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Imaging and Radiotherapy in Prostate Cancer: Advances in Biomarkers and Treatment

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Abstract

Background

Prostate cancer is a common disease, and external beam radiotherapy is a treatment often offered to men. The delivery of radiotherapy for prostate cancer needs to continue to evolve and assured to be of high quality. Simultaneously, better biomarkers are required both to help define the prognosis of a newly diagnosed man, but also to help predict who is more likely to suffer significant treatment related toxicity.

Methods

The optimisation of the management of prostate cancer is addressed in two major sections. The initial section focusses on the harnessing of newer radiotherapy technologies into the routine management of prostate cancer. One approach explores the use of a mathematical decision tool to target radiotherapy to areas at risk of subclinical disease involvement. Treatment is then delivered using a compressed 28 day schedule using larger radiotherapy doses each day than are used in a conventional 39 day regimen. The other approach uses external oversight to ensure that complex radiotherapy treatments are being planned in a safe manner. The subsequent section explores two different types of biomarker; one using serial magnetic resonance imaging (MRI) to predict for significant longer term toxicity from treatment, and the other using a blood test for circulating tumour cells (CTCs) as a potential prognostic test.

Results

Regarding treatment adaptation, the use of a mathematical decision aid was not only feasible in 27 men, but the often significantly larger radiotherapy volumes commonly targeting the pelvic lymph nodes treated on the 28 day schedule were well tolerated by patients. Furthermore, the monitoring of radiotherapy treatment for 147 men with prostate cancer treated at 12 different hospitals showed a very low rate of major protocol violations of <1%. The raises the possibility of less stringent monitoring being necessary in future studies.

As an imaging biomarker of later treatment toxicity, MRI can be used to quantify marrow structure and changes over time in a manner which has some correlation with later measured changes in bone mineral density on DEXA scanning in a cohort of 17 men ($r= -0.44$, $p=0.076$). CTCs occur relatively infrequently in men with high risk non-metastatic prostate cancer (5/36 men, 14%; 95% CI 5-30%). Counterintuitively, some men with very high risk disease such as being lymph node positive were CTC negative, whilst others with lower grade and earlier stage disease could be CTC positive.

Conclusion

The results of the publications presented in this thesis have made a contribution to improving our understanding of both how to harness new radiotherapy technologies and treatments into the management of prostate cancers as well as the emerging role of imaging and serum biomarkers in toxicity and outcome prediction. Some future directions building on these rapidly evolving fields are presented.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Martin J, Arm J, Smart J, et al. Spinal Multiparametric MRI and DEXA changes over time in men with prostate cancer treated with Androgen Deprivation Therapy: A potential imaging biomarker of treatment toxicity. European Radiology published on-line 26 May 2016.

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Keywords

radiotherapy, prostate cancer, cancer therapy, magnetic resonance imaging, tumour biomarkers.

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the MRS FF, Dixon FF and ADC percentage changes were all between baseline and the six month scans, and the average Dixon FF vertebra change looked at the gradient in FF across vertebral bodies at baseline.

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List of Abbreviations

ADC	Apparent Diffusion Coefficient
ADT	Androgen deprivation therapy
AR	Androgen Receptor
BMD	Bone Mineral Density
bNED	Biochemical no evidence of disease
BPH	Benign Prostatic Hypertrophy
CT	Computed tomography
CTC	Circulating Tumour Cell
CTV	Clinical target volume
DEXA	Dual-Energy X-Ray Absorptiometry
DVH	Dose Volume Histogram
DWI	Diffusion Weighted Imaging
ECE	Extra-capsular extension
EBRT	External Beam Radiotherapy
ECOG	Eastern Cooperative Oncology Group
EORTC	European Organisation for Research and Treatment of Cancer
FF	Fat Fraction
GI	Gastrointestinal
GS	Gleason Score
GU	Genitourinary
HNSCC	Mucosal squamous cell carcinoma of the head and neck

HRPC	High risk prostate cancer
IDEAL	Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation
IGRT	Image Guided Radiotherapy
IMRT	Intensity modulated radiotherapy
LNI	Pelvic lymph nodes involvement
LRS	Loco-regional spread
MRI	Magnetic resonance imaging
MRS	Magnetic Resonance Spectroscopy
MSKCC	Memorial-Sloan-Kettering Cancer Center
OAR	Organs at risk
OCOG	Ontario Clinical Oncology Group
P1NP	Procollagen type 1 N-Propeptide
PC	Prostate Cancer
PD	Protocol Deviation
PET	Positron emission tomography
PLN	Pelvic lymph nodes
PRESS	Point Resolved Spectroscopy
PROCITT	PROstate Cancer Imaging, Treatment and Toxicity
PROFIT	Prostate Fractionated Irradiation Trial
PRV	Planning organ at risk volume
PSA	Prostate specific antigen

PSMA	Prostate specific membrane antigen
PTV	Planning target volume
QA	Quality Assurance
RANK	Receptor activator of nuclear factor kappa-B
RCT	Randomized controlled trials
ROI	Region of Interest
RT	Radiotherapy
RTOG	Radiation Therapy Oncology Group
RTR	Real Time Review
SABR	Stereotactic Ablative Body Radiotherapy
SPARK	Stereotactic Prostate Ablative Radiotherapy using Kilovoltage Intrafraction Monitoring
SV	Seminal vesicles
SVI	Seminal vesicles involvement
SVS	Single voxel spectroscopic
TNM	Tumour Node Metastasis
TROG	Trans-Tasman Radiation Oncology Group
WPRT	Whole pelvis radiotherapy

Preface

I am currently a Staff Specialist in Radiation Oncology working at the Calvary Mater Newcastle and Genesis Cancer Care, both in Australia.

I obtained my undergraduate Bachelor degrees in Medicine and Surgery after completion of a six year full time course through the University of Otago in Dunedin, New Zealand. Following broad experience in New Zealand, England and Australia, I commenced my specialist training in Radiation Oncology at the Peter MacCallum Cancer Centre in Melbourne. After the completion of my training, I did a clinical research fellowship at the Princess Margaret Hospital in Toronto, Canada. Following a 9 month locum consultant position at the Royal Brisbane and Women's Hospital, I helped set up a new radiotherapy centre in the regional Queensland centre of Toowoomba prior to relocating to metropolitan Newcastle in 2012.

My main sub-speciality interest is genitourinary oncology. I am the national Principal Investigator in an international randomised trial in prostate cancer which has accrued over 200 men in Australia over a 3 year period, and is likely to have an impact on patient management by demonstrating it is safe and effective to halve the duration of treatment from eight weeks down to four. I established a clinical trials centre in Toowoomba which accrued more patients to national studies in its first 3 years than any other radiotherapy centre in Queensland. I set up Queensland's only regional multidisciplinary meeting for clinicians to discuss complex genitourinary oncology cases. I am currently Director of Research at the Calvary Mater Newcastle, and have overseen an expansion of research productivity including a doubling in the number of peer-reviewed publications to fifty per annum over the last three years. I also have a visiting position with Genesis Cancer Care which is Australia's largest private Radiation Oncology provider, and with whom I am helping introduce a clinical trial culture.

Motivation for this Thesis

As a junior specialist, my management of prostate cancer mimicked and was reassured by the approaches I learnt from my professional mentors. With greater

professional maturity, I began to appreciate both the limitations of some aspects of these approaches as well as the opportunities afforded by more recent findings and newer technologies. Conversations with mentors and peers demonstrated that I was far from alone in these realizations. Some form of research was clearly warranted to try to progress our collectively understanding.

I was then confronted with a range of clinically relevant issues, and a decision about which would be best to invest effort into trying to advance our knowledge. Improving tumour control, and minimizing toxicity are both key goals for any Oncologist. Each of these has multiple variations. For example, treatment often involves multiple components such as radiotherapy and androgen deprivation therapy, both of which can be monitored and modulated in ways that can impact efficacy and side effects. A key choice was whether to launch a definitive randomized study which would potentially answer one question, or a broader single armed study with multiple exploratory aspects. As is often the case, I ended up with a hybrid approach, using a subgroup from a randomized study for one part of the thesis, and a phase two cohort for the remainder.

Any Research Higher Degree is a mentoring exercise at many levels, but as a mid-career clinician who has already accumulated a reasonable research track record it is necessary to be clear as to what skills are likely to be beneficial. Modern imaging has become a cornerstone of radiation oncology, and I felt that immersion in this rapidly changing and expanding field would be valuable. Over the years of my apprenticeship in imaging I have transformed from a clinician with minimal acumen in this area to someone who has made contributions to the radiography literature, and can act with confidence as a reviewer for imaging journals as well as speak with some authority in various forums with experts in the field. Overall I would suggest that this will prove to be the greatest dividend achieved through this research journey.

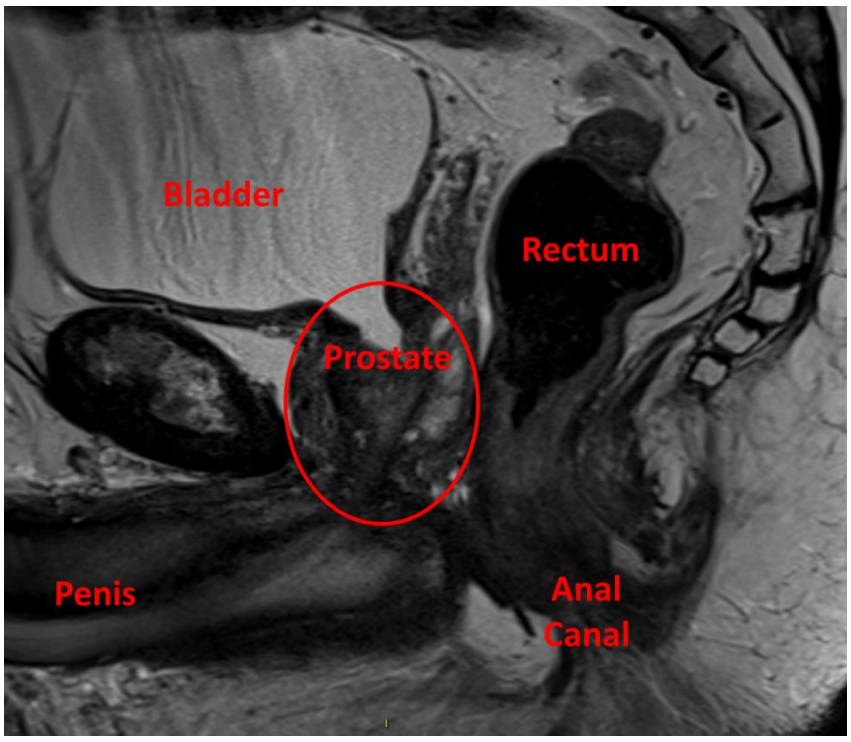
Section 1 - Prostate Cancer: Clinical Challenges and Research Opportunities

Chapter 1 – The State of the Art

The management of a man with a newly diagnosed Prostate Cancer is hugely challenging. Multiple disease, patient and treatment factors interplay with changing technologies and clinician biases. Every patient who presents for treatment needs to have consideration of treatment options as well as how best to administer them, the quality of the treatment to be delivered, their likelihood of treatment induced toxicity as well as an estimation of their likely outcomes. This thesis explores all of these issues sequentially, which, in their entirety, aims to improve the care of men with this disease.

Prostate – Anatomy, Function and Pathology

The male prostate is a pelvic organ located immediately below the bladder, in front of the rectum, and behind the pubic symphysis (see Figure 1.1, below). The urethra traverses the length of the prostate, and allows the flow of urine from the bladder towards the penis. There are also bundles of nerves and blood vessels in close proximity The prostate is largely made up of a smooth muscular central zone and a glandular peripheral zone. This glandular component produces a component of seminal fluid which supports the survival and function of sperm and is expelled at the time of ejaculation by the central smooth muscle component. These anatomical relations, especially the rectum,



urethra, neurovascular bundles and bladder, as well as the contribution to ejaculation explain many of the toxicities associated with prostate cancer treatment. With aging, under the influence of testosterone exposure, the central zone often grows in a process called benign prostatic hypertrophy (BPH). BPH is an extremely common condition in older men of European extraction, and manifests with obstructive urinary symptoms such as poor flow, hesitancy and terminal dribbling.

Prostate Cancer - Background

Prostate cancer is the most commonly diagnosed malignancy in men in developed countries, and is also a leading cause of cancer death. Like all malignancies, prostate cancer consists of mutated cells which have escaped the body's control mechanisms and have gained the ability to travel to other parts of the body. Problematically, both BPH and prostate cancer often coexist in the same gland, and both can lead to an increase in the serum Prostate Specific Antigen (PSA), necessitating further invasive investigations such as biopsy to confirm the diagnosis. Some prostate cancers have a very indolent natural history which are unlikely to cause problems in a population which is generally elderly at the time of diagnosis. Conversely, others can pursue a much more rapid clinical trajectory, necessitating more aggressive management. Since the widespread use of PSA screening in the community, it is now very common for men to present in the asymptomatic state, hence making prostate cancer treatment an exercise in later risk management. A key challenge for clinicians is therefore to use knowledge of the tumour's likely behaviour at presentation based largely on the PSA level, degree of structural organization on microscopic examination (Gleason Grading) and tumour extent, or stage, to predict later behaviour.

A wide variety of management strategies are available to treat prostate cancer. For very low risk tumours, most international guidelines recommend a policy of Active Surveillance, where men are monitored closely to select those with a less indolent natural history who are therefore more likely to benefit from consideration of local therapy.[1] At the other end of the spectrum, metastatic disease has several systemic therapy options beginning with Androgen Deprivation Therapy (ADT). ADT acts indirectly by lowering serum testosterone levels, which leads to less activation of the

Androgen Receptor (AR) and subsequently less signalling for cell growth. In the era of guidelines advocating PSA screening of men in the community, the majority of PC is diagnosed at the earlier parts of this spectrum.

For PC localized to the pelvis, the two main treatment modalities are surgery and radiotherapy. Other ablative approaches such as High Intensity Focussed Ultrasound, Cryotherapy and Electroporation are also available, but as they lack high level evidence of efficacy, are not recommended as a primary treatment approach outside of a clinical trial. In many instances, both radiotherapy and surgery can be considered as first line management strategies with approximately equal long term rates of disease control. They have very different toxicity profiles. Surgery has a high probability of erectile dysfunction and a lower chance of long term urinary leakage. Radiotherapy can cause chronic bowel and bladder symptoms, and in the longer term can affect sexual function. The two treatments are sometimes given together, or with other therapies such as ADT, which increases the potential toxicities. Psychologically, some men are better suited to the concept of surgical removal of the prostate and a rapid lowering of the PSA. Others prefer the fact that daily radiotherapy can be performed as an outpatient procedure with only a modest impact of their usual routines. Clinical trials investigating promising new treatment approaches may be available for a man to consider. Furthermore, not all men have ready access to a radiotherapy facility. In short, multiple factors come into play in the decision making process for a man newly diagnosed with prostate cancer, and their final decision is ideally arrived at after multiple consultations with various clinicians and patient advocates as well as review of tailored information.

For patients managed with radiotherapy, there are a variety of means this can be delivered. One demarcation is between external beam radiotherapy (EBRT) and brachytherapy. For brachytherapy, a radioactive source is inserted either permanently or temporarily into the prostate. Although this technique achieves excellent efficacy results in expert hands, the technical requirements and potential for severe toxicity such as urethral strictures have limited the widespread uptake of the various brachytherapy approaches.[2,3] In the case of EBRT, high energy photons are directed towards the prostate from outside the patient in a manner to concentrate energy on areas either involved with PC or at high risk of involvement. Recent

developments in EBRT include the routine use of intensity modulated RT (IMRT) to reduce unwanted dose to neighbouring critical structures such as the rectum, and image guided RT (IGRT) where soft tissue localization occurs prior to each treatment to ensure accurate treatment delivery to a mobile organ.[4,5]

EBRT Quality Assurance

Modern EBRT, encompassing IGRT and IMRT, as a complex, multistep process. There are many steps where errors can occur. There is therefore potential for flaws in treatment delivery to expose patients to risks of both increased toxicity and reduced efficacy from misdirected treatment. Compared with surgery, the indirect manner in which EBRT is given, frequent lack of pathological correlation, and delayed timeframe for both late toxicity and treatment failure to manifest present challenges in the quality assurance (QA) of EBRT. One key advantage of EBRT is that many aspects of treatment preparation and delivery can be archived for future forensic examination, in contradistinction to surgery.

Many studies have used EBRT clinical trial populations to assess the impact of suboptimal EBRT treatment delivery on disease control outcomes.[6,7] A common finding is that incorrect adherence to the trial protocol in identifying target volumes can lead to worse patient outcomes. The most famous data in this regard was from the TROG 02-02 trial in patients with head and neck cancer.[8] An attempt was made before treatment commenced to provide feedback to centres on every case, including recommendations to change target volumes if they deviated from protocol recommendations. The final plan was then reassessed, and outcomes in terms of local disease control and overall survival were presented depending on plan quality. The key finding was that comparing the plans with no major issues verses those with persisting major issues at the final review, 2-year local disease control and overall survival decrements of 24% and 20% respectively were observed. The trial was initially designed to assess the efficacy of the drug Tirapazamine, but the large effect exerted by EBRT plan quality overwhelmed the ability of the study to detect any difference. This was a sobering point of reflection regarding the importance of ensuring the baseline care delivered on clinical trials.

Extrapolating from the TROG 02-02 findings, the majority of EBRT clinical trials now undergo extensive QA.[9] This extends from site visits, use of phantoms to verify dose delivery, facility questionnaires, credentialing cases and this use of real time review (RTR).[10] The latter initiative seeks for external review of EBRT plans prior to treatment commencing, and for any major deviations to be corrected and verified via resubmission. Extensive infrastructure and staffing is required to manage such QA programs, which has driven up the cost of conducting a clinical trial involving EBRT. A key conversation now is how best to verify the benefits to be gained from each element of a QA program and how best to subsequently risk adapt the QA program for a particular clinical trial.[11]

The evidence base for EBRT QA program benefits in a prostate clinical trial population is poor. Where it exists, it can contradict conventional thinking, with one report showing the only benefit from submitting credentialing cases was that centres gained expertise in submitting good credentialing cases rather than any improvement in their performance with patients on study.[12] The most expensive element of a EBRT QA protocol is the use of RTR due to the extensive software and hardware infrastructure required as well as the need for expert involvement at several steps including plan submission, integrity checks, dose review, plan review and report generation, all under the pressure of a patient being due to commence treatment within days, if not hours. For this reason, ***one chapter of my thesis is an attempt to measure the impact of a RTR element of a comprehensive QA program in a prostate EBRT clinical trial***.[13]

Optimising EBRT - Hypofractionation

One method being explored in optimising the delivery of EBRT is altering the daily dose of radiotherapy delivered – so called hypofractionation. Observational data suggested that a larger dose of RT each day may lead to greater PC cell kill than rectal mucosal effects.[14] This is quantified by the alpha-beta ratio, which appears to be lower for PC than most other invasive tumours.[15] Many phase 2 series have explored this concept of increasing the daily RT dose beyond the standard 2 Gy, and subsequently decreasing the total number of RT sessions far below the usual 37-40 visits.[16] A number of randomized clinical trials have compared such conventional

fractionation with hypofractionation, with the experimental arm given in 19-28 fractions, with all to date suggesting approximately equivalent efficacy and toxicity.[17-19] The approach with the most mature data in the era of IGRT and IMRT is to give the treatment over 28 fractions, and thus this regimen was selected as a component of this study.[20]

Optimising EBRT – Target Individualisation

There are several different regions which can be targeted in the management of prostate cancer with EBRT. However, beyond treating the whole of the gland to a minimum dose of radiation, there is little consensus on how best to direct treatment. Gross disease detected on MRI may receive a more intense dose of treatment than the remainder of the gland which harbours a >70% risk of multifocal disease. Other areas of gross disease involvement such as the seminal vesicles also justify a higher dose of radiation.[4] The question of elective volumes is even more vexing. Here a prediction is made as to where subclinical disease may reside, and efforts made to address this by targeting them with an intermediate dose of radiation. Such approaches are standard in the management of rectal, cutaneous and head and neck cancers,[21] but have attracted varying support in the treatment of prostate cancer. This is largely due to two RCTs randomizing between whole pelvic radiotherapy (WPRT) and prostate only treatment not finding any definitive evidence of a disease control benefit from the use of WPRT.[22,23] These trials have been criticized on several grounds,[24-26] but their findings have served largely to continue the controversy about electively targeting areas at high risk of disease involvement.[4]

One approach would be to improve our ability to select which patients might benefit from the use of elective EBRT treatment volumes. Nomograms hold some promise in this regard.[27] Nomograms are multivariate decision aids which take into account all known prognostic factors and their interactions in a non-linear fashion to generate predictions on some desired endpoint. There is evidence of greater accuracy with the use of nomograms than other more coarse decision aids such as the use of single prognostic variable or risk stratification as with the D'Amico approach. Endpoints can also be allowed to vary between disease control and disease distribution. Furthermore, a nomogram can be externally validated, where it is tested

in a group separate to that used in its generation to ensure its performance can be generalized to the broader population.

The Memorial Sloan Kettering Cancer Centre Prostate Cancer Nomogram uses a database of thousands of men managed surgically for their prostate cancer, and has been externally validated.[27] It correlates baseline factors such as PSA level, stage and Gleason score on core biopsies with the risk of microscopic disease beyond the prostatic capsule, in the seminal vesicles and/or in pelvic lymph nodes. This captures some of the spatial heterogeneity of a particular patient's prostate cancer, and allows some attempt to customize the EBRT treatment volumes accordingly. It does, however, require a decision regarding a threshold of risk of disease involvement. Mirroring some past experience, a risk threshold of 15% was set in this study, although it could be argued this leads to overtreatment of approximately 85% of men. ***The use of a nomogram is not standard, and is complex, so there is a need to explore whether such an approach is feasible as well as collecting acute toxicity data in a prospective manner to determine whether the generally larger treatment volumes can be targeted in a way tolerable to patients.***

Optimising EBRT - ADT

Several large randomized trials have explored various combinations of ADT and EBRT, with several showing an improvement in overall survival for men managed with both approaches verses either ADT or EBRT alone.[28-31] The main controversies in this area are now appropriate patient selection, the duration of ADT and management of ADT related toxicities. There is some evidence that longer durations (18-36 months) of ADT lead to improved survival compared with shorter durations (4-6 months), but there continues to be uncertainty as to which subgroups of patients are destined to benefit given the certainty of greater toxicity with prolonged ADT.[32-34]

Regarding ADT toxicity, strategies are available to address issues such as vasomotor symptoms, loss of bone mineral density, weight gain, dyslipidaemia, hypertension and loss of muscle strength. Guidelines exist for management of ADT toxicity, but much of their evidence is derived from populations not managed with ADT.[35,36]

There are indications of poor levels of adherence to such guidelines, especially in the area of bone health. Level one data has shown both increased loss of bone mineral density (BMD) and fracture risk for hypogonadal men, as well as successful strategies to prevent this including the use of RANK Ligand inhibitors such as Denosumab or Osteoclast inhibiting Bisphophanates like Zolendronate.[37-40] There is also compelling population data showing reduced survival for men who have an osteoporotic fracture whilst being managed with ADT.[41,42]

Despite such an iatrogenic toxicity with proven therapeutic solutions, management of bone health is poor. A gold standard is to perform a DEXA scan at baseline, and manage anyone with low BMD with anti-resorptive agents. Population based data linking ADT prescriptions with requests for DEXA imaging show that only approximately 15-20% of men who commence ADT have a DEXA performed within 12 months of treatment initiation.[43,44] There are several potential ways to explain this gap between evidence and practice. There is the delayed manifestation between ADT administration and an osteoporotic fracture. On a related point, there is the issue that osteoporotic fractures become increasing common with age, making it easy to ascribe any such events to the population background rate rather than the use of ADT. A more subtle point may be that Radiation Oncologists and Urologists have traditionally focussed on disease eradication rather than late toxicity management. Compare this culture with the analogous situation in breast cancer where women managed with an Aromatase Inhibitor will routinely have a bone management plan instigated.[45] The main difference here is that physician trained Medical Oncologists manage the bone health of such patients.

Potential Biomarkers of BMD Changes on ADT

A biomarker (or biological marker) is a measurable characteristic which can identify associated disease or physiological processes. They are commonly used to either predict outcomes to particular approaches and hence adapt management, or prognostic, in where outcomes can be estimated independent of treatment. Promising biomarkers are initially explored to determine if there are potential associations between its level and an outcome of interest. This is the main approach

with biomarkers in this thesis. Subsequently, once a biomarker appears to have utility through this initial step, validation needs to occur, where the same biomarker is tested in an independent cohort.

One approach which could assist with bone health management would be improved identification of a high risk population. Currently, this is largely dictated by the baseline BMD, although clinical factors such as smoking status and use of concurrent medications such as glucocorticoids are also incorporated into predictive models.[35] All of this is static data from a single point in time, and doesn't look at the effects of ADT on a particular individual's BMD. Indeed, there is a wide variation in rates of loss of BMD on ADT, with averages in different studies ranging from 2-8% per annum, depending on the imaging modality used (DEXA verses quantitative CT – see figure 1.2).[37,46] Even with such variation across studies, there are extremes within studies, with some men losing >10% of their BMD within one year, and others paradoxically experiencing improvements in their BMD measurements. There is therefore merit in exploring whether any data available within the first 6 months of treatment with ADT predicts which men are likely to experience more rapid loss in BMD. If such a subgroup can be identified early, there may be an argument in exploring more aggressive management of these selected patients.

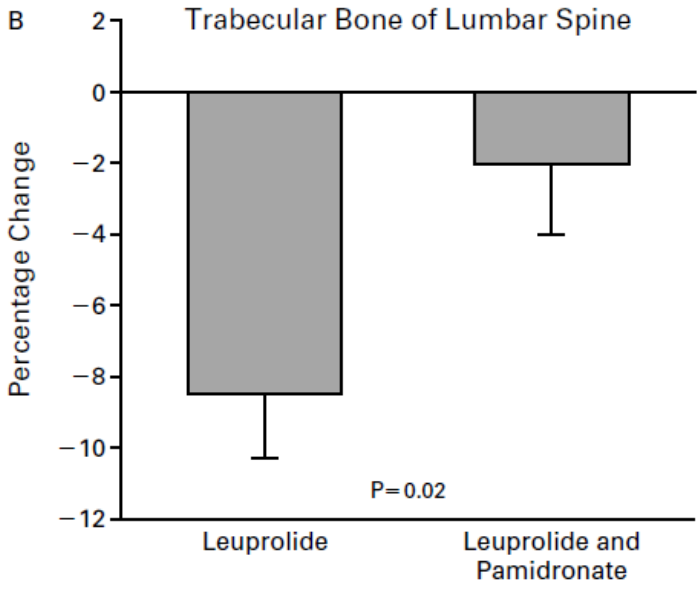


Figure 1.2: Data estimating loss of BMD using quantitative CT in a cohort of men with prostate cancer managed with ADT (Leuprolide) either with placebo (left) or an oral bisphosphonate (pamidronate). Note the 8% loss of trabecular BMD of the Lumbar spine for men managed with ADT after 48 weeks. (from Smith MR, McGovern FJ, Zietman AL, et al. Pamidronate to prevent bone loss during androgen-deprivation therapy for prostate cancer. N Engl J Med 2001;345:948-55.)

