. Three patients (6%) eventually had to discontinue nicotinamide despite dose reduction and treatment break. One of them (no 15) stopped after 2 fractions and had to take a four days break from his course of radiotherapy to recover. One patient (No 29) stopped after 7 fractions of radiotherapy. One (No 18) stopped after 35 fractions when he became jaundiced, as mentioned already, in addition to feeling nauseous. Figure 3.2 shows the percentage of planned nicotinamide dose received by our cohort of patients during their treatment. 74% (37 patients) of our cohort completed their planned course of nicotinamide with no dose reduction or delay, and only 18% (9 patients) received less than 80% of their planned dose.

Carbogen had to be abandoned in one patient after nine fractions of radiotherapy because he was unable to tolerate the tight fitting face mask; he also could not use the mouthpiece because he was unable to breath through his mouth without also breathing through his nose at the same time. All fifty patients received neoadjuvant androgen deprivation therapy and completed their prescribed course of radiotherapy 74Gy/37#. Their median duration of radiotherapy were 52 days, ranging from 48 to 56 days. The most common reason for treatment interruption was public holidays.

3.3 PSA progression free and overall survival

Survival data was last updated on 25-1-2019. The median duration of follow up was sixty months. Five patients had died. The cause of death was established for three of them (myeloma, gastric cancer and aspiration pneumonia). The five year overall survival rate for the entire cohort was 92% (figure 3.3). The PSA values for all five patients had remained suppressed prior to their deaths hence in terms of PSA progression free survival they were all censored at the time of their last PSA measurement. Three patients were lost to follow up with suppressed PSA at their last appointment, and they were censored at 18, 24 and 36 months respectively.

Six patients experienced biochemical progression. One patient (No 14) developed PSA progression at 30 months and subsequently radiologically confirmed metastatic disease 36 months from the end of his radiotherapy. The 3, 4, and 5 years PSA progression free survival rate are 93%, 90% and 87 % respectively as shown on figure 3.4.

3.4 Quality of Life

The number of patients who have completed their FACT-P quality of life questionnaires are shown in figure 3.5. As expected, this droped over time, but data was available for at least 40 patients up to two years after the completion of radiotherapy. The mean scores at each follow up time point for the entire cohort are shown in figure 3.6. The baseline value was 1.7 (0-5), reaching a peak of 3.2 (0-6) two weeks after the end of treatment before recovering to 1.6 (0-4) at the two year time point.

3.5 Discussion

Compliance and toxicities associated with nicotinamide and carbogen treatment in our trial population is comparable to the other larger studies involving the use of CON. Nausea was reported in 40% of the patients who received 60mg/kg of nicotinamide in the ARCON trial for head and neck cancer [188]. In the BCON trial, 63%, 35%, and 12% of patients in the 64Gy/32# cohort developed at least grade 1, 2 and 3 nausea and vomiting [189]. The corresponding figures for our study are 50%, 38% and 24%. Our higher incidence of grade 3 nausea compared to the BCON trial is likely to be due to the larger volume irradiated with the use of whole pelvic nodal radiotherapy, and possibly higher radiation dose given to the prostate. Our rate of nicotinamide discontinuation of 6% is similar to the 10% reported by the ARCON in which patients were prescribed prophylactic anti-emetics routinely. They introduced this practice after 32% of their second cohort of patients had to discontinue nicotinamide. Despite our higher incidence of grade 3 nausea compared to BCON, our nicotinamide stoppage rate of 6% is lower than their reported rate of 33-35%. The rate of anti-emetics' use was not reported for the BCON trial, and it is not possible to ascertain if our lower rate of discontinuation may be due to the earlier use of anti-emetics in our patients.

The rate of grade 1 and grade 2 genitourinary and gastrointestinal toxicities in our trial are compared to those reported in the ChiPP, Dutch HYPRO and RTOG 0126 trials and summarised in table 5. These are all trials in which patients received at least 74Gy of radiation to their prostate. In the CHHiP trial, the prevalence of genitourinary and gastrointestinal toxicities peaked at the end of the course of

radiotherapy in all three arms. We did not collect acute toxicity data during the course of radiotherapy, but the week 10 time-point (10 weeks form the start of radiotherapy) for patients in the 74Gy/37# arm of CHHiP roughly corresponds to our week 2 and week 4 time-points. Toxicity data at the corresponding time points were available three months after the completion of radiotherapy in Dutch HYPRO and the RTOG 0126 studies. Except for grade 1 genitourinary toxicity rate of 58% at week 12, our toxicity rates were broadly in line with those observed among the patients recruited into the other three trials. This is despite our larger irradiated volume to cover pelvic lymph nodes. Our use of IMRT is likely to have reduced the volume of rectum, bowel and bladder receiving high dose of radiation (>50Gy), at the expense of increasing the volume receiving lower dose of radiation (<50Gy). This may explain the higher prevalence of grade 1 urinary toxicities three months from the end of treatment.

As expected, the IPSS scores increased in most patients soon after they had completed their course of radiotherapy. Nearly 90% of patients experienced grade 1 or grade 2 urinary toxicities. Even though more than 60% of patients were still troubled by urinary side effects three months after they had completed their course of radiotherapy, albeit mostly grade 1 toxicities, the drop in their IPSS scores would be suggestive of an improvement in their urinary flow.

The increase in the FACT-P quality of life score soon after treatment has finished is likely to have been driven by the associated increase in physical discomfort. The disappointing drop in the completion rate of the FACT-P questionnaires beyond two years may partly be due to the loss of patients to follow up; the long time required to complete this questionnaire might have also discouraged patients. Using an

alternative quality of life instrument, such as the shorter version of the expanded prostate index composite (EPIC-26) [190], which has been recommended by the International Consortium for Health and Outcome Measurements and is more suitable for use in the routine clinical setting, might have resulted in more data being collected [191].

In terms of assessing the tolerability of combining radiation treatment with carbogen and nicotinamide, the incidences of acute gastrointestinal and urinary toxicities have been the focus of this trial. However, long term late side effects which occur months to years after the completion of treatment are also important toxicity endpoints. Unfortunately, these have not been captured systematically in this cohort of patient.

The five-year biochemical progression free survival rate of 87% for our cohort is in line with our pre-trial expectation of 70% and comparable to those reported for patients with high risk prostate cancer in other trials who have received 74-78Gy of radiotherapy; they ranged from 57% to 86.5% [51,180,192]. Eleven patients (22%) had either died (5 patients) or developed PSA progression (6 patients). This is comparable to the progression event or death rate of 17-21% reported in the CHHiP trial [50].

One of the main purpose of a phase 2 trial is to provide efficacy data to decide if the new treatment on trial should be evaluated further in a larger phase 3 trial [193]. Whilst we have established that it is tolerable and safe to administer CON concurrently with a course of prostate radiotherapy, the single arm design of this study renders us unable to estimate the degree of any potential therapeutic benefit with the addition of CON to the standard of care. A randomised phase 2 design with a

control arm not receiving CON would have provided us with a contemporaneous comparator to evaluate the efficacy of the new approach, provided the two arms are well balanced. Bearing in mind that cross trial comparison may lead to misleading conclusion due to the differences in study design and heterogeneity in the patients' population, the 5 year PSA progression free survival of 87% in our study is comparable to that reported for the high-risk cohort in the CHHiP trial which was 86%. However, patients in the CHHiP trial did not receive any hormone treatment post radiotherapy, in contrast to the three years of adjuvant ADT mandated in our study. This would have given our result an unfair comparative advantage when PSA progression was the primary event of interest. On the basis of this indirect comparison, taking our current study further to a phase 3 trial for unselected patients with high risk prostate cancer is unlikely to result in an improvement in the biochemical progression free survival for these patients. More targeted selection of patients on the basis of their hypoxic biomarker is likely to be necessary in any future trial with the addition of hypoxic modification. Plans are in place to consider incorporating the design of PROCON into the Pivotal Boost trial which is currently open [194]. Some of the therapeutic effect of nicotinamide may be mediated by its inhibition of the PARP enzyme [195], and we had planned to substitute nicotinamide with olaparib to evaluate its safety when combined with radiation before the support was withdrawn by AstraZeneca. In view of the recently announced positive result for the PROfound trial [145], it may be worth re-exploring this approach.

Table 3.1 – rate of patients recruitment from Dec 2011 to Sept 2013

Phase	Period	Number of patients recruited	No of patient recruited/month
1	Dec 2011	3	3
1	April 2012	3	3
2	Oct 2012 to Sept 2013	44	4

Table 3.2 – summary of patients' characteristics

Gleason Score (ISUP grade group)	No of patients (percentage)
6 (1)	5 (10%)
3+4=7 (2)	9 (18%)
4+3=7 (3)	16 (32%)
8 (4)	14 (28%)
9 (5)	6 (12%)
MRI T stage	
T2a-2c	12 (24%)
T3a-T3b	38 (76%)
PSA level at presentation	
<10	20 (40%)
10-20	15 (30%)
>20	15 (30%)
Median PSA = 12.85 (range: 2 to 54.4)	

Table 3.3 – prevalence of genitourinary and lower gastrointestinal toxicities within 3 months from the end of treatment

No of patients (%)

	Grade	Baseline (pre-RT)	2 weeks post RT	4 weeks post RT	12 weeks post RT
GI toxicity	Missing data	0	0	0	2 (4%)
	0	37 (74%)	29 (58%)	37 (74%)	36 (72%)
	1	11 (22%)	16 (32%)	12 (24%)	10 (20%)
	2	2 (4%)	5 (10%)	1 (2%)	2 (4%)
GU toxicity	Missing data	0	0	0	2 (4%)
	0	27 (54%)	7 (14%)	11 (22%)	17 (34%)
	1	19 (38%)	35 (70%)	33 (66%)	29 (58%)
	2	4 (8%)	8 (16%)	6 (12%)	2 (4%)

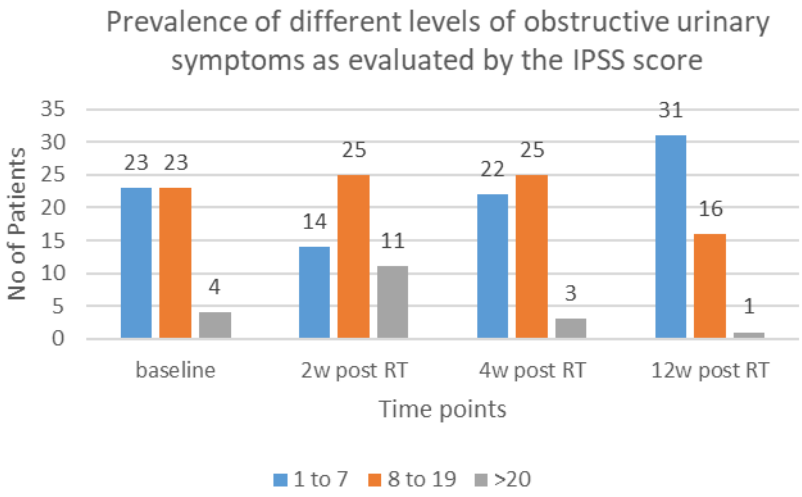
Table 3.4 – median IPSS score at each time point post radiotherapy

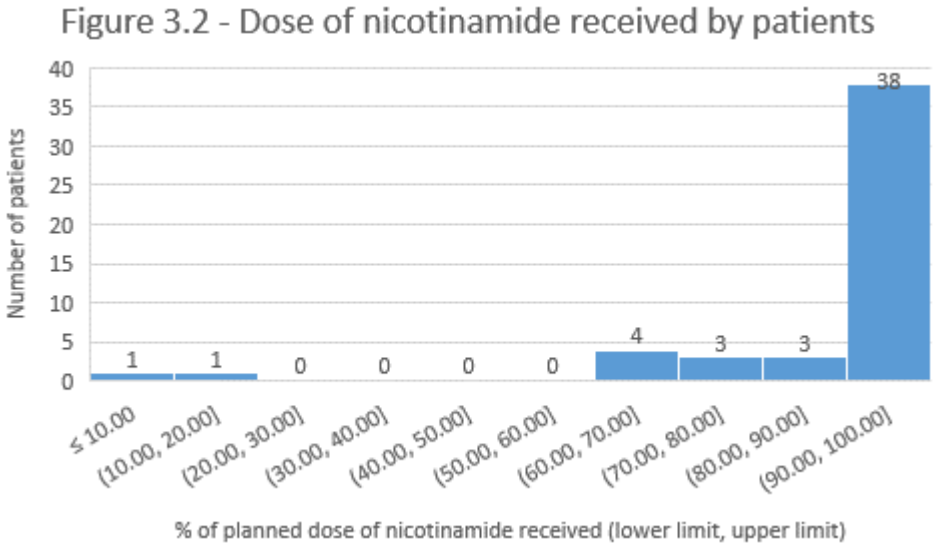
	Time point			
	baseline	Week 2 post RT	Week 4 post RT	Week 12 post RT
Median IPSS score (range)	8 (1-28)	13 (3-34)	9 (1-25)	6 (0-19)
p-value		vs baseline (<0.00001)	vs baseline (0.02884) vs week 2 (<0.00001)	vs baseline (0.02664) vs week 4 (0.0001503)

Table 3.5 – prevalence of grade 1 or 2 gastrointestinal and genitourinary toxicities in other trials

	grade	CHHiP [51]		Dutch HYPRO [192]		RTOG 0126 [196]		PROCON	
GI	G1	week 10	40%					weeks 2, 4	32%, 24%
	G2	week 10	10%					weeks 2, 4	10%, 2%
	G1	3m	20%	3m	14%	3m	8%	week 12	20%
	G2	3m	5%	3m	12%	3m	5%	week 12	4%
GU	G1	week 10	70%					weeks 2, 4	70%, 66%
	G2	week 10	20%					weeks 2, 4	16%, 12%
	G1	3m	30%	3m	28%	3m	20%	week 10	58%
	G2	3m	<5%	3m	16%	3m	15%	week 10	4%

Figure 3.1 – prevalence of different levels of obstructive urinary symptoms as evaluated by the IPSS score





Percentage of planned dose of nicotinamide for each patient is calculated as below:

$$\frac{[(\text{number of days on 60mg of nicotiamide x 60}) + (\text{number of days on 40mg of nicotiamide x 40})] \times 100}{60 \times 37}$$

Figure 3.3 – Kaplan Meier Curve of the overall survival

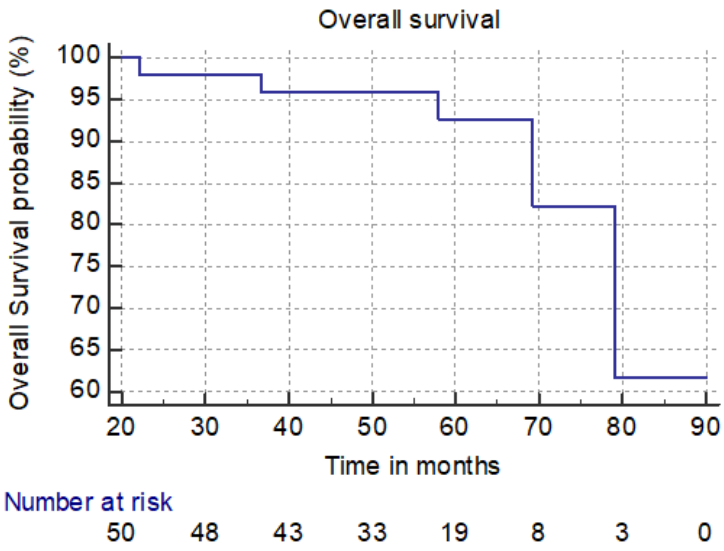
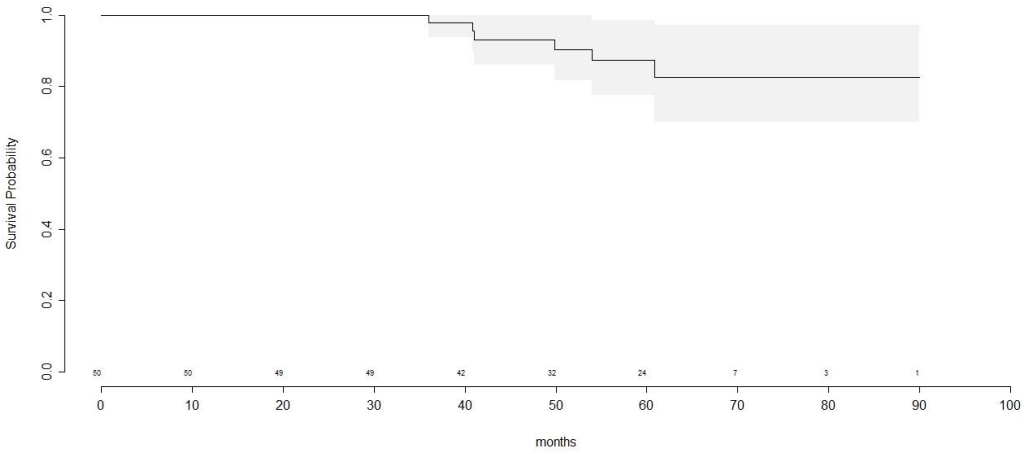


Figure 3.4 – Kaplan Meier Curve of the PSA progression free survival (number of patients at risk at each time point are listed above the x-axis; confidence interval shaded in grey)