Given the large number of randomized controlled trials exploring therapeutics for men on ADT with low baseline BMD, here is relatively little work in exploring predictive biomarkers of accelerated loss of BMD on ADT. One approach is to do serial DEXA scanning,[47] and only deploy anti-resorptive interventions for men experiencing more significant reductions in BMD. A similar approach has been explored in a RCT comparing either continuous use of a bisphosphonate verses a six month course of treatment either at baseline or commencing six months after starting ADT, finding the continuous approach to be superior in maintaining BMD.[48] A potential criticism of this approach is that rather than assessing timing of the intervention, it ended up primarily assessing the duration of exposure to the intervention, and hence is relatively uninformative regarding the primary question of risk adaptation. An alternative approach is to use serum and urine markers of bone turnover. There is evidence from a small study that changes in levels of P1NP can predict eventual changes in BMD, but this was the result of multiple analyses of several potential markers with varying thresholds and these findings are yet to be replicated (see Figure 1.3).[49]

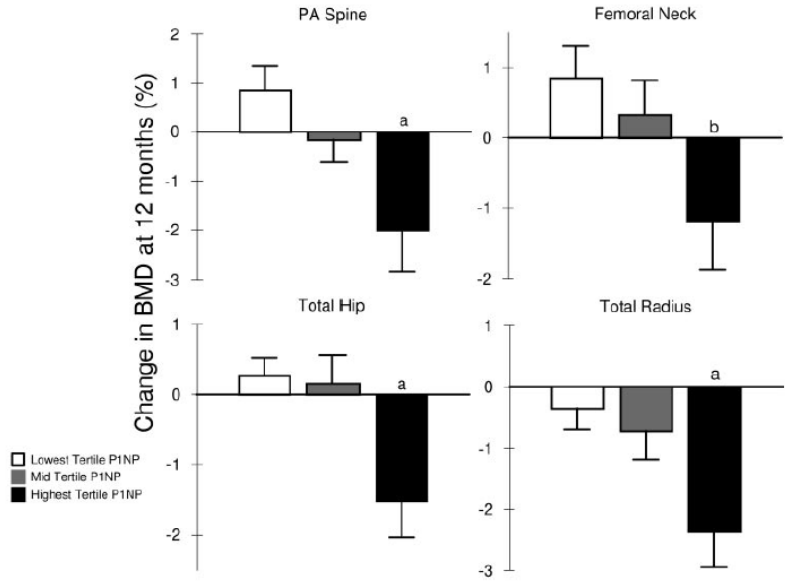


Figure 1.3: Tertiles of P1NP six months after starting ADT compared with changes in BMD at 12 months showing patients in the highest tertile of bone turnover according to this biomarker have a larger degree of later bone loss. (from Greenspan SL, Coates P, Sereika SM, Nelson JB, Trump DL, Resnick NM. Bone loss after initiation of androgen deprivation therapy in patients with prostate cancer. J Clin Endocrinol Metab 2005;90:6410-7.)

A completely different strategy would be to use novel imaging approaches to assess bone health over time. Both ultrasound and CT have some data in this regard, but

suffer from issues of interobserver reproduction for the former, and radiation dose for the latter.[50] MRI has the advantage of no radiation dose and the acquisition of 3-dimensional information, unlike DEXA, which suffers from artifacts given the 2-dimensional nature of the modality.[47] Furthermore, various MRI sequences can be selected tailored to the underlying biological processes being examined. For example, either MR Spectroscopy (MRS) or In-Out Phase imaging are able to quantify the proportion of marrow make up of adipose tissue, the Fat Fraction (FF).[51] Osteoporosis has been termed 'Obesity of the Bones', as due to redirected cellular differentiation within a confined region, increased fat should conversely mean reduced Osteoblasts and haematopoietic progenitors (see figure 1.4).[52] Furthermore, consistent correlations have been observed between MRI assessed FF and DEXA measured BMD (figure 1.5, 1.6).[51,53-55]

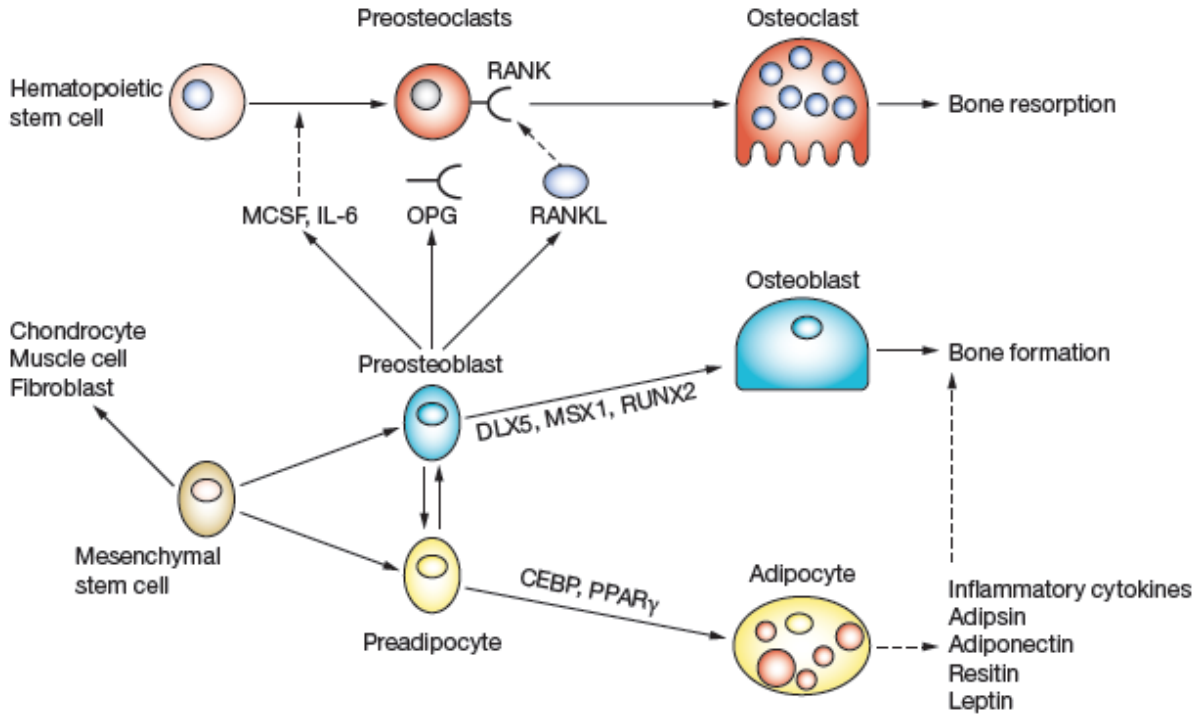


Figure 1.4: Schematic showing crosstalk between mesenchymal and haematopoietic stem cells. This mechanism underpins the observed inverse correlation between MRI Fat Fraction from increased differentiation into the adipocyte lineage, and corresponding reduced bone formation through lower rates of osteoblast activation and hence bone formation. One hypothesis is that reduced levels of testosterone and oestrogens increases differentiation from preosteoblasts into preadipocytes (from Rosen CJ, Bouxsein ML. Mechanisms of disease: is osteoporosis the obesity of bone? Nature clinical practice Rheumatology 2006;2:35-43.)

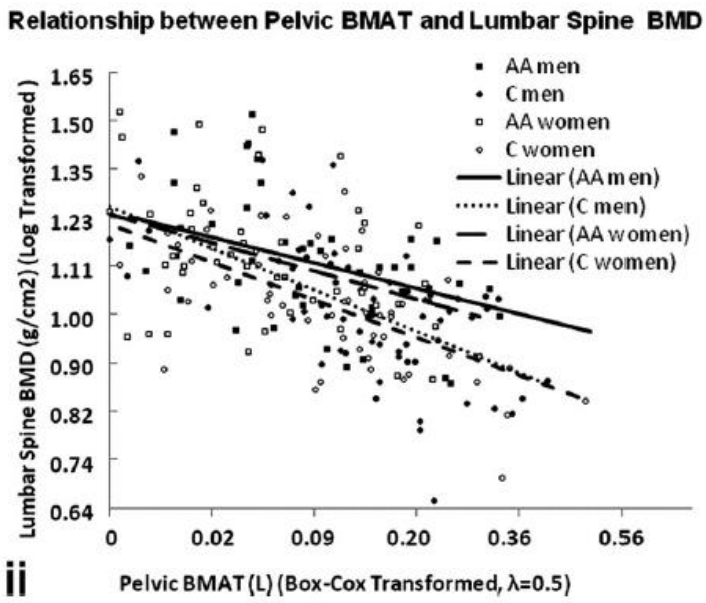


Figure 1.5: A 210 person cohort of men and women of either African American (AA) or Caucasian (C) ethnicity comparing lumbar spine BMD with MRI computed Bone Marrow Adipose Tissue (BMAT), also referred to as the Fat Fraction. Note the strong inverse correlation of lower BMD as the FF increases irrespective of race or gender. (from Shen W, Scherzer R, Gantz M, et al. Relationship between MRI-measured bone marrow adipose tissue and hip and spine bone mineral density in African-American and Caucasian participants: the CARDIA study. J Clin Endocrinol Metab 2012;97:1337-46.)

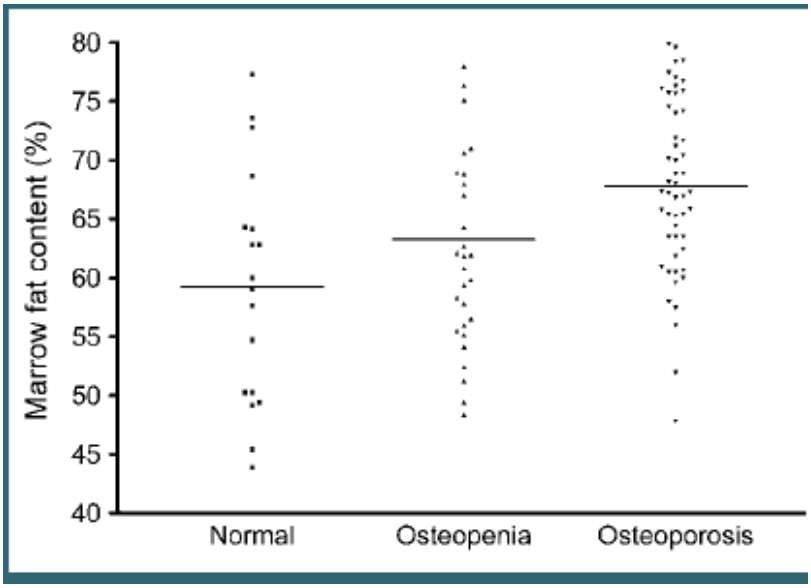


Figure 1.6: Correlation between MRI measured Fat Fraction on the y-axis and worsening bone mineral density. Note, however, the large degree of overlap despite the statistically significant difference in the median values.
(Griffith JF, Yeung DK, Antonio GE, et al. Vertebral bone mineral density, marrow perfusion, and fat content in healthy men and men with osteoporosis: dynamic contrast-enhanced MR imaging and MR spectroscopy. Radiology 2005;236:945-51.)

A key aspect of this thesis explores the question of whether MRI can be used as an imaging biomarker to identify which men managed with ADT experience accelerated BMD loss on serial DEXA scans. In addition to MRS and In-Out Phase imaging, Apparent Diffusion Coefficients (ADC) have also been explored (see figure 1.7).[56] ***Chapter 3 is a methods paper, looking at interobserver variations in five patients and initial findings.[57] Chapter 4 builds on this with a separate cohort of 28 men who while treated with ADT had two MRIs six months apart and two DEXA scans a year apart, and seeks correlations between the various sequences and scans with the aim of trying to identify an early imaging biomarker of later toxicity.***

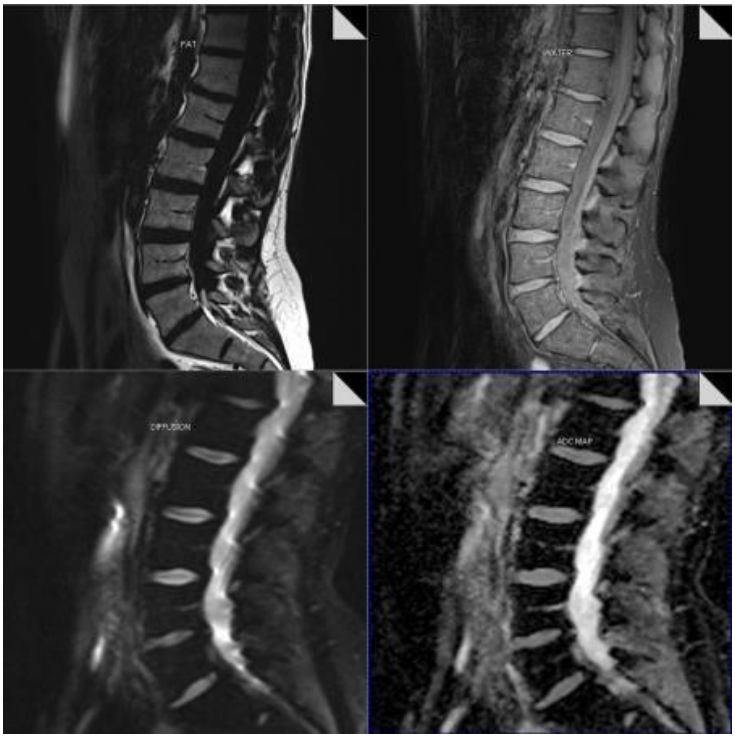


Figure 1.7: Mid-sagittal MRI of the lumbosacral spine of the author showing (clockwise from upper left) Fat only, Water only, Diffusion and Apparent Diffusion Coefficient (ADC) map. The first two are Dixon sequences derived from In and Out of phase images, and the ADC map is derived from various diffused weighted b-values from 0 to 750 in three orthogonal planes.

Efficacy Biomarkers

Whilst a core component of this thesis is trying to determine which men are likely to experience greater toxicity, the vast majority of work with biomarkers has focussed on their utility in predicting tumour control outcomes.[58-60] Some are well validated across multiple tumour types, including TNM tumour staging and tumour grade, which in the case of prostate cancer, uses the Gleason grading system. Unique to prostate cancer is the use of Prostate Specific Antigen, or PSA. PSA continues to create controversy in some settings, most notably in the screening setting where a

large proportion of men found with asymptomatic indolent disease are destined to die of other causes, and hence subjecting them to interventions only leads to increased morbidity and cost.[61,62] However, once prostate cancer is diagnosed, the PSA level proves to be a very strong prognostic factor as it tends to be a good, although still imperfect, surrogate for the volume of disease. Indeed, other accurate biomarkers of prostate cancer outcomes such as PSA velocity heavily rely on serial PSA measurement.[63,64]

One promising alternative avenue of efficacy biomarker research is the direct measurement of circulating tumour cells (CTCs).[65] These are tumour cells which have detached from the primary and gained access to the systemic venous circulation (see Figure 1.8). They have been investigated in several tumour types, and found to have prognostic power in the setting of established metastatic disease in a range of histologies including breast, colorectal and non-small cell lung cancers.[66] The data in prostate cancer is more emergent, but a substudy of a RCT looking at the efficacy of the agent Abiraterone in men with metastatic disease previously treated with Docetaxel chemotherapy showed an inverse correlation between CTC levels and overall survival.[67]

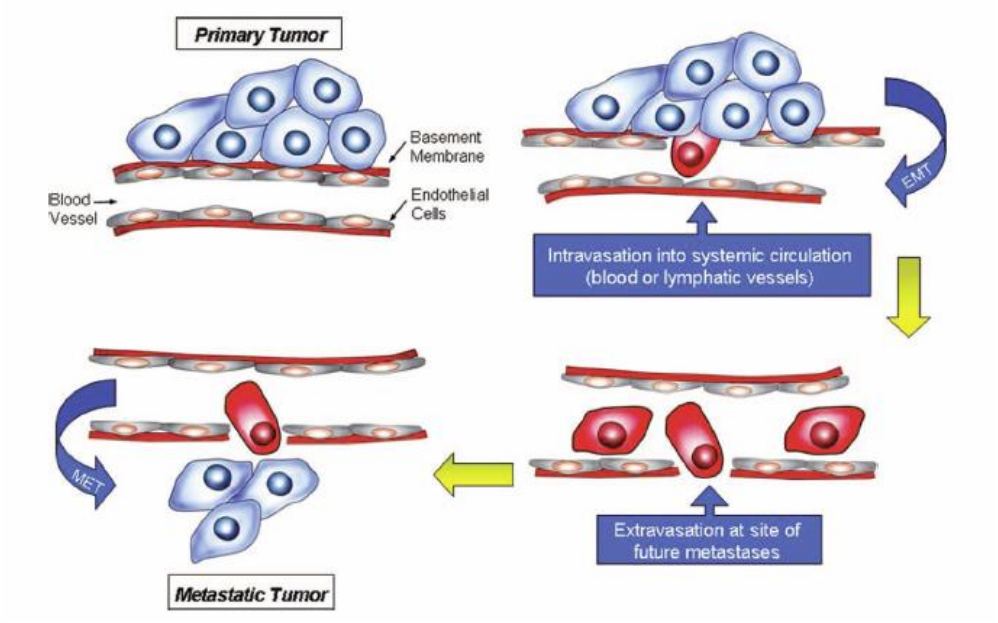


Figure 1.8: Schematic demonstrating some of the steps of how a primary tumour eventually can establish a metastasis. Note the intermediate step of circulating tumour cells in red, which can be directly enumerated at low volume. (from Dotan E *et al*. Circulating Tumor Cells: Evolving Evidence and Future Challenges. The Oncologist 2009;14:1070-82).

Men with High Risk Prostate Cancer (HRPC) have unfavourable baseline characteristics such as a high Gleason score of 8-10, a PSA >20, or stage T3-4, without evidence of established metastatic disease. The ability of any investigation to detect metastatic disease is subject to a threshold effect, below which a test can prove to be insensitive by giving a false negative result. Moderate cellular tumour burdens can exist despite a normal scan. For example, abdominopelvic CT imaging is still recommended as the standard scan to assess for metastatic disease to regional lymph nodes.[68] Any lymph node with a short axis diameter of less than 10 mm is considered negative. Given the size of a cell of approximately 10 microns, up to 10^9 or one billion cells may be present despite the scan being called normal. This is undoubtably a large part of the reason that a large proportion of men with HRPC are destined for metastatic relapse: the disease is already micrometastatic at the time of diagnosis, but at a volume too low to detect with conventional imaging.

A more sensitive biomarker of low-volume metastatic disease in men with HRPC would have multiple uses. It may present men from receiving morbid and ultimately futile treatment to the pelvis if more disseminated disease is detected. Conversely, it may help identify a subgroup for whom more aggressive systemic therapy could be justified at the time of initial therapy delivery. Given the use of CTCs in the metastatic setting, and the observation that some men with HRPC really represent a watershed between localized and metastatic disease, it was felt that this population would warrant further exploration with this promising new biomarker.[69] ***Chapter six aims to quantify the incidence of CTC positivity in men with HRPC, with further follow-up necessary to assess the impact this may have on efficacy.***

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Section 2: Optimising treatment for High Risk Prostate Cancer

The use of modern radiotherapy has opened a host of options for men with prostate cancer. The increased complexity of safe treatment delivery is both a risk and an opportunity. Key risks relate to the potential for suboptimal treatment, which can compromise the chance of tumour control, and increase the potential toxicities. This is particularly important within the context of a clinical trial, where unproven regimens are being used. These experimental approaches inherently carry risk due to the uncertainty of their efficacy and long term toxicity profiles. Furthermore, large clinical trials need to accrue from many centres, some of which may not necessarily be at the forefront of innovation. It is also possible for poor treatment to confound the key results being measured within a clinical trial. In chapter two we present a report on an aspect of a strict quality assurance program which was designed to mitigate many of these risks. A key finding was that we were appearing to reach a limit of useful risk management, and subsequent work has sought to be much more risk adaptive in the intensity of quality assurance required.

An opportunity is to try to harness complexity to benefit prostate cancer patients. We designed an innovative approach using a multivariate nomogram to help tailor the radiotherapy treatment based on a patient's specific risk factors. Such treatments could be safely delivered in 5 ½ weeks rather than the standard 8 week regimen, and were well tolerated by the men. However, some aspects of the treatment were needlessly complex, without proof of any additional benefit to be gained. Once again, we appeared to be approaching a limit where more processes were not necessarily helpful, leading to a more measured approach in subsequent work.

Chapter 2 – Prostate radiotherapy clinical trial Quality Assurance

Modern radiotherapy is a highly technical and complex treatment. As treatment and toxicity outcomes can be delayed by years, it is essential to have robust quality assurance processes in place to ensure radiotherapy is delivered in an optimal manner. However, it is difficult to determine exactly what degree of quality assurance is necessary, with a temptation existing to add further processes without necessarily having any evidence of the additional benefit of such extra steps. Within the confines of an international clinical trial, a natural experiment occurred where men in Canada had a moderate degree of quality assurance performed, whilst men in Australia had extra layers applied. This allowed us the opportunity to assess any additional value derived from the extra quality assurance processes used in Australia. Our results showed no discernible impact from such extra steps. As such, subsequent studies have taken a more risk adaptive approach where the intensity of quality assurance is more risk managed and titrated against a range of factors including the complexity of target volumes and number of patients enrolled at a particular centre.

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Prostate Radiotherapy Clinical Trial Quality Assurance: How Real Should Real Time Review Be? (A TROG-OCOG Intergroup Project)

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Abstract

Background and Purpose

Review of plans early in treatment offers the potential to reduce the chance of sub-optimal treatment delivery. We compare the use of Real Time Reviews (RTR) either before randomization (pre-rand 3D RTR) or following randomization (post-rand 2D RTR).

Materials and Methods

PROFIT is an international randomized trial for men with prostate cancer which had credentialing via multiple dummy runs. In Australia, but not Canada, all plans were submitted for pre-rand 3D RTR using 3D software, and resubmission was requested if significant protocol deviations (PD) were seen. All plans from Canada and Australia then underwent post-rand 2D RTR using a 2D assessment.

Results

For 147 Australian patients, pre-rand 3D RTR was fast (median 1 day, 95% range 0-4 days). 51 minor and 5 major PD were observed and 15 of the 147 cases (10%) required resubmission. Of the 5 major PD, 4 were remedied on resubmission and 1 was withdrawn from study. For the post-rand 2D RTR, reports from 147 Australian cases and 193 Canadian cases were reviewed. No major PD were reported from Australian cases, but 3 were seen in Canadian cases (0% v 1.5%; p=0.26). There was also no difference in the rate of minor PD (14.3% v 15.3%; p=NS).

Conclusions

In a study using relatively simple treatment volumes after comprehensive credentialing, pre-rand 3D RTR offers only modest benefits compared with post-rand 2D RTR. In the future the intensity of RTR may need to vary according to protocol and site specific factors.

Introduction

Modern radiotherapy requires the accurate delivery of radiation dose to a defined tissue volume. Dose escalated radiotherapy for prostate cancer requires complex planning techniques including intensity modulated radiotherapy (IMRT) to reduce dose to neighbouring critical structures.[1] A suboptimal radiotherapy treatment plan can be responsible for under-dosing of the planning target volume (PTV) or overdosing of neighbouring critical structures. These in turn can have consequences for both tumour control probability, as well as the incidence of normal tissue complications.[2] More fundamentally, poor delineation of target volumes can cause errors that no amount of planning expertise can circumvent.[3]

In the context of a clinical trial, it is important to monitor for such errors, as incorrect contouring or suboptimal planning can cause a greater effect on outcomes than the intervention under investigation.[2] This has led to greater attention being paid to the area of quality assurance (QA), which can be applied to many stages of the patient's treatment journey, although the historical trend has been to introduce interventions earlier in the treatment process.[4] The ultimate aim would be to introduce a thorough QA process for every treatment plan prior to the patient starting treatment.[5] Through the process of audit, such a real time review (RTR) process must be shown to provide tangible benefits to justify the effort required to establish and maintain it.

PROFIT (PROstate Fractionated Irradiation Trial) (NCT00304759) is a randomized controlled trial designed to determine whether a 78 Gy in 39 fraction course of radiotherapy can be safely compressed into a 60 Gy in 20 fraction regimen with non-inferior clinical-biochemical control. Building on phase 2 data for both treatment arms, this Ontario Clinical Oncology Group (OCOG) trial originated in Canada, and subsequently opened in Australia under the auspices of the Trans-Tasman Radiation Oncology Group (TROG).[6,7] All cases are submitted and reviewed in 2-Dimensions (2D) after randomisation by OCOG. For Australian sites, there is additional 3-Dimensional (3D) review which is completed prior to randomisation by TROG. Here we explore whether pre-randomization (pre-rand) 3D RTR have enough impact to justify the additional resources required to sustain such a process.[8] This

is a novel question, as true RTR is a relatively recent phenomenon. If additional benefit is seen compared with post-randomization (post-rand) 2D review, pre-rand review may be considered as a standard of care for future prostate radiotherapy trials.

Methods and Materials

Hypothesis

Performing 3D real time review (RTR) prior to randomization reduces the incidence of protocol deviations (PD).

Credentialing

Prior to activation for PROFIT, all centres have to submit data for 5 ‘dry-run’ cases with plans conforming to the protocol constraints for the 60 Gy arm of the trial (table 1, also showing the 78 Gy constraints). These cases were deidentified patient datasets previously treated at each of the submitting centres. In Australia, 2 centres which were outreach clinics of a larger centre rotating the same staff were only required to submit 2 dry run cases each. Being the experimental arm of the trial, the dose constraints for the 60 Gy regimen were more difficult to achieve than the standard 78 Gy arm. Contouring instructions are outlined in table 2, similar to those validated in the preceding phase 2 protocols.[6,7] The data requested included a Dose-Volume Histogram (DVH) and screen captures of the three orthogonal axial, sagittal and coronal planes through the centre of the prostate. Centres received educational material and presentations on protocol requirements, including dose constraints. A QA helpdesk was available to facilitate rapid resolution of any protocol queries or technical planning issues. Any deviations noted would result in a request for the case to be replanned prior to being reassessed. In addition, TROG centres submitted the same 5 cases for 3D review (described subsequently), usually at the same time as 2D submission. Other accreditation components such as Image Guided Radiotherapy credentialing and site visits for dosimetric verification of IMRT treatment delivery have been reported separately.[9,10]

Plan Generation

Any patient who consented to entering PROFIT needed to have a 60 Gy plan generated. Centres were instructed to ensure that contouring and dosing conformed to protocol requirements prior to proceeding towards randomization (tables 1 and 2).

3D Real Time Review

For Australian centres, all plans needed to be assessed by a Radiation Therapist and a Radiation Oncologist not involved in the direct care of the patient prior to randomization. Plans were made anonymous and DICOM data of the radiotherapy plan (including DVH) remotely uploaded into the TROG Central Quality Management System (CQMS). The data transfer was assessed at the TROG Central Operations Office prior to the assessing centre being notified of the readiness of the plan for review. The SWAN software was used to review the plan in 3D including contouring and adherence to dose constraints. Results were compared with protocol constraints and any minor deviations were recorded and a recommendation was made to the treating centre to address these prior to randomization. Any major deviations led to personal contact with the treating centre to discuss the case directly and randomization was not permitted until such problems had been remedied. PROFIT 60 Gy arm dose constraints and protocol contouring instructions are listed in tables 2.1 and 2.2. In general, a minor dosimetric deviation was defined at the start of the trial as being up to 102.5% of the target threshold while a major deviation was anything beyond that.

Table 2.1: PROFIT protocol 60 Gy arm dose constraints. All contouring (Prostate±Seminal Vesicles, Correct CTV-PTV expansion, Rectal Wall, Bladder Wall, Proximal Femurs) also had to comply with protocol for a plan to be passed.

Structure and Metric	**Acceptable Dose**	**Minor Deviation**	**Major Deviation**
CTV D99	≥60 Gy	<60 Gy	<59.4 Gy
PTV D99	≥57 Gy	<57 Gy	<56.4 Gy
PTV Max Dose to 1 cc	≤63 Gy	>63 Gy	>64.5 Gy
Rectal Wall D30	≤47.15 Gy	>47.15 Gy	>48.3 Gy
Rectal Wall D50	≤37.93 Gy	>37.93 Gy	>38.85 Gy
Bladder Wall D30	≤47.15 Gy	>47.15 Gy	>48.3 Gy
Bladder Wall D50	≤37.93 Gy	>37.93 Gy	>38.85 Gy
Left or Right Femur D5	≤44.08 Gy	>44.08 Gy	>45.15Gy

Table 2.2: Contouring instructions for PROFIT.

Structure	Instruction
CTV	Contour Prostate. Add proximal 10 mm of seminal vesicle if risk of seminal vesicle invasion >15% via Partin tables.
PTV	Non-uniform expansion around CTV of 10 mm in all directions, except for 7 mm posteriorly.
Rectal Wall	2-3 mm thick wall extending from 8 mm inferior to the PTV to 8 mm superior to the PTV.
Bladder Wall	2-3 mm thick wall extending from bladder base to 8 mm superior to the PTV.
Femoral Necks	All of femur superior and medial to a plane through the greater and lesser trochanters.

2D Real Time Review

Following randomization, the final plan from either Australia or Canada (either 60 Gy or 78 Gy) was submitted via e-mail for central review by a Canadian Radiation Oncologist. The submission took a similar form to the credentialing dry-runs ie orthogonal screen captures, a screen capture of the DVH, and the centre supplying data on requested DVH parameters. Submission was encouraged to occur prior to the 3rd fraction of treatment having being delivered. If any dosing or contouring deviations were noted, the centre was advised to address these; however no formal mechanism was in place to ensure this occurred. As all patients were anonymized and five Australian patients were removed from the study between the two reviews, the outcomes of the 2D and 3D reviews can only be reported as a pooled result.

Endpoints

Firstly a baseline measurement of the rate of deviations from each centre was established from the 2D credentialing phase with the 5 dry runs. The resubmission rate was defined as the total number of resubmissions divided by the total number of submissions separately for OCOG and TROG centres.

Secondly, the incidence of minor and major deviations for the Australian and Canadian centres were recorded separately for the pre-rand (Australian only) and post-rand (both Australian and Canadian) RTRs. As some Canadian centres had contributed >200 patients and were activated up to 4 years before other centres, the maximum number of cases from any one center was capped at 20. This is also in keeping with the observation that centres which accrue heavily to a trial have demonstrated a lower overall rate of deviations.[2]] The characteristics of each deviation were recording including the structure affected, whether contouring or dosimetry was involved, and whether the deviation was major or minor. For the pre-rand 3D RTR, as an indicator of the feasibility of this process, the number of working days from submission to report was also recorded.

Results

Baseline Measures

In Australia, 54 cases were submitted from 12 sites for initial credentialing. In total, 8 resubmissions were required for a range of contouring and dosimetric deviations. The Australian resubmission rate was therefore 8/(54+8)=12.9%. For Canada, 70 cases were submitted from 14 sites, and 10 resubmissions were required, giving a Canadian resubmission rate of 10/(70+10)=12.5%. There was no significant difference in the resubmission rates between the two countries (Chi-squared p=0.84).

Pre-Randomization Real Time Reviews

Pre-rand 3D RTR were achievable within the required timeframes, with a median time from receipt of treatment plan to completion of review 1 day, and 95% of case reviews being performed within 4 days. Of the first 147 Australian cases submitted for pre-rand 3D RTR, a total of 3202 dosimetric, contouring, technique and verification parameters were assessed. Of these, there were 51 minor and 5 major deviations recorded. Of the 56 deviations the most common were minor deviations in the maximum dose to 1 cc of the PTV (17, or 33%), and incorrect contouring of the bladder or rectal walls (15, or 29%). 15 of the 147 cases (10%) required resubmission with the reasons for resubmission request detailed in table 2.3. The five major deviations all occurred in different patients giving a major PD rate of 3.4%. Of the 5 major deviations, 4 were corrected prior to randomization and 1 was withdrawn from the study due to inability to meet dose constraints following CTV recontouring. Images from one patient whose CTV was assessed to require correction are shown in figure 2.1, with figure 2.2 showing the changes made after initial reivew. At final review 16 deviations had been reversed leaving 40 minor deviations for patients approved to proceed to randomization. Centres had all deviations high-lighted to them, and were recommended to remedy them prior to randomization and submission for the post-rand 2D RTR.

Table 2.3: Reasons for the 15 resubmission requests in Australian pre-randomization real time review cases. Impact is graded as Likely, Possible and Unlikely to reflect the perceived probability of an adverse clinical outcome had the major deviation not being addressed.

Deviation	Impact	Outcome
CTV was central zone of prostate on MRI only	Likely	Remedied, Resubmitted, Passed
Prostate not fully included in CTV	Likely	Patient withdrawn from study, as following recontouring 60 Gy dose constraints could not be satisified.
Bladder Wall D30 Major Deviation	Possible	Remedied, Resubmitted, Passed
CTV D99% was 59 Gy = Major deviation	Possible	Remedied, Resubmitted, Passed
Excessive CTV contouring including penile bulb	Possible	Remedied, Resubmitted, Passed
CTV to PTV margin 7 mm instead of 10 mm inferior and/or superior (4 cases)	Unlikely	Remedied, Resubmitted, Passed
Bladder and/or Rectal Wall contouring (3 cases)	Unlikely	Remedied, Resubmitted, Passed
CTV should have included seminal vesicles	Unlikely	Remedied, Resubmitted, Passed
CTV should not have included seminal vesicles	Unlikely	Remedied, Resubmitted, Passed
CTV should not have included seminal vesicles	Unlikely	Not Remedied, Passed after discussion with treating clinician who elected to treat SV.

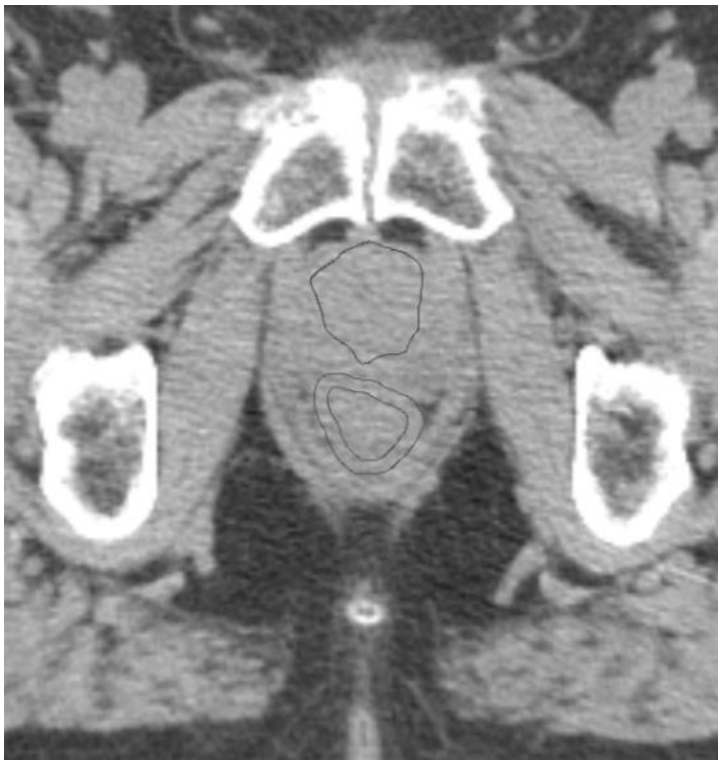

Figure 2.1: Pre-randomization real time review where the CTV was assessed to not include the entire prostate. Further investigation showed that the CTV was defined using an MRI and that the central zone was mistaken for the entire gland.

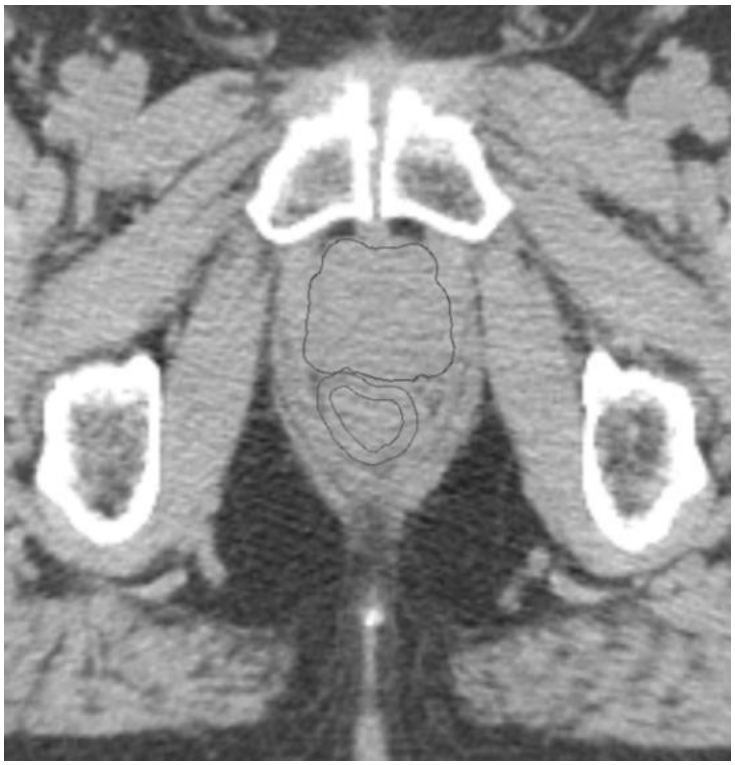

Figure 2.2: This was remedied, replanned, the new contouring and plan reassessed then passed prior to randomization.

Post-Randomization Real Time Reviews

For the post-rand 2D RTR, reports from 147 Australian cases from 11 centres (one of the 12 centres credentialled did not accrue any patients) and 193 Canadian cases from 13 centres were reviewed. No major deviations were reported from Australian cases and 3 from Canadian cases (0 v 1.5%; $p>0.15$ for relevant equality of proportions exact tests). The nature of the major deviations in the 3 Canadian cases related to insufficient prostate CTV contouring, >7.5% dose inhomogeneity across the PTV and the Bladder Wall D30 dose constraint. All were seen in patients randomized to the 60 Gy arm of the study.

Table 2.4: Frequencies and percentages of major and minor deviations on the post-randomization real time review. Note that one Canadian patient had both a major and a minor deviation recorded, hence the row adds up to 197 deviations for 196 patients.

	Total Cases	No Deviations	Major Deviations	Minor Deviations		
				One	Two	≥Three
Australia	147	126 (85.7%)	0	18 (12.2%)	2 (1.4%)	1 (0.7%)
Canada	196	166 (84.7%)	3 (1.5%)	25 (12.8%)	2 (1.0%)	1 (0.5%)

Table 2.4 shows the relative frequencies of minor deviations within the two countries, once again showing no significant differences (exact chi-square $p > 0.95$). For both countries the largest proportion of minor deviations noted related to maximum PTV dosing (Australia 48% and Canada 88% of cases exhibiting this deviation, sometimes in conjunction with other deviations). Combining both minor and major deviations together, 14.3% of Australian cases and 15.3% of Canadian cases exhibited at least one deviation (exact chi-square $p > 0.95$). There was also no difference in the frequency of 2 or more deviations being recorded in the same case, at 2% in both groups.

A total of 26 minor deviations were noted in 21 Australian cases, compared with the 40 minor deviations commented on in the final pre-rand 3D RTR. This suggests that although approximately 35% of minor deviations were remedied as recommended, the remaining 65% were not. Further analysis showed that no bladder or rectal wall deviations were noted for Australian patients on the post-rand 2D RTR, suggesting that this was the main area where rectification occurred.

Discussion

Dose escalated radiotherapy has become a standard treatment option for men with prostate cancer because of proven benefits demonstrated in randomized trials.[11,12] However a higher rate of rectal toxicity has generally been reported in these trials, emphasizing the importance of improving the accuracy and conformality of treatment delivery. There are many steps where errors can occur, including CTV and organ at risk contouring, margin generation, treatment planning, plan transfer to the linear accelerator, patient positioning and treatment delivery. This justifies the implementation of quality assurance processes to try to minimize the impact of such errors on treatment outcomes.

We have shown that it is feasible to conduct 3-dimensional pre-rand RTR in a timely and effective manner. Four major deviations were remedied and one managed with conventional fractionation off-study for Australian patients through the pre-rand 3D RTR process. Although the incidence of major deviations on post-rand 2D RTR are low in Canada at 1.5%, they have been completely eliminated in Australia. This may be the greatest value of the pre-rand 3D RTR process, as treatment can be delayed until remedial action has been taken to correct the most egregious radiotherapy planning issues.

Compared with post-rand 2D RTR, the additional gains from pre-rand 3D RTR appear to be modest in this trial of prostate radiotherapy. There are several potential explanations for this. One is that the study was not a truly randomized comparison, either of RTR timing or 2D versus 3D, and that for methodological reasons undetected biases have prevented an existing advantage from being observed. Another is that prostate radiotherapy is relatively simple, and that the true advantage from pre-rand 3D RTR may be achieved in more complex scenarios such as head and neck radiotherapy. It is also plausible that the strict credentialing process educated clinicians in how to avoid deviations, making subsequent deviations less likely to occur. Overall, the data does not support the routine integration of pre-rand 3D RTR into prostate radiotherapy trials with credentialing and post-rand 2D RTR similar to PROFIT, and that such an approach should be further investigated in more complex treatments. This adds to the debate regarding the optimal QA framework,

as recent data from the EORTC regarding dry runs suggests that the main benefit from doing them is an improvement in future dry run results rather than affecting trial outcomes.[13]

It might be argued that no minor deviations should have been observed in Australian cases given the potential to identify and correct these during the pre-rand 3D RTR process. The relatively low incidence of PD overall may be due to the need to perform 5 dry runs as part of the credentialing process. The vast majority of the minor deviations noted in the Australian cases were either very close to thresholds (eg CTV D99 of 7790 cGy rather than 7800 cGy) or in PTV dose homogeneity metrics, usually the maximum dose to 1 cc of the PTV (48% of cases where a deviation was seen). Given the slight differences in dose calculation across different treatment planning systems, it is clear that some degree of latitude is needed in applying dose thresholds when plans are imported into 3-dimensional software for RT QA case reviews, particularly on the interface between 'acceptable' and 'minor deviation'. The deviation classifications includes a tolerance range to take this into account, and screen captures from the treatment centres' planning system were also used to clarify and confirm 'borderline' deviations identified at QA case review.

Pioneering work in the early 1990s on the pre-treament QA of a large Hodgkin's Disease clinical trial concentrated on advising centres about appropriate fields after reviewing baseline pathology and imaging.[14] Prior to electronic submission of radiotherapy plans the only plan review that was feasible was often performed following the completion of treatment.[15] This approach clearly left no opportunity to intervene for the plan and patient in question and instead served mainly to educate clinicians in order to hopefully avoid similar errors in the future. Real time review has evolved to allow an opportunity for intervention and has now been around in progressively more technologically elaborate forms for more than 15 years.[5]

Major radiotherapy PDs for patients on clinical trials have been linked to adverse tumour control outcomes.[16] It has previously been reported for children with medulloblastoma receving craniospinal radiotherapy that RTR and intervention to correct diagnosed planning errors are feasible and reduces relapses associated with incorrect eye shield placement compared with historical controls.[17] There is some

evidence in the setting of head and neck cancers that RTR can correct plans with significant deviations.[2] Indeed, that study suggests local control and survival benefits of the order of 20% in the favour of protocol compliant plans. There is clearly momentum building for RTR to be performed prior to study entry, leaving the actual structure of the RTR program as a key issue to be addressed.

Australian investigators clearly used some discretion to which deviations were clinically significant. Issues of PTV dosing were more common while deviations to critical structures were relatively rare. This perhaps reflects the stronger evidence for negative consequences from exceeding dose constraints for the rectum.[12] Given the relatively generous margins in PROFIT in the IGRT era, reduced PTV coverage may have been considered during the planning process and deemed clinically acceptable and an appropriate means of achieving the rectal constraints. Consensus agreement on clinically relevant dose parameters and contouring are helpful although problems arise when different recommendations are presented. For example, although QUANTEC ("Quantitative Analysis of Normal Tissue Effects in the Clinic") recommends the rectum be contoured as a solid structure, ICRU-83 presents an argument to contour the rectum as a walled structure.[18,19] Both arms of the PROFIT study have dose constraints and contouring guidelines derived from published prospective series with low late toxicity rates giving some validation for their use.[6,7]

Contouring of the prostate using planning CT datasets involves a degree of interobserver variation in practice.[3]] Judging where 'reasonable practice' ends and a PD begins can be difficult to quantify in all but the extreme cases. This emphasizes the importance of trying to select and callibrate the cut off points for deviations in a clinically meaningful manner and developing guidelines for reviewers on how best to apply them.

Although the timing of the RTR may have some importance in selected scenarios, the advantage of 3D v 2D methods are less evidence based. The former is much more complex and resource intensive to administer and the latter should detect all but the most subtle issues for prostate radiotherapy. Although feasible in the setting of 1-2 patients needing review per week, upscaling of this to 1-2 patients per day

would require a full time reviewer with significant cost implications. Most of the issues rectified following 3D review appeared to relate to contouring of the critical structures, which should have been apparent on 2D review. Overall, 2D review may be all that is justified in some situtations, although further effort will be required to clarify in what cases this may be.

A limitation of this study is that a pre-selected patient population had access to the pre-rand 3D RTR. The similar rates of major deviations on the credentialing dry runs and pre-intervention RTRs support our claim that it was the pre-rand 3D RTR which was likely to be responsible for the elimination of major deviations. A further limitation is that the 147 Australian cases were not matched exactly between the two reviews due to some patients being managed off trial after the first RTR and them being anonymous prior to randomization. Due to only 5 patients dropping out of the trial, >95% of the cohorts will have been the same patients and by selecting the same number of cases over the same timespan for the two reviews, we aimed to minimize this issue.

The integration of various digital-imaging modalities into treatment planning, the facilities to transfer large data files relatively quickly and QA review systems which support rapid analysis and reporting of RT QA results make Real Time Reviewing likely to become central to future clinical trials. The leaders in integrating this into clinical trial RT QA processes have been the Image-Guided Therapy QA Center (ITC) and the Quality Assurance Review Center (QARC) in the US, and TROG and the SWAN software group in Australasia.[20,21] The EORTC now also has a similar facility available via the VODCA platform.[18] Pre-treatment RTR is feasible, and intervention possible, but only with a significant investment of effort with instigation of an appropriate informatics platform.[22] Now that such facilities exist, a key question raised in our study is whether all studies benefit from a very comprehensive QA approach, or whether a more flexible structure is required where the intensity of QA is titrated against the complexity of treatment delivered, extent of credentialing and number of patients accrued by a site to a particular trial.

Conclusion

With appropriate central QA facilities including IT infrastructure, the ability to utilise the RTR approach to enable intervention when a major PD is identified and modification of a treatment plan prior to treatment commencing is feasible in modern clinical trials. It is possible, and has been demonstrated within the PROFIT trial, that the inclusion of pre-rand 3D RTR to the QA process can eliminate major deviations. In this study we did not show significant superiority of the pre-rand 3D RTR approach compared with a post-rand 2D RTR. It may be that a more flexible RTR is needed for future clinical trials, where the intensity of review is dependent on a range of trial, credentialing and tumour site specific factors.

Conflict of Interest: The Authors have no conflict of interest to declare in relation to this work.

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Chapter 3 – Nomogram based prostate radiotherapy target volumes

Most guidelines suggest a generally similar approach to the treatment of prostate cancer with radiotherapy. This ignores some of the heterogeneity of disease behaviour, particularly with regarding to the varying patterns of microscopic spread. We explored the use of a nomogram as a decision aid to assist in defining regions at risk of subclinical disease involvement. This lead to an adaptation of radiotherapy treatment volumes, which were delivered using varying doses per day. Our main interest was in the feasibility of applying this different approach to target delineation and planning, as well as patient tolerability of this novel treatment regimen. The main findings were that although the voluming of targets was largely protocol compliant, the excessive complexity of some of the organs at risk proved more challenging for clinicians. An important lesson here was not to engage with needless complexity. Furthermore, acute toxicity was similar to what would be expected from more standard treatments, suggesting that the regimen is tolerable. Only more mature follow-up will reveal efficacy and any significant late toxicity of this schedule.

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A prospective study of nomogram-based adaptation of prostate radiotherapy target volumes

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Abstract

Background:

A prospective clinical trial was conducted to evaluate the feasibility of a novel approach to the treatment of patients with high risk prostate cancer (HRPC) through the use of a nomogram to tailor radiotherapy target volumes.

Methods:

27 subjects with HRPC were treated with a mildly hypofractionated radiotherapy regimen using image-guided IMRT technique between Jun/2013-Jan/2015.

A set of validated prognostic factors were inputted into the Memorial-Sloan-Kettering Cancer Center (MSKCC) prostate cancer nomogram to estimate risk of loco-regional spread (LRS). The nomogram risk estimates for extra-capsular extension (ECE), seminal vesicles involvement (SVI), and pelvic lymph nodes involvement (LNI) were used to adapt radiotherapy treatment volumes based on a risk threshold of ≥15% in all cases. A planning guide was used to delineate target volumes and organs at risk (OAR). Up to three dose levels were administered over 28 fractions; 70Gy for gross disease in the prostate +/- seminal vesicles (2.5Gy/fraction), 61.6Gy for subclinical peri-prostatic disease (2.2Gy/fraction) and 50.4Gy to pelvic nodes (1.8Gy/fraction).

Data regarding protocol adherence, nomogram use, radiotherapy dose distribution, and acute toxicity were collected.

Results:

Nomogram use

100% of patients were treated for ECE, 88.9% for SVI, and 70.4% for LNI. The three areas at risk of LRS were appropriately treated according to the study protocol in 98.8% cases. The MSKCC nomogram estimates for LRS differed significantly between the time of recruitment and analysis.

Contouring protocol compliance
Compliance with the trial contouring protocol for up to seven target volumes was 93.0% (159/171). Compliance with protocol for small bowel contouring was poor (59.3%).

Dose constraints compliance
Compliance with dose constraints for target volumes was 97.4% (191/196). Compliance with dose constraints for OAR was 88.2% (285/323).

Acute toxicity
There were no grade 3 acute toxicities observed. 20/27 (74.1%) and 6/27 (22.2%) patients experienced a grade 2 genitourinary and gastrointestinal toxicity respectively.

Conclusions:
We have demonstrated the feasibility of this novel risk-adapted radiation treatment protocol for HRPC. This study has identified key learning points regarding this approach, including the importance of standardization and updating of risk quantification tools, and the utility of an observer to verify their correct use.

Trial registration:
ClincialTrials.gov identifier NCT01418040
Hunter New England Human Research Ethics Committee (HNEHREC) reference number 12/08/15/4.02

Keywords
Prostatic Neoplasms, Radiotherapy, Nomograms.

Background

Radiotherapy (RT) has been shown to independently improve overall survival for men with high risk prostate cancer (HRPC) managed with androgen deprivation therapy (ADT)[1, 2]. The traditional approach to radiotherapy for HRPC is to treat the prostate alone. However, there is extensive surgical pathological literature demonstrating the risk of subclinical disease infiltration of HRPC into pelvic lymph nodes (PLN), seminal vesicles (SV), and in an extra-prostatic distribution [3–5]. With the increased uptake of intensity modulated radiotherapy (IMRT), there is a potential opportunity to tailor treatment to such areas at significant risk of loco-regional spread (LRS) rather than managing all men with HRPC in an identical manner. In other sub-sites, for example in the treatment of mucosal squamous cell carcinoma of the head and neck (HNSCC), this treatment approach has long been accepted as standard of care.

Uncertainty regarding the role of whole pelvis radiotherapy (WPRT) in high risk prostate cancer is reflected in various clinical guidelines, in which the elective treatment of pelvic nodes is left up to the treating clinician's discretion [6, 7]. Two randomized controlled trials (RTOG 9413 and GETUG-01) have failed to convincingly demonstrate improvement in progression free survival with the use of WPRT versus prostate-only treatment [8, 9], although later results from the RTOG study show improved biochemical control in a subset of patients receiving neo-adjuvant hormonal therapy. Reasons for a lack of benefit from WPRT have been described, including insufficient radiation dose, inadequate coverage of at-risk nodes, and poorly targeted patient selection [10, 11]. Despite the lack of level I evidence for WPRT, this practice has been incorporated into the standard treatment of HRPC in multiple practice-defining randomized controlled trials (RCT) [12–14].

WPRT in this setting is not without its risks; there is mixed evidence to suggest increased acute and late grade 3 gastrointestinal toxicity and decreased bowel quality of life [8, 9, 15, 16]. Despite reductions in dose to critical structures and late GI adverse effects achieved through the use of IMRT over 3D-conformal RT [17, 18], WPRT is still likely to result in increased toxicity compared to treatment of the prostate alone. It is therefore important to reserve the use of WPRT, and to a lesser

extent irradiation of the SV and peri-prostatic regions, for those patients that are most likely to experience improved tumour control outcomes.

We conducted a prospective clinical trial to assess the feasibility and tolerability of a hypofractionated radiotherapy treatment protocol for HRPC that employed the use of a widely accessible and externally validated online nomogram to estimate risk of LRS and accordingly adapt delineation of target volumes.

Methods

Study design and participants

This prospective phase two single institution study enrolled patients with high-risk prostate cancer for 18 months (Jan 2013-June 2014). Patients were eligible for the study if they met the following inclusion criteria: histologically confirmed adenocarcinoma of the prostate, high risk disease (defined by any one of baseline prostate-specific antigen (PSA) ≥ 20 µg/L, Gleason Score (GS) 8-10 and/or clinical stage T3-T4), and conventional staging imaging negative for distant metastases (technetium-99m whole body bone scan and CT of abdomen and pelvis). Exclusion criteria included: previous pelvic radiotherapy, history of prior malignancy within the last 5 years (excluding non-melanomatous skin cancers), Eastern Cooperative Oncology Group (ECOG) performance status ≥ 2, or any contraindication to insertion of intra-prostatic fiducial markers or planning MRI prior to RT simulation. All patients were administered a total of 18 months of ADT in the form of Leuprorelin 22.5mg every 3 months.

All patients gave written informed consent. The study was reviewed and approved by the Hunter and New England Human Research Ethics Committee (HNEHREC Ref: 12/08/15/4.02). The trial was registered with ClincialTrials.gov (identifier NCT01418040).

Nomogram use

A set of parameters (age, PSA, tumour GS, clinical stage and percentage of positive biopsies) were inputted into the Memorial-Sloan-Kettering Cancer Center (MSKCC) prostate cancer nomogram [19] prior to radiotherapy and again at time of analysis to estimate risk of LRS. The pre-RT nomogram risk estimates for extra-capsular extension (ECE), seminal vesicles involvement (SVI), and pelvic lymph nodes involvement (LNI) were used to adapt radiotherapy treatment volumes based on a risk threshold of ≥15% in all cases.