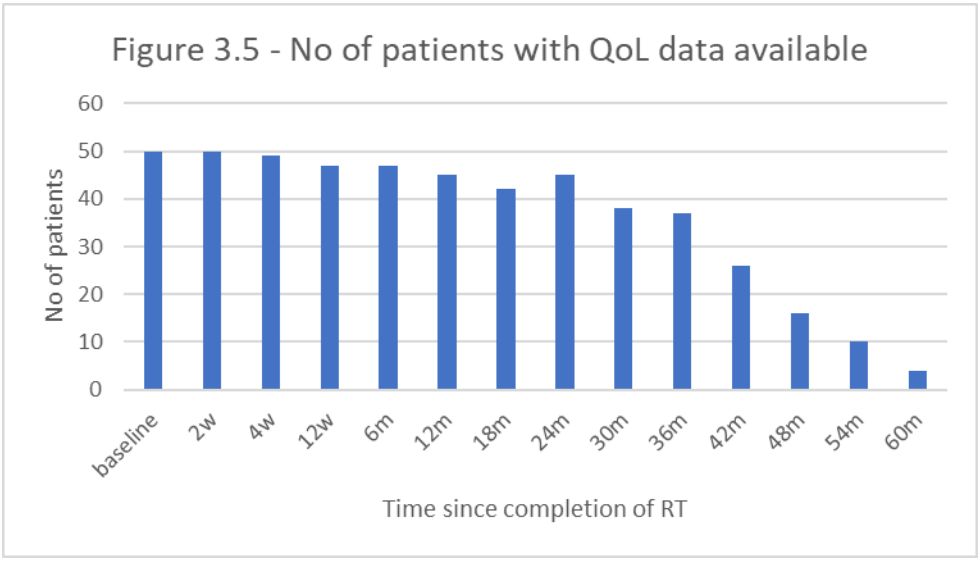


Figure 3.5 - No of patients with QoL data available
No of patients
60
50
40
30
20
10
0
baseline
2w
4w
12w
6m
12m
18m
24m
30m
36m
42m
48m
54m
60m
Time since completion of RT

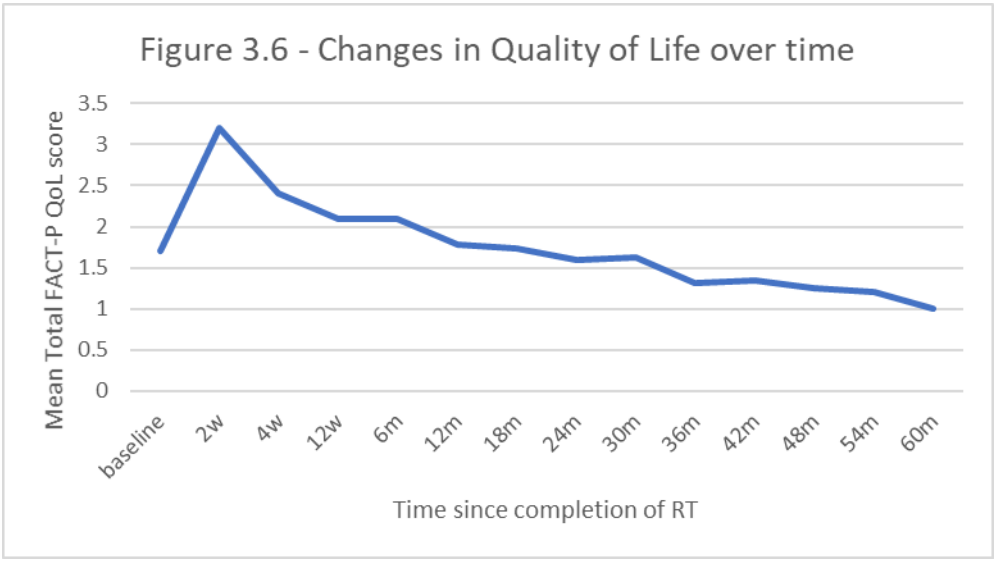


Figure 3.6 - Changes in Quality of Life over time
Mean Total FACT-P QoL score
3.5
3
2.5
2
1.5
1
0.5
0
baseline
2w
4w
12w
6m
12m
18m
24m
30m
36m
42m
48m
54m
60m
Time since completion of RT

4.1 Practical and technical difficulties with data collection and analysis

Twenty patients consented participated in the imaging part of the PROCON study. One patient (patient 35) subsequently withdrew his consent from this part of the study but remained in the clinical part of the study and he was not replaced. One patient (patient 34) with metallic hips had his DCE sequences suspended because the specific absorbed ratio of his artificial hips would have been breached had he completed all 6 DCE-MR scans; however he was able to complete 5 BOLD and dwMRI scans (D1, D2, D3, D4 and D5) before withdrawing from further imaging. The day 2 DCE sequences was omitted in error in patient 27. The day 3 dwMRI sequences was omitted in error in patient 43. Three patients missed one day of their scans for logistic reasons (patient 19 missed day 3; patient 14 missed day 4; patient 32 missed day 5). Hence fourteen patients in total completed all the MRI sequences at all six time points. The total number of datasets generated for analyses were 110 for BOLD-MRI, 109 for dwMRI and 105 for DCE-MRI, as summarised in the table 4.1.

BOLD signals captured using fast-gradient-echo imaging can suffer from substantial signal dropout caused by inhomogeneities in the static magnetic field. These field inhomogeneities occur near air/tissue interfaces because they are generated by variations in magnetic susceptibilities [197]. Susceptibility artefacts increase with the main magnetic field strength, and are slightly larger at 3T compared with 1.5T MR imaging [198]. van Buuren et al demonstrated that rectal gas could distort the measurement of R2* in the prostate causing an increase in the R2* value [199].

For the 110 BOLD-MRI datasets, 25 of them were excluded from the paired pre and post carbogen analysis because either both the pre and post carbogen scans (19 datasets), or their post carbogen scan alone (6 datasets), were too distorted by susceptibility artefact due to rectal peristalsis for the tumour region of interest to be accurately delineated. This left 85 pairs of pre and post carbogen datasets suitable for analysis. For analysing the changes in pre-carbogen R2* over the course of treatment, data from 91 datasets were used – these include the 85 datasets used for the paired analysis plus the 6 datasets in which the pre-carbogen, but not the post-carbogen, scans were suitable for region of interest definition.

For DCE-MRI analysis, the Ktrans value for patient 27 on day 2 could not be calculated due to failure of the model to fit the data points. This left 104 datasets suitable for analysis. No technical difficulty was encountered in calculating the ADC value from the 110 dwMRI datasets. The number of patients whose datasets were suitable for analysis at each time point and for each MR sequences are shown in table 4.2.

4.2 Results

4.2.1 The changes in Ktrans during the course of treatment

Figure 4.1 and table 4.3 show the mean percentage change in the Ktrans value of the tumour region of interest over the course of treatment compared to the baseline values. Figure 4.2 shows the number of patients with changes in their Ktrans at each time point.

The mean absolute Ktrans values between Day 2 and Day 3 (D2-D3 mean), measured within one week of each other after three months of androgen deprivation therapy but before the start of radiotherapy, for each patient were calculated and used to represent the change after three months of androgen deprivation therapy. These were then compared to their corresponding values at baseline (before the start of ADT), day 4 (after 5# of RT), day 5 (after 15# of RT) and day 6 (end of RT) using the Wilcoxon signed rank test. The changes in Ktrans were significant at all time points (decrease after three months of ADT, and increase after the start of radiotherapy), with the following p values - 0.04828 (D2-D3 mean versus baseline), 0.0006561 (D2-D3 mean versus D4), 0.004639 (D2-D3 mean versus D5) and 0.01823 (D2-D3 mean versus D6).

4.2.2 The changes in R2* in response to carbogen administration

Figure 4.3 shows the mean percentage change in R2* after the administration of carbogen at the six time points. Figure 4.4 shows the number of patients with changes in their R2* after breathing in carbogen. Table 4.4 shows the p-value in the comparison between the paired pre and post carbogen R2* values at each time points.

For the thirteen pairs of data collected prior to the use of androgen deprivation therapy, the administration of carbogen resulted in a drop in the R2* value in 6/13 (46%) patients. The mean percentage change in R2* for all thirteen pairs of data after the administration of carbogen was an increase of 4.6% (p=0.29). For the subsequent time points, the number (percentage of patients) whose tumour R2* dropped following administration of carbogen were 11/14 (79%) on Day 2, 5/15 (67%) on Day 3, 9/14 (64%) on Day 4, 9/16 (56%) on Day 5 and 9/13 (69%) on Day 6. Whilst none of the changes at each individual time point was statistically significant (Table

4.4), by pooling together the seventy two pairs of data collected after three months of androgen deprivation therapy (Day 2 to Day 6), the drop in R2* in 48/72 paired measurements following the administration of carbogen was highly statistically significant ($p<0.0001$). The mean percentage change in R2* for all seventy two pairs of data was a decrease of 5.8%.

4.2.3 Evaluating potential imaging biomarker to predict response to the application of carbogen

The responses to carbogen in the 85 pairs of pre and post carbogen R2* data were dichotomised (reduction in R2* value versus no reduction in R2* value). A logistic regression model was constructed to evaluate whether patients' baseline pre-carbogen R2*, ADC and ktrans levels might predict response to carbogen application. None of them reached a statistically significant level of $p<0.05$; the p values was 0.868 for ktrans, 0.510 for ADC and 0.191 for pre-carbogen R2*.

4.2.4 The changes in the pre-carbogen R2* value over the course of treatment

Figure 4.5 shows the mean percentage change in the pre-carbogen R2* level at the different time points, compared to the baseline value. The trend was suggestive of an increase in R2* after three months of androgen deprivation; the R2* then plateaued during the course of radiotherapy before coming down towards the end of treatment. However, none of the differences between each time point and the baseline was statistically significant (Table 4.5)

4.2.5 The changes in the tumour ADC value over the course of treatment

Figure 4.6 and table 4.6 show the percentage changes in the ADC values over the course of treatment as compared to the baseline value. After three months of

androgen deprivation therapy, it increased in 14/19, 16/17, 16/18, 15/18 and 18/18 patients on day 2, 3, 4, 5 and 6 respectively.

The differences in the ADC value between Day1 and rest of the time points were all statistically significant, with the corresponding p-values in their comparison summarised in table 4.7.

4.2.6 Correlation between patients' imaging parameters and the clinical characteristics

No correlation was found between the levels of ADC, ktrans and pre-carbogen R2*. In their pairwise tests, the Spearman correlation co-efficient values were -0.03 (pre-carbogen R2* and ADC), -0.19 (ktrans and ADC) and -0.10 (pre-carbogen R2* and ktrans).

No correlation was found between the percentage changes in the levels of ADC and ktrans. In their pairwise test, the Spearman correlation co-efficient value was -0.09.

A weak negative correlation was found between the percentage changes in the levels of ADC and pre-carbogen R2* on univariate analysis; the Spearman correlation co-efficient value was -0.49 (p=0.002251).

A weak negative correlation was also found between the percentage changes in the levels of ktrans and pre-carbogen R2* on univariate analysis; the Spearman correlation co-efficient value was -0.33 (p= 0.04408).

No statistically significant correlation was found among the baseline imaging parameters and the clinical characteristics on univariate analysis. The Spearman rho values for the corresponding pairwise tests are shown in table 4.8.

4.2.7 Cox regression analysis

Three patients out of the cohort of 19 who had taken part in the imaging section of this study experienced PSA progression. Univariate Cox regression analysis was conducted to evaluate the potential of the following imaging biomarker at baseline to predict for PSA progression free survival: ADC, PreCarbogen R2* and Ktrans of the tumours. None of the tests conducted was statistically significant (p-value = 0.9 for PreCarbogen R2*, 0.96 for Ktrans and 0.84 for ADC).

4.2.8 Reproducibility analysis

4.2.8.1 Statistical methods for reproducibility calculations

Within subject coefficient of variation (wCV) is calculated using the root mean square method as shown below [175]:

The mean squared difference (dsd) = $\sqrt{\frac{\sum d^2}{n}}$ where d is the difference between the two measurements, and n is the total number of paired samples.

The within-patient standard deviation (wSD) = $\frac{dsd}{\sqrt{2}}$

For original data, the within patient coefficient of variance (wCV) = $\frac{wSD}{m}$ where m = mean of d^2

For log transformed data, wCV=e^{wSD}-1

Variance ratio (F) is the ratio of the between patient variance and within patient variance. A parameter with a larger variance in the patient population but a small variance within individual patients (wCV) will have a higher variance ratio. Intra-class correlation measures the reliability of measurements. An intra-class correlation co-efficient (ICC) close to 1 indicates that the pair of data obtained are highly similar (equation 3), and is calculated as below:

ICC = $\frac{m \times SSb - SSr}{(m-1) \times SSr}$ where m is the number of observations per subject, SSb is the sum of squared between subjects, and SSr is the total sum of squares.

4.2.8.2 Results of reproducibility analysis

wCV, F and ICC for each of the three parameters discussed (R2*, Ktrans and ADC) have been calculated based on the paired data collected at the two time points within one week of each other (Day 2 and Day 3) after three months of androgen deprivation therapy treatment, and are as shown below.

	number of paired data	wCV	F	ICC
R2*	10	11.90%	16.72	0.875
Ktrans	16	90%	10.16	0.81
ADC	17	7.29%	10.73	0.825

4.3 Discussion

The main translational hypothesis this study has set out to test is that the application of carbogen will result in improved tumour oxygenation, as measured by a drop in R2*, despite the disruptive effect of three months of androgen deprivation therapy on the tumour vasculature. Before the start of ADT, less than half the patients had a

reduction in their tumour R2* following the administration of carbogen, and the mean percentage change in R2* for all thirteen patients was an increase of 4.6% though this change was not statistically significant. This is in contrast to our previous report [131], and may be explained by differences in the patients' intrinsic tumour characteristics between the two cohorts. However, after three months of ADT, more than half (56-79%) of patients experienced a drop in their tumour R2* post carbogen; the lack of statistical significance in the reduction at each individual time points may be due to small number of patients involved resulting in statistical under-powering. When the seventy two pairs of datasets at all five post ADT time points were pooled together the mean percentage reduction of 5.8% in the R2* value post carbogen became statistically significant ($p<0.0001$). A mean reduction in R2* of 21.6% in response to carbogen was previously reported [131]. The differences in the magnitude of reduction between that study and our finding may be related to the differences in the magnetic field strength of the MR scanners used for the BOLD imaging; in the previous study a 1.5T scanner was employed, whereas in the present study a 3T scanner was used. None of the baseline imaging biomarkers predict response to carbogen application. A possible reason why a response to carbogen was observed in more patients after three months of ADT than prior to the start of ADT is that the antiangiogenic effect of ADT worsens perfusion related acute hypoxia, which particularly benefits from the pro-vasodilation property of the carbon dioxide contained in carbogen to overcome transient occlusion of tumour related blood vessels.

An increase, albeit statistically insignificant, in the mean percentage changes in pre-carbogen R2* value after three months of hormone treatment, associated with a reduction in the tumour blood supply as reflected by the corresponding drop in

Ktrans level, was once again observed, consistent with a previous report [107]. As already discussed in the introduction, clinical studies have yielded discordant results in terms of the impact on tumour oxygenation status by androgen deprivation therapy [107,112]. In support of the finding by Milosevic et al [112], Mainta et al [200] showed a reduction in the 18F-misonidazole (18F-miso) uptake in regions of prostate cancer already identified to be hypoxic pre treatment after three months of ADT among 6 patients with Gleason 8+ prostate cancer. This would be suggestive of a reduction in tumour hypoxia. In their animal model, Ming [111] showed that the change in tumour oxygenation status in response to anti-androgen was time dependent. Tumour became hypoxic within 24 hours of starting ADT but less hypoxic after 28 days of treatment secondary to re-vascularisation. This would however not account for the discordant findings from the three clinical studies quoted [107,112,200] because in all of them the assessments to evaluate tumour oxygenation status were done no later than four months from the start of ADT. A possible explanation is that the increase in R2* in BOLD-MRI following ADT reflects a worsening in acute hypoxia, whereas the reduction in F-miso uptake and the increase in pO_2 taken from direct electrode measurements reflect an improvement in the chronic hypoxic state of the tumour. ADT improves chronic hypoxia in tumour by reducing the oxygen demand though its cytotoxic effect, but exacerbates acute hypoxia due to its anti-angiogenic effect. A rise in the R2* signal in BOLD-MRI is due to the accumulation of deoxyhaemoglobin in red cells; acute hypoxia occurs because of transient occlusions of poorly formed tumour vasculature. This primarily affects areas in close proximity to the vessels and can drive the dissociation of oxygen from the red cells to the hypoxic areas. Cyclic fluctuations in the intrinsic susceptibility signals over minutes, reflective of the changes in tumour perfusion, have been reported in animal models and patients with head and neck cancer

[201,202], and their frequency were reduced with the application of carbogen and nicotinamide [201]. In contrast, chronic hypoxia is caused by critical limitations in oxygen diffusion from tumour vessels into the surrounding tissues [203], and tends to be present in parts of the tumour further away from blood vessels. Thus it is less likely to be reflected by BOLD because the red cells in the vessels are too distant to be affected the area of hypoxia [204]

A drop in Ktrans was observed after three months of androgen deprivation therapy. As Ktrans reflects both the vascular permeability and blood flow, its reduction would be consistent with the disruptive effect of androgen deprivation therapy on the tumour vasculature as reported previously [107,108,176]. It rose after the start of radiotherapy, possibly due to the inflammatory effect of radiation which had resulted in an increase in the blood flow and possibly vascular permeability. At the end of radiotherapy, a reduction in Ktrans was observed. This may be explained by the "normalisation" of the chaotic tumour vasculature by radiation, resulting in a reduction in the overall blood flow and vascular permeability. A similar trend of early increase in Ktrans during the course of treatment followed by a reduction in its value soon after treatment was reported for patients who underwent concurrent chemo-radiotherapy for cervical cancer [205]. A weak negative correlation was found between the changes in Ktrans and the corresponding change in pre-carbogen R2* during the course of treatment, possibly suggestive of an improvement in the tumour oxygenation level (drop in R2* level) with an increase in blood supply (increase in Ktrans). This may account for the statistically insignificant drop in pre-carbogen R2* from the start to the end of radiation treatment. At 90%, the within subject coefficient of variation for Ktrans in this study is much higher than what has been reported previously [175]. This does raise the possibility that some of the observed changes in

Ktrans may simply be a natural variation and does not reflect any significant physiological change. If the data from the two extreme outliers for Ktrans are removed, the wScv falls to 26% which is more consistent with previously reported result [175].