Simulation and planning protocol

Radiotherapy commenced after 6 months of neo-adjuvant ADT, in keeping with the results from the TROG 96.01 randomised trial showing superiority of this duration

verses 3 months or no ADT [20]. Following insertion of three intra-prostatic gold fiducials, all patients underwent CT simulation (Aquilion LB TSX-201A, Toshiba Medical Systems Corporation) in the supine position with customized immobilization. Patients were instructed to have a comfortably full bladder and an empty rectum for simulation and treatment. A 3-tesla non-contrast planning MRI scan (MAGNETOM Skyra, Siemens) using 2mm slices and T2 weighting was completed on the same day and co-registered with the simulation CT by matching to the fiducial markers.

A standardized planning guide was developed and used to direct contouring of target volumes and organs at risk (OAR) on the CT and MRI imaging. Target volumes were contoured as listed in table 1. Elective irradiation of extra-capsular disease extension, proximal seminal vesicles, and/or pelvic lymph nodes was completed if the risk of involvement of each respective region exceeded 15%, as estimated by the MSKCC nomogram.

The rectum, bladder, neck of femur, small bowel and penile bulb were contoured as organs at risk (OAR). The rectum was contoured as a solid organ from the ano-rectal junction to the recto-sigmoid flexure. The entire bladder was also contoured as a solid organ. The small bowel was contoured as any visible small bowel as well as peritoneal contents within 8mm of the superior margin of the PTV. This volume was expanded 3mm in all directions to create a small bowel planning organ at risk volume (PRV).

Radiotherapy technique

Radiotherapy was administered over 28 daily fractions, given five fractions per week using an image-guided dynamic IMRT technique. Pre-treatment image guidance was conducted using matching of kilovoltage electronic portal imaging to the three intra-prostatic gold fiducial markers with a 1 mm action threshold for a translational shift. Extrapolating from the HNSCC literature, and given the expectation of reduced clonogen density in imaging-negative areas, reduced radiotherapy dosing was administered to elective regions. Up to three dose levels were treated in 28 fractions using a simultaneous integrated boost:

- All patients received radiotherapy to the prostate +/- seminal vesicles (if grossly involved on clinical examination or MRI) to a dose of 70 Gy (2.5 Gy per fraction).
- If the nomogram estimate for ECE ≥15%, an additional volume (formed by a 3mm isotropic expansion from the prostate excluding overlap with the rectum) was treated to 61.6 Gy (2.2 Gy per fraction).
- If the nomogram estimate for SVI ≥15%, the proximal 20mm of the seminal vesicles was treated to 61.6 Gy (2.2 Gy per fraction).
- If the nomogram estimate for LNI ≥15%, the pelvic lymph nodes were treated to 50.4 Gy (1.8 Gy per fraction), contoured according to RTOG consensus guidelines [21] with a modified upper border of 10mm inferior to the sacral promontory.

Dose constraints

Planning objectives and field arrangement were optimized to achieve the best dosimetry to satisfy dose constraints for target volumes and OAR (listed in table 2). All planning was performed using the Eclipse Treatment Planning System v12 (Varian Medical Systems). In particular, dosing to the PTVs aimed to deliver 100% of the prescribed dose to 98% of the target volume as per ICRU 83 [22].

Data collection and analysis

Genitourinary and gastrointestinal toxicity were assessed on a weekly basis during radiotherapy, at 1.5 months and 4.5 months post radiotherapy, then at 6 monthly intervals thereafter. Scoring of toxicity was completed using the Radiation Therapy Oncology Group (RTOG) acute and late radiation morbidity scoring criteria.

Efficacy of treatment will be assessed by biochemical no evidence of disease (bNED) as defined by the ASTRO Phoenix definition (nadir + 2.0mcg/L) [23]. bNED was and will be assessed at each post-RT review. Treatment efficacy outcome results will be presented at a later date when longer follow-up has been achieved.

Evaluation of compliance with the trial protocol for nomogram-directed target volume delineation, contouring, and dose constraints were conducted after the final patient

completed radiotherapy. To demonstrate feasibility, we aimed to achieve ≥90% protocol compliance rate with each of these parameters.

Up to seven target structures (CTV_P, CTV_{ECE}, $CTV_{SVA/SVI}$, CTV_{LN}, PTV70, PTV61.6, and PTV50.4) were generated for each patient according to the protocol outlined in table 3.1. At the time of analysis, each patient's plan was reviewed to determine whether appropriate target structures were treated according to the threshold of ≥15% risk of involvement as estimated by the MSKCC nomogram. Each target structure was assessed for strict adherence to the contouring protocol by a third party not involved in the original planning process (RW). This assessment was repeated for contouring of OAR.

Table 3.1: Contouring protocol for target volumes

Structure	**Contouring protocol**	**Condition**
CTV_P	Prostate as defined using CT and MRI imaging + any extra-prostatic extension as noted on examination or pre-ADT imaging	All patients
CTV_{ECE}	3mm isotropic margin from CTV_P, excluding overlap with rectum	If ECE risk ≥ 15%
CTV_{SVI}	Entire bilateral seminal vesicles (only contoured if known seminal vesicle involvement)	If SV involved
CTV_{SVA}	Proximal 20mm of SV, measured obliquely along long axis of SV (only contoured for adjuvant treatment of seminal vesicles)	If SVI risk ≥ 15%
CTV_{LN}	Pelvic nodes: 7mm margin around obturator, pre-sacral, and external and internal iliac vessels contoured as per RTOG consensus guidelines [21], up to 10mm inferior to the sacral promontory)	If LNI risk ≥ 15%
PTV70	If no SV involvement: 5mm margin around CTV_P If SV involvement: 5mm margin around CTV_P + 7mm margin around CTV_{SVI} anteriorly and posteriorly and 5mm otherwise	All patients

Structure	Contouring protocol	Condition
PTV61.6	If SV involvement or SVI risk <15%: 5mm margin around CTV_{ECE} If no SV involvement and SVI risk ≥15%: 5mm margin around CTV_{ECE} + 7mm margin around CTV_{SVA} anteriorly and posteriorly and 5mm otherwise	If CTV_{ECE} or CTV_{SVA} contoured
PTV50.4	5mm margin around CTV_{LN}	If CTV_{LN} contoured

Dose constraints for target volumes and OAR were assessed according to the objectives listed in table 3.2. Values exceeding the 'mandatory' limits were termed 'major variations'. Values in between the 'mandatory' and 'ideal' limits were termed 'minor variations'. In all cases, descriptive statistics generated from Microsoft Excel are presented.

Table 3.2: Dose constraints for target volumes and organs at risk

Target volumes	**Mandatory**	**Ideal**
PTV70 D98%	≥70.0 Gy	-
PTV70 D1cc	≤77.0 Gy	≤74.9 Gy
PTV61.6 D98%	≥61.6 Gy	-
PTV50.4 D98%	≥50.4 Gy	-
CTV_P	≥70.0 Gy	-
CTV_{SVI} D99%	≥70.0 Gy	-
CTV_{ECE} D99%	≥61.6 Gy	-
CTV_{LN} D99%	≥50.4 Gy	-
Organs at risk	**Mandatory**	**Ideal**
Rectum D15%	≤74.0 Gy	≤74.0 Gy
Rectum D25%	≤69.0 Gy	≤60.0 Gy
Rectum D35%	≤64.0 Gy	≤50.0 Gy
Rectum D50%	≤59.0 Gy	≤40.0 Gy
Bladder D15%	≤79.0 Gy	≤74.0 Gy

Bladder D25%	≤74.0 Gy	≤60.0 Gy
Bladder D30%	≤69.0 Gy	≤50.0 Gy
Bladder D50%	≤64.0 Gy	≤40.0 Gy
Neck of femur D5%	≤44.0 Gy	-
Small bowel PRV D99%	≤52.0 Gy	-
Small bowel V45Gy	≤195 cc	-
Penile bulb mean dose	-	≤51.0 Gy

Sub-studies examining the use of imaging to predict risk of ADT-induced loss of bone mineral density, and the prognostic significance of circulating tumour cells were completed concurrently using the same patient cohort and are reported on separately [24, 25].

Results

Patient characteristics

28 patients were enrolled onto the trial, of which 27 (96.4%) completed the planned treatment without unscheduled breaks. The remaining patient was not suitable for treatment due to an acute myocardial infarction prior to radiotherapy. Two patients were enrolled to the trial despite baseline characteristics not fulfilling the inclusion criteria for high risk disease. Median follow-up at the time of analysis was 11.4 months. The patient characteristics are shown in table 3.3.

Table 3.3: Baseline patient characteristics

	Median (range)
Age	70.6 years (54.6 – 78.9)
PSA	12.4 ng/mL (4.0 – 52.1)
% biopsy cores positive	50% (25 – 100)
Gleason Score	**Number of patients (%)**
3 + 4	2 (7%)
4 + 3	3 (11%)
4 + 4	3 (11%)
4 + 5	16 (59%)
5 + 4	4 (15%)
T stage	**Number of patients (%)**
T1b	1 (4%)
T1c	3 (11%)
T2a	1 (4%)
T2b	7 (25%)
T2c	4 (14%)
T3a	9 (32%)
T3b	3 (11%)

Nomogram use and radiotherapy treatment volumes

The MSKCC nomogram was used to estimate risk of loco-regional spread (ECE, SVI and LNI) both prior to radiotherapy (to direct treatment), and later at the time of data analysis. There was a difference in nomogram outputs between these two time points (table 3.4). Student's t-test demonstrated significant increases for ECE and LNI (both $p<0.001$), but no change for SVI ($p=0.35$). If current nomogram outputs were used instead of those obtained prior to RT, 9 of 27 patients would have received different treatment; 7 patients using larger volumes and 2 patients using smaller volumes.

Table 3.4: Nomogram estimates for risk of LRS at pre-radiotherapy and at time of data analysis

	Pre-RT	**At analysis**	**Paired t-test**
ECE	70.7% (20.8)	87.1% (13.8)	$p < 0.001$
SVI	47.0% (24.1)	43.5% (24.7)	$p = 0.356$
LNI	32.05% (27.7)	50.4% (26.9)	$p < 0.001$

Data are presented as mean (standard deviation)

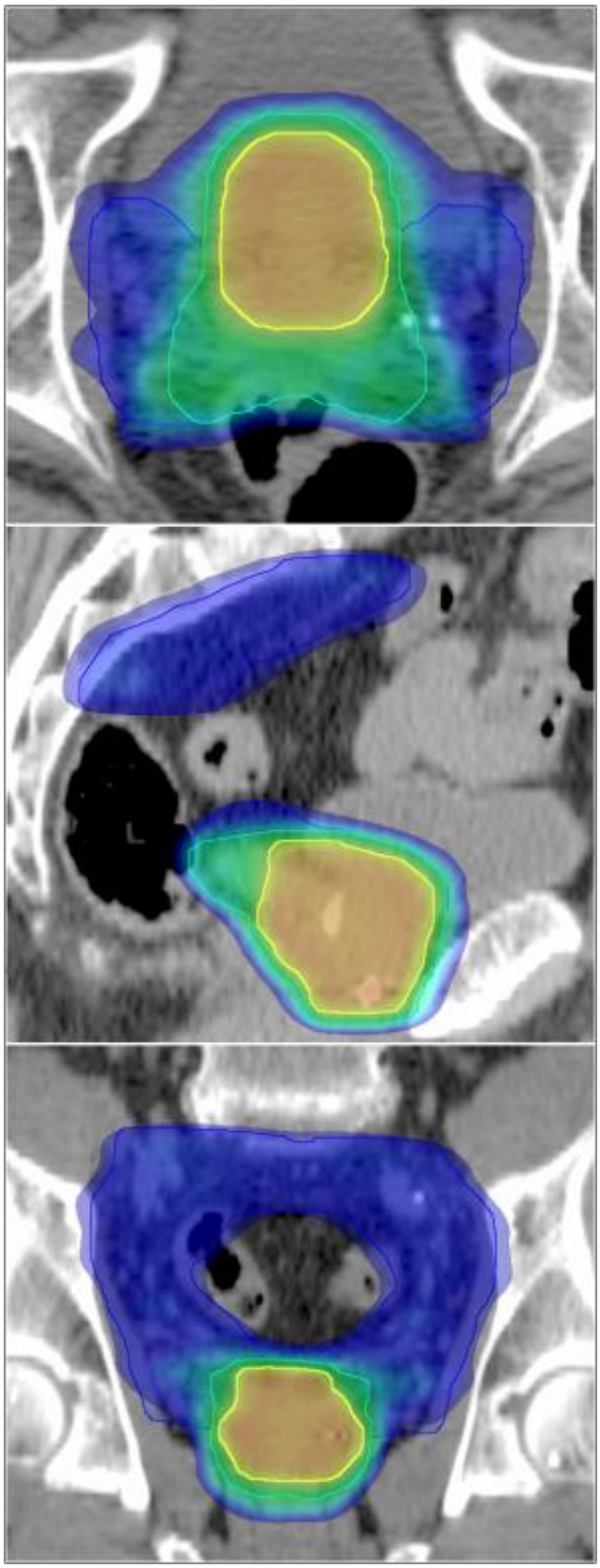
Two patients with known pelvic lymph node metastases were entered as 100% risk of LNI

Radiotherapy target volumes were expanded to account for risk of ECE in 27/27 patients (100%), SVI in 24/27 (88.9%), and LNI in 19/27 (70.4%). An example of the volumes treated to three different dose levels is shown in figure 3.1. Areas at risk of LRS were appropriately included/omitted from treatment according to the study protocol in 98.8% of cases. However, error in inputting post-ADT rather than pre-ADT PSA into the nomogram for two patients resulted in falsely low estimates of LRS, and the incorrect omission of treatment of both SV and PLN.

Contouring of target volumes and OAR

The seven target volumes were correctly delineated according to the trial contouring protocol in 94.1% of cases (160/170). Compliance with protocol for contouring of OAR (rectum, bladder, small bowel) was 70.4% (57/81), although 22/24 variations were due to small bowel contouring that was incorrect or omitted.

Figure 3.1: Typical radiotherapy dose distribution. Legend: PTV70 (yellow), PTV61.6 (cyan) and PTV50.4 (dark blue) are displayed with dose colour wash overlay



Dose constraints

Each patient's radiotherapy plan was assessed for adherence to 5-8 target volume dose constraints and up to 13 OAR dose constraints, dependent upon the volumes treated. Compliance with dose constraints for target volumes was 97.1% (170/175), with 1.7% minor variations and 1.1% major variations. Compliance with dose constraints for OAR was 88.2% (285/323), with 9.9% minor variations and 1.9% major variations.

Acute toxicity

There was no grade ≥3 genitourinary (GU) or gastrointestinal (GI) acute toxicity observed (table 3.5). 20/27 (74.1%) patients experienced grade 2 GU toxicity at some point during radiotherapy. In all cases this was either increased obstructive or irritative urinary symptoms managed with supportive measures such as Tamsulosin or urinary alkalinisation respectively. 6/27 (22.2%) patients experienced grade 2 GI toxicity in the form of increased bowel frequency managed with Loperamide.

Table 3.5: Maximal acute toxicity (during radiotherapy)

	Grade 0	**Grade 1**	**Grade 2**	**≥ Grade 3**
Genitourinary	2 (7.4%)	5 (18.5%)	20 (74.1%)	0 (0%)
Gastrointestinal	12 (44.4%)	9 (33.3%)	6 (22.2%)	0 (0%)

Data are presented as number of patients (% of total cohort)

Discussion

We have demonstrated the feasibility of a risk-adjusted radiotherapy treatment protocol that adapts target volume delineation based on nomogram estimates of risk of LRS. This treatment was shown to be technically feasible, clinically practicable, and resulted in acceptable levels of acute toxicity in line with standard of care.

It is important to appreciate the natural patterns of spread of disease when determining target volumes to be treated. A rich surgical pathological literature is available to inform this approach, demonstrating the frequency, and often extent of disease involvement. For example, the risk of SV involvement in patients with T2 disease has been described, as has the fact that in 90% of such cases disease is confined to the proximal 20 mm of the SV measured along the axis of the structure [4]. It is perhaps noteworthy that in the HNSCC setting, such data regarding pathological risk of loco-regional involvement is deemed appropriate to allow clinical implementation without prospective clinical trial validation [26]. Yet in the prostate radiotherapy setting, clinical trials attempting to quantify the benefit of WPRT continue to be performed (e.g. RTOG 0534 and RTOG 0924). In the era of improved imaging, integration of new systemic agents, and highly conformal radiotherapy, it will be challenging for such studies to definitively answer such questions for all patients, which is part of the reason that most modern protocols simply mandate the extent of elective volume treatment [27].

The twenty-eight treatment hypofractionated radiotherapy regimen used in this study was first described by the Cleveland Clinic [28]. This original protocol has been adapted to form the experimental arm in the RTOG 0415 study, a multi-centre phase III randomized controlled trial examining modest hypofractionation for treatment of favourable risk prostate cancer. Neither of these treatment regimens included elective WPRT. Two separate groups in the US have published their experiences administering conventionally fractionated WPRT concurrently or sequentially with hypofractionated prostate irradiation [16, 29]. Early data regarding biochemical control and toxicity from these four groups have demonstrated encouraging results with the modestly hypofractionated treatment.

The frequency of grade ≥2 acute GU toxicity (74.1%) observed in this trial was slightly higher than that recorded by the aforementioned studies of McDonald et al. (52%) and Pollack et al. (approximately 56%) [16, 29]. This difference may be accounted for by the increased dose to the seminal vesicles (61.6 Gy vs. 56 Gy or 50 Gy respectively) or more likely, a lower threshold for the use of interventions. The increase in toxicity was limited in severity to RTOG grade 2, and it remains to be seen whether this will translate into more meaningful differences in late toxicity. The absence of grade 3 acute toxicity in this study is reassuring and consistent with the published data using similar treatments. The incidence of grade ≥2 acute GI toxicity (22.2%) was in the same range as the levels seen in the University of Alabama at Birmingham series (37%) [16]. Their series treated all HRPC men with the same radiotherapy doses to the primary disease and pelvic lymph nodes as in our cohort, and have reported efficacy and late toxicity rates similar to conventional treatment. Our data adds to the literature that supports the feasibility of moderately hypofractionated radiotherapy treating the prostate and pelvis concurrently for men with HRPC.

The question remains as to how best to select patients for radiotherapy volume adaptation. Some guidelines such as from the EORTC recommend using the D'Amico risk stratification. This would probably lead to overtreatment, as some patients designated as high risk actually have very favourable outcomes, illustrating the heterogeneity of such risk groupings [30]. Clinical tools such as the 'Partin tables' [31] have analysed historical data from large cohorts of patients undergoing radical prostatectomy to demonstrate the correlation between LRS and prognostic factors such as PSA, GS, and clinical staging. These data could provide an individualized estimate for risk and degree of LRS, which may then be used to adapt the extent of treatment. The use of a web-based nomogram (such as the MSKCC nomogram) allows further refinements to this approach. The clinical tool is widely accessible, simple to use, considers the additional variable of tumour volume, and considers relevant prognostic factors as continuous rather than discrete variables. Furthermore, as the calculations are not completed manually, the underlying algorithm can be sufficiently complex to achieve maximal accuracy. For these reasons, a computer-based nomogram is a powerful tool that facilitates risk-adapted treatment individualization.

There are, however, a number of limitations in using a nomogram in this fashion. First of all, the nomogram is dependent upon historical data that may not be suitable for extrapolation to the current population. Changes in disease epidemiology, staging, and screening practices mean that the effect of prognostic factors may differ between contemporary and historical populations, and the estimates may therefore be inaccurate. A key example of this was the upward migration of Gleason scoring in recent years, partly due to the altered definitions of the core biopsy grading system introduced in 2005 [32]. There is therefore a need to regularly review the applicability of historical results to current populations and update the nomogram algorithms accordingly (which then also prompts the need for external re-validation). The degree to which this affects results is illustrated in the difference in nomogram outputs between the time of planning and analysis (table 4).

Secondly, most clinical tools used to estimate the risk of LRS in prostate cancer are based upon radical prostatectomy series that employed limited lymph node dissection. It has been demonstrated that standard/limited pelvic lymph node dissections may result in false negative rates for pathological involved nodes of over 50% compared to extended dissections [33]. Nomogram algorithms that have been derived from these data may therefore generate estimates of LNI that are deceptively low.

Thirdly, there is a danger that data entry errors may result in grossly inaccurate estimates of LRS and incorrect clinical decision-making. For example, a misplaced decimal point, or inputting the post-ADT PSA rather than PSA at diagnosis may alter the nomogram estimates considerably. The latter error occurred twice in our study and resulted in artificially low estimates of LRS and incorrect non-treatment of seminal vesicles and pelvic nodes in these patients. Simple safeguards would prevent such errors from occurring, for example, a second observer to verify correct data entry and nomogram use.

Fourthly, there are small sub-groups of prostate cancer patients who are not suitable for nomogram-directed adaptation of treatment. Outcomes for PSA-negative tumours for example are not correctly predicted with current nomograms. This group however

represents only a very select subset of patients (1% or less of total prostate cancer cases) who very often present late with metastatic disease that is not suitable for curative treatment [34]. Neuroendocrine carcinoma of the prostate is another group for which standard prognostic tools are similarly unsuitable.

A further lesson from our experience was appreciating the danger in over-complicating treatment.

The novel treatment regimen used in this study involves a number of features that increase its complexity compared to standard practice. These include the use of a nomogram to define risk of LRS and adapt target delineation, protocolised generation of multiple target structures to be treated using up to three different dose levels, and a hypofractionated regimen with many unfamiliar dose constraints. Added complexity is liable to increase the likelihood of errors and protocol non-compliance and must be justified with a benefit to clinical outcomes. We identified a number of examples of this, including rotation of the simulation CT images to contour the proximal seminal vesicles along their axes, or the use of multiple, redundant dose constraints for rectum and bladder. Here, excessive and unfamiliar processes are unlikely to improve outcomes and should be simplified. If additional complexity is value-adding, it may be necessary to implement further safeguards such as peer review of contouring and the use of checklists to maximise protocol compliance.

We have demonstrated feasibility and deliverability of a complex risk-adapted treatment for patients with HRPC. Many future directions are being pursued along similar lines. The use of more extreme hypofractionation coupled with pelvic radiotherapy is increasing, for example in the ‘SATURN’ trial, in which stereotactic radiotherapy treatment is administered over 5 fractions to both the prostate and pelvic lymph nodes. A similar protocol used in the earlier ‘FASTR’ trial however resulted in unacceptable levels of late toxicity, suggesting caution in using such an approach [35].

In contrast, emerging imaging modalities such as PSMA PET are likely to detect early metastatic spread with increased sensitivity, which may reduce the number of at-risk patients with negative staging investigations who are therefore candidates for elective loco-regional treatment. If this does eventuate, however, we would then face the question of how to treat this growing group of patients with early loco-regional or

oligometastatic disease, an area where there is again a paucity of evidence to guide management. It is likely that the management of prostate cancer will shift further towards a risk-adapted approach as the results of current trials and integration of new imaging into clinical practice continues.

Conclusions

We have demonstrated the feasibility of this novel risk-adapted radiation treatment protocol for HRPC. This study has identified key learning points regarding this approach, including the importance of standardization and updating of risk quantification tools, and the utility of an observer to verify their correct use.

Competing Interests

The authors declare that they have no competing interests.

Author's contributions

RW carried out analysis of data and radiotherapy plans, and drafting of the manuscript. HW participated in collection and analysis of data. AC participated in drafting of the trial protocol, subject recruitment, and prescription of treatment. PH participated in development of the treatment protocol and planning guide, and directed radiotherapy planning. GC participated in conception and design of the study. KHT and PN assisted in drafting and critical review of the manuscript. PC assisted in subject recruitment and data acquisition. JM conceived the study, directed study design and coordination, recruited subjects and assisted in drafting of the manuscript. All authors have read and approved the final manuscript.

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Section 3: Biomarkers of toxicity and efficacy

There is great interest in oncology in trying to predict outcomes early in the management of patients. Various approaches can be taken to achieve this. One touched on in the previous chapter is to use a mathematical model incorporating information available at the time of diagnosis. Another avenue is to use the patient's own inherent reaction to treatment. This can be assessed in many ways, but two general approaches are to either use samples from the patient (either tumour, blood or other specimens), or non-invasive imaging. In the next three chapters, we explore both methods. Chapters 4 and 5 assess the utility of multiparametric MRI as an imaging biomarker of later treatment toxicity, in this case, the risk of rapid loss in bone mineral density with the use of androgen deprivation therapy. Chapter 6 assesses Circulating Tumour Cells, a serum biomarker, to determine their sensitivity in a high risk prostate cancer population.

Chapter 4 – MRI Assessed Vertebral Fat Fraction: A Pilot Study

Based on prior work showing a relationship between MRI derived fat fraction in bone and DEXA scan measured bone mineral density, we performed a local pilot study. Our main aims were to assess inter- and intra-observer variability in calculating the fat fraction, as well as exploring a Dixon technique as an alternative to MR Spectroscopy to assess the fat fraction. Although only five men were assessed, we found that there was minimal variability in assessing the fat fraction, and that the fat fraction tended to increases the more inferiorly in the spine the measurements were taken. These findings allowed us to proceed with the more comprehensive study presenting in Chapter 5.

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Rapid Determination of Vertebral Fat Fraction Over a Large Range of Vertebral Bodies

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Running Title: Spinal Fat Fraction Gradient

Abstract

Introduction

Vertebral body Fat Fraction (FF) has been found to vary between lumbar vertebrae using Magnetic Resonance Spectroscopy (MRS). We aim to more quickly assess a larger number of adjacent vertebrae using a single T2-weighted iterative decomposition of water and fat with echo asymmetric and least squares estimation (IDEAL) sequence.

Methods

Five males had DEXA and 1.5 Tesla MR scans performed. MRS was performed at L3, and a sagittal IDEAL sequence resulting in separate pure fat and pure water readings from T10 to S2. For the IDEAL measurements, two independent observers followed a set reading protocol with five observations each per vertebra. Intra- and Inter-observer variability was assessed as deviations from the mean respectively within and between observers.

Results

For FF measurements there was limited intra-observer variation, with observers being on average within 3.4% of the pooled mean value. Similarly, there was good interobserver agreement with an average variation of 2.1%. All men showed a reduced FF between L5-S1 of between 1.6–7%. Otherwise, there was a trend for increasing FF moving inferiorly from T10 to S2. This averaged 2.7%/vertebra (range 1.1-3.8%), and may not be dependent of MRS measured FF at the L3 level. There was poor correlation between MRS FF at L2-4 and BMD using DEXA (R^2=0.06).

Conclusion

IDEAL measurements are generally reproducible between observers following a set protocol. There appears to be a gradient in FF moving from T10 to S2 with S1

showing a consistent decrease. This variation may better describe overall marrow function than a single vertebral reading.

Key Words:

Magnetic Resonance Imaging
Magnetic Resonance Spectroscopy
Bone Marrow
Fat Fraction
Fat Imaging
Osteoporosis

Introduction

Bone marrow contains hematopoietic stem cells, generating circulating blood and osteoclasts as well as mesenchymal stem cells which can mature into osteoblasts and adipocytes/fat [1] . The ratio of these respective red and yellow cells is not constant and changes with age, gender and anatomical location [2, 3]. The fat fraction (FF) is ratio of fat-to-water-plus-fat and has been determined by magnetic resonance spectroscopy (MRS) and imaging (MRI) [3, 4]. Marrow Fraction is the inverse of FF (ie 100% - FF), and is thought to correlate with marrow function [5].

The FF is known to vary according to anatomical site. This has been indirectly demonstrated in work with 18F-fluoro-L-deoxythymidine Positron Emission Tomography (FLT-PET) imaging looking at the inverse of FF in the form of marrow proliferation [6]. This group reported high marrow proliferation in the thoracic spine (19.9%), intermediate in the lumbar spine (16.6%) decreasing in the sacrum (9.2%). However, PET/CT studies are not suitable for repeated measures or acquiring normative data due to the radiation exposure. Early experience measuring FF via MRS at two separate lumbar vertebrae showed increasing FF for the more inferior vertebra [3]. This observation is consistent with a later report assessing the four vertebrae from L1 to L4, showing the same trend, even between neighbouring vertebrae [2]. It is therefore plausible that a point estimate of FF in a single vertebra doesn't completely describe marrow function. A more comprehensive measurement of not only FF but also change in FF according to anatomical site and time may provide a more complete description.

Preliminary work suggests there is a correlation between the Fat Fraction (FF) estimated by Magnetic Resonance Spectroscopy (MRS) in Lumbar vertebrae and the accepted standard of Bone Mineral Density (BMD) measured by DEXA [7]. However, there is a wide degree of overlap between subjects classified as normal, osteopaenic and osteoporotic on DEXA and their corresponding FF values. Some of this variability would be explained by clinical factors such as age and gender [4], which are routinely incorporated into fracture risk nomograms such as FRAX [8]. However, due to the complex physiology of bone beyond what can be quantified through DEXA

imaging and clinical parameters, it is plausible that some of this variability may be due to other factors which MRI would be well placed to assess.

The T2-weighted iterative decomposition of water and fat with echo asymmetric and least squares estimation (IDEAL) image sequences produce separate water and fat only images, and have previously been shown to correlate very strongly with MRS derived FF measurements in bone [3, 9]. The advantage of imaging approaches applied to a sagittal section of vertebrae is that the FF can be estimated for a larger number of structures in a much shorter time than performing MRS in multiple Regions of Interest (ROI). We aim to use the IDEAL sequence in a group of older male patients entering onto a clinical trial investigating the effects of androgen deprivation on marrow function.

Evaluation of normal and pathological changes in bone marrow FF would ideally use non-invasive methods that do not require x-rays or radiotracers. The variation of FF along the vertebral column suggests the requirement of assessing a large a range of the spine as possible. MRI methods including IDEAL imaging as investigated in this study show some promise in this regard.

Methods

Participants

Eligible patients were men with localized prostate cancer who were to receive androgen deprivation therapy (ADT) and prostate radiotherapy. None had a history of lower back problems. Screening thoracolumbar X-Rays were performed to exclude osteoporotic compression fractures, advanced degenerative changes or any other gross abnormalities. A whole body 99m-Technicium bone scan was performed and needed to be negative for the presence of metastatic bony disease. Signed informed consent was obtained from five participants aged between 70 to 75 years, without any destroy of bisphosphonate, corticosteroid or ADT treatment, nor any history of low trauma fracture or osteoporosis. This project received ethics approval from the Toowoomba and Darling Downs Human Research Ethics Committee.

Imaging details

Protons in water and lipids have unique chemical environments, resulting in particular MRI characteristics such as resonance frequencies at each magnetic field strength. Immediately after the excitation pulse the proton spins of the water and lipids are in–phase, resulting is addition of the water and fat signal. As the TE increases the difference in frequency results in the water and lipid spins having different phase off-set until they are 180° out-of-phase, resulting in subtraction of the water and fat signals. This results in periodic changes in image intensity, dependent on the phase offset of the water and lipid. The change in image intensity of a series of images can be modelled to determine the separated water and lipid contents.

All MR examinations were performed on a GE 1.5 Tesla Signa Excite system using a lumbar phased-array coil. Patients were positioned using a knee rest to minimise lumbar lordosis. Initial scout images were followed by axial In-phase and Out-of-phase imaging (5 mm slice thickness, 0 mm inter-slice gap, 36 cm field of view, 22 sec) were acquired as a breathhold from T10 – S2. An IDEAL-Fast Spin Echo (FSE) sequence (TR 3000 ms, TE 60 ms, 32 cm field of view, 20 slices, 3.0 mm slice thickness, 0.5 mm interslice gap, 6.35 min) was performed. The IDEAL sequence is

a three point Dixon technique which has been demonstrated to provide uniform and reliable fat suppression[10, 11] Online processing of the raw data of the sagittal IDEAL produced water only, fat only, and recombined in-phase and out-phase images. Twenty sagittal images of the spine from T10 to the mid-Sacrum were obtained using this approach for each patient.

DEXA scanning was performed on all individuals with individual readings from L2, L3 and L4 vertebral bodies. Z- and T-Scores were also calculated based on Australian data.

Data Extraction

Offline two independent observers (JM and WW) extracted data from the sagittal Fat T2 IDEAL images using proprietary software (Voyager Telerad Picture Archive and Communication System, Intellrad Solutions Pty Ltd, Melbourne, Australia). A set protocol was followed with separately drawn ROIs on five adjacent sagittal slices per vertebra. The observers were instructed to begin at the sagittal slice 5 mm from cortical bone and to make freehand ROIs to provide measurements of vertebral fat content for all vertebral bodies within the field of view. To limit peripheral artefact, the most superior and inferior vertebrae were not assessed. Observers were instructed to exclude bony cortex, or any anatomical abnormalities observed on the T2 images. They would then proceed from right to left on serial sagittal slices repeating the measurements five times overall. An example of this is illustrated in figure 4.1.

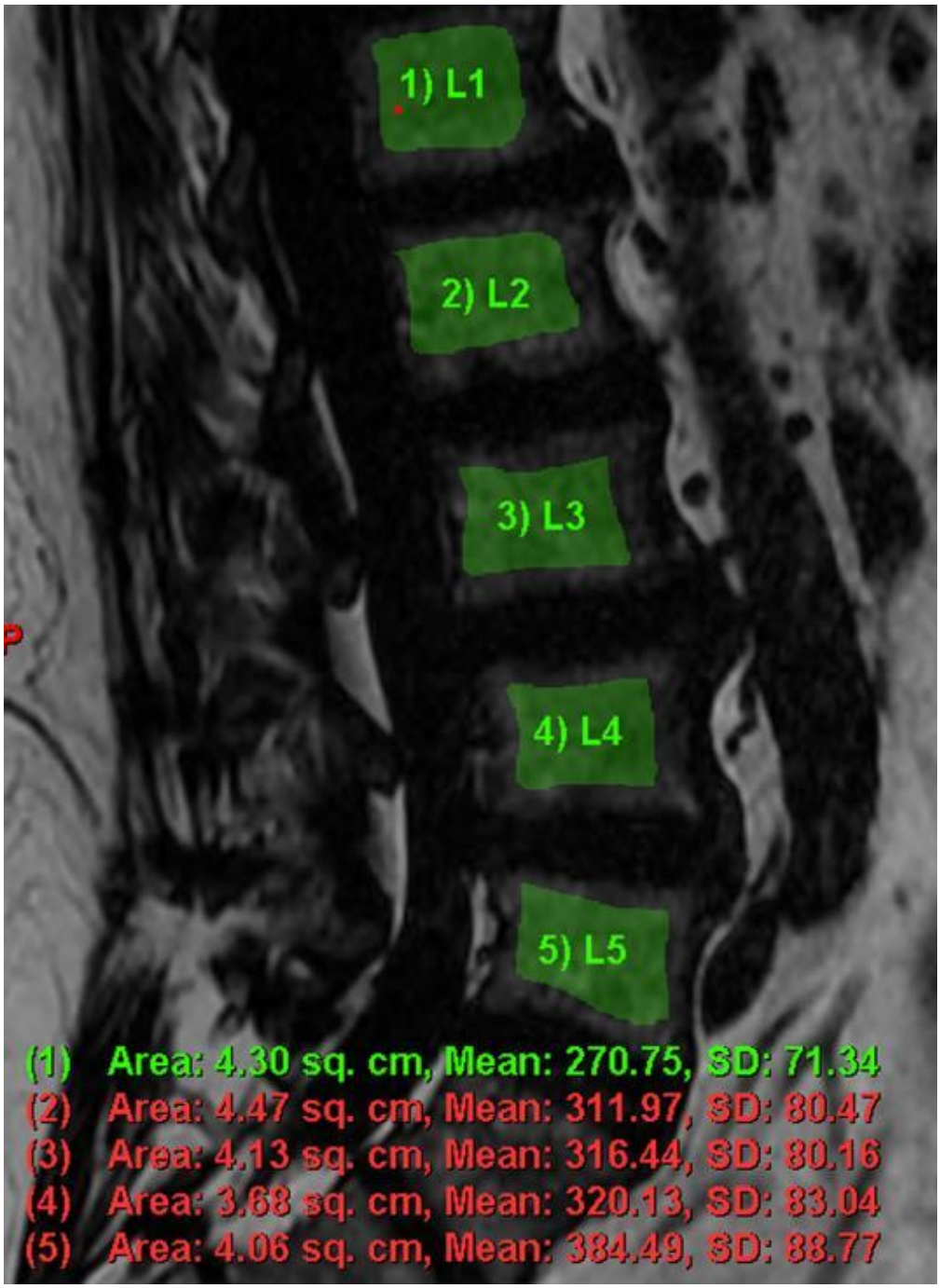

Figure 4.1: Sample sagittal view of a Fat T2 IDEAL image with Regions of Interest (ROIs) on L1-L5 showing the mean fat content at each vertebral level.

Magnetic Resonance Spectroscopy

MR spectroscopy (MRS) was performed at the L3 level on all five patients. MRS was acquired using the point resolved spectroscopy sequence (PRESS) (TR 2000ms, TE 35ms, 2.20min). Non-fat suppressed spectra were obtained by setting the fat suppression settings to zero. A single voxel was prescribed of approximately 2.0 cm^3 in size within the trabecular bone of L3. Particular care was taken to ensure that the voxel did not 'protrude' outside the confines of the vertebral body. Presaturation

bands were placed posteriorly, superiorly, inferiorly and anterior to the vertebral body to help eliminate unwanted signal contamination from outside the voxel. The software package SAGE (GE Medical Systems) was used to extract the areas under the peaks for separate fat and water peaks (A_f and A_w respectively). Post processing time was approximately 15 minutes per voxel using the SAGE software package. Fat fraction (FF) was calculated as $A_f / (A_w + A_f)$. As this has very high correlation with FF using the IDEAL approach (R^2 values of 0.85-0.9 [3, 9]), this figure was used to calculate a measurement for pure fat on the IDEAL: Fat images. This was done by dividing the mean measure from the IDEAL:Fat image at L3 by the FF figure from the MRS for the same vertebral body. The FF for all other vertebrae were then calculated by dividing the Fat: IDEAL measure for that vertebra by the pure fat measure. For example, if the MRS gave a FF at L3 of 0.4, and IDEAL:Fat ROI gave a reading of 300 for L3, pure fat was calculated as 300 / 0.4 = 750. Then if the IDEAL;Fat ROI reading for another vertebra was 400, FF would be calculated as 400 / 750 = 0.53.

Data Analysis

Regarding intra-observer variation, for each observation, the percentage deviation from the mean for that vertebra and observer was calculated. Similarly, inter-observer percentage deviation was calculated as the absolute value for Observer A – Observer B divided by the average of the two. If minimal deviations were noted, then a pooled mean value using data from all ten observations would be used in the subsequent analyses.

Descriptive plots were constructed to assess for trends in observer variation as well as FF across adjacent vertebral bodies. Linear regression using a mixed model was used to model the changes observed in FF within each participant. This approach allowed investigation of any trends across vertebrae, any differences in these trends between patients, and any anomalous measures between vertebrae. R version 2.15.1 was used for this analysis. For the comparison between MRS FF and DEXA bone mineral density, a Pearson correlation coefficient was calculated, with the square of this being R^2.

Results

Reproducibility of Marrow Fat Fraction Measures

Figure 4.2: Colour enhanced Fat T2 IDEAL sagittal image from a patient demonstrating vertebrae from T9 to S2 where green represents a lower FF than red. Note how the more rostral vertebrae are generally more green coloured than the redder caudal vertebra suggesting increasing fat fraction moving caudally.

Figure 4.2 shows an example of a false colour LINEAR Fat MRI sagittal image. To ensure stability of the FF measures, tests were done of intra- and inter-observer variation. Between the two observers, 94 vertebral body Fat measurements were obtained, with a median of 5 observations per vertebral body. A total of 464 measurements were recorded. Figure 4.3 shows the percentage deviation of each observation from the mean for the corresponding vertebral level and observer. The overall average intra-observer variation was 3.4%, and in greater than 95% of

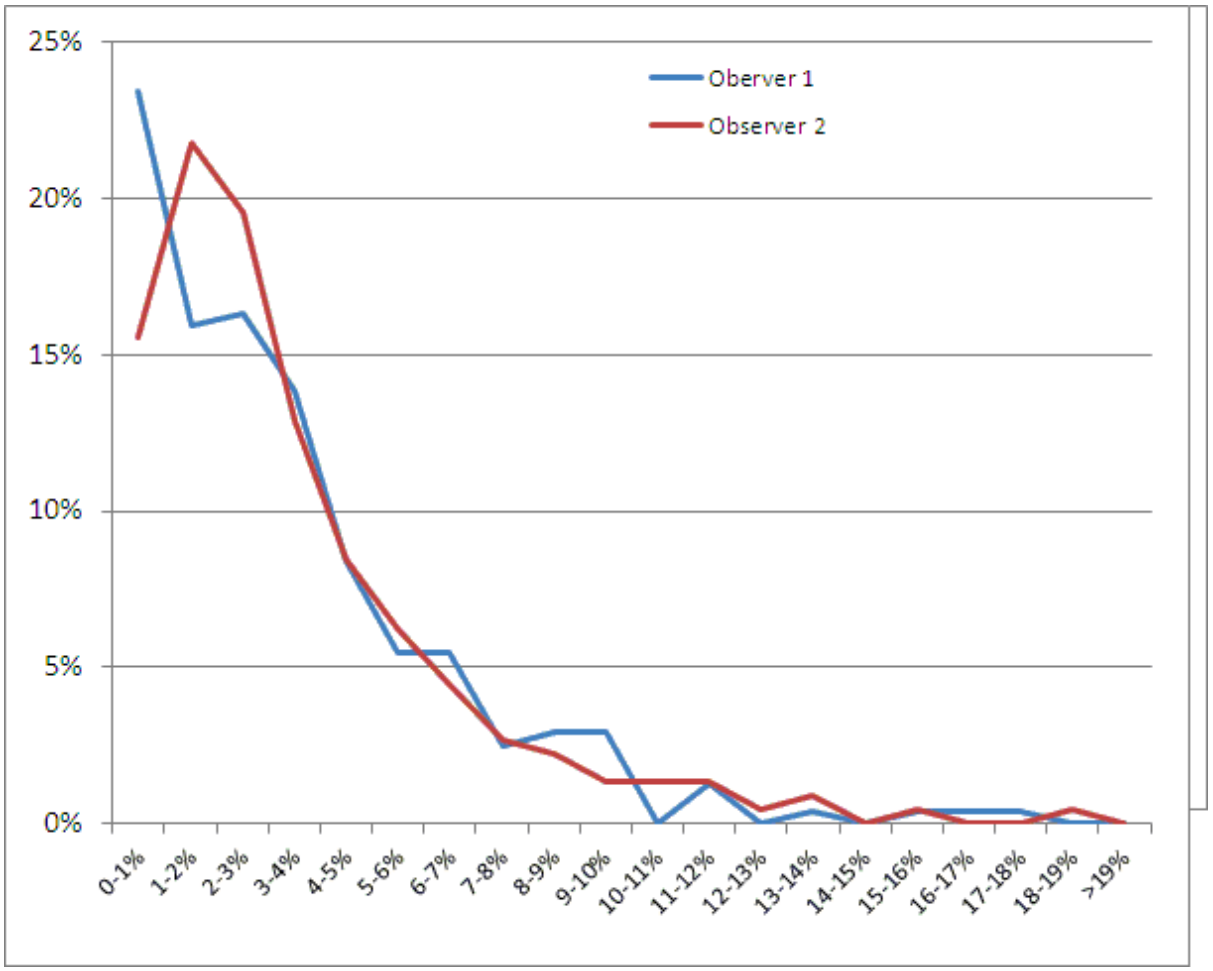

Figure 4.3: Line graphs showing low intraobserver variation in Fat measurements across all observations for both observers and nine vertebral bodies.

instances, observations were within 9% of the mean. No one patient's vertebra had two separate measurements greater than 9% from the mean, nor was any individual deviation greater than 18%. It was therefore concluded that there would be little effect from outliers on the data, and that the mean rather than the median would be a robust measure of data location.

Similarly, inter-observer variation was compared across 45 different vertebral levels (nine vertebral bodies in five patients each) between the two observers. Of the total of 45 observations, the overall average inter-observer variation was 2.1%, with 21 of the vertebrae within 1% between the two observers, 33 within 3% and 39 within 5%. The maximal variation was 8.1%. This suggests that the reading protocol lead to consistent results within and between observers, and justified using a pooled mean value in the subsequent analyses.

Fat Fraction

Figure 4.4 shows FF at each vertebral level for each of the five patients. Note that all show a gradual trend of increasing FF moving from the most rostral towards the most caudal vertebral body. Table 4.1 quantifies the average differences in FF between adjacent vertebrae. Note the anomaly in the trend that all patients show a reduced FF in S1 compared with L5. This 'L5-S1 Dip' is noted in all five patients with figures of -4.9%, -6.1%, -2.4%, -7.0% and -1.6% respectively. Fitting a mixed linear model to the data with patients as a random factor and vertebrae as a fixed factor, with S1 excluded there is a highly significant upward linear trend in mean FF from T11 to S2 ($p<0.0001$). The L5-S1 change is significantly different to that observed at the other levels ($p=0.0007$).

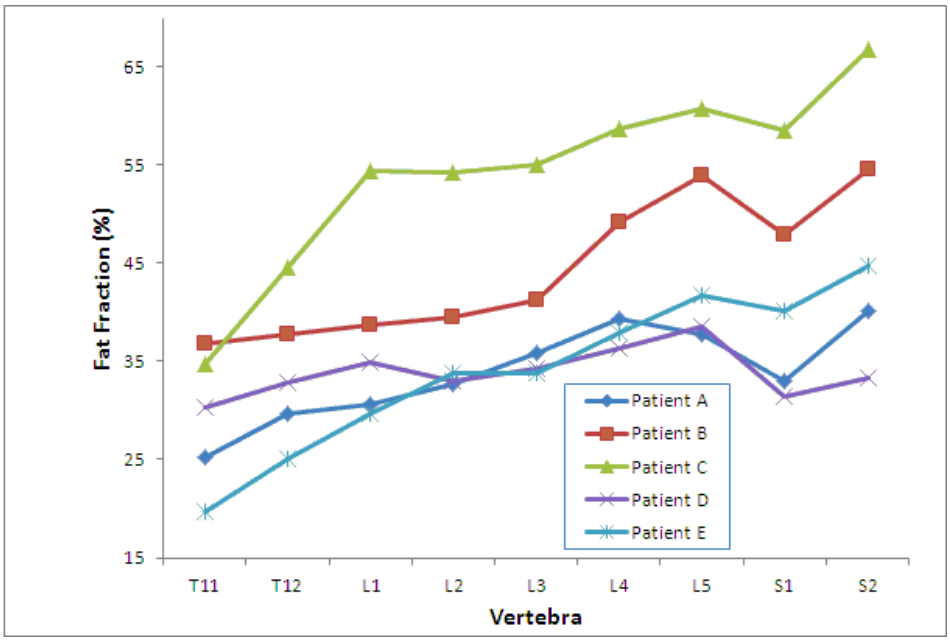

Figure 4.4: Fat fraction from T11 to S2 for the five patients measured. Most patients demonstrate a trend of increasing fat fraction moving caudally.

Table 4.1: Difference in Fat Fraction between adjacent vertebrae. Note the steady increase at all levels except for L5-S1.

Vertebra	Fat Fraction Mean (Range)	Difference to previous
T11	29.4% (19.7 – 36.9)	
T12	33.9% (25.1 – 44.5)	4.6%
L1	37.6% (29.6 – 54.4)	3.7%
L2	38.6% (32.7 – 54.2)	1.0%
L3	40.0% (33.8 – 55.1)	1.4%
L4	44.3% (36.3 – 58.7)	4.3%
L5	46.6% (37.8 – 60.8)	2.3%
S1	42.2% (31.5 – 58.5)	-4.4%
S2	47.9% (33.3 – 66.7)	5.7%

Due to one patient being shorter than the others, it was possible for a larger range of twelve vertebral bodies to be measured, and his FF per vertebrae is shown in Figure 4.5. The trend of increasing FF moving inferiorly appears even more pronounced, with a 50.9% difference in FF seen between T10 (29.1%) and S4 (80.0%) seen. Even excluding the most peripheral vertebral bodies because of the possibility of peripheral artifact, the range of FF from T11 to S3 is greater than 43%.

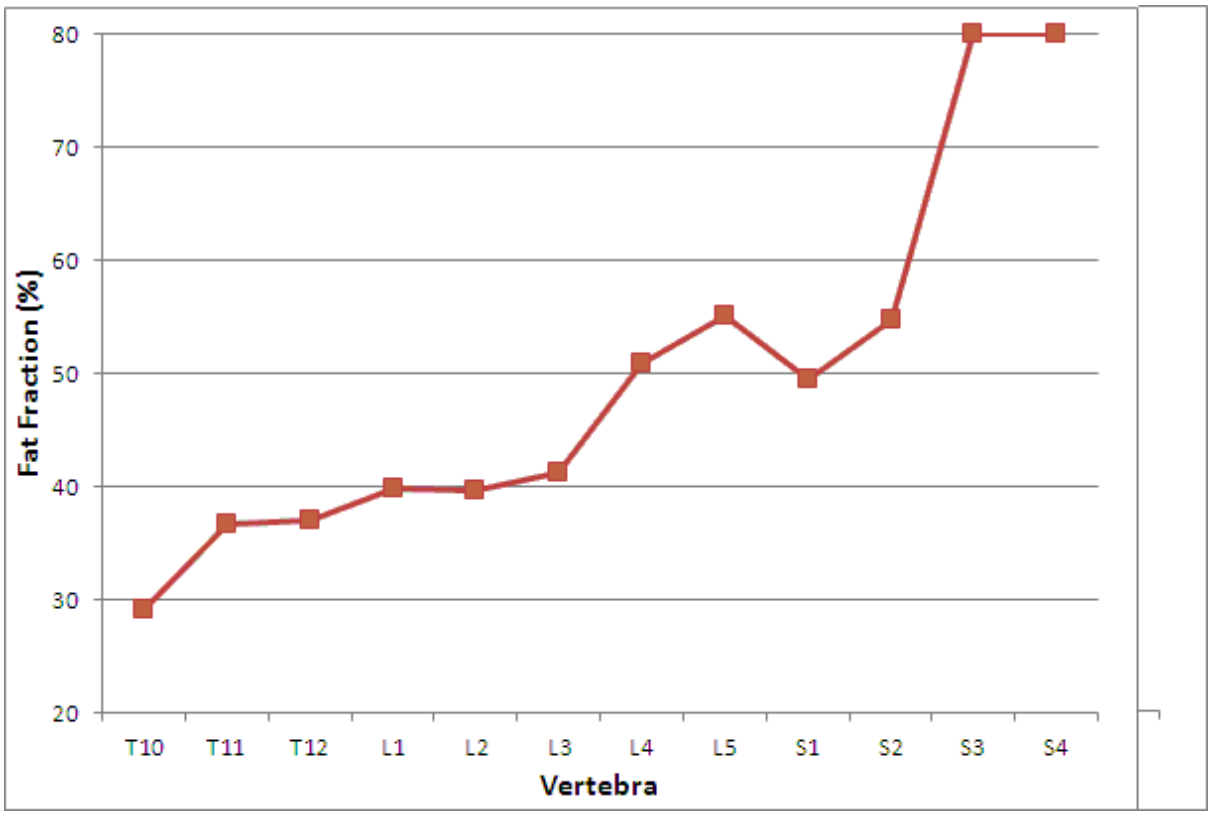

Figure 4.5: The patient who due to his smaller size was able to have FF estimated for 12 adjacent vertebral bodies showing a range of values of over 50% across the field of view.

Table 4.2: L3 MRS FF, with linear regression slope (an indicator of gradient in FF across the adjacent vertebral bodies), R^2 values and T-Scores. Note that normal BMD corresponds to a T-score of > -1, osteopaenia is between -1 and -2.5, and <-2.5 indicates osteoporosis.

Patient	MRS FF at L3	FF Slope (FF%/vertebra)	R^2	T-Score
1	35.8	2.23	0.94	0.7
2	41.2	2.74	0.82	-2.1
3	55.1	3.84	0.83	-0.2
4	34.2	1.10	0.81	-0.2
5	33.8	3.44	0.96	2.3

Fat Fraction Gradient

The gradient in FF between T11 to L5 was fitted to a linear regression model. For all five patients, a linear regression model proved a very good fit of the data, with R^2 values of between 0.81 and 0.96 (see Table 4.2). The slope of the regression line varied from 1.1%/vertebra to 3.8%/vertebra (see Table 4.2). Note how patients with similar L3 MRS FF readings can have different FF gradients. Patients 1, 4 and 5 have L3 MRS FF of 34.8+/-1%, yet despite this similarity the FF gradient varies by a factor of 3.5 across the full range of observed FF gradient values for these 3 patients. This suggests that a FF reading at a single vertebral level does not completely describe the functional marrow distribution. However, the gradients were not significantly different in the mixed linear model used in the previous section ($p=0.15$).

DEXA versus MRS FF

The T-scores for the Lumbar vertebra were -2.1, -0.2, 2.3, -0.2 and 0.65, suggesting that 4 of the participants had normal BMD and one was osteopaenic. The isolated DEXA BMD reading at L3 was compared with the MRS FF reading at the same vertebral level. Only a weak negative correlation was noted between the two readings ($R^2=0.17$). Similarly weak negative correlations were noted at L2 and L4

(R^2=0.12 and 0.11 respectively). Considering all 15 of the individual L2, L3 and L4 vertebral bodies together, although there is a negative correlation overall between BMD and FF, the relationship is weak (R^2=0.06, Figure 4.6).