Consistent with previous reports [156,157,160,161,183,184], a rise in the ADC value of the tumour region of interest was observed from baseline to the end of treatment. This may reflect the cytotoxic effect on the tumour cells of both the hormone treatment and radiotherapy. A weak negative correlation was observed between the changes in R2* of the tumour region of interest and the corresponding change in ADC. A reduction in the cancer burden, as reflected by an increase in the ADC values, might have resulted in a reduced demand for oxygen hence the improvement in the oxygenation state of the tumour. This may explain the observed reduction in R2* at the end of the course of radiotherapy.

The total number of patients we could carry out functional MRI scans on was determined by the financial support we had received from Prostate Cancer UK. This was set at twenty patients in our protocol. The subsequent withdrawal of patients' consent and their non-attendance meant we were only able to collect data from between seventeen and eighteen patients. The motion artefacts associated with rectal peristalsis, which is worse in a 3T scanner with its stronger magnetic field strength when compared to 1.5T scanner in which previous similar studies had been carried out [198], further compromised the number of scans suitable for analysis. With the benefit of hindsight, we should have implemented a standardised procedure to promote rectal emptying prior to patients undergoing their MRI scans so that rectal peristalsis could have been minimised. Another approach we can employ if the study

were to be conducted now is the use of SpaceOAR hydrogel which provides a physical barrier to separate the prostate from the rectum and minimise the impact of gaseous filling in the rectum on the R2* value of the prostate [206].

In summary, the results from the imaging translational component of the PROCON trial supported our hypothesis that the application of carbogen would improve the hypoxic state of the tumour despite the disruptive effect of hormone treatment on the tumour vasculature prior to the start of radiotherapy.

Table 4.1 Number of patients who completed each MRI sequence and generated dataset for analysis at each time point

MR sequence	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Total
BOLD	19	19	18	18	18	18	110
DCE-MRI	18	18	17	17	17	18	105
dwMRI	19	19	17	18	18	18	109

Table 4.2 – the number of patients whose datasets are suitable for analysis at each time point

MR sequences	D1	D2	D3	D4	D5	D6	Total
PreBOLD	14	14	15	16	17	15	91
PostBOLD	13	14	15	14	16	13	85
DCE	18	17	17	17	17	18	104
dwMRI	19	19	17	18	18	18	109

PreBOLD – BOLD scan pre carbogen; PostBOLD – BOLD scan post carbogen

Table 4.3 – the mean in % change in Ktrans value compared to baseline

Time point	Day 2	Day 3	Day 4	Day 5	Day 6
% change	-25%	-18%	+32%	+36%	+11%

Table 4.4 - The p-values of the comparison between the pre-carbogen and post-carbogen R2* at each time point

Time point	Day 1	Day 2 (preRT1)	Day 3 (preRT2)	Day 4 (#5)	Day 5 (#15)	Day 6 (#37)
No of pairs of data	13	14	15	14	16	13
p-value	0.2879	0.05038	0.2447	0.3627	0.07733	0.07653

Table 4.5 - the p-value of the comparisons in the pre-carbogen R2* value between Day 1 and the other time points

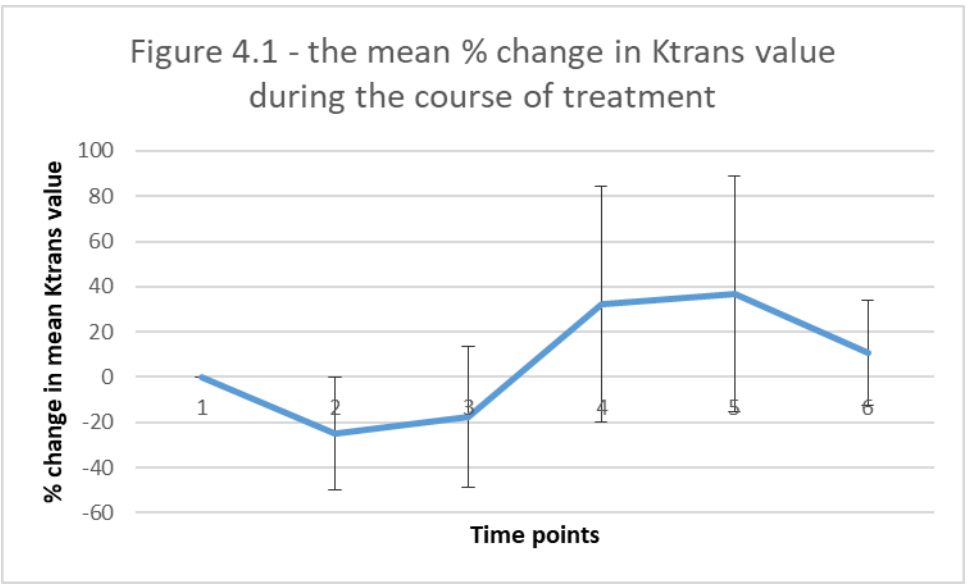
Time point	Day 2	Day 3	Day 4	Day 5	Day 6
p-value	0.2353	0.06602	0.7218	0.6462	0.4494

Table 4.6 – the mean % change in ADC value compared to baseline

Time point	Day 2	Day 3	Day 4	Day 5	Day 6
% change	23%	28%	34%	33%	52%

Table 4.7 – the p-value of the comparisons in the ADC value between Day 1 and the other time points					
Time point	Day 2	Day 3	Day 4	Day 5	Day 6
p-value	0.005673	0.0008416	0.001706	0.001005	<0.001

Table 4.8 – correlation coefficients in the pairwise Spearman test between each of the clinical and imaging parameter.			
	ADC	ktrans	Pre-carbogen R2*
Gleason score	0.038	0.199	0.147
T staging (dichotomised to stage 2 and stage 3)	0.134	-0.442 (p=0.07)	-0.39 (p=0.17)
Presenting PSA	-0.063	0.036	0.073



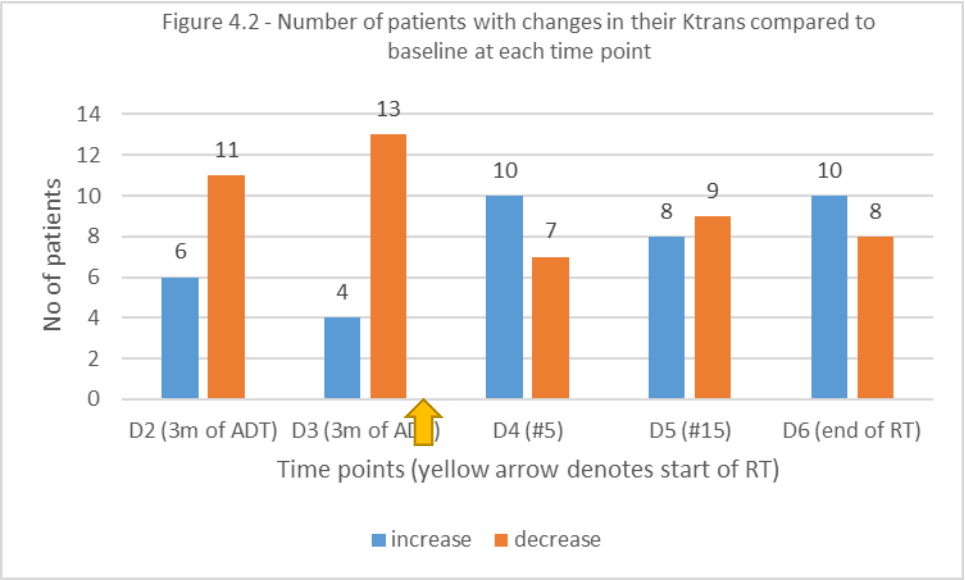


Figure 4.2 - Number of patients with changes in their Ktrans compared to baseline at each time point
No of patients
14
12
10
8
6
4
2
0
6
11
4
13
10
7
8
9
10
8
D2 (3m of ADT)
D3 (3m of ADT)
D4 (#5)
D5 (#15)
D6 (end of RT)
Time points (yellow arrow denotes start of RT)
increase
decrease

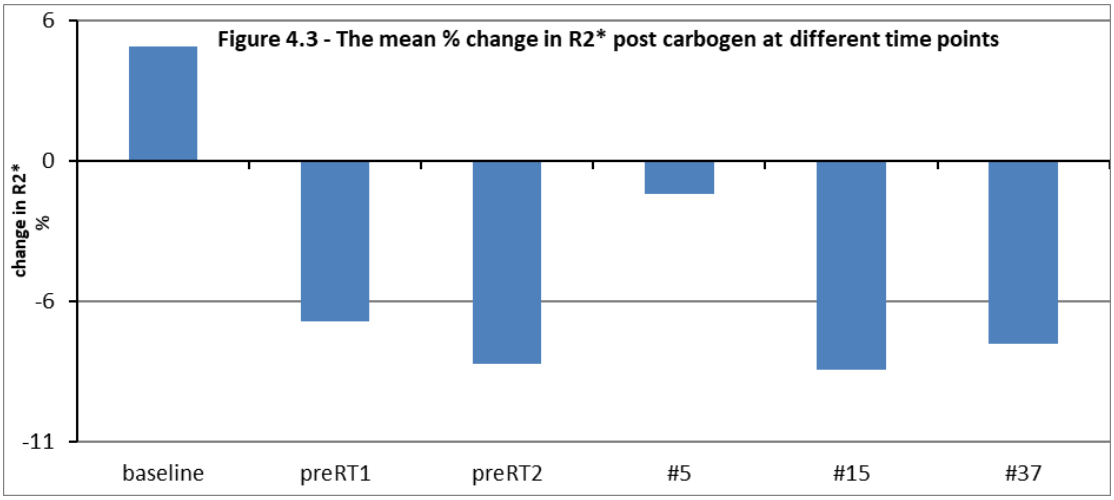


Figure 4.3 - The mean % change in R2* post carbogen at different time points
change in R2* %
6
0
-6
-11
baseline
preRT1
preRT2
#5
#15
#37

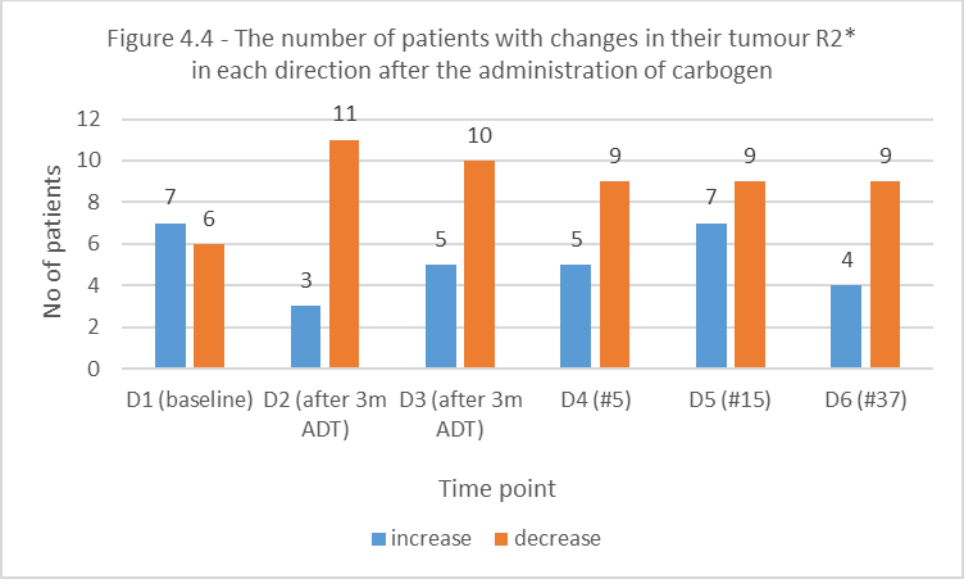


Figure 4.4 - The number of patients with changes in their tumour R2* in each direction after the administration of carbogen
No of patients
12
10
8
6
4
2
0
7
6
3
11
5
10
5
9
7
9
4
9
D1 (baseline)
D2 (after 3m ADT)
D3 (after 3m ADT)
D4 (#5)
D5 (#15)
D6 (#37)
Time point
increase
decrease

Figure 4.5 - the mean % change in the pre-carbogen R2* value compared to baseline over the course of treatment

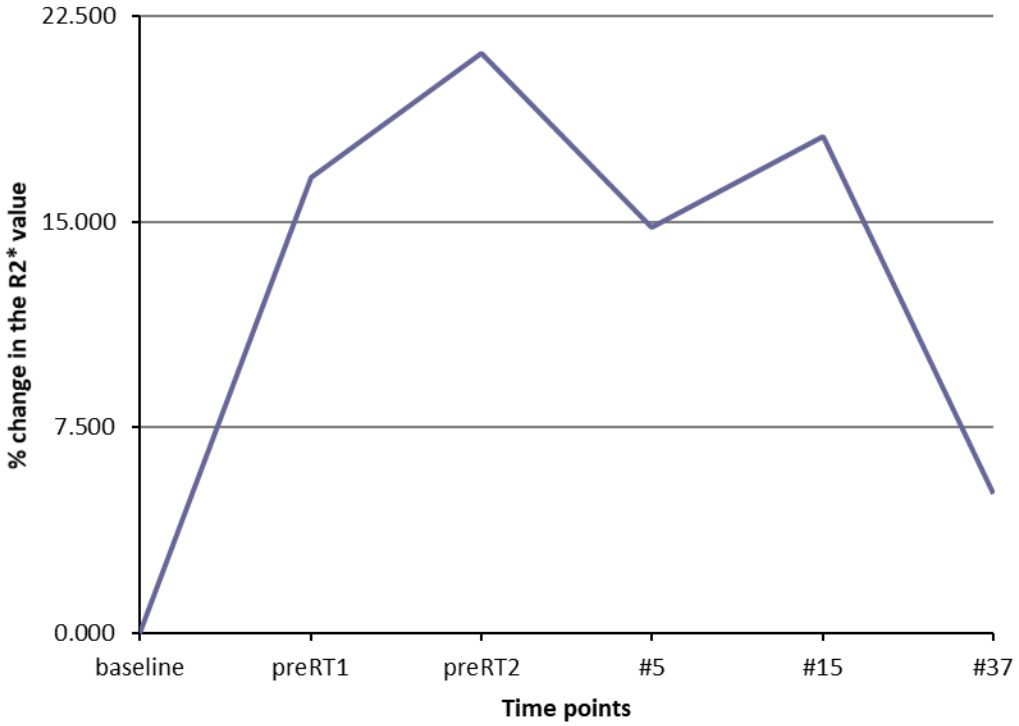
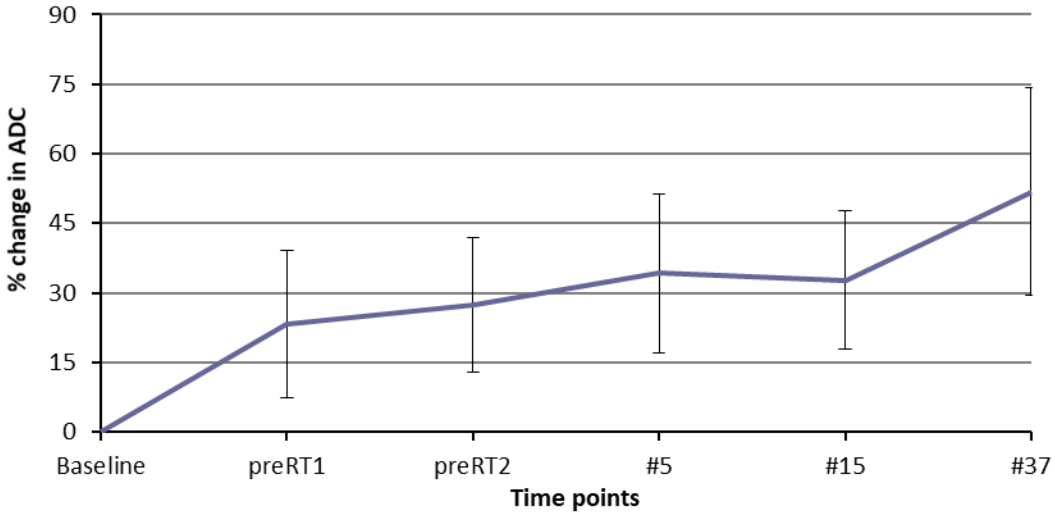


Figure 4.6 - the mean % change in ADC over the course of treatment



Chapter 5 - Concluding discussion and future directions

The initial improvement in biochemical progression free survival observed in the dose escalated cohort of 74Gy/37# when compared to the control group of 64Gy/32# in the RT01 trial did not translate into better overall or metastases free survival [207]. This was the case regardless of the patients' risk categories. The indolent nature of PSA progression in some of the patients, and the much higher mortality risk from non-cancer related causes in this elderly population might account for the lack of overall survival benefit. For patients who developed metastatic disease following their biochemical progression the recent advances in the systemic treatment for metastatic prostate cancer would have prolonged their survival rate, thus confounding the effect of dose escalation [208–211]. Patients with hypoxic prostate cancer are more likely to develop metastatic disease [212], and this may be related to the development of more aggressive cancer phenotype driven by hypoxia and mediated by chromosomal instability [197,198] and the upregulation of the HIF-1 pathway which promotes downstream processes involved in the formation of metastases [29]. Whilst studies have suggested that these patients are also more likely to benefit from receiving escalated radiation dose in terms of PSA control [115,116], their improved biochemical progression free survival rate may simply reflect better local control rather than a reduced risk in the development of metastases. This may provide another reason why the improved biochemical progression free survival rate in the RT01 trial did not translate into better overall or metastatic free survival. Moreover, dose escalation alone is unlikely to be sufficient to improve the survival rate of patients who harbour hypoxic prostate cancer. Prostate cancer is more responsive to treatment with a hypofractionated schedule due to its low alpha/beta ratio. 60Gy/20# is now the standard radiotherapy regime in the UK since the publication of the results

from the CHHiP trial [51]. If the results from the PACE trial [54] are positive, further hypofactionation will likely follow. As discussed in the introduction, the use of hypofractionated radiotherapy schedules may disadvantage patients with hypoxic tumours [55]. These patients are likely to have the most to gain from receiving CON concurrently with their course of radiotherapy.

Patients with advanced head and neck cancer whose tumour harboured the 15 gene signature for hypoxia [215] were more likely to benefit from concurrent treatment with nimorazole during radiotherapy in the DAHANCA 5 trial. In the Dutch ARCON trial for advanced laryngeal cancer, patients with tumours judged to be hypoxic on the basis of their high level of pimonidazole staining were the ones who had benefited from treatment using ARCON [153]. In the BCON trial, the presence of necrotic or HIF-1 positive tumours were found to predict response to the addition of CON to radiotherapy in patients with muscle invasive bladder cancer [216,217]. When the 28 gene hypoxic signature designed for prostate cancer patients was applied to these BCON patients, those classified as having highly hypoxic tumours by being in the top quartile of the hypoxic gene scores had improved survival rate with CON treatment, whilst those with the lowest quartile scores had poorer outcome following CON [212].

Results from our imaging translational study and a previous study [131] showed that improvement in the tumour oxygenation status was observed in 50-80% of patients when CON was used. It is likely that only patients who harbour hypoxic prostate cancer will derive clinical benefit from undergoing hypoxic modification, and the use of a hypoxic gene signature score may allow these patients to be identified to enrich any future trial population involving hypoxic modification. Plans are in place to

validate the 28 gene hypoxic assay [196] in patients who took part in the PROCON study. Had there been a control arm in our trial in which patients received ADT and radiotherapy only with no hypoxic modification, an improvement in the survival outcome of patients judged to have hypoxic tumour who received CON compared to those who received standard treatment would have provided strong support to the CON approach. With our single arm design, we shall be confined to comparing the outcome of patients with hypoxic tumours against those with normoxic tumour; a better outcome in the former group will still lend support to the CON approach, as patients with hypoxic tumour are expected to have worse prognosis. However, the limited number of PSA progression events in our study, due to the short median follow up period and the small population size of our cohort, may result in inconclusive findings. The level of reduction in the tumour R2* level in response to carbogen may serve as a useful surrogate endpoint, with the hypothesis that only those judged to have hypoxic tumour would experience a drop in their tumour R2* with the application of CON.

The oxygenation status of tumour is likely to be a continuous variable, yet when any predictive biomarker is used to aid with decision making, a cut off value will have to be applied to dichotomise tumours into the hypoxic or non hypoxic group. In the case of the 28 gene signature already discussed, the median value for each trial population used for its validation was chosen as the cut off. Clearly, this can only be done retrospectively and varies among different populations. To show its predictive value in the BCON population, the top and quartile scores were used instead of the median value [212]. The trade off between false positive and false negative rates in prospectively designating the cut off value for any predictive biomarker test for entry into a clinical trial, and possibly to guide specific hypoxic therapeutic interventions in

routine clinical practice in the future, is to an extent a subjective decision. If the bar is set too high there may be insufficient patients suitable for trial recruitment and the benefit of any hypoxic intervention may be restricted to a very small population; if the bar is set too low any potential predictive power of the chosen biomarker will be lost. Strong correlation has been demonstrated among nine independently developed mRNA based hypoxic signatures [218], and at least one of these should be explored as a screening tool in any future trial involving hypoxic modification.

None of the imaging biomarkers (pre-carbogen R2* level, ADC, Ktrans) was predictive of response to carbogen in our study. Whilst changes in R2* value in response to carbogen has been shown to correlate with changes in tumour pO_2 measured directly by electrodes in animal models [219,220], there is no correlation between the absolute R2* values and pO_2 levels [221]. Alternative MR based imaging biomarker, such as oxygen enhanced MRI (OE-MR) in conjunction with DCE-MRI, should be explored further. OE-MR is based on the fact that dissolved O_2 in blood can result in an increase in the R1 measurement (R1=1/T1). When excess oxygen is inhaled, R1 values in voxels of normoxic tissues with saturated haemoglobin are increased as the excess oxygen remains dissolved. Conversely, in hypoxic tissues no change in R1 will be seen as there will be no dissolved oxygen. The advantage of OE-MR over BOLD-MR is that the measured change in R1 is proportional to the partial pressure of oxygen, unlike R2* measurement [221].

This study was first conceived ten years ago. In the intervening period one of the biggest advance in the systemic management for cancer has been the introduction of immune check point modulators in the routine clinical practice for other cancers. Unprecedented improvement in the overall survival rates were seen in patients with

melanoma, lung cancer, renal cancer, bladder cancer, breast cancer, head and neck cancer and lymphoma. Results to date for prostate cancer, however, have not been as promising [222–224]. Gajewski et al hypothesized that patients with a non-immunogenic tumour are unlikely to respond to immunotherapy alone [225]. Factors, intrinsic to the tumour itself, such as its mutational burden [226,227], neo-antigen heterogeneity [228], microenvironment [229], and those related to its host such as the HLA-phenotype, germline polymorphisms in immune cell receptors and the gut microbiota can impact on the immunogenicity of the tumour [229]. The lower tumour mutational burden found in prostate cancer [230,231] compared to other cancers may explain the relative lack of efficacy of immune checkpoint blockade for these patients. Strategies to promote immunogenic cell death and activate the immune system to prime the T cells may help to convert immunologically "cold" tumours, into tumours with a more "inflamed phenotype", thus improving their response to checkpoints modulation [21]. Combining immune check point modulators with radiotherapy may be one way to achieve this [232,233]. In the STAMPEDE trial involving patients with metastatic prostate cancer, Parker et al demonstrated the therapeutic benefit of local radiotherapy to the prostate primary in patients with low burden of metastatic disease. A short course of palliative radiotherapy 36Gy/6# or 55Gy/20# resulted in an improvement in the progression free and overall survival for these patients. Immune modulation by radiation was proposed as one potential mechanism to account for this beneficial effect [234].

However, hypoxia may counteract any potential synergistic effect between radiation and immunotherapy, as it can impair the functions of cells involved in both the adaptive and innate immune systems. This may be due to the accumulation of adenosine and the acidic tumour microenvironment resulting from lactic acid build

up. Moreover, hypoxia promotes immune suppression by attracting inhibitory cells such as myeloid derived suppressor cells and tumour associated macrophages into the tumour micro-environment. In their animal model, Hatfield et al. [235] showed that applying respiratory hyperoxia could promote the intra-tumour infiltration of tumour reactive CD-8 T cells, increase the amount of proinflammatory chemokines being released, decrease the amount of immunosuppressive molecules being secreted, and weaken immune suppression by regulatory T cells. Scharping et al showed that the co-administration of metformin and a PD-1 inhibitor led to an improvement in the functioning of intratumoral T cells and tumour clearance by reducing tumour oxygen consumption and improving tumour hypoxia [236]. Jayaprakash et al demonstrated in their TRAMP-C2 prostate mouse model that the addition of TH-302 (evofosfamide), a hypoxia activated pro-drug of an alkylating agent, to combined CTLA-4 and PD-L1 blockade, significantly improved the overall survival of the mice. Moreover, this improvement was mediated by hypoxia ablation rather the cytotoxicity of evofosfamide because the synergistic effect was not seen when evofosfamide was substituted with ifosfomode, and the use of Th302 in combination with immune checkpoint blockaders resulted in a reduction in the percentage of hypoxic tumour area [237]. As discussed in the introduction, the down-regulation of DNA repair mechanisms in hypoxic tumours makes the combination of radiotherapy and a PARP inhibitor (PARPi) an attractive therapeutic strategy to pursue [39,139]. This synergism may be further enhanced with the concurrent delivery of CON which can result in more DNA damage. The use of PARP inhibitors can however lead to an adaptive up-regulation of PD-L1 expression in tumour cells to allow them to avoid immunogenic cell death. Thus the addition of a PD-1 immune check point inhibitor to the CON-RT-PARPi regime should be considered [238].

We have demonstrated that the administration of CON can improve tumour hypoxia despite the anti-vascular effect of androgen deprivation therapy. However, whether the transient improvement in hypoxia will lead to a clinically meaningful increase in DNA double strand break, tumour cytotoxicity, promotion in anti-cancer immune response and ultimately long term survival for patients with high risk prostate cancer can only be answered by a randomised trial similar to BCON. Given the commercial interest vested in the development of immunotherapy and drugs targeting DNA damage repair and cell cycle pathway, it is difficult to envisage a randomised trial, in which CON is the only comparator being evaluated, will be endorsed with enthusiasm. However, the favourable toxicity profile associated with CON will allow its combination with other therapeutics to be explored. Such studies will also provide the opportunity to explore the impact of hypoxia on response to immunotherapy, alone or in combination with radiation and possibly other therapeutics which target DNA damage repair mechanisms and cell cycle modulators. For too long hypoxic modification has been adored and ignored [56], but the simplicity of the CON approach may prove its synergistic value to the other more glamorous and expensive modern therapeutic strategies.

Reference

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Appendix 1 – functional MRI data and clinical characteristics for each patient

Units are s^{-1} for PreCarbo and PostCarbo R2*, min^{-1} for Ktrans and mm^2/s for ADC

				Day1			
Trial ID	Gleason	PSA	T stage	PreCarbo	PostCarbo	ktrans	ADC
9	7	21.4	2c	47.603599	46.3415438	0.149673	87.97071
13	6	12	3b	32.8315714	33.441661	0.111009	59.78903
14	9	7.65	3a	23.4490133	21.665061	0.229969	81.35472
16	8	13.2	3a	25.0591638	27.719061	0.065101	108.457
18	7	15.2	3b	N/A	N/A	0.218138	98.14693
19	7	16.9	3a	51.2193505	57.6240229	0.056151	113.9325
21	6	20.3	3a	20.7132171	N/A	0.069569	84.0552
22	8	54.4	2a	28.9970076	32.6753476	0.170074	75.7892
23	8	4.7	2a	46.4594276	53.6925429	0.167086	111.9456
25	7	12.6	3b	38.4540857	53.6047	0.217663	87.5786
27	9	25.1	3a	N/A	N/A	0.11711	81.62502
32	7	15.7	3b	24.5583724	21.988339	0.10591	84.8213
33	6	37.3	2c	25.3622333	23.937581	0.442741	67.33319
34	8	19.1	2c	N/A	N/A	N/A	63.85359
36	8	29.7	2a	62.0041486	54.56714	0.243544	70.55084
37	8	14.3	3b	N/A	N/A	0.152363	83.60313
42	7	7.9	3a	27.3726552	23.7411238	0.362361	56.38879
43	7	9.3	3a	39.6654229	47.1666133	0.092553	53.73265
44	6	13.9	3b	N/A	N/A	0.08695	91.16602

	Day2			
Trial ID	PreCarbo	PostCarbo	ktrans	ADC
9	44.03224	42.01243	0.124156	88.69729
13	29.37791	23.49402	0.075578	102.5305
14	23.36125	25.50839	0.172835	80.03647
16	32.29937	21.66963	0.01465	122.6624
18	29.53731	26.92198	0.285196	99.94582
19	50.06387	32.77295	0.011156	114.4923
21	N/A	N/A	0.086552	118.4649
22	54.23079	52.23473	0.080769	86.90083
23	N/A	N/A	0.033605	116.7908
25	N/A	N/A	0.35311	96.48899
27	N/A	N/A	N/A	76.25127
32	29.87567	27.97927	0.033705	117.7812
33	24.72317	32.10389	0.143856	96.70485
34	48.97366	34.06925	N/A	44.03181

36	60.86108	61.61379	0.165621	92.06543
37	34.20986	32.90586	0.167823	80.40057
42	29.11764	26.5661	0.01	112.4765
43	47.96678	45.83449	0.096637	103.0199
44	N/A	N/A	0.150588	111.9831
	Day3			
Trial ID	PreCarbo	PostCarbo	ktrans	ADC
9	36.01166	38.12513	0.218413	100.5459
13	27.67943	23.86021	0.108591	117.1529
14	24.0904	32.69411	0.141499	84.86775
16	45.16386	43.56144	0.017696	118.5672
18	33.52293	24.31791	0.167586	100.1298
19	N/A	N/A	N/A	N/A
21	31.14222	48.37096	0.086637	122.4197
22	49.45121	42.27719	0.004313	89.86839
23	56.32063	56.23083	0.08173	101.0195
25	N/A	N/A	0.525546	103.871
27	23.29364	22.29842	0.080172	94.8202
32	N/A	N/A	0.036084	120.185
33	30.86448	31.79317	0.135849	91.45096
34	36.61654	24.47651	N/A	65.80239
36	67.77452	50.89883	0.163158	102.3929
37	29.83881	26.4608	0.13272	89.87362
42	N/A	N/A	0.048433	111.4783
43	54.83735	43.39435	0.060113	N/A
44	22.69463	24.56701	0.180977	110.8964
	Day4			
Trial ID	PreCarbo	PostCarbo	ktrans	ADC
9	41.03518	36.25146	0.297549	96.15437
13	24.1035	23.15026	0.13688	103.1905
14	N/A	N/A	N/A	N/A
16	36.13449	31.99842	0.038592	115.2272
18	38.3907	N/A	0.231008	99.86879
19	58.52119	50.43184	0.096848	102.2341
21	N/A	N/A	0.177515	131.0107
22	40.06388	39.08103	0.038789	100.9446
23	22.91102	23.1469	0.079363	100.5189
25	23.17415	36.43738	0.907692	115.7843
27	25.32502	19.94984	0.163724	94.31977
32	34.86191	41.82704	0.046809	120.5551
33	34.46851	25.17587	0.208471	86.07518
34	49.14917	N/A	N/A	74.57041
36	48.82682	51.20987	0.38724	116.2171
37	43.37321	33.25375	0.172474	85.78385
42	N/A	N/A	0.15967	123.5939
43	36.15495	41.25228	0.004181	110.3781
44	19.66237	17.28518	0.255719	118.8077

	Day5			
Trial ID	PreCarbo	PostCarbo	ktrans	ADC
9	39.96026	33.21592	0.082618	113.3721
13	33.0614	26.76712	0.145646	108.2761
14	24.80939	24.29474	0.178766	75.15601
16	43.34165	16.31358	0.043838	118.2149
18	24.18253	25.187	0.206438	122.6226
19	63.93644	47.21972	0.114335	97.1667
21	N/A	N/A	0.098228	123.4619
22	39.45646	34.95613	0.204845	146.8492
23	23.24475	25.35986	0.206734	107.4596
25	35.31597	32.17132	0.982181	108.9558
27	24.28074	26.4703	0.09637	92.80752
32	N/A	N/A	N/A	N/A
33	31.77209	32.81902	0.212339	89.83888
34	49.72945	N/A	N/A	84.74349
36	51.70197	55.64384	0.41392	112.6362
37	29.32365	26.39962	0.13516	90.31532
42	26.5688	22.66366	0.103563	106.0286
43	49.12602	49.61787	0.08685	104.7613
44	20.1812	20.19725	0.299812	124.3698

	Day6			
Trial ID	PreCarbo	PostCarbo	ktrans	ADC
9	45.22258	39.07825	0.189458	254.6286
13	21.83571	20.23015	0.106286	127.9559
14	40.99	39.94527	0.386091	107.2982
16	22.60774	24.35952	0.042289	116.1857
18	27.82326	N/A	0.238376	123.4974
19	37.06437	29.2725	0.072468	116.2706
21	29.90796	30.18531	0.10076	112.2511
22	35.80211	35.44184	0.172145	105.7559
23	23.46058	N/A	0.224616	116.8637
25	38.04872	34.92332	0.359975	107.0292
27	N/A	N/A	0.06381	102.6266
32	24.13723	17.56248	0.045972	129.7011
33	25.57905	22.5214	0.211957	96.33653
34	N/A	N/A	N/A	N/A
36	55.0219	58.43877	0.46465	114.4555
37	N/A	N/A	0.137776	105.7235
42	26.8037	32.31026	0.239839	114.2564
43	N/A	N/A	0.053859	109.7242
44	27.40175	20.13251	0.178865	108.5154

Appendix 2 – protocol for the PROCON trial

Prostate Radiotherapy in Conjunction with Carbogen and Nicotinamide. A phase Ib/II Study. (PROCON)

Protocol identification number: RD2010-48
EudraCT number 2010-021886-63

Principal Investigator: Dr Roberto Alonzi
Phone [redacted]
Email [redacted]

Co-Investigator: Prof Peter Hoskin
Phone [redacted]
Email [redacted]

1 Background and introduction

1.1 Background Disease Information

Worldwide, more than 670,000 men are diagnosed with prostate cancer every year. In the UK, it is the most common cancer in men, with a lifetime risk of being diagnosed with the disease of 1 in 10, accounting for nearly a quarter (24%) of all male cancers.[1-5] Substantial increases in incidence have been reported in recent years,[6] which have been closely linked to the emergent use of trans-urethral resection for urinary outflow symptoms during the 1980s and the subsequent introduction of PSA testing in the 1990s.[7-9] In 2007 there were 36,101 new cases of prostate cancer diagnosed in the UK.[5] and this figure will undoubtedly rise as the population at risk grows with the lengthening of life expectancy.

In the Western world most patients present with localised disease. The only potentially curative, evidence-based treatment options are radical prostatectomy and radiotherapy (in the form of either external beam therapy or brachytherapy). Although these modalities have never been compared in a randomised trial, comparative series suggest that there is little to choose between them in terms of survival and disease control.