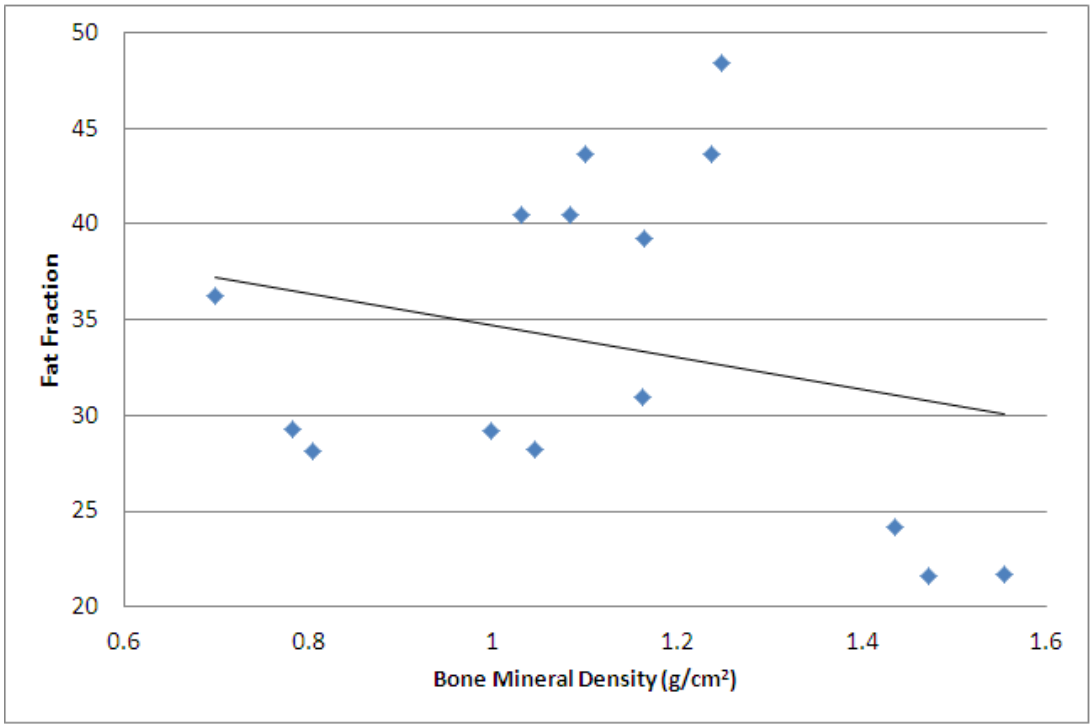

Figure 4.6: Scatterplot of DEXA measured BMD verses Fat Fraction which shows a weak negative correlation.

Discussion

The use of IDEAL imaging, with ROI analysis, proved to be a rapid and reliable method for determination of vertebral FF. This method enables a large series of vertebral bodies to be measured following a single rapid acquisition achieved in under seven minutes. This contrasts with spectroscopy, which normally evaluates a single vertebra per acquisition, with significant post processing time required to obtain the relevant data. There was minimal inter- and intra-observer variation for these measurements for independent observers following our set protocol. As such, it should be possible to extract relevant data from only one ROI per vertebral body, which will make post-processing FF calculation relatively simple. The most striking observation was that the more inferior the vertebral location, the more likely the FF would steadily increase. This FF gradient appears to be largely independent of an isolated measure of FF using MRS at a single vertebral body level. This result has not been reported using the IDEAL approach, but is in agreement with reports in the literature using MRS to estimated FF [2, 3].

An early report touches on the possibility of a marrow gradient noting a trend toward increasing FF for more inferior vertebral bodies measured with MRS [3]. In this study the mean FF value at L1 was 40.5%, and at L5 it was 51.3%, albeit with wide ranges due partially to only ten patients being examined in this manner. This trend was not consistent, and may have been overwhelmed by the stronger relationships noted with both age and gender [4]. Even with these caveats, it is worth noting that the corresponding figures from our series were broadly similar at 37.7% and 46.7%.

Another previous report again using MRS to quantify FF focussed on post-menopausal women, which would be expected to reduce the impact of age and gender on the results [2]. Vertebral levels from L1 to L4 were all measured individually for 40 women, some of whom were known to have low BMD. The FF gradient increased by an average of 2.2%/vertebra moving inferiorly in the patients with low BMD, although no strong evidence of a gradient was seen in the healthy controls. The average figure in our series was 2.7%/vertebra, although only one of our five patients had a low BMD reading. A subsequent report from the same group looking at diabetic women, also noted a trend towards reducing FF from L1 to L3 [12].

It would appear that our results using the IDEAL approach are consistent with some earlier observations reported using MRS suggesting a gradient in FF moving caudally down the lumbar spine. While the two approaches correlate well with each other, the latter sequence has the advantage of been able to examine more vertebral bodies simultaneously in a shorter time [3, 9]. Given the observed gradient in FF, it would seem that future investigators will either need to examine multiple vertebral bodies with MRS, or use an alternative approach such as IDEAL.

There have been several reports suggesting a correlation between FF measured at a single vertebral body level and BMD measured by DEXA imaging [3, 5, 7]. However, there is extensive overlap between normal, osteopaenic and osteoporotic individuals. Part of the reason for this may be anatomical variations such as osteophytes which can interfere with DEXA readings [13, 14]. Although this might be circumvented with the use of qualitative CT, the increased radiation dose and lack of widely validated population data for this modality may curtail its widespread use [15]. Our hypothesis is that the complex functional anatomy, physiology and biology of bone is poorly captured using a single parameter such as Fat Fraction at a single vertebral level [16-18]. Additional factors such as the FF gradient may be helpful in separated people into distinct BMD categories, and as such this is an area that we are continuing to investigate.

A second finding from our study was that there is a consistent reduction in FF moving from L5 to S1. This contrasts with a result published using FLT-PET, although that study did not resolve to the same degree of anatomical precision as in the current report[6]. This may be due to other subtle degenerative pathology at this level such as spondylosis relating to the unique mechanics of the L5-S1 joint compared with the more superior thoracic and lumbar articulations. Further work will be required to clarify both the consistency of the reduction, as well as trying to gain a greater understanding of the underlying causes. If the FF does not change consistently and at an equal rate along the spine, individual measures by spectroscopy may miss changes.

Loss of BMD is a common problem for men managed with ADT for prostate cancer [19]. This results in a higher rate of fractures for these men [20]. Previously, ADT was only used for men being managed palliatively for metastatic disease and hence with relatively low life expectancy. Two key developments now make the long term toxicity of ADT more pertinent. One is the evidence of efficacy of adjuvant ADT in the curative setting, meaning many men expecting to be cured of their prostate cancer will survive long enough to potentially experience the chronic effects of ADT exposure [21, 22]. The second is the increasing number of effective systemic therapies, extending the life of men in the metastatic setting [23]. Abiraterone Acetate in particular requires long term exposure to not only ADT, but also low dose prednisone, which would be expected to further accelerate loss of BMD [24].

Although our small study is in a relatively homogeneous patient population, we have demonstrated the potential to measure large regions of the spine revealing some consistent findings. Following from the results from this study, we have initiated a larger prospective trial to investigate the capacity of lumbar spine MRI to predict which men are at higher risk of accelerated loss of BMD while on ADT as treatment for their prostate cancer. Several randomized studies have shown improvements in BMD for unselected men on ADT treated with bisphosphonates, RANKL inhibition or selective oestrogen reuptake modulators [25]. Although there are numerous guidelines recommending pharmacological intervention for such men mainly on the basis of their T-score on DEXA imaging, given potential toxicities like osteonecrosis of the jaw as well as the expense of such agents there is scope to further target therapy to men most likely to benefit [25-27].

Our current study focuses on men with prostate cancer being managed with curative intent with an 18 month course of ADT and pelvic radiotherapy. Due to improved signal to noise ratio and shorter image acquisition time to reduce motion artefact, we will use a 3 Tesla system for this successor study [12]. We aim to investigate whether multiparametric MRI of the lumbar spine at baseline including In:Out phase, pure fat/water as well as diffusion weighted imaging might contribute to a model combined with clinical and DEXA findings to identify a subgroup of patients at risk of accelerated loss of BMD [28].

Conclusions

Rapid acquisition of a large range of vertebral bodies with accurate determination of FF with ROI was demonstrated. We have observed the existence of a gradient in Fat Fraction from T10 to S2. There is also a consistent dip in Fat Fraction between L5 and S1, which may be due to the different anatomy and degenerative changes at this level. These findings will be explored in a larger prospective study attempting to use such extra information available on MRI to determine which men are at risk of more rapid loss of BMD while on ADT.

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Chapter 5 – Serial Spinal MRI and DEXA changes while on ADT: A potential toxicity biomarker.

Changes over time in bone mineral density are variable for men with prostate cancer being managed with androgen deprivation therapy. Expanding on the foundations set in the previous chapter, we explored in a larger cohort whether changes in serial multiparametric MRI of the spine correlated with later changes in DEXA measured bone mineral density. If a reliable early imaging biomarker of later loss of bone mineral density could be identified, further research would be justified in exploring a risk adapted approach to bone health incorporating these findings.

Several observations were forthcoming. An increase in fat fraction (FF) was observed from T11 to S2. There was a positive correlation between baseline MR spectroscopy (MRS) FF and Dixon FF as well as a negative correlation between MRS FF and the apparent diffusion coefficient (ADC). Over six months, MRS FF increased by a median of 25% in relative values, Dixon FF increased and ADC values decreased. Men with >5% loss in bone mineral density after one year had triple the percentage increase in MRS FF at six months. Although these observations are of interest, given the complexity associated with obtaining them, they are unlikely to be of significant clinical utility in the short term.

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Spinal Multiparametric MRI and DEXA changes over time in men with prostate cancer treated with Androgen Deprivation Therapy: A potential imaging biomarker of treatment toxicity.

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Abstract

Objectives

To explore changes in Bone Mineral Density (BMD) measured by DEXA and MRS Fat Fraction (FF), Dixon FF and ADC in lower spinal vertebral bodies in men with prostate cancer treated with Androgen Deprivation Therapy (ADT).

Methods

28 men were enrolled onto a clinical trial. All received ADT. DEXA imaging was performed at baseline and 12 months. L-spine MRI done at baseline and six months.

Results

The number of patients who underwent DEXA, Dixon, ADC and MRS at baseline/follow-up were 28/27, 28/26, 28/26 and 22/20. An increase in FF was observed from T11 to S2 (average 1%/vertebra). There was a positive correlation between baseline MRS FF and Dixon FF ($r=0.85$, $p<0.0001$) and a negative correlation between MRS FF and ADC ($r= -0.56$, $p= 0.036$). Over six months, MRS FF increased by a median of 25% in relative values ($p=0.0003$), Dixon FF increased ($p<0.0001$) and ADC values decreased ($p=0.0014$). Men with >5% BMD loss after one year had triple the percentage increase in MRS FF at six months (61.1% v 20.9%, $p=0.19$).

Conclusions

Changes are observed on L-spine MRI after six months of ADT. Further investigation is warranted of MRS change as a potential predictive biomarker for later BMD loss.

Keywords:

Magnetic Resonance Imagining
Bone Density
Prostate Neoplasms
Biomarkers
Toxicity

Key points:

- Spinal marrow fat fraction increases after 6 months of Androgen Deprivation Therapy.
- More inferior vertebral bodies tend to have higher fat fractions
- MRS Fat Fraction changes were associated with later changes in DEXA BMD.

Acronyms

ADT = Androgen Deprivation Therapy
BMD = Bone Mineral Density
DEXA = Dual-Energy X-Ray Absorptiometry
DWI = Diffusion Weighted Imaging
FF = Fat Fraction
HRPC = High Risk Prostate Cancer
PRESS = Point Resolved Spectroscopy
PROCITT = PROstate Cancer Imaging, Treatment and Toxicity
SVS = Single voxel spectroscopic

Introduction

Some men with apparently non-metastatic prostate cancer at diagnosis have a high probability of developing widespread disease following local therapy.[1] This entity of high risk prostate cancer (HRPC) has been extensively studied, and multimodality treatment with pelvic radiotherapy and androgen deprivation therapy (ADT) has been shown in multiple randomized controlled trials to have a survival advantage compared to either treatment alone.[2,3]

ADT reduces testosterone levels and leads to lower amounts of peripherally converted estrogens, which has a direct effect on bone mineral density (BMD). Numerous studies have demonstrated that this leads not only to changes on DEXA imaging, but also osteoporotic fracture rates and even overall survival.[4-6] Current consensus guidelines recommend annual DEXA monitoring of BMD for men on ADT and intervention with anti-resorptive agents for those found to have osteopaenia or osteoporosis.[7] Population based data suggests that less than 20% of men on ADT have a DEXA performed, suggesting a low awareness of the importance of managing bone health for such patients.[8]

There is a wide variety in the rate of BMD change for men on ADT, quoted between 0 and 8% in the first year of treatment.[6,9] It is therefore plausible that a risk adapted approach is better targeted not only to men with osteopaenia or osteoporosis at baseline, but also the subgroup with more rapid loss in BMD. Concurrently, literature has begun to emerge suggesting MRI Fat Fraction (FF) has a correlation with DEXA measured BMD.[10] There is a biological rationale for this given the common stem cell progenitors for both bone forming osteoblasts and fat containing adipocytes and the effect of estrogen on driving the relative proportions of cellular differentiation.[11] MRI also has the advantages of being a fully 3-dimensional approach better suited to understanding changes within a multifunctioning organ such as bone which is not susceptible to artefacts which can confound the interpretation of DEXA imaging.[12]

We hypothesize that early changes on serial MRI of the spine may correlate with the rate of change in DEXA measured BMD for men on ADT. If this is the case, further

investigation of early selective intervention with anti-resorptive therapy for such higher risk men would be warranted.

Materials and Methods

Patient Recruitment

A prospective clinical trial (PROCITT: PROstate Cancer Imaging, Treatment and Toxicity) was offered to men with HRPC between January 2013 and July 2014. Eligible men needed to have non-metastatic HRPC features (any one of: PSA>20, Gleason score of 8-10 or Stage of T3-T4 or N1), and be appropriate for an 18 month course of ADT and definitive prostate radiotherapy. The Hunter New England Human Research Ethics committee provided ethical approval (12/08/15/4.02). The project was funded by an unrestricted investigator initiated study grant by Abbvie Pharmaceuticals. 28 men with high risk prostate cancer were recruited to the study over an 18 month period. The median age was 70 years (range 54-78), median PSA was 12.4, and 23 of the men had Gleason score 8 or 9 disease.

Management

After providing informed consent, all men had baseline imaging including a MRI, plain films of the thoracic-lumbar spine and DEXA imaging. They then commenced an 18 month course of ADT. As per national bone health consensus guidelines, they were all recommended to commence oral Vitamin D and Calcium supplementation as well as moderate physical activity.[7] At the six month time point, men had a repeat MRI just prior to commencing a course of prostate+/-pelvic radiotherapy, the technical details of which have been reported.[13] Men continued to be followed after radiotherapy including receiving annual DEXA imaging for three years. An MRI at 6 months was selected as a compromise between sufficient time to assess any changes whilst still providing an opportunity for possible bone health interventions, while a DEXA at 12 months complies with consensus guidelines as well as the period of most rapid loss in BMD.[7, 14]

Imaging Protocols

MRI Dixon Method

All patients underwent morphological imaging of the lumbar spine in supine and feet first orientation on a 3 Tesla whole body scanner (Magnetom Skyra, Siemens AG, Erlangen, Germany) with the combination of a dedicated 18 Channel body matrix and 32 channel phase array spine receiver coils. After localiser scans, a 3-point Dixon Turbo Spin Echo T1 weighted scan was performed in sagittal plane to assess fat fraction. Four series of images (in/opposed phases, fat/water only) generated by the system were used for FF analysis. The sequence parameters were TR/TE=600/9.5ms, Slice thickness/gap=3mm/10%, FOV=340mm, Matrix=384x384 with 0.9x0.9 in-plane resolution, iPAT=2, Number of slices=28 and Average=1.

Regions of interest (ROIs) were manually applied in 2-dimensions on the mid-sagittal slice for all vertebral bodies within the field of view. Each ROI would typically require a polygon with 6-10 points of at least 3 cm^2, with the same ROI copied onto the corresponding Fat and Water images. Mean readings from the Fat and Water Dixon images were recorded, and Dixon FF calculated for individual vertebral bodies as Mean Fat/(Mean Fat+Mean Water).

Diffusion Weighted Imaging (DWI)

After six patients had been accrued, a protocol amendment was made to allow more routine acquisition of DWI and MR Spectroscopy. Following T1 weighted scan, Echo planar spin echo based (Single shot EPI) DWI sequences with a pair of rectangular motion probing gradient pulses along three orthogonal directions (phase, frequency and slice) were obtained with b-values equal to 0, 250, 500 and 750 s/mm^2.[15] Six sagittal slices were acquired. The sequence parameters include TR/TE=1400/87ms, Slice thickness/Gap=10mm/10%, FOV=260mm, Matrix=156x156mm with 1.7mmx1.7mm in-plane resolution, iPAT=2.
The quantitative analysis of diffusion was performed by calculating the Apparent Diffusion Coefficient (ADC) values. The ADC value was derived from the equation ADC = -1/b ln (S(b)/S(0)) where Sb and S0 are signal intensities from each voxel with and without diffusion gradients and b is the sensitizing parameter. The three directional diffusion images were used to generate an average ADC map using Syngo (Siemens Healthcare, Erlangen, Germany). The ADC values were measured

for each patient at all four b-values from 0 to 750 s/mm^2 by drawing a ROI similar to the Dixon ROI within each vertebral body from T12 to S1 on all the DWI images.

^{1}H-Magnetic Resonance Spectroscopy (MRS)

Single voxel spectroscopic (SVS) technique was employed in the transverse plane to generate non-water suppressed 1H spectra, and the voxel was placed within the marrow of the L3 vertebral body seen on T1 weighted images. The spectroscopic data was acquired using a double echo, slice selective technique based on Point Resolved Spectroscopy (PRESS) with an echo time of 30ms, TR of 2000ms, 64 signal averages, 1024 complex data points, and bandwidth of 2000Hz and automatic image based shimming. A voxel size of 8cm^3 was used.

Spectra were reconstructed using Syngo which involved water referencing for frequency shift correction, a Gaussian filter of 125ms was applied to the time domain data. Following the fourier Transform, phase correction and baseline correction was applied to the spectrum. A series of peaks, including water at 4.7 ppm and five fat peaks at 1.1, 1.39, 1.9, 2.5 and 5.36 ppm was used to model the spectrum using the Syngo curve fitting routine (see Figure 5.1). The lipid peak area (LPA) was the sum of the dominant fat peaks at 1.1 (-CH3) and 1.39 (-CH2) ppm and quantified relative to the sum of the water peak area (WPA) to give a MRS FF using LPA/(LPA+WPA).

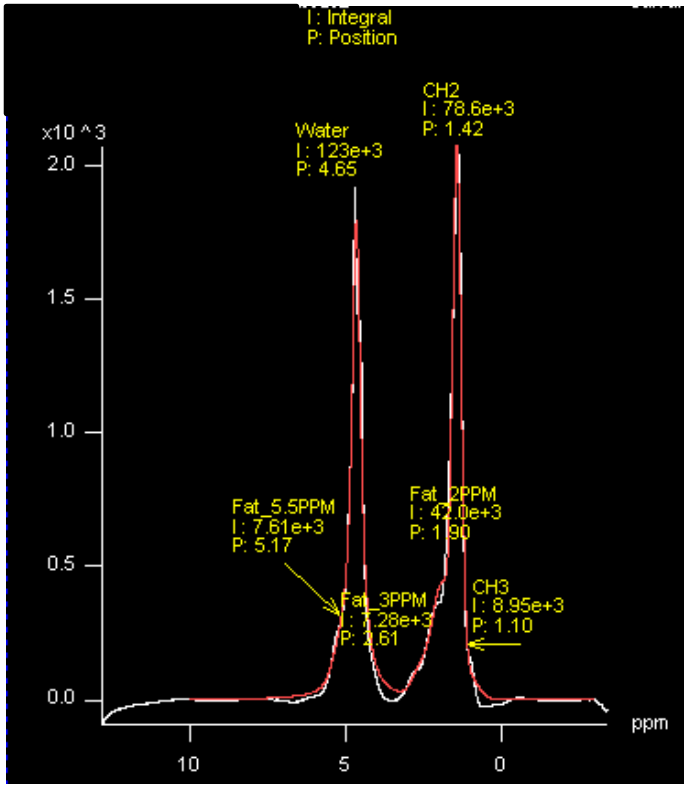

Figure 5.1: Example of curve fitting for a L3 MRS showing the water (left) and fat (right) peaks along with the area under the curve for each of the six respective positions.

One male subject not managed with ADT was scanned twice within one week to assess reproducibility. This showed an average coefficient of variation of 3.5% for Dixon FF, 4.0% for ADC and a 3% variation in MRS FF.

DEXA

A DEXA scan was performed on all subjects within one week of their baseline MRI. Readings of BMD in g/cm^2 were obtained individually for L1-L4 vertebral bodies as well as both necks of femurs (NOFs). These were translated into age and gender matched T-Scores as per WHO recommendations. Serial imaging was performed 12 months later on the same DEXA scanner that the baseline imaging occurred on.

Statistical Analysis

DEXA Lumbar spine T-score scan values were averaged across L1-L4. For Dixon and ADC, all fully visualized vertebrae were used, usually from T10 or T11 down to S1 or S2. Due to evidence of a statistical interaction both over time and between vertebral body level and various scan parameters, it was not appropriate for all data to be pooled together for analysis, and hence the various analyses are presented separately.

Correlations between scans/parameters were examined using Pearson correlation. Changes between baseline and follow-up scan values for each vertebra were examined using linear mixed modelling with robust standard errors. Mean changes with 95% confidence intervals from baseline and p-value are presented. All statistical analyses were performed using SAS v9.4 (SAS Institute, Cary, North Carolina, USA).

Results

Baseline DEXA Results

All men had baseline DEXA imaging. Baseline DEXA T-Scores averaged from L1-L4 spanned a range of -2.63 to 4.05, with a mean of 0.07. Baseline DEXA showed a correlation between raw BMD measured at the NOF and L-Spine averaged from L1-L4 (r= 0.62, p=0.0004). An inverse correlation was noted between increasing age and raw BMD at the NOF (r= -0.41, p=0.03 – see Figure 5.2), with a weaker correlation between age and raw BMD at the L-Spine (r= 0.26, p=0.19). The latter may be due to some confounding from degenerative changes in the L-Spine on DEXA imaging.[12]

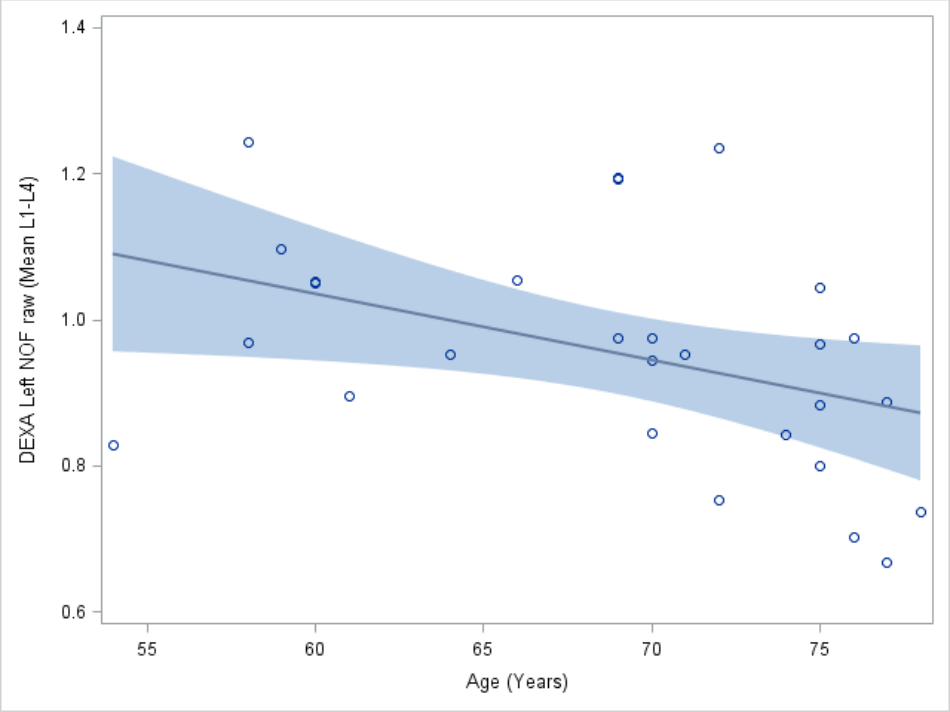

Figure 5.2: Association between age and DEXA BMD at the neck of femur (r= -0.41, p=0.03, 95% CI limits shown).

Two men, aged 76 and 77, had a new diagnosis of osteoporosis on their baseline DEXA imaging with NOF T-scores of -2.8 and -2.7 respectively. As per national consensus guidelines, they were commenced on oral bisphosphonate therapy.[7] All 28 men had no insufficiency fractures on thoraco-lumber spinal plain film imaging. No metastases were observed, but two men had evidence of haemangiomas of the vertebral bodies which were excluded from ROI delineation.

Baseline MRI Results

The number of patients who underwent DEXA, Dixon, ADC and MRS at baseline/follow-up were 28/27, 28/26, 28/26 and 22/20, respectively. Examining individual Dixon FF measures from T11 to S2, there was evidence of increasing values with more inferior vertebral bodies by 1%/vertebral body on average (Figure 5.3). The only exception was the L5-S1 vertebral bodies where a slight decrease was noted, possibly due to the different mechanical stress at this level. For ADC, no strong trend was noted between the individual vertebral measurements from T11 to S2.

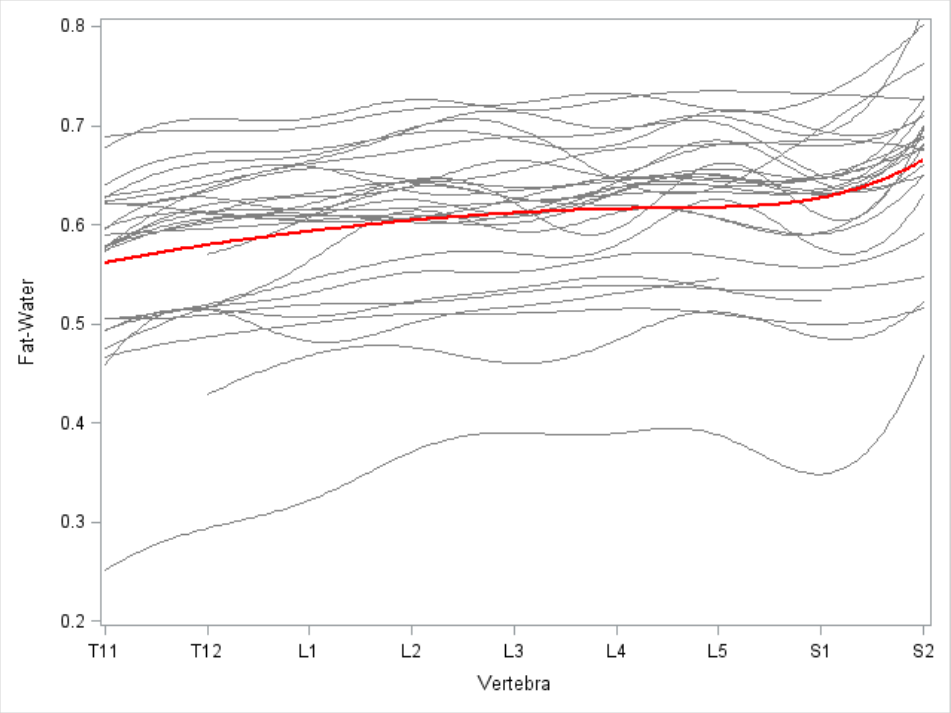

Figure 5.3: Trend for Dixon FF to increase by approximately 0.01/vertebral body moving inferiorly. Note that most of the results are clustered around the fitted lines and that the slopes for the individual lines are generally similar to the fitted lines, both suggesting relatively small variations between individuals in this population.

MRI Correlations

There was evidence of a correlation between baseline MRS FF and Dixon FF ($r=0.85$, $p<0.0001$). For the 14 men who had both an MRS FF and ADC at L3 performed at baseline, there was a negative correlation between these parameters ($r= -0.56$, $p= 0.036$).