It is now well established that radiotherapy dose escalation results in improved outcomes from radiotherapy. A large phase III trial run by the Medial Research Council (MRC RT01) demonstrated the superiority of 74Gy radiation dose compared with 64Gy in achieving tumour control.[10] Dose escalation is particularly important for patients with more advanced disease. It is now standard practice to treat patients to these dose levels and frequently even higher doses using techniques such as Intensity Modulated Radiotherapy (IMRT) or High Dose Rate (HDR) brachytherapy. Despite these advances, many patients treated with curative intent do not remain disease free in the long term, with disease-free 5-year survival rates for T1-T2 disease of 63-96% for external beam radiotherapy[11] and 83-92% in equivalent risk patients treated with external-beam plus a brachytherapy boost.[12,13] For T3 disease the corresponding 5-year disease-free survival rates are 32-87% and 31-56% respectively. The two principle causes for this failure remain inadequate initial staging leading to the incorrect assumption that the disease is localised at presentation and biological resistance which may reflect varying degrees of hypoxia and intrinsic resistance. Strategies to combat the causes of radioresistance are urgently required.

1.2 Background Therapeutic Information

The detrimental effects of hypoxia in human tumours was first shown over fifty years ago.[14,15] Hypoxia is an important factor in radiotherapy treatment failure and has been associated in clinical studies with poor local tumour control and relapse in many cancer sites.[16-20] Hypoxic regions exist in human prostate carcinoma.[21,22] The outcome of radical radiotherapy for prostate cancer is influenced by the presence of hypoxia. A study that prospectively analysed 57 patients with localized disease demonstrated that hypoxic tumours had a significantly worse biochemical relapse-free survival at 2 years (31% versus 92%, $p < 0.0001$).[19] Another study used three different immunohistochemical markers of hypoxia and showed that increased expression of VEGF and HIF-1 alpha identified patients at high risk of biochemical failure in 201 patients that received radical radiotherapy. Furthermore, increased expression of osteopontin identified patients at high risk of biochemical failure in 289 patients that received radical surgery.[23] Oxygenation

status is therefore an additional prognostic factor beyond the classic prognostic factors (age, clinical stage, Gleason score and prostate specific antigen) that predicts for radiation treatment failure in prostate cancer. A modelling study based on these clinical data predicts an oxygen enhancement ratio for prostate cancer of 1.4 (95% confidence interval of 1.2 – 1.8), which is consistent with *in vitro* measurements of human tumour cell lines under chronic hypoxia conditions.[24] These data taken together suggest that hypoxia is likely to be a valid therapeutic target in prostate cancer.

Hypoxia-modification has been shown to improve radiotherapy outcomes in several tumour sites.[25] One strategy to achieve this is to increase the inspired oxygen concentration. Carbogen is a high oxygen content gas mixture (98%O_2/2%CO_2), which has been shown to improve the oxygenation of both experimental and human tumours.[26-29] This gas mixture increases intravascular oxygen availability resulting in greater oxygen uptake by tumours. The addition of nicotinamide (vitamin B3) is thought to prevent the transient, short-term fluctuations in blood flow, thereby potentiating the oxygen-enhancing effect.

CARBOGEN

Carbogen is a normobaric gas mixture of carbon dioxide and oxygen, which is usually administered at one of two concentrations (95% O_2 with 5% CO_2 or 98% O_2 with 2% CO_2). Carbogen has been demonstrated to improve the oxygenation of both experimental and human tumours. However, there is evidence to suggest that, at least in some tumours, enhanced blood flow may also contribute to its action. This gas mixture increases intravascular oxygen availability resulting in greater oxygen uptake by tumours. Carbogen also transiently opens non-functional blood vessels, likely to be a result of the CO_2 component of the gas mixture. This further increases oxygen delivery to regions of perfusion-limited hypoxia and also causes an increased leakage of molecules from the plasma to the extracellular space. Extracellular tumour pH has been shown to decline in response to carbogen gas breathing, in particular if the tumours are large and hypoxic.[30]

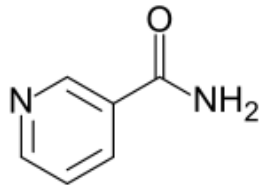
The rationale for the use of a high oxygen-content gas to improve tumour oxygenation is that the resulting increase in arterial pO_2 will enhance the diffusion of oxygen into the tissue. The addition of 2% or 5% CO_2 was originally proposed to counteract any vasoconstriction induced by pure oxygen breathing. A study in a murine tumour model was performed to determine how the CO_2 content of the inspired gas influences radiosensitivity.[31] Gas mixtures containing 0, 1, 2.5 and 10% CO_2, balanced with oxygen, were compared with 5% CO_2 + 95% O_2. Measurements of tumour oxygenation and perfusion were also made during the breathing of each gas. The results showed that the level of radiosensitisation achieved is dependent on both the CO_2 content of the inspired gas and the duration of gas breathing. No radiosensitisation was evident following inhalation of 90% O_2 + 10% CO_2. All other gases elicited radiosensitisation. However, that achieved with 100% O_2 disappeared at the extended pre-irradiation breathing time of 45 min. Changes in oxygenation, as measured by pO_2 electrodes, did indicate improved oxygenation status following the inhalation of the gases. However, the time course and extent of the changes did not mirror accurately the changes in radiosensitization. All the gases with a CO_2 content of 2.5% or greater induced a 10-20% reduction in microregional blood flow.

This study implies that the decreased radiosensitisation seen at extended breathing times of 100% oxygen is unrelated to blood flow changes. The fact that radiosensitisation is seen with extended breathing times of gases containing 2.5% & 5% CO_2, despite blood flow

decreases, is indicative of other overriding physiological changes, perhaps related to oxygen utilization. The studies overall indicate that, at least in the tumour investigated, radiosensitisation is maintained if the CO_2 content of the inspired gas is reduced from 5% to 2.5 or even 1%.

In humans, tolerance to carbogen can be a problem with patients feeling flushed and breathless during inhalation. These symptoms are considerably reduced if 2% CO_2 is used instead of 5% CO_2. A study comparing tumour oxygenation during inhalation of hyperoxic gas containing either 2% or 5% CO_2 has been performed.[27] Tumour pO_2 was measured in 16 patients using the Eppendorf pO_2 histograph. After breathing gas containing either 5% or 2% CO_2 an increase in median pO_2 was measured in every tumour, the frequency of low pO_2 values (less than or equal to 10 mmHg) fell from 47% to 29% in the 5% group and from 55% to 17% in the 2% group. This confirms that breathing 2% CO_2 and 98% O_2 is well tolerated and effective in increasing tumour oxygenation.

NICOTINAMIDE



Nicotinamide, also known as niacinamide and nicotinic acid amide, is the amide of nicotinic acid (vitamin B3 / niacin). Nicotinamide is a water-soluble vitamin and is part of the vitamin B group. Nicotinic acid, also known as niacin, is converted to nicotinamide in vivo, and, though the two are identical in their vitamin functions, nicotinamide does not have the same pharmacologic and toxic effects of niacin, which occur incidental to niacin's conversion. Thus nicotinamide does not reduce cholesterol or cause flushing. In cells, nicotinamide is incorporated into nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). NAD+ and NADP+ are coenzymes in a wide variety of enzymatic oxidation-reduction reactions. Overall, it rarely causes side effects, and is considered generally safe as a food additive, and as a component in cosmetics and medication. In the UK, nicotinamide can be purchased over-the-counter from many high-street retailers and pharmacies for use as a nutritional supplement. The British National Formulary lists no drug interactions for nicotinamide.

Radiotherapy with Carbogen and Nicotinamide

In terms of hypoxia modification, nicotinamide appears to reduce impaired tumour blood flow and thus affects the proportion of acutely hypoxic cells. A major component of its activity is the improvement of tumour oxygenation resulting from a reduction in microregional ischaemia. Nicotinamide is known to reduce arterial blood pressure in rodents, suggesting a vascular component in its mechanism of action. A direct effect on supplying blood vessels probably contributes to the oxygenating action of nicotinamide in tumours. Athough the precise mechanism remains obscure, studies on murine tumours suggest that nicotinamide, at least at high doses, reduces the occurrence of transient decreases in microregional perfusion.[32]

Both carbogen and nicotinamide have radiosensitizing properties. When used together in animal models they produce enhancement ratios of 1.9 with conventionally fractionated

radiotherapy and 2.8 with accelerated radiation schedules.[33,34] Over the past 15 years a number of early phase human studies have been conducted using accelerated radiotherapy with carbogen and nicotinamide (ARCON) in a variety of tumour sites.[35-39] Some of these studies encountered significant nausea and vomiting due to the nicotinamide resulting in reduced patient compliance. The common conclusion is that a dose of 80mg/kg or higher is not feasible and future studies should proceed with a starting dose of 60mg/kg.

As none of these studies were randomised, it was not possible to formally evaluate efficacy. However two studies had sufficient patient numbers and length of follow up to draw tentative conclusions in comparison with historical controls. A study in glioblastoma multiforme, ARCON seemed to have no apparent benefit and concluded with the suggestion that a phase III trial was not justified.[40] In contrast, a larger study of 215 patients with locally advanced head and neck cancer indicated very favourable rates of locoregional tumour control and overall survival.[41] The actuarial 3-year local control rates were 80% for larynx, 69% for hypopharynx, 88% for oropharynx, and 37% for oral cavity tumors. Regional control rates were 100% for N0, 93% for N1, and 74% for N2 disease.

Phase III evidence

A large improvement survival has recently been demonstrated using this approach during radiotherapy for bladder cancer.[42] The randomised multicentre phase III BCON trial, designed and coordinated at Mount Vernon Cancer Centre, demonstrated a 13% benefit in overall survival at three years from randomisation when radiotherapy was combined with carbogen and nicotinamide compared to radiotherapy alone (hazard ratio for death from any cause was 0.8613, CI 0.7445–0.9955, p=0.04) **(figure 1)**. Furthermore, there was minimal additional morbidity in the experimental arm.

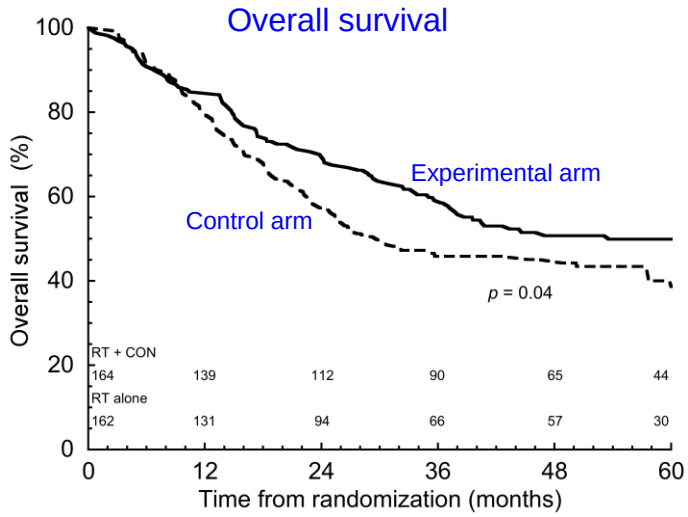


Figure 1: A plot of the Kaplan-Meier estimate of overall survival in the BCON trial of radiotherapy alone (control arm) versus radiotherapy plus carbogen and nicotinamide (experimental arm). The hazard ratio for death from any cause was 0.8613, CI 0.7445–0.9955, p=0.04.

Evidence in prostate cancer

Our group has also recently provided the first evidence that carbogen gas breathing can improve tumour oxygenation in prostate cancer.[43] This translational research in murine xenografts and humans showed a mean reduction tumour hypoxia of 6.4% (p = 0.003) for DU145 xenografts and 5.8% (p = 0.007) for PC3 xenografts. 64% of the human tumours

showed an increase in tumour oxygenation during carbogen inhalation with a mean improvement of 21.6% (p = 0.0005). **(figure 2)**

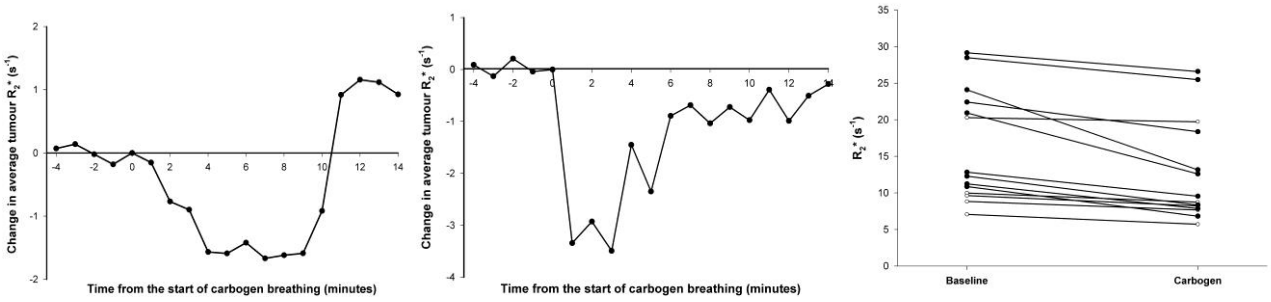


Figure 2: The temporal effect of carbogen breathing on the oxygenation of the 23 DU145 xenografts (left panel) and 17 PC3 xenografts (middle panel). Hypoxia was measured using blood oxygen level dependent MRI (R_2*). R_2* values fall rapidly after the commencement of carbogen breathing (at time 0), as tumour oxygen levels increase. R_2* values return to baseline after carbogen exposure was terminated. The right panel shows the effect of carbogen gas breathing on the median R_2* value of 14 individual human prostate cancers (age 56-76 years old, Gleason grade 6-8, PSA 1.9-32.0 ng/ml). Each line represents an individual tumour, lines with filled black circles represent tumours with a change in R_2* that was statistically significant at the 95% confidence level. There was a mean reduction in R_2* of 21.6% (p = 0.0005).

The overall sequence of evidence has demonstrated that hypoxia results in poor outcomes following radiotherapy for prostate cancer; that prostate tumour hypoxia is amenable to modification using carbogen gas and that the combination of carbogen and nicotinamide with pelvic radiotherapy can significantly improve survival without added toxicity. On this basis we believe that there is sufficient expectation that the success of the BCON trial in bladder cancer can be repeated in prostate cancer.

The fact that the cost of carbogen and nicotinamide is little over £100 per patient, makes this an extremely economical way of improving outcomes for prostate cancer patients. Furthermore, the versatility of this treatment means that if successful there is no reason why this strategy could not be combined with other developments in prostate radiotherapy such as hypofractionation, stereotactic radiosurgery or high-dose rate brachytherapy.

2 Objectives of the trial

2.1 Primary objective

- To determine the efficacy (as measured by PSA relapse-free survival) of carbogen gas breathing and nicotinamide tablets, given during a 74Gy course of intensity-modulated radiotherapy to the prostate gland in previously untreated patients with prostate cancer.

2.2 Secondary Objectives

- To describe the toxic effects of the combination of radiotherapy with carbogen and nicotinamide.
- To describe the time to progression, the proportion achieving PSA control following radiotherapy (PSA<1ng/ml), the local control rate and overall survival.
- Translational imaging research will explore: (1) Whether hypoxia modification benefits androgen deprived tumours to an even greater extent that hormone-naive cancers. (2) The way radiotherapy alters the extent and distribution of hypoxia within prostate tumours, which will be important for the development of hypoxia-targeted radiotherapy. (3) Whether imaging biomarkers can predict which patients may benefit most from hypoxic modification.
- Translational immunohistochemistry research will attempt to determine: (1) Whether immunohistochemical markers of hypoxia and angiogenesis can be used to determine prognosis following radiotherapy for prostate cancer. (2) Whether immunohistochemical markers of hypoxia and angiogenesis in prostate cancer can be used to predict which patients may benefit from hypoxia modification.

2.3 End-points

Primary: Biochemical Relapse Free Survival

Secondary: Time to Progression
Local Control
Overall Relapse Free Survival (Biochemical + Clinical)
Overall Survival
Safety Profile (As measured by CTCAE v 4)
Quality of Life (As measured by IPSS, FACT-P, IIEF-5, use of alpha blockers, and use of PDE5 inhibitors)

3 Patient selection criteria

Inclusion Criteria

- Histological diagnosis of prostate adenocarcinoma of Gleason grade 3+3 or higher
- Radical radiotherapy is considered to be appropriate treatment
- Any of: PSA > 20ng/ml, Gleason grade ≥ 8, T3 disease on MRI
- Patients must have radiographically documented measurable disease on pelvic MRI scan within 3 months of trial entry
- Age over 18 with no upper age limit
- Before patient registration, written informed consent must be given according to GCP and local regulations.
- Life expectancy of more than 5 years based on other co-morbidities

Exclusion Criteria

- Metastatic disease (including pelvic lymph node metastases) on conventional imaging including pelvic MRI scan and isotope bone scan within 3 months of trial entry
- PSA > 50ng/ml
- T4 disease on pelvic MRI scan within 3 months of trial entry
- Prior treatment for prostate cancer, either local or systemic (other than neo-adjuvant androgen deprivation for a period of less than 3 months)
- Current active malignancy other than prostate cancer or non-melanomatous skin cancer
- Previous radiotherapy to the pelvis
- Co-morbid conditions such that the technique of external beam radiotherapy is inappropriate
- Contraindication to MRI (only applicable to patients that are being considered for entry into the imaging component of the study)
- Current treatment with an ACE inhibitor
- Psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule; those conditions should be discussed with the patient before registration in the trial

4 Trial Design

A single centre, open-label non-randomised Phase Ib/II study. The protocol will accrue up to 50 patients with high-risk prostate cancer.

Phase Ib

Although the doses of radiotherapy, carbogen and nicotinamide are established from previous studies, this combination has never before been tested in prostate cancer. Minimal toxicity was seen in the BCON study for bladder cancer and we do not expect appreciable differences in PROCON. Although the normal tissues at risk are similar, radiotherapy will be delivered to a higher overall dose, albeit to smaller volumes. We therefore initially propose to confirm the tolerability of the regime:

- Toxicity will be assessed using CTCAE version 4, gastrointestinal and genitourinary scores (see appendix B)

- 3 patients will be recruited initially. If ≤1 experiences ≥grade 3 urinary, bowel or pain toxicity within 4 weeks of the end of treatment then recruitment will continue to 6 patients.

- If no further patients experience ≥grade 3 urinary or bowel or toxicity within 3 months from the end of treatment then phase II recruitment can proceed.

- If 2 of the first 6 patients experience ≥grade 3 urinary or bowel toxicity within 3 months from the end of treatment then recruitment will be allowed to continue to 12 patients. If no further ≥grade 3 urinary or bowel toxicity within 3 months from the end of treatment is seen then phase II recruitment can proceed.

- If >1 of the first 3 or >2 of the first 12 patients experience ≥grade 3 urinary or bowel toxicity within 3 months from the end of treatment then phase II testing will be abandoned.

Phase II

Current PSA relapse-free survival (PSA-RFS) estimates at 3 years for high-risk disease treated with radiotherapy range between 40% and 70%. The PROCON strategy would be rejected for phase III testing if the PSA-RFS rate was <40% at 3 years. The null hypothesis is that the PSA-RFS rate (H0) is 40% versus alternating hypotheses that the rate (Ha) is 60%. For a significance level (i.e., the probability of rejecting H0 when it is true) of $\alpha=0.05$ and a power (i.e., the probability of deciding the strategy is effective) of 80% the expected sample size with this design is 48 using a beta-approximation of a one sample test of proportions.

We therefore seek ethical approval to recruit 50 patients in total to account for withdrawals.

Patient Replacement

At least six patients in the phase Ib trial must complete their course of radiotherapy and a 3-month follow up period before accrual to phase II may begin.

If a patient is withdrawn from the phase Ib study prior to completing their course of radiotherapy, or the 3 month follow up period, without experiencing ≥grade 3 urinary or bowel toxicity prior to withdrawal, an additional patient may be added. Such patients will still be included in the analysis of secondary endpoints and, wherever possible, will be followed-up in accordance to protocol.

If a patient is withdrawn from the phase II study prior to completing their course of radiotherapy, or the 3 month follow up period, they will not be replaced.

End of Trial Definition

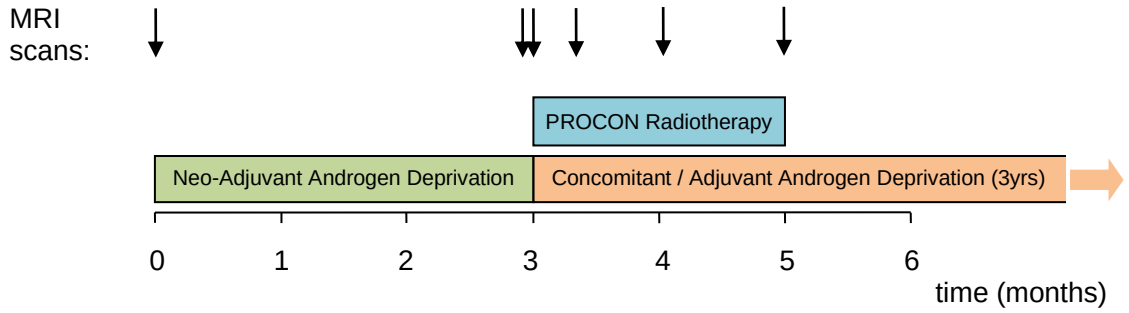
The end of the trial is defined by the last visit of the last subject undergoing the trial, which will be the 5 year follow up appointment of the last participant.

Storage of Data

Research data generated by this study and any histological specimen related to this study will be stored for 10 years. Personal data will be stored for 12 months after the end of the study.

5. Therapeutic regimens and expected toxicity

5.1 Overview



The overall treatment scheme is represented in the above diagram. 20 of the 50 patients will be invited to participate in the imaging component of the study. These patients will each be examined with a total of six MRI scans. The remaining 30 patients will receive the same PROCON radiotherapy schedule without any imaging. The description of the treatment schedule below includes the imaging component, which can be disregarded for those patients not included in the MRI component.

1) Following recruitment, patients will receive a baseline multi-functional MRI scan.

Details of the imaging research and MRI protocols can be found in Section 5.3

2) Patients will commence androgen deprivation on the day after the MRI scan
3) Androgen deprivation will continue for a total of 36 months
4) After 3 months of androgen deprivation patients will receive two further multi-functional MRI scans on consecutive days to measure the reproducibility of the imaging biomarkers prior to radiotherapy. These scans will be performed during the week prior to the commencement of PROCON radiotherapy.
5) PROCON radiotherapy commences after 3 months of androgen deprivation, lasting for 7½ weeks
6) Three further multi-functional MRI scans will be performed at the end of the first and third weeks of radiotherapy and at the end of the treatment course
7) Following radiotherapy, patients will then be seen in the outpatients department at 2 weeks, 4 weeks, 12 weeks, 6 months, 12 months and then 6 monthly for 5 years. At each visit they will be clinically assessed, have blood taken for PSA analysis and evaluated for toxicity using CTCAE v 4, IPSS, IIEF-5, use of alpha blockers, and use of PDE5 inhibitors

5.2 Androgen Deprivation

Androgen deprivation for 36 months is standard therapy for all high-risk prostate cancer patients that receive radiotherapy and would occur whether or not patients decide to enter the study. The drugs used for androgen deprivation will be used in their usual manner in accordance with the terms of their regulatory approval.

All patients will receive bicalutamide 50mg orally (AstraZenca, London, UK), once daily for a total of 28 days. After 14 days of bicalutamide treatment, patients will receive their first injection of goserelin 10.8mg subcutaneous depot injection (AstraZenca, London, UK). Goserelin injections will then be given every 3 months for 3 years (12 injections in total).

5.3 MRI Scanning

20 of the 50 patients will be asked to participate in the MRI aspect of the study; each will receive a total of 6 MRI scans. The timing of these scans is depicted in the treatment overview (section 5.1). One scan will be performed before starting androgen deprivation to give overall baseline values. Two scans will be performed on consecutive days after three months of androgen deprivation during the week prior to radiotherapy. These scans will demonstrate the effects of androgen deprivation and give reproducibility and repeatability assessments that can be used to evaluate the significance of any changes seen in subsequent scans. Further scans will be performed at the end of the first, third and last weeks of radiotherapy to determine the changes that occur during PROCON radiotherapy.

Patients will all be asked to complete the MRI safety questionnaire to ensure that they are suitable for the MR imaging examination (See Appendix C)

Before the imaging examination, patients will have a 20 gauge peripheral intravenous cannula inserted into the dorsum of the hand using aseptic procedures. Carbogen breathing apparatus will be assembled however the patient will begin the examination breathing room air (see figure 3, section 5.5).

The following imaging sequences will be performed:

1) Anatomical images
2) Diffusion Weighted Imaging (DWI)
3) Intrinsic Susceptibility Weighted MRI (ISW-MRI) Breathing Room Air
4) Intrinsic Susceptibility Weighted MRI Breathing Carbogen
5) Dynamic Contrast Enhanced MRI (DCE-MRI)
6) Dynamic Susceptibility Contrast MRI (DSC-MRI)

Carbogen breathing will commence after the first ISW-MRI scan. Patients will breathe carbogen for 10 minutes before the second ISW-MRI scan to allow oxygen equilibration to occur. Carbogen breathing will stop after the second ISW-MRI scan and the carbogen breathing apparatus will be disassembled. Patients will complete the imaging series breathing room air.

An intravenous bolus of 0.1 mmol/kg of gadopentetate dimeglumine (Gd-DTPA, Bayer-Schering) will be administered at 4 ml/s during the DCE-MRI acquisition using a power injector followed by 20ml of normal saline. Similarly, bolus of 0.2 mmol/kg of Gd-DTPA will be administered at 4 ml/s during the DSC-MRI acquisition followed by 20ml of normal saline.

The entire imaging procedure will take 50-55 minutes

5.4 Radiotherapy

Radiotherapy will be planned and administered according to the existing current Mount Vernon Cancer Centre policy (version 5, June 2010, appendix D):

Patients will all be asked to follow a low-residue diet from 2 weeks before radiotherapy planning until the completion of radiotherapy (appendix E). Internal audit has demonstrated that this diet significantly reduces the need for repeated imaging for radiotherapy planning, reduces prostate motion during radiotherapy and reduces the incidence of treatment related diarrhoea.

Radiotherapy Planning

Target volume: Prostate, seminal vesicles and pelvic lymph nodes

Volume Definition: CTVp: Prostate and seminal vesicles

CTVn: Pelvic nodes below bifurcation of common iliac vessels to include internal iliacs, obturator, presacral and external iliac nodes. To follow vessels as defined by contrast CT with asymmetric manual expansions to nodes along tissue planes as defined by Taylor *et al.* Clinical Oncology 2007; 19: 542-550

CTVb: Enlarged lymph nodes to be boosted.

Expansions: PTVp: CTVp expanded 10mm except posteriorly where 5mm
PTVn: CTVn + 5mm expansion all dimensions
PTV1: PTVp + PTVn
PTVb: CTVb expanded 5mm in all dimensions

Normal Tissues: Rectum, bladder and bowel to be outlined

Prescription: Conventionally Fractionated
PTVn 60Gy in 37 fractions
PTVp 74Gy in 37 fractions
PTVb 63Gy in 37 fractions

Normal Tissue Dose Constraints:

	Dose(%)	Total dose (74Gy)	Max Vol(%)
Rectum	68%	50Gy	60%
	81%	60Gy	50%
	88%	65Gy	30%
	95%	70Gy	15%
	100%	74Gy	3%
	102%	75.5Gy	0%
Bladder	68%	50Gy	50%
	81%	60Gy	25%
	95%	70Gy	5%

Small Bowel	61%	45Gy	78ml
	68%	50Gy	17ml
	74%	55Gy	14ml
	81%	60Gy	0.5ml
	88%	65Gy	0ml
Sigmoid	61%	45Gy	78ml
	68%	50Gy	17ml
	74%	55Gy	14ml
	81%	60Gy	0.5ml
	88%	65Gy	0ml

5.5 Carbogen Gas Breathing

Carbogen gas will be administered using a tight-fitting facemask at a flow rate of 10 litres/min. Patients will be given the opportunity to get used to the experience of carbogen breathing before the first exposure (figure 3a). Patients will breathe carbogen gas for 10 minutes before and during each fraction of radiotherapy. Patients taking part in the imaging component of the study will also be asked to breathe carbogen gas for approximately 15 minutes during each MRI scan (figure 3b).

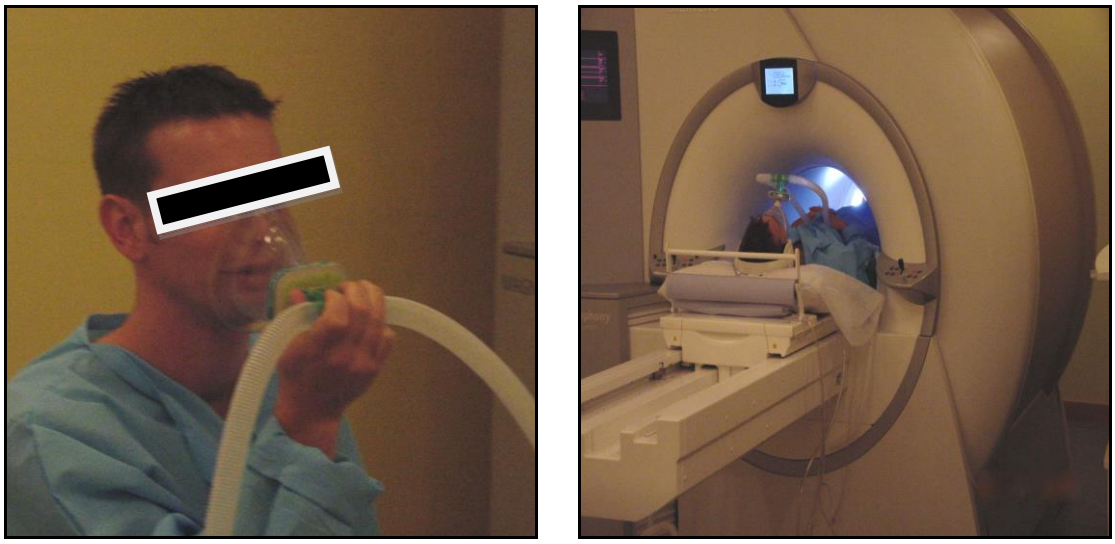


Figure 3. Carbogen Gas breathing. (A) Patients will be allowed to get used to the feeling of breathing carbogen gas by holding the mask and controlling the exposure themselves. (B) The mask will be securely fitted during MRI scanning and radiotherapy. (photographs with permission)

5.6 Nicotinamide (NICOBION®, Mawdsleys Clinical Services, Doncaster, UK)

Patients will receive oral nicotinamide tablets at a dose of 60mg/kg on each radiotherapy day (37 treatment days in total). Tablets should be taken each morning before attending for radiotherapy. Dose will be capped at 4g per treatment day.

5.7 Dose Adjustments

Nicotinamide

The major adverse effect of nicotnamide which limits dose is nausea. Nausea will be graded using the NCI Common Terminology Criteria for Adverse Events Version 4.0 (appendix B)

Grade of Nausea	Management
≤ grade 1	Administer anti-emetic – no change in nicotinamide dose
Grade 2	Administer anti-emetic – reduce nicotinamide dose to 40mg5.7kg
≥ grade 3	Administer anti-emetic. Hold nicotinamide until < grade 2 then resume at 40mg/kg. Patients requiring a delay of more than 1 week should discontinue nicotinamide for the remainder of the study period.

Carbogen

Some patients experience a sensation of breathlessness and claustrophobia when breathing carbogen. This is usually resolved with reassurance and practice, however if persistent and debilitating the patient will continue without carbogen breathing.

5.8 Concomitant therapy

Other anti-cancer or investigational therapies are not permitted from the time of recruitment until the completion of the course of PROCON radiotherapy.

5.9 Expected adverse events

All known toxicities from each component of the treatment are listed below and should thus be exempted from being recorded as adverse drug reaction via the yellow card scheme. All grades are in accordance with CTCAE version 4.

- Radiotherapy: All grade 1/2 gastrointestinal and genitourinary side effects as listed on CTCAE version 4
- Goserelin: hot flushes and sweating, sexual dysfunction, gynaecomastia, hypersensitivity reactions (rashes, pruritus, asthma, and rarely anaphylaxis), injection site reactions, headache, visual disturbances, dizziness, arthralgia and possibly myalgia, hair loss, peripheral oedema, gastro-intestinal disturbances, weight changes, sleep disorders, and mood changes.
- Bicalutamide: nausea, diarrhoea, cholestasis, jaundice; asthenia, weight gain; gynaecomastia, breast tenderness, hot flushes, impotence, decreased libido; anaemia; alopecia, dry skin, hirsutism, pruritus, vomiting, abdominal pain, dyspepsia, interstitial lung disease, pulmonary fibrosis, depression, haematuria, thrombocytopenia, hypersensitivity reactions including angioneurotic oedema and urticaria, cardiovascular disorders (including angina, heart failure, and arrhythmias), and hepatic failure
- Carbogen: transient sensation of dypsnoea
- Nicotinamide: allergic reactions, grade 1/2 nausea, vomiting, diarrhoea

All other toxicities not listed above should be considered as adverse drug reactions, and be reported to the chief investigator, who will report to the MHRA as indicated.

5.10 Urgent Safety Measures

In the event of patients becoming unwell, they will be advised to seek urgent medical advice from either the chief investigator or his deputy (Dr Kent Yip, Clinical Research Fellow) during office hours (9am-5pm Monday-Friday), or the duty oncology registrar at Mount Vernon Cancer Centre out of hours.

6. Clinical evaluation, laboratory tests, follow-up

6.1 Before treatment start

Stage and Prognostic Information

Histological evaluation	at any time point before treatment start
Isotope bone scan	within 3 months of trial entry
MRI pelvis	within 3 months of trial entry
PSA	within 1 month of trial entry
Blood for genetic analysis	within 3 months of trial entry
Urine for gene fusion-protein analysis	within 3 months of trial entry

Evaluating patient eligibility (within 1 month of trial entry)

Clinical Examination & Digital Rectal Examination (DRE)

Assessing baseline values (all within 14 days of trial entry)

- CTCAE version 4.0 Gastrointestinal, Genitourinary and Erectile Dysfunction scores (appendix B)
- International Prostate Symptom Score (IPSS) (appendix F)
- Functional Assessment of Cancer Therapy – Prostate (FACT-P) quality of life measurement (appendix G)
- IIEF-5 sexual health questionnaire (appendix H)
- Initial Form (appendix I)

Imaging

Patients taking part in the imaging aspect of the study will have an MRI scan before commencing androgen deprivation and two scans during the week prior to the start of radiotherapy according to the imaging protocol (see above).

6.2 During treatment

No investigations are required during treatment other than in response to specific medical complications as determined by the attending medical practitioner. Patients taking part in the imaging aspect of the study will have MRI scans at the end of the first, third and seventh week of radiotherapy according to the imaging protocol (see above).

6.3 After the end of treatment (Follow-up)

Patients will be seen at 2, 4, 12 and 26 weeks after treatment. Thereafter they will be seen at six monthly intervals for a total of five years in accordance with our standard protocol.