Some correlation was noted between Age and increased MRS FF ($r=0.37$, $p=0.09$). Exploring a relationship between age and individual vertebral body Dixon FF and ADC values showed only weak positive correlations without any strong evidence of

statistical association. There was no evidence of a relationship between baseline MRS FF, Dixon FF or ADC values per vertebral body verses baseline DEXA BMD at the L-spine or NOF.

DEXA and MRI Changes over time

On serial DEXA 12 months apart, similar relative median changes in raw BMD were observed both for the L-Spine (-3.2%, IQR -1.7 – -5.7, p<0.0001) and NOF (-3.4%, IQR -0.4 – -6.4, p=0.0003). Due to our series focussing on the L-spine changes on MRI, and the potential for spurious relationships if excessive correlations were attempted, further analysis of BMD focussed on the L-Spine rather than NOF. Out of the 26 patients who had 2 DEXAs performed, four had an increase in L-Spine DEXA raw BMD ranging from 0.1% to 7.4%, and ten had large decreases of greater than 5% ranging from -5.2% to -11.7%.

Men managed with ADT had MRS FF increase by an absolute median value of 0.092 over six months. This corresponded to a median 25% relative increase (IQR 17% - 89%, p=0.0003). Dixon FF also showed median increases over six months from a minimum of 6.3% at S2 to a maximum of 10.4% at T11 (p<0.0001 – table 5.1 and figure 5.4). Conversely, ADC values tended to decrease over six months (p=0.0014 – table 5.2).

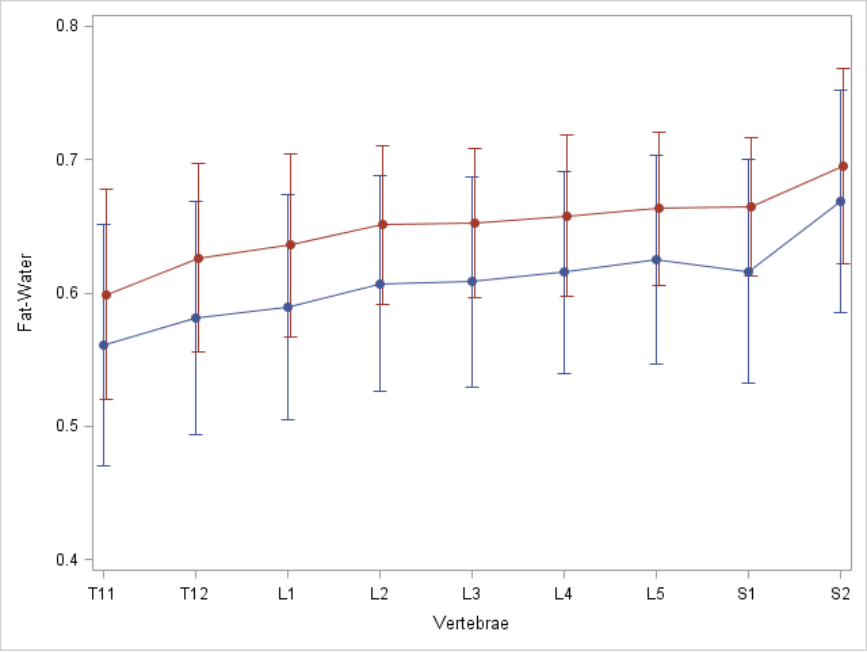

Figure 5.4: Line graph showing Dixon FF at baseline and six months per vertebral body. Note again the trend for higher FF with more inferior vertebral body and the consistent increase in mean FF for every vertebral body over time.

Vertebral Level	Dixon change		p-value
	Percentage (95% CI)	Absolute (95% CI)	Overall <0.001
T11	10.40 (3.8, 14.6)	0.045 (0.027, 0.062)	<.0001
T12	7.25 (2.6, 15.0)	0.048 (0.031, 0.064)	<.0001
L1	9.50 (1.8, 15.7)	0.049 (0.035, 0.064)	<.0001
L2	8.80 (0.8, 14.4)	0.046 (0.030, 0.061)	<.0001
L3	8.80 (2.7, 16.3)	0.046 (0.028, 0.063)	<.0001
L4	7.95 (1.7, 13.7)	0.044 (0.027, 0.060)	<.0001
L5	6.82 (1.2, 11.6)	0.040 (0.022, 0.058)	<.0001
S1	8.61 (-0.1, 13.5)	0.051 (0.029, 0.073)	<.0001
S2	6.33 (3.8, 9.8)	0.035 (0.015, 0.054)	0.0005

Table 5.1: Change in MRI Dixon Fat Fraction between baseline and after six months of ADT. P-values are adjusted for bisphosphonate use and age at baseline.

Vertebral Level	ADC Change		p-value
	Percentage (95% CI)	Absolute (95% CI)	Overall 0.0014
T12	-50.42 (-63.6, -33.3)	-0.00012 (-0.00019, -0.00005)	0.0012
L1	-29.63 (-46.9, 19.2)	-0.00007 (-0.00012, -0.00001)	0.0120
L2	-58.57 (-78.2, -18.5)	-0.00011 (-0.00017, -0.00005)	0.0002
L3	-17.09 (-61.5, 65.0)	-0.00004 (-0.00009, 0.00001)	0.0952
L4	-28.41 (-62.5, 14.3)	-0.00005 (-0.00010, -0.00001)	0.0284
L5	23.05 (-34.5, 90.9)	-0.00003 (-0.00009, 0.00004)	0.4436
S1	-50.00 (-73.1, 4.5)	-0.00011 (-0.00018, -0.00004)	0.0026

Table 5.2: Change in MRI ADC between baseline and after six months of ADT. P-values are adjusted for bisphosphonate use and age at baseline.

Predictors of DEXA Changes

We wished to investigate whether there were any parameters at baseline or within the first six months of treatment which predicted for larger changes in BMD at one year. We defined a larger change in DEXA L-spine raw score as a loss of BMD of at least 5% over 12 months, which is double the mean change in this parameter reported in the literature for men with prostate cancer on ADT.[16] The univariate analysis of potential predictive factors is presented in table 5.3. Note that the patients with >5% loss in BMD at one year had nearly triple the percentage increase in their MRS FF at L3 (61.1% v 20.9%). Exploring DEXA BMD change and MRS FF change as continuous variables amongst the 17 patients who had all four relevant scans performed show that only three patients had a reduction in MRS FF, and these were the only three to exhibit an eventual increase in DEXA BMD of between 3.2 and 7.4%. The correlation between these variables is negative (r= -0.44, p=0.076), and is shown in figure 5.5).

Factor		Large decrease (>5%) of DEXA raw score		
		No (n=18)	Yes (n=10)	p-value
Age at baseline [mean (SD)]		69 (8)	69 (6)	0.84
Bisphosphonate Use	No	16 (62%)	10 (38%)	0.27
	Yes	2 (100%)	0	
DEXA T-score at Baseline [mean (SD)]		1.216 (0.197)	1.260 (0.158)	0.55
MRS FF percent change [median (IQR)]		20.89 (-3.5, 39.01)	61.10 (22.48, 92.51)	0.19
Dixon FF percent change [median (IQR)]		8.12 (1.41, 15.11)	9.22 (1.39, 12.23)	0.90
ADC percent change [median (IQR)]		-24.21 (-50.8, 10)	-58.89 (-62.65, 5.57)	0.44
Average Dixon FF vertebra change [median (IQR)]		0.01 (0.007, 0.012)	0.009 (0.005, 0.012)	0.41

Table 5.3: Univariate associations between various factors and DEXA percent decrease >5%. Age, bisphosphonate use and DEXA T-Score are all from baseline, the MRS FF, Dixon FF and ADC percentage changes were all between baseline and the six month scans, and the average Dixon FF vertebra change looked at the gradient in FF across vertebral bodies at baseline.

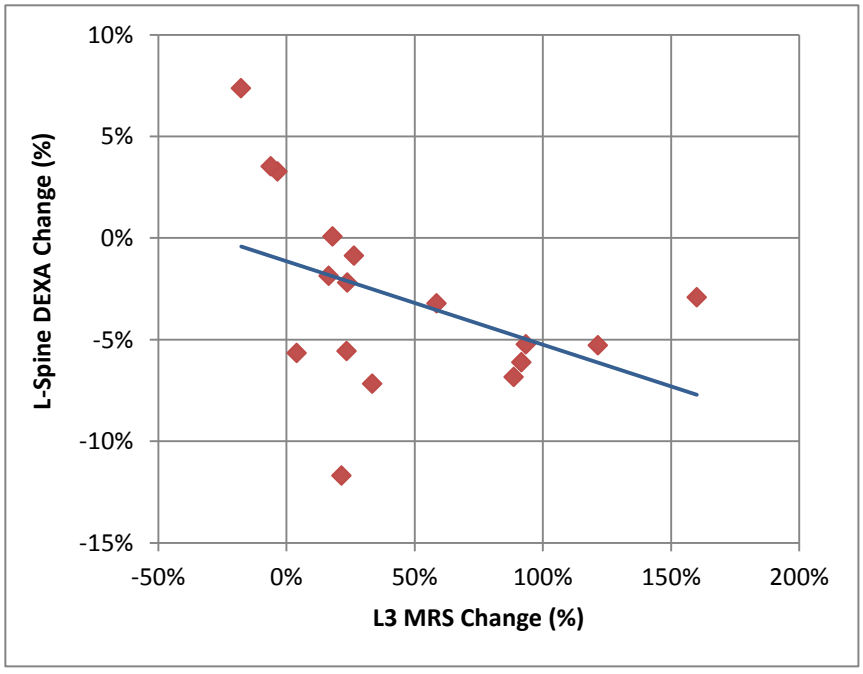

Figure 5.5: Percentage change in MRS FF over six months plotted against percentage change in DEXA L-Spine raw score over 12 months. Note the small number of men exhibiting a positive change in both factors, and the moderate inverse relationship between the two variables (r= -0.44, p=0.076).

Discussion

Our work shows correlations between various baseline MRI sequences, changes in FF between adjacent vertebral bodies and over time, and a correlation between changes in MRI FF at six months verses DEXA BMD changes after 12 months. Some of these replicate earlier work, particularly the correlations between MRS FF with both Dixon FF and ADC[17] as well as changes in FF between adjacent vertebral bodies on MRS and using In-Out Phase techniques.[18] The increased FF is plausible given the known effects of ADT on lipogenesis, with the increased adipocyte volume also potentially causing restricted diffusion. The changes in MRI sequences over time under the influence of various oncological interventions and their correlation with DEXA BMD changes is a more emergent area which our work helps lay a stronger foundation for.

Previous studies looking at changes in serial MRI FF have tended to be small and have heterogeneous interventions. One series used a 9 patient cohort with gynaecological cancers managed with either chemotherapy or various pelvic radiotherapy regimens and showed over 6 months increases in In-Out Phase FF in L4 by an absolute average of 16.1%.[19] A second series used a 19 patient cohort with a range of pelvic malignancies managed with several chemotherapy regimens in concert with various types and doses of pelvic radiotherapy.[20] They observed using an In-Out FF measure that increases were more marked in the L4-S2 region than at other spinal levels, and also influenced by the myelotoxicity of the chemotherapy regimen. Given the treatment variations, it can be challenging to be confident of the specific effect of the dose-volume response effect of either radiotherapy or a particular chemotherapy agent in this setting, as well as their interaction. Our work shows that in the face of a standardised intervention, there is approximately a 25% relative increase in MRI FF over six months of ADT, but with a wide range, and there are some patients demonstrating much larger changes of 50% or more. As this appears to have some correlation with later DEXA BMD changes, such patients may represent a subgroup where more aggressive early intervention with antiresorptive agents such as denosumab or bisphosphonates might be investigated.

There is now a growing body of work showing a correlation between MRI FF measured using either MRS or In-Out Phase, as well as ADC with DEXA BMD in various populations.[10] This is not a relationship which we were able to confirm in our cohort. Possible reasons for this include that some of the positive series were relatively small and included both men and women with a wide age range. An early report examined 16 volunteers with an age range of 8-57, of whom only 2 males and 4 females had both a DEXA and MRI performed.[21] This series showed an inverse correlation between the two scans with a p-value of 0.076, but considered each vertebral body as a separate entity despite evidence of a strong within patient relationship across adjacent vertebrae. Despite this, these initial findings have been largely confirmed in several subsequent series, including a 560 subject cohort spanning a wide age range and including both males and females.[22] As outlined in the Introduction, here is also a biological rationale for this.[11] We hypothesize that our inability to detect such a relationship in our cohort was a function of the relatively low number of patients, who were all male with a limited range of largely normal baseline BMDs.

Several small studies have reported on the use of serial multiparametric MRI and changes in various imaging parameters as correlating with treatment response in several malignancies including rectal, prostate and head and neck cancers.[23-25] Many studies are ongoing exploring multiparametric MRI and other functional imaging such as PET as an early biomarker of later tumour response.[26] Such concepts have the appeal of potentially allowing treatment adaptation, either intensification or de-escalation, while it is still being delivered. Care is needed in the interpretation of such studies however, given the often large number of parameters explored in multiple physical locations over serial scans increasing the chance of a type I statistical error ie finding a statistical relationship when no real relationship exists.

To our knowledge, the use of MRI as an early biomarker of treatment toxicity is a much less investigated entity. Given that ADT can cause mild anaemia and fatigue, both of which could plausibly be mediated via effects at the level of bone marrow, there is potential for imaging to predict a suite of ADT related toxicity. The PROCITT

study prospectively collected serial data on fatigue and blood counts, and once these data collection is complete we intend to explore whether such relationships exist.

Our series has the advantages of being prospective and hence relatively standardised in patient population, treatment delivered and imaging protocols. There are clear limitations. It is a relatively small series, although larger than others looking at serial imaging of patients undergoing cancer treatment. Although a comparison between Dixon and DEXA changes were the main aim of the study, the ADC and MRS sequences were not uniformly applied for all patients, reducing our power to detect a meaningful impact from these sequences. Given the highly targeted patient cohort, further work will be required to assess whether similar observations occur in the broader population. Although gross lesions seen on MRI were excluded from ROIs, degenerative changes may have affected the DEXA outputs; the lack of any major disc loss on plain films and MRI make this less likely to be a major confounder. The use of manual techniques to define ROIs is common, but does introduce additional variability into assessments. We also used several DEXA platforms in the community, although our concentration on relative changes over time would be considered to be platform independent.

There are several other potential future directions. The utilisation rates of DEXA imaging for men on ADT is suboptimal, and a multifaceted Implementation science approach is being explored to try to use patients and their local medical officer to try to correct this. It is possible that the inclusion of additional clinical factors such as serum and urine markers of bone turnover, and anthropomorphic parameters such as height, weight and BMI may lead to greater predictive power regarding men at risk of more rapid loss in BMD. Some very preliminary work in this area is promising.[16] We have collected such data, and hope to analyse this as our BMD data matures with further serial DEXA imaging.

Acknowledgements

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Chapter 6 – Circulating Tumour Cell Detection in Non-Metastatic Prostate Cancer

Over the last decade there has been rapid development of various platforms to assay for biomarkers of tumour activity in the blood. These have evolved from proteins derived from cancer cells such as PSA through to the cancer cells themselves, so called Circulating Tumour Cells (CTCs). Given the higher tumour burden, much of this work has occurred in the metastatic setting. Since a moderate proportion of men with high risk prostate cancer are destined for metastatic failure, we were interested in the incidence of CTC positivity in such men. This manuscript details our approach to this question, showing a CTC positivity rate of only 14%, which is not greatly different to what has previously been reported in a healthy control population. Although only published in late 2014, by mid-2016 seventeen citations had already been made referencing this work, demonstrating the ongoing interest in this field. With more mature follow-up in upcoming years, we also hope to correlate tumour control outcomes with CTC positivity.

Loh J, Jovanovic L, Lehman M, et al. Circulating tumor cell detection in high-risk non-metastatic prostate cancer. Journal of cancer research and clinical oncology 2014;140:2157-62.

Abstract

Aim

The detection of Circulating Tumor Cells (CTCs) provides important prognostic information in men with metastatic prostate cancer. We aim to determine the rate of detection of CTCs in patients with high-risk non-metastatic prostate cancer using the CellSearch® method.

Method

Samples of peripheral blood (7.5mL) were drawn from 36 men with newly diagnosed high-risk non-metastatic prostate cancer, prior to any initiation of therapy and analyzed for CTCs using the CellSearch® method.

Results

The median age was 70 years, median PSA was 14.1, and the median Gleason score was 9. The median 5-year risk of progression of disease using a nomogram was 39%. Five out of 36 patients (14%; 95% CI 5%-30%) had CTCs detected in their circulation. Four patients had only 1 CTC per 7.5mL of blood detected. One patient had 3 CTCs per 7.5mL of blood detected, which included a CTC cluster, also termed circulating tumor microemboli. Both on univariate and multivariate analysis, there were no correlations found between CTC positivity and the classic prognostic factors including PSA, Gleason score, T-stage and age.

Conclusion

This study demonstrates a low CTC detection rate using the CellSearch® method in high-risk non-metastatic prostate cancer, which is consistent with the limited literature available for non-metastatic prostate cancer. Further follow-up will explore disease related outcomes for our patient population, with CTCs potentially helping to identify a very-high risk non-metastatic population based on early tumor behavior rather than solely on classic clinicopathological predictors.

Introduction

Since the isolation of PSA from prostate tissue in 1979(Wang et al. 1979), there has been no other molecular marker widely accepted for clinical use in the diagnosis, staging, and monitoring of disease response for prostate cancer. Despite baseline PSA at diagnosis having demonstrated prognostic significance, its usefulness is limited by its imperfect prognostic accuracy and poor correlation to pathological stage. Risk categorization tools and multivariate nomograms that combine pre-treatment clinical and pathologic factors are improvements over PSA alone but none are able to accurately predict which individual patients may already harbor micrometastatic disease.

A key event in haematogenous dissemination of carcinoma is intravasation of cancer cells into vasculature. Cancer cells in blood are called circulating tumor cells (CTCs) and are important markers of distant metastases. In metastatic breast, prostate and colorectal carcinomas, CTC numbers as detected using the CellSearch® CTC test, are associated with overall survival (OS) and progression-free survival (PFS)(Cohen et al. 2008; Cristofanilli et al. 2004; Danila et al. 2007). CTC counts were also found to be superior to PSA in predicting OS in metastatic castrate resistant prostate cancer (de Bono et al. 2008). Based on clinical validation, the CellSearch® CTC testing has been approved by the Food and Drug Administration (FDA) for monitoring disease progression and treatment response in patients with metastatic breast, prostate and colorectal carcinoma.

The clinical utility of CTCs in patients with high-risk prostate cancer which appears to be non-metastatic by conventional imaging remains uncertain, with limited research evidence published in this area. The potential applications for CTCs in this setting include their use to differentiate true localized disease from those with occult metastatic disease, for prognostication, and to identify patients that may benefit from more aggressive treatment strategies.

Methods and materials

Patients with newly diagnosed high-risk, non-metastatic prostate cancer were eligible for this prospective study. Inclusion criteria include histological diagnosis of prostate cancer, and high risk disease defined by any one of: baseline PSA≥20; Gleason grade 8-10 disease; Clinical stage T3-T4 or N1. All patients had staging investigations negative for disease outside of the prostate with the exception of peri-prostatic pelvic lymph nodes including a ^{99m} Technetium (^{99m} Tc) whole body bone scan and computed tomography (CT) of the abdomen and pelvis. Patients were excluded if they had previous pelvic radiotherapy. All patients were planned for definitive treatment with radical radiotherapy to the prostate and eighteen months duration of androgen deprivation therapy.

This study received approval from a human research ethics committee (NSW HREC Reference no: HREC/12/HNE/268). The trial recruited patients from three Australian hospitals, with all CTC assays performed at the same centre. The clinical trial is registered on clinicaltrials.gov [ClinicalTrials.gov Identifier: NCT01418040]
The objective of this study was to examine the CTC detection rate in high-risk non-metastatic prostate cancer patients using the CellSearch® method.

CTC isolation and detection

Blood was collected in CellSave® tubes and processed within 96h of collection. CTC capture and assessment was performed using the CellSearch® platform, in accordance with manufacturer's protocols and as previously described in detail elsewhere(de Bono et al. 2008) . In brief, immunomagnetically captured EpCAM (epithelial cell adhesion molecule)-positive cells were immunofluorescently stained for cytokeratins 8, 18 and 19 and nuclear stain DAPI (4'-6-diamidino-2-phenylindole). Contaminating leukocytes were identified as allophycocyanin (APC)-labelled, CD45-positive cells. CTCs were identified as ≥4μm in size, cytokeratin-positive, DAPI-positive and CD45-negative events.

Data Collection

Baseline patient and tumor related factors were recorded including patient age, initial PSA, tumor stage and core biopsy Gleason score based on the 2005 ISUP

consensus. Estimates of the 5-year risk of biochemical or metastatic failure for each individual patient were generated using the Memorial Sloan Kettering Cancer Centre (MSKCC) nomogram.

Analysis

A 95% exact (Clopper-Pearson) binominal confidence interval was constructed around the point estimate of CTC positivity. The distribution of categorical variables based on CTC positivity was compared using Fishers exact test, and continuous variables using the Mann-Whitney U-test. Logistic regression was used to estimate the joint effects of all predictors and linear discriminant analysis was used to explore clusters of clinico-pathological factors which may predict for CTC positivity.

Results

Thirty-six patients had their peripheral blood collected for CTC testing. Six patients had multiple samples tested for CTCs; five had consecutive samples tested, and one had three samples examined over a period of 10 months. Patient characteristics are summarized in Table 6.1. The median age was 70 years, median PSA was 14.1, and the median Gleason score was 9. Median 5-year risk of progression of disease was 39%.

Variable	Categories	Total (N=36)	CTC negative (N=31)	CTC positive (N=5)	P
Clinical Stage (T)	T1	9 (25%)	9	0	0.19
	T2	14 (39%)	10	4	
	T3	13 (36%)	12	1	
N stage	N0	34 (94%)	30	4	0.26
	N1	2 (5.6%)	1	1	
PSA	median (min, max)	14.1 (5.9, 52.7)	14.1 (6.2, 52.7)	10.0 (5.9, 33.3)	0.59
Gleason score	7	7 (19.4%)	5	2	
	8	7 (19.4%)	7	0	
	9	19 (52.8%)	16	3	
	10	3 (8.3%)	3	0	
	median (min, max)	9 (7, 10)	9 (7, 10)	9 (7, 9)	0.55
Age	median (min, max)	70 (54, 78)	70 (54, 78)	74 (69, 78)	0.12
5 year risk of progression (%)	median (min, max)	39 (19, 73)	40 (19, 73)	37 (21, 47)	0.67

Table 6.1: Patient characteristics and summary of results.

Five of the 36 patients (14%, 95% CI 5%-30%) presented with CTCs. No CTCs were detected in any of the patients where multiple samples were tested. Four patients had 1 CTC per 7.5mL of blood detected. A single patient presented with 3 CTC events, which included a CTC cluster (Figure 6.1a), or circulating tumor microemboli (CTM). Table 6.2 shows the characteristics of the five patients with CTCs detected. Unexpectedly patients with very high risk disease had no CTCs, including all three patients with Gleason 10 tumors, and the patient with a positive lymph node.

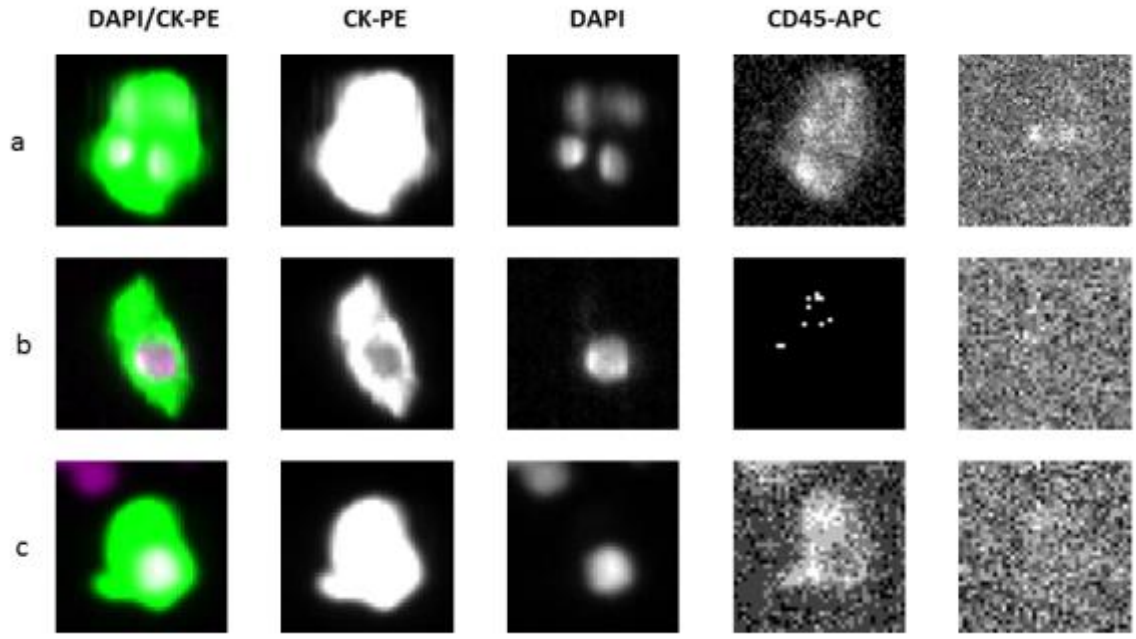

Figure 6.1. Three CTC events detected in Patient 9. Circulating tumor cluster (6.1a). Note four individual nuclei in the DAPI channel and overlapping intensive cytokeratin staining in the CK-PE channel. Individual CTCs presented with a single nucleus per cell (6.1b and 6.1c).

No. of CTCs	No. of patients	Age	PSA	Gleason score	Stage
1 CTC detected	4	78	18.5	7	T2
		76	33.3	7	T2
		74	5.9	9	T3
		69	9.7	9	T2N1
3 CTCs detected	1	70	10	9	T2

Table 6.2: Characteristics of the CTC positive patients

Analysis results

Univariate analysis showed that there were no clinico-pathological factors strongly predicting for CTC positivity (Table 6.1). The 5-year risk of progression score based on a validated multivariate (MSKCC) nomogram also showed no statistically significant association. Multivariate methods failed to identify an apparent pattern in the data points. There was no difference between CTC-positive and CTC-negative patient groups when compared for PSA, Gleason score, T-stage and age.

Discussion

Our results are consistent with, and add to the current literature that demonstrate low levels of CTCs detected using the CellSearch® assay in non-metastatic prostate cancer. Detection rate of 14% is within the range reported by three other studies investigating CTCs in non-metastatic prostate cancer (5 to 27%)(Davis et al. 2008; Resel Folkersma et al. 2012; Thalgott et al. 2013). The patient cohort examined in the MD Anderson study (Davis et al. 2008) consisted of mainly low to intermediate risk prostate cancers. Their slightly higher rate of CTC detection at 21% could be due to false positives, which is supported by their observed CTC positivity rate of 20% in a group of prostate biopsy negative controls. In the study from Spain, again the majority of the patients were mainly intermediate risk prostate cancers. They had a CTC detection rate of 27% (7 of 26 men with the localised prostate cancer). Three of ten healthy volunteers (10%) were found to have CTC in their circulation. In comparison, the 20 patients included in the German series (Thalgott et al. 2013) represented a higher risk group, with a median PSA of 21 and Gleason score of 7.5. The majority also had clinical T3 disease. Only one patient (5%) had 1 CTC per 7.5mL of blood detected. The healthy controls in that series had no CTCs detected, suggesting improved specificity of the CellSearch® assay since the earlier study was conducted.