At 2, 4 and 12 weeks the following will be performed:

- CTCAE version 4.0 Gastrointestinal and Genitourinary scores
- International Prostate Symptom Score (IPSS)

At each visit thereafter the following will be performed:

- CTCAE version 4.0 Gastrointestinal, Genitourinary and Erectile Dysfunction scores
- International Prostate Symptom Score (IPSS)
- Functional Assessment of Cancer Therapy – Prostate (FACT-P) quality of life measurement
- IIEF-5 sexual health questionnaire
- Follow up form (Appendix J)
- Serum PSA

Each patient will be followed up for 5 years after the end of their treatment

6.4 Summary table

	Baseline	2 weeks	4 weeks	12 weeks	26 weeks	every 6 months thereafter
Bone Scan	✓					
MRI	✓					
Blood / Urine	✓				✓	✓
Clinical Exam	✓				✓	✓
DRE	✓				✓	✓
CTCAEv4	✓	✓	✓	✓	✓	✓
IPSS	✓	✓	✓	✓	✓	✓
FACT-P	✓				✓	✓
IIEF-5	✓				✓	✓
Initial Form	✓					
Follow-up form		✓	✓	✓	✓	✓

7 Criteria of evaluation

Biochemical relapse-free survival

- The time from 1st treatment to biochemical relapse or
- The time from 1st treatment to censorship

Patients will be censored at the time they are withdrawn from the trial for any reason or in the case of them being lost to follow-up, at the time of their last assessment.

Patients will be assessed for biochemical relapse at 2, 4, 12 and 26 weeks after treatment. Thereafter they will be assessed at six monthly intervals for a total of five years.

Biochemical relapse is defined according to the RTOG-ASTRO 'Phoenix' definition of relapse and is defined as PSA nadir +2ng/ml, with time to relapse being defined as the time from the first day of androgen deprivation to the date of the PSA blood sample that exceeds the nadir +2ng/ml threshold.

Overall relapse-free survival

includes both *clinical* and *biochemical* relapse.

- The time from 1st treatment to overall (clinical or biochemical) relapse or
- The time from 1st treatment to censorship

Patients will be censored at the time they are withdrawn from the trial for any reason or in the case of them being lost to follow-up, at the time of their last assessment.

Patients will be assessed for overall relapse at 2, 4, 12 and 26 weeks after treatment. Thereafter they will be assessed at six monthly intervals for a total of five years.

Clinical relapse includes any confirmed diagnosis of recurrent prostate cancer that has occurred either locally or distant from the prostate. The diagnosis can be made by; 1) biopsy or 2) radiological assessment including isotope bone scan, MRI scan or CT scan. Suspicion raised by abnormal plain radiography should be confirmed with isotope or axial imaging, or a PSA test.

Overall survival

- The time from registration to death from any cause.
- All patients are evaluable from the date of their 1st day of androgen deprivation.
- Patients will be censored at the time they are withdrawn from the trial for any reason or in the case of them being lost to follow-up, at the time of their last assessment.
- Patients will be assessed for survival at 2, 4, 12 and 26 weeks after treatment. Thereafter they will be assessed at six monthly intervals for a total of five years.

Safety profile

- Safety profile will be assessed baseline and then at 2, 4, 12 and 26 weeks after treatment. Thereafter it will be assessed at six monthly intervals for a total of five years.
- CTCAE version 4.0 criteria will be used to assess safety (see appendix B)

Quality of Life

- Quality of life will be assessed at baseline and then at 12 and 26 weeks after treatment. Thereafter it will be assessed at six monthly intervals for a total of five years.
- International Prostate Symptom Score (IPSS), Functional Assessment of Cancer Therapy – Prostate (FACT-P) quality of life measurement, the IIEF-5 sexual health questionnaire and questions regarding drug use will be used for this assessment.

8 Statistical considerations

8.1 Statistical design

A single centre, open-label non-randomised Phase Ib/II study. The protocol will accrue up to 50 patients with high-risk prostate cancer (as described in section 4 above).

- The primary outcome is biochemical relapse
- The primary endpoint is biochemical relapse-free survival

- Secondary outcomes include clinical relapse, death from any cause, toxicity and quality of life
- Secondary endpoints are overall relapse-free survival, overall survival, safety profile (as measured by CTCAEv4.0) and quality of life (as measured by IPSS, FACT-P, IIEF-5 and drug use)

8.2 Statistical Analysis

Baseline characteristics will be summarised using descriptive statistics

Relapse-free survival and overall survival for all patients will be determined using the Kaplan-Meier method and specified in terms of median survival, 2-year survival and 5-year survival estimates. Survival plots will be presented.

Safety profile, as measured by CTCAEv4, will be analysed and presented in terms of prevalence and incidence across dose levels.

All grade 3, 4 and 5 adverse events will be described.

Each quality of life measure will be presented as an increase / decrease from baseline using descriptive statistics (mean, standard deviation & range).

Functional MRI parameters that are investigated as part of translational research will be analysed as follows:

Predictors of Survival: Cox Proportional Hazards Regression
Predictors of Response: Logistic Regression

All tests will be two-sided and significance will be defined as a p-value of ≤0.05.

9 Translational Research

1) Imaging

Magnetic Resonance Imaging provides the gold-standard for anatomical prostate assessment together with the ability for simultaneous multi-functional acquisition of a host of validated biomarkers related to hypoxia, proliferation, apoptosis, cellular density, blood flow, vascular integrity as well as tumour grade, invasion and metastatic potential. Previous studies have demonstrated the potential for individual or combinations of these imaging parameters as predictive and prognostic markers. Correlations with immunohistochemical markers of hypoxia, proliferation and angiogenesis have suggested a biological basis for the measurements.

Our group has shown that androgen deprivation causes a 5-fold reduction in prostate cancer blood flow and an associated 41% reduction in tumour oxygenation.[44] Using imaging we have also demonstrated the oxygen enhancing effect of hypoxia modification in hormone naive patients.[43] This study provides the perfect opportunity to further this line of investigation. We aim to demonstrate:

i) Whether hypoxia modification benefits androgen deprived tumours to an even greater extent that hormone-naive cancers.
ii) The way radiotherapy alters the extent and distribution of hypoxia within prostate tumours, which will be important for the development of hypoxia-targeted radiotherapy.
iii) Whether imaging biomarkers can predict which patients may benefit most from hypoxic modification.

2) Immunohistochemistry (IHC)

A recent report has suggested that certain IHC markers (VEGF, HIF1 and osteopontin) may be able to predict failure following both radiotherapy and prostatectomy surgery and may therefore play a role in patient selection.[23] These results have not yet been verified in an independent data set, as is required for validation of any new biomarker. Furthermore, there are several other IHC markers of hypoxia, angiogenesis and proliferation that are as yet untested in prostate cancer. This research aims to:

i) Determine whether immunohistochemical markers of hypoxia and angiogenesis can be used to determine prognosis following radiotherapy for prostate cancer.
ii) Determine whether immunohistochemical markers of hypoxia and angiogenesis in prostate cancer can be used to predict which patients may benefit from hypoxia modification.

This work will be performed at University College London. Tissue blocks from the diagnostic biopsy will be collected. Sections from each subject will be stained for markers of hypoxia (Glut1, HIF-1□□□Osteopontin), vascularity (VEGF, CD34) and cellular proliferation (Ki67). IHC scores will then be combined with the outcome data. Survival curves will be calculated for each of the IHC stains, using the Kaplan–Meier method and compared with the log rank test using a two-way p-value.
To predict which patients may benefit from hypoxia modification, patients will be stratified into two groups based on their MRI-derived hypoxia response score (i.e good response v.

poor response) for each parameter, using median values as the threshold. Chi-squared tests will be performed to assess significance.

3) Genomic analysis

Tissue blocks will be sent to Prof Catharine West at the Patterson Institute in Manchester (see collaborator's letter, appendix K).

4) Germline genetic analysis

Whole Blood will be collected for DNA extraction (this can be performed at any point during the study). Blood will be collected into EDTA –BD Vacutainer KTE 10.8mg, 6ml tubes (sterile, EDTA to prevent clotting, plastic, for DNA extraction). No processing is required. Tubes will be transfered to a -80^{o}C freezer as soon as possible. (The samples can be stored at 4oC for up to 24 hours). Blood tubes will then be sent for analysis to Professor Ros Eeles at the Cancer Genetics Unit, The Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey, SM2 5PT.

5) Urine gene fusion protein analysis

Urine samples will be transferred to the Department of Cancer Studies and Molecular Medicine, Clinical Sciences Unit, University of Leicester for inclusion in their collection for an analysis of urinary TMPRSS2:ETS gene fusion to determine their diagnostic utility in prostate cancer.

6) Serum osteopontin analysis

Whole blood will be collected into EDTA-BD Vacutainer KTE 10.8 mg, 6 ml tubes (sterile, EDTA to prevent clotting). The sample will need to be centrifuged within 30 minutes of collection at 1000 x g to obtain plasma. This can then be assayed immediately or aliquoted and stored at <-20 ^{o}C. Repeated freeze-thawing cycles should be avoided. Analysis of serum osteopontin levels will be made using an ELISA method. Comparison with the immunohistochemical assessment of osteopontin from the prostate biopsy specimen will be made to see whether serum measurements can substitute for biopsy measurement.

10 Patient registration procedure

Once a patient has been identified and has agreed to consider trial entry the referring physician should contact either of:

Information redacted in e-thesis

PATIENTS MUST NOT BE COMMENCED ON ANDROGEN DEPRIVATION UNTIL TRIAL REGISTRATION IS COMPLETE

Some patients will require imaging *before* androgen deprivation starts

The following information should be faxed to the Marie Curie Research Wing at Mount Vernon Cancer Centre on [redacted]

- Patient Details (Name, Date of Birth, Address, Phone Numbers, GP details, NHS number)
- A copy of the patient's PSA blood test report
- A copy of the patient's prostate biopsy histopathology report
- A copy of the patient's bone scan report
- A copy of the patient's MRI scan report

11 Reporting adverse events

11.1 Definitions

An **Adverse Event (AE)** is defined as any untoward medical occurrence or experience in a patient or clinical investigation subject which occurs following the administration of the trial medication regardless of the dose or causal relationship. This can include any unfavourable and unintended signs or symptoms, an abnormal laboratory finding or a disease temporarily associated with the use of the protocol treatment.

A **Serious Adverse Event (SAE)** is defined as any undesirable experience occurring to a patient, whether or not considered related to the protocol treatment. Adverse events and adverse drug reactions which are considered as **serious** are those which result in:

- death
- a life threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- hospitalization or prolongation of hospitalization
- persistent or significant disability/incapacity
- a congenital anomaly/birth defect
- any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above)

An **Adverse Reaction (AR)** is defined as any response to a medical product, that is noxious and/or unexpected, related to any dose. **Response to a medicinal product** means that a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

An **Unexpected Adverse Reaction** is any adverse reaction for which the nature or severity is not consistent with the applicable product information.

A **Serious Adverse Reaction (SAR)** is a Serious Adverse Event (SAE) which is considered related to the protocol treatment.

A **Suspected Unexpected Serious Adverse Reaction (SUSAR)** is a Serious Adverse Event (SAE) which is considered related to the protocol treatment where a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility and for which the nature or severity is not consistent with the applicable product information.

11.2 Reporting procedure

The following information must be collected for all reported adverse events:

- participant details
- reporter details
- adverse event description
- start date of event
- end date of event
- outcome of event
- severity of event
- relationship to study drug (i.e. causality/relatedness)
- action taken with study drug
- whether subject withdrawn due to adverse event
- whether the event is serious
- expectedness (especially important for Serious Adverse Reactions)

Reporting Requirements:

Adverse Events (AE)	All adverse events will be recorded as above
Adverse Reactions (AR)	All adverse reactions will be recorded as above and will be followed until resolution or the event is considered stable.
Serious Adverse Events (SAE)	All SAEs must be reported to the sponsor immediately (within 1 working day)
Serious Adverse Reaction (SAR)	All SARs must be reported to the MHRA and the relevant ethics committee, with copies to the clinical trials group (CTG) in the form of an Annual Safety Report on the anniversary of the Clinical Trials Authorisation (CTA).
Suspected Unexpected Serious Adverse Reaction (SUSAR)	SUSARs which are fatal or life-threatening must be reported to the MHRA, to the competent authorities and relevant ethics committees within 7 calendar days of awareness. Other SUSARs must be reported within 15 calendar days of awareness.

12 Ethical considerations

12.1 Patient protection

The principle investigator will ensure that this study is conducted in agreement with either the Declaration of Helsinki (Tokyo, Venice, Hong Kong, Somerset West and Edinburgh amendments) or the United Kingdom laws and regulations, whichever provides the greatest protection of the patient.

The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (*J Postgrad Med.* **2001**. 47(1): 45-50)

The protocol will be approved by the Local and National Ethics Committees.

12.2 Subject identification

Patients will be identified in the trial database using a unique trial reference number. This reference number will be independent of the patient's hospital number, NHS number, date of birth or any other identifiable information. Only the researchers named in this protocol will have access to the de-identification list that may be used to identify individual patients. Only anonymous data will be seen by the other members of the research team (such as statisticians, pathologists, immunohistochemistry technicians etc.)

12.3 Human tissue

No human tissue will be *collected* as part of this trial, however participants will be asked for permission to allow further analysis of their existing prostate biopsy samples. Patients will be asked to sign a specific consent for this aspect of the study (see consent form). If a patient refuses to consent to further analysis of their histological material, they can still proceed in the trial without participating in the immunohistochemical translational research. This is stated in the patient information sheet.

All human tissue will be handled and stored in accordance with the legislation that is set out in the Human Tissue Act (2004) and the Human Tissue Authority Revised Codes of Practice (Sept 2009) www.hta.gov.uk/legislationpoliciesandcodesofpractice.cfm

12.4 Informed consent

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. An example of a patient informed consent statement is given as an appendix to this protocol.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered in the study, in accordance with national and local regulatory requirements.

Also, patients will be specifically asked to consider and specifically sign the following statements:

'I consent to provide a blood sample, which will be used for laboratory studies looking into the genetic basis of prostate cancer.'

'If any gene changes of clinical significance are found in my samples in the future then I / my next of kin would like to be informed.'

The informed consent procedure will conform to the ICH guidelines on Good Clinical Practice. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

13 Publication Policy

Results of this study will be reported in national and international peer reviewed journals and be presented in national and international conferences.

14 Insurance and Indemnity

The potential legal liability for harm to participants arising from this study will be covered by the NHS indemnity scheme.

15 Data monitoring

This trial will be subject to routine monitoring by East and North Hertfordshire NHS Trust via the GCP Compliance Officer. During the study, the GCP Compliance Officer will regularly check the completeness of patient records, the accuracy of entries on the CRFs, the adherence to the protocol and to Good Clinical Practice, the progress of enrolment, and to ensure that study treatment is being sorted, dispensed, and accounted for according to specifications.

The investigator must maintain source information for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data and the results of any other tests or assessments. All information on CRFs must be traceable to these source documents in the patient's file. The investigator must also keep the original informed consent form signed by the patient (a signed copy is given to the patient). The investigator must give the GCP Compliance Officer access to all relevant source documents to confirm their consistency with the CRF entries. No information in source documents about the identify of the patients will be disclosed.

The data monitoring committee will consist of representatives from the R&D department, the pharmacy and the study team. It will meet after 3, 6, 12 patients have been recruited, and at regular intervals as required thereafter.

16 Trial sponsorship and financing

Sponsor

East and North Hertfordshire NHS Trust

Information redacted in e-thesis

Research and Development Contact:

Information redacted in e-thesis

Financing

This study is funded by The Prostate Cancer Charity (charity number 1005541)

The Prostate Cancer Charity
First Floor Cambridge House
Cambridge Grove
London
W6 0LE

Grant number:	**PG09-38**
Grant title:	A Phase Ib/II trial of prostate radiotherapy in conjunction with carbogen and nicotinamide (PROCON)
Award:	£240,721.00
Grant Start Date:	6th September 2010
Grant End Date	5th September 2013

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Appendix B: Common Terminology Criteria for Adverse Events (CTCAE) version 4

Gastrointestinal

Adverse Event	Grade				
	1	2	3	4	5
Abdominal pain	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	-
Anal mucositis	Asymptomatic or mild symptoms; intervention not indicated	Symptomatic; medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Anal pain	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	-
Anal ulcer	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; altered GI function	Severely altered GI function; TPN indicated; elective operative or endoscopic intervention indicated; disabling	Life-threatening consequences; urgent operative intervention indicated	Death
Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental ADL	Obstipation with manual evacuation indicated; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Diarrhoea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of ≥7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Faecal incontinence	Occasional use of pads required	Daily use of pads required	Severe symptoms; elective operative intervention indicated	-	-
Haemorrhoidal haemorrhage	Mild; intervention not indicated	Moderate symptoms; medical intervention or minor cauterization indicated	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated	-	-
Proctitis	Rectal discomfort, intervention not indicated	Symptoms (e.g., rectal discomfort, passing blood or mucus); medical intervention indicated; limiting instrumental ADL	Severe symptoms; fecal urgency or stool incontinence; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Rectal fistula	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; altered GI function	Severely altered GI function; TPN or hospitalization indicated; elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Rectal hemorrhage	Mild; intervention not indicated	Moderate symptoms; medical intervention or minor cauterization indicated	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Rectal mucositis	Asymptomatic or mild symptoms; intervention not indicated	Symptomatic; medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL	Life-threatening consequences; urgent operative intervention indicated	Death
Rectal pain	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	-
Rectal ulcer	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; altered GI function (e.g. altered dietary habits, vomiting, diarrhoea)	Severely altered GI function; TPN indicated; elective operative or endoscopic intervention indicated; disabling	Life-threatening consequences; urgent operative intervention indicated	Death

Urinary

Adverse Event	Grade				
	1	2	3	4	5
Cystitis noninfective	Microscopic hematuria; minimal increase in frequency, urgency, dysuria, or nocturia; new onset of incontinence	Moderate hematuria; moderate increase in frequency, urgency, dysuria, nocturia or incontinence; urinary catheter placement or bladder irrigation indicated; limiting instrumental ADL	Gross hematuria; transfusion, IV medications or hospitalization indicated; elective endoscopic, radiologic or operative intervention indicated	Life-threatening consequences; urgent radiologic or operative intervention indicated	Death
Haematuria	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; urinary catheter or bladder irrigation indicated; limiting instrumental ADL	Gross haematuria; transfusion, IV medications or hospitalization indicated; elective endoscopic, radiologic or operative intervention indicated; limiting self care ADL	Life-threatening consequences; urgent radiologic or operative intervention indicated	Death
Urinary frequency	Present	Limiting instrumental ADL; medical management indicated	-	-	-
Urinary incontinence	Occasional (e.g., with coughing, sneezing, etc.), pads not indicated	Spontaneous; pads indicated; limiting instrumental ADL	Intervention indicated (e.g., clamp, collagen injections); operative intervention indicated; limiting self care ADL	-	-
Urinary retention	Urinary, suprapubic or intermittent catheter placement not indicated; able to void with some residual	Placement of urinary, suprapubic or intermittent catheter placement indicated; medication indicated	Elective operative or radiologic intervention indicated; substantial loss of affected kidney function or mass	Life-threatening consequences; organ failure; urgent operative intervention indicated	Death
Urinary tract pain	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	-
Urinary urgency	Present	Limiting instrumental ADL; medical management indicated	-	-	-

Erectile Dysfunction

Adverse Event	Grade				
	1	2	3	4	5
Erectile dysfunction	Decrease in erectile function (frequency or rigidity of erections) but intervention not indicated (e.g., medication or use of mechanical device, penile pump)	Decrease in erectile function (frequency/rigidity of erections), erectile intervention indicated, (e.g., medication or mechanical devices such as penile pump)	Decrease in erectile function (frequency/rigidity of erections) but erectile intervention not helpful (e.g., medication or mechanical devices such as penile pump); placement of a permanent penile prosthesis indicated (not previously present)	-	-

Appendix C: MRI Safety Questionnaire

These questions are necessary for your safety, please answer them all.

Name: _____Date of Birth_____

Please tick Yes or No

	YES	NO
Have you got a pacemaker, defibrillator or programmable shunt?		
Have you had **ANY** heart surgery e.g. bypass, surgery or heart valve replacement?		
Have you **EVER** had surgery on your brain e.g. shunts, clips on your arteries or blood clots removed?		
If you have a shunt is it programmable?		
Have you **EVER** had metal fragments in your eyes even if the fragments have been removed?		
Do you have **ANY** shrapnel or bullet injuries?		
Have you had **ANY** surgery or biopsies?		
For women of childbearing age, could you be pregnant?		
Do you have an IUD/COIL or sterilisation clips or have you had a hysterectomy?		
Do you have any metal or electronic implants e.g. ear implants, joint replacements, pins, clips, plates or screws?		

If you have answered YES to any of the above questions please ring the MRI unit now.

You could save yourself a wasted journey.

Do you have dentures or a hearing aid?		
Do you suffer from epilepsy?		
Do you have any tattoos, piercing or permanent eyeliner?		
Are you breast-feeding at the moment?		
Do you have any allergies?		
Do you suffer from asthma, eczema or hay fever?		
Have you got a history or family history of glaucoma?		
Do you have chest, heart or kidney problems?		
Are you a diabetic?		

It is important that you remove all metal objects such as hair slides, jewellery, metallic body piercing and watches for your scan.
Please leave as many of these items at home as possible. Keys, money and credit cards must be kept outside of the scanning room.

IF YOU HAVE ANY QUESTIONS, PLEASE RING THE MRI UNIT ON 01923 844 283
Please sign below if you have understood and answered all the questions.

Patient signature_____
Date_____

Questionnaire checked
by_____
____

Would you consent to your images being used for: education and/or research purposes? If yes please sign below.

_____ _____
_____

Appendix D: Mount Vernon Cancer Centre Prostate Radiotherapy Protocols: v 5.0 June 2010

Low/intermediate risk
T1c T2a-c; PSA <15; Gleeson 7 or less

Target Volume: Prostate + seminal vesicles

Imaging: CT simulator with contrast imaging from anterior superior iliac spine to 3cm below ischial tuberosity

PLANNING PROTOCOL

CTV = Prostate + seminal vesicles

PTV = CTV + 10mm all directions except 5mm posterior

Normal tissues: Rectum and bladder whole organ to be outlined
Femoral heads NOT outlined
Small bowel NOT outlined (accept 110% to $2cm^3$)

PRESCRIPTION: 57Gy in 19f

57 x 1+ 3/3.5 = 57 x 1.86 = 106.02Gy $_{3.5}$

Dose constraints to rectum

Max volume	Max dose (Gy)
60%	42Gy
50%	49Gy
30%	53Gy
15%	57Gy
3%	61Gy

High Risk <70yrs
Clinical T3a, MRI T3b; PSA >20; Gleason 8-10 (any one)

Target volume: Prostate, seminal vesicles and pelvic nodes

Imaging: CT simulator with contrast whole pelvis
L2/3 to 3cm below ischial tuberosity

PLANNING PROTOCOL

CTVp Prostate and seminal vesicles

CTVn Pelvic nodes below bifurcation of common iliac vessels to include internal iliacs, obturator, presacral and external iliac nodes.

CTVb Enlarged lymph nodes to b boosted.

Lymph node volumes should follow vessels as defined by contrast CT using asymmetric manual expansions to nodes along tissue planes as defined in table below from Taylor et al Clinical Oncology 2007; 19: 542-550

Recommended modifications to margins

Lymph node group	Recommended margins*
Common iliac	7-mm margin around vessels; extend posterior and lateral borders to psoas and vertebral body
External iliac	7-mm margin around vessels; extend anterior border by additional 10-mm anterolaterally along iliopsoas muscle to include lateral external iliac nodes
Obturator	Join external and internal iliac regions with 18-mm-wide strip along pelvic sidewall
Internal iliac	7-mm margin around vessels; extend lateral borders to pelvic sidewall
Presacral	10-mm strip over anterior sacrum

*Also include any visible nodes.

PTVp CTV1 expanded 10mm except posteriorly where 5mm

PTVn CTV2 + 5mm expansion all dimensions

PTV1 PTVp + PTVn

PTVb CTVb + 5mm expansion all dimensions

Normal tissues: Rectum and bladder whole organ to be outlined
Femoral heads NOT outlined
Small bowel NOT outlined (accept 110% to 2cm^3) unless IMRT is being used

PRESCRIPTION

Six schedules are in use depending upon whether IMRT is used, whether the entire treatment is to be with external beam or an HDR boost will be used and whether the patient elects to take part in the HDR monotherapy study:

1. **EXTERNAL BEAM ALONE NON IMRT**
   PHASE I: PTV1: 46Gy in 23 fractions
   PHASE II: PTVp: 28Gy in 14 fractions external beam
2. **EXTERNAL BEAM ALONE IMRT (60)**
   PTVn: 47Gy in 20 fractions
   PTVp: 60Gy in 20 fractions
   PTVb: 52Gy in 20 fractions
3. **EXTERNAL BEAM ALONE IMRT (74)**
   PTVn: 60Gy in 37 fractions
   PTVp: 74Gy in 37 fractions
   PTVb: 63Gy in 37 fractions
4. **EXTERNAL BEAM NON IMRT + HDR BOOST**
   PTV1: 46Gy in 23 fractions
   PTVb: 52Gy in 23 fractions
   HDR Boost: 15Gy single fraction
5. **EXTERNAL BEAM IMRT + HDR BOOST**
   PHASE I: PTV1: 46Gy in 23 fractions
   PHASE II: PTVp: HDR 15Gy single dose
6. **HDR Monotherapy**
   26Gy in 2 fractions

Normal tissue constraints (based on CHIPP protocol) using 2Gy fractions

	Dose(%) Max Vol(%)	Total dose		
		60Gy/20f	46Gy/23f	74Gy/37f
Rectum	68% 60%	40.6	31.1	50
	81% 50%	48.7	37.3	60
	88% 30%	52.7	40.4	65
	95% 15%	56.8	43.5	70
	100% 3%	60	46	75
	102% 0	60.8	46.6	
Bladder	68% 50%	40.6	31.1	50
	81% 25%	48.7	37.3	60
	95% 5%	56.8	43.5	70
Small Bowel	61% 78ml	36.5	28.0	45
	68% 17ml	40.6	31.1	50
	74% 14ml	44.6	34.2	55
	81% 0.5ml	48.7	37.3	60
	88% 0	52.7	40.4	65
Sigmoid	61% 78ml	36.5	28.0	45
	68% 17ml	40.6	31.1	50
	74% 14ml	44.6	34.2	55
	81% 0.5ml	48.7	37.3	60
	88% 0	52.7	40.4	65

Appendix E: Low Residue Diet Sheet

A low residue diet is designed to reduce the *frequency* and *volume* of stools. It is similar to a low fibre diet but also restricts foods that increase bowel activity

It has been recommended that you follow a low residue diet during your radiotherapy.

We would like you to start this diet two weeks prior to your radiotherapy planning appointment and continue until your radiotherapy treatment has finished. **After treatment** you should **return to a normal diet** that includes a wide variety of nutritious foods

General Guidelines

- Remove fruit and vegetable skins where possible
- Cook vegetables well
- Caffeine stimulates bowel activity, therefore consume caffeine containing foods in moderation (eg cola, coffee, tea, energy drinks, chocolate)
- Alcohol stimulates bowel activity, therefore reduce alcohol consumption
- Ensure you have an *adequate fluid intake* (6-8 glasses/day) to assist with regular bowel motions

Foods to include

- White bread, buns and bagels
- Plain cereals (Rice Krispies, Corn Flakes, Cheerios, Special K)
- White rice, white pasta and noodles
- Fruit juices without pulp*
- Ripe, soft fruit* (eg bananas, apricots, grapes, peaches, melon, canned fruit)
- Well cooked, non-stringy vegetables* (eg carrots, potato (skinless), pumpkin (seedless and skinless), squash, aubergine)
- Well cooked tender meat and fish
- Eggs
- Milk and plain yoghurt, cheese

- Butter, mayonnaise, vegetable oils, margarine, gravies and dressings

* Can include these foods but aim to minimise intake

Foods to avoid

- Wholegrain breads, pasta and bran
- High fibre breakfast cereals (eg Bran Flakes, Muesli, Porridge, cereals containing dried fruit or nuts)
- Fruit/vegetable juices with pulp
- Fruit skins, seeds, pips
- High fibre fruit (eg berries, pears, figs, dates, prunes)
- Dried Fruit (eg sultanas, raisins, dried apricots)
- Beans and pulses (including canned beans, baked beans, cooked beans)
- Raw, fibrous vegetables (eg peas, sweet corn, celery, salads, brussel sprouts)
- Tough meat with gristle
- Nuts and seeds
- Popcorn
- Yoghurt/cheese containing fruit, seeds or nuts

Appendix F: International Prostate Symptom Score (IPSS)

	Not at all	Less than 1 time in 5	Less than half the	About half the time	More than half the	Almost always	**Your score**
Incomplete emptying Over the past month, how often have you had a sensation of not emptying your bladder completely after you finish urinating?	0	1	2	3	4	5	
Frequency Over the past month, how often have you had to urinate again less than two hours after you finished urinating?	0	1	2	3	4	5	
Intermittency Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
Urgency Over the last month, how difficult have you found it to postpone urination?	0	1	2	3	4	5	
Weak stream Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5	
Straining Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5	

	None	1 time	2 times	3 times	4 times	5 times or more	**Your score**
Nocturia Over the past month, many times did you most typically get up to urinate from the time you went to bed until the time you got up in the morning?	0	1	2	3	4	5	

Total IPSS score	

Quality of life due to urinary symptoms	Delighted	Pleased	Mostly satisfied	Mixed – about equally satisfied and dissatisfied	Mostly dissatisfied	Unhappy	Terrible
If you were to spend the rest of your life with your urinary condition the way it is now, how would you feel about that?	0	1	2	3	4	5	6

Total score: 0-7 Mildly symptomatic; 8-19 moderately symptomatic; 20-35 severely symptomatic

Appendix G: Functional Assessment of Cancer Therapy – Prostate (FACT-P) quality of life measurement

Below is a list of statements that other people with your illness have said are important**. Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

EMOTIONAL WELL-BEING

	Not at all	A little bit	Some-what	Quite a bit	Very much
I feel sad	0	1	2	3	4
I am satisfied with how I am coping with my illness	0	1	2	3	4
I am losing hope in the fight against my illness	0	1	2	3	4
I feel nervous	0	1	2	3	4
I worry about dying	0	1	2	3	4
I worry that my condition will get worse	0	1	2	3	4

FUNCTIONAL WELL-BEING

	Not at all	A little bit	Some-what	Quite a bit	Very much
I am able to work (include work at home)	0	1	2	3	4
My work (include work at home) is fulfilling	0	1	2	3	4
I am able to enjoy life	0	1	2	3	4
I have accepted my illness	0	1	2	3	4
I am sleeping well	0	1	2	3	4
I am enjoying the things I usually do for fun	0	1	2	3	4
I am content with the quality of my life right now	0	1	2	3	4

ADDITIONAL CONCERNS

	Not at all	A little bit	Some-what	Quite a bit	Very much
I am losing weight	0	1	2	3	4
I have a good appetite	0	1	2	3	4
I have aches and pains that bother me	0	1	2	3	4
I have certain parts of my body where I experience pain	0	1	2	3	4
My pain keeps me from doing things I want to do	0	1	2	3	4
I am satisfied with my present comfort level	0	1	2	3	4
I am able to feel like a man	0	1	2	3	4
I have trouble moving my bowels	0	1	2	3	4
I have difficulty urinating	0	1	2	3	4
I urinate more frequently than usual	0	1	2	3	4
My problems with urinating limit my activities	0	1	2	3	4
I am able to have and maintain an erection	0	1	2	3	4

Appendix H: IIEF-5 sexual health questionnaire

SCORE	1	2	3	4	5
How do you rate your confidence that you could get and keep an erection?	Very low	Low	Moderate	High	Very high
When you had erections with sexual stimulation, how often were your erections hard enough for penetration?	never or almost never	a few times	sometimes	most times	almost always or always
During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	never or almost never	a few times	sometimes	most times	almost always or always
During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	extremely difficult	very difficult	difficult	slightly difficult	not difficult
When you attempted sexual intercourse, how often was it satisfactory for you?	never or almost never	a few times	sometimes	most times	almost always or always

Total: (5-25)……

Appendix I: Initial Form

Name: Hospital No: Trial No:

Referring MVH Consultant		NAME
Date of birth		XX/XX/XX
Date of diagnosis		XX/XX/XX
Date of first symptoms		XX/XX/XX
Date first seen by Treating Clinic		XX/XX/XX
Presentation status		0 – Asymptomatic 1 - Symptomatic
Biopsy date		XX/XX/XX
IPSS score		Maximum of 35
Date of Initial IPSS score		XX/XX/XX
FACT P quality of life score		XX
Start and end dates for alpha blocker treatment (this is in addition to IPSS alpha blockers)		XX/XX/XX XX/XX/XX
Performance status		0 - Normal activity 1 - Light tasks 2 - Bed bound 50% 3 - Bed bound > 50% 4 - Bed bound
Adeno-carcinoma		0 - No 1 - Yes
T stage		XX 1-4
Grade		X 1-10
Volume		XX.X mls
Turp		0 - No 1 - Yes
Needle biopsy		0 - No 1 - Yes
PSA		XXX.X
Date of PSA		XX/XX/XX
Bone scan		0 - Not done 1 - Normal 2 - Equivocal 3 - Abnormal 3 - Metastases
MR Scan		0 – No 1 - Yes
MRI Stage		T1-3 / a-c
Pelvic nodes		0 - Not done 1 - Normal 2 - Enlarged
Para-aortic nodes		0 - Not done 1 - Normal 2 - Enlarged

Appendix J: Follow-up Form

PROCON FOLLOW UP: **Month ……**
Site Number …….. **Patient Number ……..**

Anti androgens Yes / No
Alpha Blockers Yes / No
PdE5 Inhibitors Yes / No

IPSS …… **PSA ……** **IIEF5 ……**

GI SYMPTOMS						
Abdominal pain	0	1	2	3	4	5
Anal mucositis	0	1	2	3	4	5
Anal pain	0	1	2	3	4	5
Anal ulcer	0	1	2	3	4	5
Constipation	0	1	2	3	4	5
Diarrhoea	0	1	2	3	4	5
Faecal incontinence	0	1	2	3	4	5
Haemorrhoidal haemorrhage	0	1	2	3	4	5
Nausea	0	1	2	3	4	5
Proctitis	0	1	2	3	4	5
Rectal fistula	0	1	2	3	4	5
Rectal hemorrhage	0	1	2	3	4	5
Rectal mucositis	0	1	2	3	4	5
Rectal pain	0	1	2	3	4	5
Rectal ulcer	0	1	2	3	4	5
URINARY SYMPTOMS						
Cystitis	0	1	2	3	4	5
Haematuria	0	1	2	3	4	5
Frequency	0	1	2	3	4	5
Incontinence	0	1	2	3	4	5
Retention	0	1	2	3	4	5
Pain	0	1	2	3	4	5
Urgency	0	1	2	3	4	5
Urethral stricture	No	Yes:	Symptomatic; no intervention			
				Symptomatic; surgery required		
SEXUAL FUNCTION						
Erectile dysfunction	0	1	2	3	4	5

Appendix K: Collaborator's Letter

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