Our series is the first to our knowledge to include only patients with high risk disease who could potentially already have micrometastases not detectable by conventional imaging. Patients in our series had higher risk disease than in either of the previously published reports (median Gleason score 9, 39% 5-year risk of progression according to the MSKCC nomogram) which potentially explains the higher proportion of CTC positive patients (14% *vs.* 5%) when compared with the German cohort (median 5-year risk of progression of 10%).However the confidence interval in our series is 5 to 30%, and thus the results from the three series may well represent the true population estimate. Multivariate analysis did not identify a discernible pattern in the CTC positive patients according to PSA, Gleason score, T-stage or age. This could reflect the small sample size and small number of events in this study, but does agree with the MD Anderson series findings. However, some patients with very high risk disease were CTC negative, including all three patients with Gleason 10

tumors, and one patient with a positive lymph node. This is in keeping with the literature showing that some patients with such high risk disease are successfully cured with local therapies. This raises the possibility that CTC positivity may help discriminate disease with a tendency towards early metastasis, although further follow-up of clinical outcomes will be essential to explore this hypothesis more fully.

None of the CTC positive patients in our study had > 3 CTCs detected. This is in keeping with the findings of the Spanish study which did not detect >3 CTCs in their cohort of localised prostate patients. In that study, they identified a cutoff point of ≥3 CTCs/7.5mL that best distinguished between localised and metastatic prostate cancer. None of the CTC positive localised prostate patients in that Spanish study developed biochemical progression following radical prostatectomy (RP), although the length of follow-up at a median of 42 months may be insufficient (Resel Folkersma et al. 2012). Assessment of the long-term disease control outcomes of the five CTC-positive patients in our study would inform if a worse prognosis is associated with CTC detection pre-treatment. It remains unclear at this stage what is the true biological potential of CTCs detected in peripheral blood of patients with localised prostate cancer. If prognostic significance emerges, more aggressive treatment strategies may potentially improve outcomes in patients positive for CTCs. Possible strategies include radiotherapy dose escalation and incorporation of systemic therapies including adjuvant chemotherapy, biologic agents and novel agents targeting the hormonal axis. CTCs may therefore offer the potential to biologically adapt initial treatment options, although further investigation of this issue is essential.

Most guidelines still recommend the use of ^{99m}Tc bone scans and CT as staging investigations to detect metastatic disease in newly diagnosed high risk prostate cancers. These techniques have limitations with low specificity and sensitivity. Newer imaging techniques such as whole body Magnetic Resonance Imaging(Lecouvet et al. 2012), and [18F]-Fluoride positron emission tomography-CT (Even-Sapir et al. 2006) have been demonstrated to outperform conventional imaging in the detection of bone metastases in high-risk prostate cancer patients, but still suffer from needing a threshold of millions of cancer cells in one area to be

positive. The incorporation of biologic data such as from CTCs remains a promising area of interest in optimising baseline prognostic accuracy.

In one patient we detected 3 CTCs, including a CTM (Figure 1a). CTMs have been reported in peripheral blood of patients with a number of malignancies (Hou et al. 2012; Kats-Ugurlu et al. 2009; Molnar et al. 2001). Animal model studies observed that intravenously injected CTM are more likely to form metastases than single tumor cells(Liotta et al. 1976). One study demonstrated an association of CTM with worse survival in small cell lung cancer patients (Hou et al. 2012). An absence of proliferating cells within a CTM has been observed, making them potentially resistant to chemotherapy(Frisch and Francis 1994). The exact significance of CTM is currently not known.

The low levels of detectable CTCs using the CellSearch® system in high-risk non-metastatic prostate cancer may not be an accurate reflection of the true CTC frequency in this patient population. The CellSearch® platform is the only platform with the FDA approval for detection of EpCAM- and cytokeratin-positive CTCs in metastatic malignancies; however, CTCs from tumors with down regulated or absent EpCAM and/or cytokeratins go undetected. Although the majority of prostate cancers show overexpression of EpCAM, about 11% of prostate adenocarcinomas show no or weak expression of EpCAM(Spizzo et al. 2011). Furthermore, CTCs that have undergone epithelial-mesenchymal transition (EMT) escape detection due to down-regulation of epithelial markers EpCAM and cytokeratins.

Other techniques for detection and isolation of CTC have been used to capture EpCAM-negative cells, however none has been approved for clinical use so far. The CTC-Chip is a microfluidic device that requires a much lower volume of blood (1-2 mL), is very sensitive and maintains viability of the captured cells. In one study, CTCs were detected in 8 of 19 (42%) patients with localized prostate cancer (median 95 CTCs per mL; range 38 to 222) using this method (Stott et al. 2010). Nucleic acid-based methods indirectly detects the presence of CTCs by identifying tumor-specific DNA or mRNA in peripheral blood. It is a highly sensitive method, but false positive results can occur due to detection of non-malignant cells that carry the same gene expression. Each of these techniques has its advantages and limitations, and

comprehensive validations across the different techniques have yet to be performed in clinical settings. As such, the CellSearch® platform remains the gold standard for CTC detection.

Moving beyond the enumeration of CTCs, the molecular characterization of these cells could serve as a real-time “liquid biopsy” to guide therapeutic decisions informed by the biology of a patient’s cancer. One study demonstrated a significant association between expression of the TMPRSS2-ERG gene fusion in CTCs and PSA response to Abiraterone(Attard et al. 2009). Another study suggests that the monitoring of androgen receptor nuclear localization in CTCs might predict clinical responses to taxane chemotherapy (Darshan et al. 2011). However contamination with other normal blood cells remains a significant challenge for the molecular analyses of CTCs, and further technological improvements and optimization of isolation techniques are required.

The pre-treatment presence of disseminated tumor cells (DTC) in bone marrow of non-metastatic prostate cancer patients have been shown to predict clinical outcomes (Lilleby et al. 2013). In a systematic review, there was a suggestion that CTCs may be better at predicting prognosis than DTCs (Ma et al. 2014). The invasive nature of this method renders it less user-friendly compared to a peripheral blood draw for CTC detection. Of the different forms of circulating nucleic acids, cell free circulating DNAs (cfDNA) and microRNAs (miRNAs) show promise as potential biomarkers for prostate cancer. Higher levels of serum cfDNA concentration were found to be associated with increased risk of PSA recurrence within 2 years of RP (Bastian et al. 2007) . Significant associations were also observed for a range of histopathological prognostic factors in relation to total serum cfDNA concentration(Bastian et al. 2007). A relationship between the occurrence of CTCs and circulating tumor-associated DNA in blood has also been reported, which may provide a new tool for the monitoring of disease progression(Schwarzenbach et al. 2009). One study suggests that circulating miRNAs measured at the time of RP could predict for future disease progression in men with intermediate risk prostate cancers(Selth et al. 2013).

Conclusion

This study demonstrates that patients with high-risk, non-metastatic prostate cancer present with small number of CTCs in peripheral blood. This finding is consistent with the limited literature available in this setting. Other CTC isolation and detection technologies with improved sensitivity and specificity may enable detection of CTCs with mesenchymal phenotypes, although none as yet have been validated for clinical use. Newer assays are emerging for detection of new putative biomarkers for prostate cancer. Correlation of disease control outcomes with CTC detection will be important.

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Section 4 – Future Directions

Chapter 7 – Where to from here?

Introduction

This thesis has addressed several aspects of the management of men with high risk prostate cancer. The main thrust has being the use of imaging[1] and serum biomarkers[2] as predictors of treatment toxicity and efficacy respectively. In both cases, a promising approach has been explored, with further follow-up necessary to ascertain any longer term potential of the respective biomarkers. Regarding the delivery of EBRT, a novel approach integrating nomograms for risk prediction and a hypofractionated regimen has been shown to be both feasible and tolerable.[3] Furthermore, a key element of an EBRT quality assurance program has been assessed, with a suggestion that the extra effort and cost is unlikely to always be justified.[4] In the five years since this project was initiated, the field has continued to evolve. This chapter attempts to add some context as to where the findings of this thesis sit in the wider and continually evolving prostate cancer management milieu, acknowledging that these areas still only represent a fraction of the wider field.

Radiotherapy

In the subsequent biomarkers section we will touch on some of the potential expanded roles of EBRT for men with oligometastatic disease, nodal metastases at diagnosis, and irradiation of the primary tumour in the presence of more widespread disease. Relevant to this thesis are also are the continuing evolution in the areas of hypofractionation, quality assurance and integration of systemic therapies.

Hypofractionation

This trial concentrated on moderate hypofractionation of prostate EBRT, where the fraction size varies between 2-3 Gy per day. Phase 3 data are beginning to emerge from several large RCTs that this approach is likely to result in approximately equivalent disease controlled compared to a more conventionally fractionated cohort. After presenting the phase two data of the experimental arm, the author lead the Australian arm of a Canadian RCT in this field where a four week regimen of 60 Gy in 20 fractions was compared with a standard arm of 78 Gy in 39 fractions using modern IMRT and IGRT.[5,6] Over 200 Australian men participated in this 1200 patient study, with the final analysis due in 2016.

Beyond this, hypofractionation has embraced the principles of SABR to give very high doses of 7-8 Gy per fraction in as few as five fractions, so called stereotactic monotherapy. Promising single institution and multi-institution data has been presented of large cohorts treated in this manner, although generally with relatively short follow-up.[7-9] In Australia, the author is the clinical principal investigator on a multicentre collaborative phase 2 study via the Trans-Tasman Radiation Oncology group (TROG) using advanced technology to track prostate motion while treatment is being delivered in a five fraction stereotactic monotherapy regimen – the SPARK trial.[10] The first patient on this study was recruited by the author in February 2016, with technical data likely to be presented from 2017 onwards, and efficacy as well as toxicity data due in 2020. Quality assurance is clearly becoming more adaptive on trials such as SPARK, based at least partially on the evidence presented in this thesis.

Integration of systemic therapy

This study used an 18 month course of ADT, largely on the basis of modelling suggesting this gave the best compromise between maximising efficacy and reducing toxicity.[11] Since then, two key studies have helped consolidate the evidence base in this regard. TROG 03-04 was a RCT which compared a 6 and 18 month course of ADT for men with high risk prostate cancer, and found a small survival advantage for the longer treatment course.[12] Conversely, a RCT from Quebec has

compared 18 months with 36 months, suggesting modest if any additional efficacy from the 3 year duration of ADT. This later study has generated some controversy, because it was powered for superiority of the longer treatment course rather than having the largely number of patients needed to be convinced of the non-inferiority of the 18 month regimen. Overall, the intermediate duration of ADT appears to be gaining favour, with the main current international collaborative RCT for men with high risk disease using a 24 month treatment course in both the standard and experimental arms.[13]

The integration of agents other than ADT into the management of men with high risk prostate cancer treated with EBRT will continue to be an area of energetic research. This has not historically proven to be a fruitful area of inquiry, with one notable trial of chemotherapy using Paclitaxel, Estramustine and Etoposide adjuvantly following EBRT showing excessive thromboembolic events requiring it to be abandoned prior to reaching target accrual.[14] However, there is renewed enthusiasm since the presentation of data showing the early use of Docetaxel in men with newly diagnosed metastatic disease confers a large survival advantage (see Figure 7.1).[15,16] A single conflicting French RCT has been reinterpreted as being consistent with these positive trials based on differing patient populations and excessive treatment toxicity.[17] This approach has quickly become a standard treatment approach in Australia, and has renewed interest in bringing Docetaxel into the initial treatment setting. One RTOG study has recently been presented in abstract form suggesting an overall survival advantage for adjuvant Docetaxel following EBRT.[18] The esteemed discussant at this meeting who pioneered this use of Docetaxel in prostate cancer criticised these findings on the grounds of the toxicity and one sided p-value testing deployed, meaning that any uptake will need to await full peer review.[19] Newer agents such as Enzalutamide are also being integrated with standard hormo-radiotherapy in currently accruing RCTs.[13] Informed by the results of such studies, there will be the potential for the standard management of such men to continue to evolve in the near future.

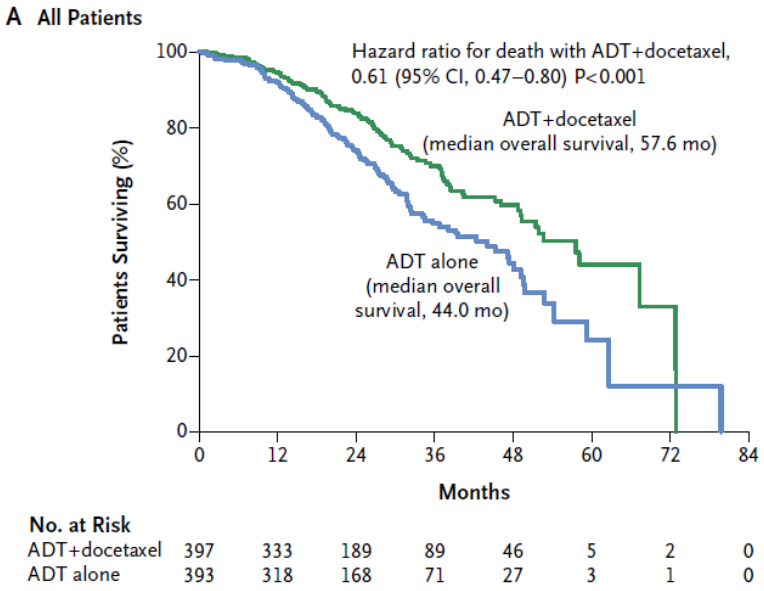

Figure 7.1: Overall Survival curves from the CHAARTED study showing 13.6 month improvement in median survival for patients with castrate naïve metastatic disease treated with early docetaxel at the time of initiation of ADT. (Sweeney et al. N Engl J Med 2015;373:737-46.)

Biomarkers

Bone Health

Given the widespread availability, population data and reproducibility of DEXA imaging, it is unlikely that this will be usurped as the gold standard in BMD assessment in the near future. The failure of either ultrasound or qualitative CT in this regard suggest that although MRI provides useful information, DEXA will continue to reign supreme. One aspect being currently explored in collaboration with the CSIRO in Brisbane is the potential for automatic segmentation of spinal MRI data rather than manually defining regions of interest.[20,21] This offers the likelihood of much faster and more reproducible data extraction from the myriad sequences and regions examined. Although further work will incorporate serum and urine markers of bone turnover with anthropomorphic data to try to improve the predictive accuracy of MRI, even if such an approach provides useful information, it is unlikely to be simple enough to lead to widespread adoption. In light of this, rather than exerting significant additional energy into finding a better test, the main effort in this area should be how best to increase the awareness of bone health amongst clinicians caring for men with prostate cancer on ADT. There are several avenues which can be explored in this regard.

A bone health initiative was developed in conjunction with a pharmaceutical company which has Australia's largest share of the ADT market. Astra Zeneca convened an Advisory Board where I presented the data relating to the effects of ADT on bone health. From this sprung a patient support program to allowed expanded access to bisphosphonate therapy for patients who fell between eligibility for these medications on the pharmaceutical benefits scheme and national consensus guidelines (see figure 7.2). Although some degree of promotion accompanied this initiative, uptake has been low. This would suggest interaction with clinicians caring for prostate cancer patients needs to occur at a more fundamental level.

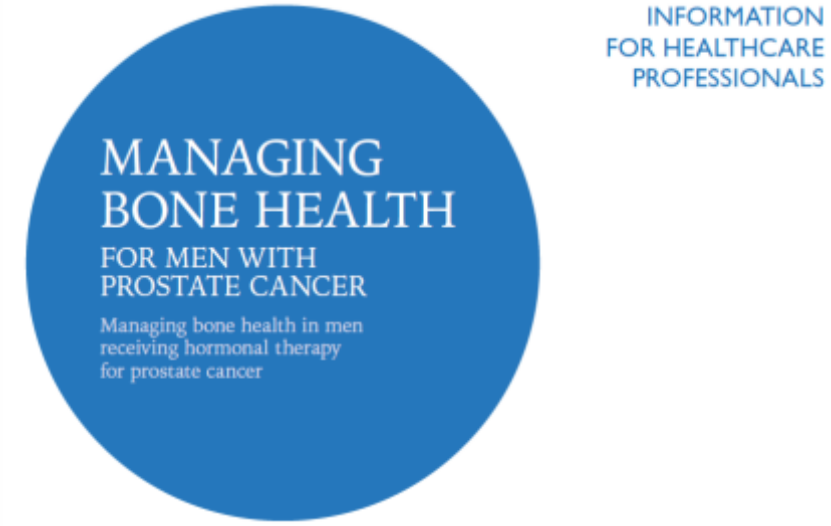

Figure 7.2: Front page of Astra Zeneca booklet on the management of bone health for men on ADT.

A South Australian medical oncologist embarked on a TRIPP fellowship in early 2014 focussing on bone health of women with breast cancer managed with endocrine manipulation. I joined her management committee, and introduced men with prostate cancer treated with ADT into the conversation. Rather than targeting radiation oncologists and urologists, this intervention targets the patients, asking them to engage with their general practitioners with a specific suite of bone health initiatives. This work is ongoing, and there is optimism that mobilising the patients and their primary health carers will prove a more fruitful avenue in improving bone health.[22]

Efficacy

Much of the effort with efficacy biomarkers falls into two distinct areas. These are the identification of subgroups with different prognoses, as well as the attempting to

predict subpopulations likely to respond to different treatments. Most of this work is occurring in patients with more advanced, metastatic disease, but any positive findings will be worth exploring in the high risk non-metastatic population.

Regarding circulating biomarkers, a wide plethora of options have emerged. Trying to adapt systemic therapy according to CTC response has been trialled without success in breast cancer patients, but has yet to be reported on in a prostate cancer population.[23] In addition to CTCs, cell free circulating DNA (cfDNA) and micro-RNA (miRNA) have also gained favour, largely due to their greater sensitivity and quantification abilities.[24,25] Perhaps more exciting, is rather than pure enumeration of circulating tumour markers, it is now also possible to derive their function. This work is most advanced in the case of the Androgen Receptor splice variants, in particular ARv7.[26] This entity has the binding site for ligand excised yet retains constitutional activity (see figure 7.3). As such, agents such as Abiraterone and Enzalutamide appear to have minimal efficacy in such patients, perhaps suggesting a greater role of cytotoxics like Cabazitaxel or Docetaxel.[27] This is being prospectively explored,[28] although the large number of splice variants, their low individual frequency and the dynamic nature of phenotype selection through the use of therapeutics make this an enormously challenging area.

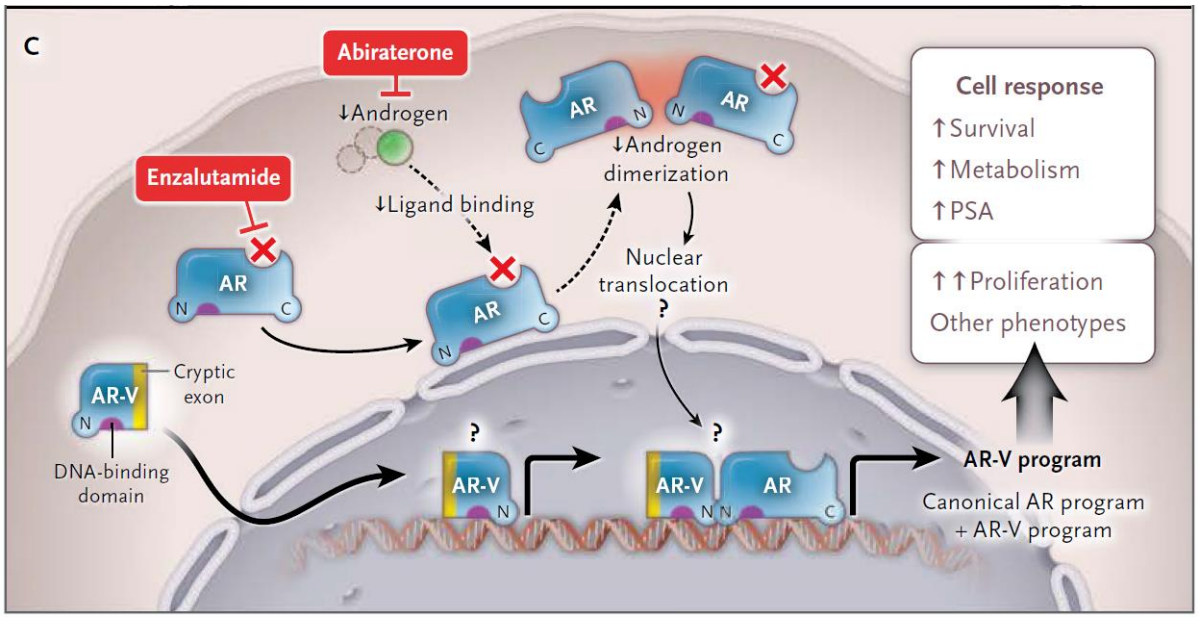

Figure 7.3: Diagram showing the lack of a ligand binding region on the androgen receptor slice variants (AR-V), and how this constitutionally active pathway comes to dominant the cell response independent of the presence of ligands such as testosterone or enzalutamide. (From Nelson N Eng J Med 2014; 371: 1067-69.)

The use of more advanced imaging at the time of diagnosis for men with apparently localized disease is also a rapid emerging area. Pelvic MRI including diffusion weighted imaging (DWI) has become relatively routine at the time of initial diagnosis both to assist in targeting of biopsies, definition of local extent of disease and hence operability, as well as affording more accurate staging of pelvic lymph nodes than achieved through the use of CT imaging. [29-31] Simultaneously, whole body MRI including DWI has gained some enthusiasm for better defining the presence of low volume metastatic disease, particularly in the axial skeleton.[32,33] Although this technology has being available for a decade, it has failed to enter widespread use, partly due to the specialised coils need for whole body imaging, the time required for such studies, and the difficulty in interpreting them given the huge volume of data to be analysed. There has been some work on using serial mpmMRI of the prostate as a patient progresses through a course of ADT to gauge early indications of later disease control.[34] One of my PhD students is currently exploring the use of serial mpmMRI for patients with anal canal squamous cell carcinoma with the aim of defining subgroups for whom either treatment intensification or de-escalation may be appropriate, with similar work occurring in a wide range of mucosal tumours.[35,36]

PSMA PET imaging has recently become available, with early data suggesting superiority to other imaging modalities in the sensitivity and specificity of detecting low volume nodal or metastatic prostate cancer (see figure 7.4).[37,38] The relative ease of image interpretation and widespread access have led to the rapid uptake of this modality in Australia ahead of a firm evidence base emerging on the longer term impacts on disease control outcomes.[39,40] The recent availability of combined PET-MRI machines offers the potential to harness the advantages already outlined from both platforms in a single scanning session. In conjunction with the Prostate Cancer Foundation of Australia, grant applications are currently underway both in the initial staging scenario as well as the metachronous recurrence setting (ie following efinitive therapy to the prostate) to try to rectify this.

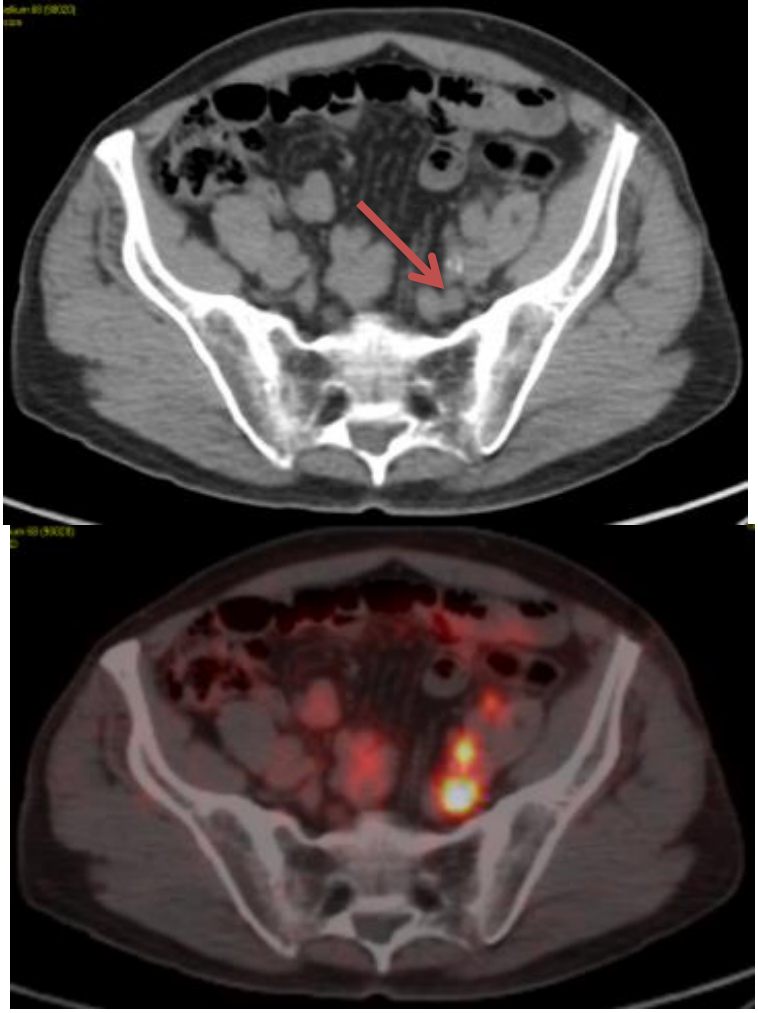

Figure 7.4: CT and PSMA PET imaging of a man with high risk prostate cancer. CT imaging showed a lymph node in the left common iliac chain which was borderline by size criteria. PSMA shows obvious avidity in this lesion, as well as two much smaller lymph nodes in close proximity. PSMA PET also showed much more widespread nodal disease throughout the pelvis, abdomen and chest, all of which appeared normal on CT imaging.

Oligometastatic State

In conjunction with the above imaging findings, a greater awareness has emerged of the continuum between organ confined disease and widespread metastatic disease. The entity of oligometastatic disease where a relatively small number of established metastatic lesions are present has relatively recently emerged in the oncology literature.[41-45] In particular, the threshold between high risk apparently non-metastatic prostate cancer and synchronous oligometastatic disease (ie low volume metastases detected at the time of initial diagnosis) has been recognized as a function not only of the disease, but also the increasing sensitivity of the imaging modalities utilised. Evidence is beginning to emerge regarding the disease control

benefit of EBRT for men with nodal metastases at diagnosis, a group previously through to be beyond the benefit of local treatment.[46] Irradiation of the primary tumour in the presence of widespread metastases is also the subject of an ongoing RCT,[47] largely based on evidence from the renal cell carcinoma of the survival benefit of a cytoreductive nephrectomy for selected patients with established metastatic disease.[48]

Simultaneously, therapeutics for oligometastatic prostate cancer have evolved both in the form of surgical intervention for pelvic nodal deposits as well as stereotactic ablative radiotherapy (SABR – see figure 7.5). It is an open question whether the greater sensitivity of PSMA PET imaging is truly a step forward as the more aggressive therapeutics available raise the potential for greater toxicity without any evidence of long term efficacy.[49] Grant applications and clinical trials are currently underway exploring both the utility of PSMA PET but also whether any meaningful disease free survival advantage is afforded by aggressively managing oligometastases.[50-52] SABR, delivered in 1-5 sessions non-invasively, achieves local tumour control in approximately 90% of patients with <1% risk of medium term significant toxicity (see figure 7.6).[53] There will clearly be a great need for clinical trials to validate this approach and ongoing rapid evolution in this space in the near future.

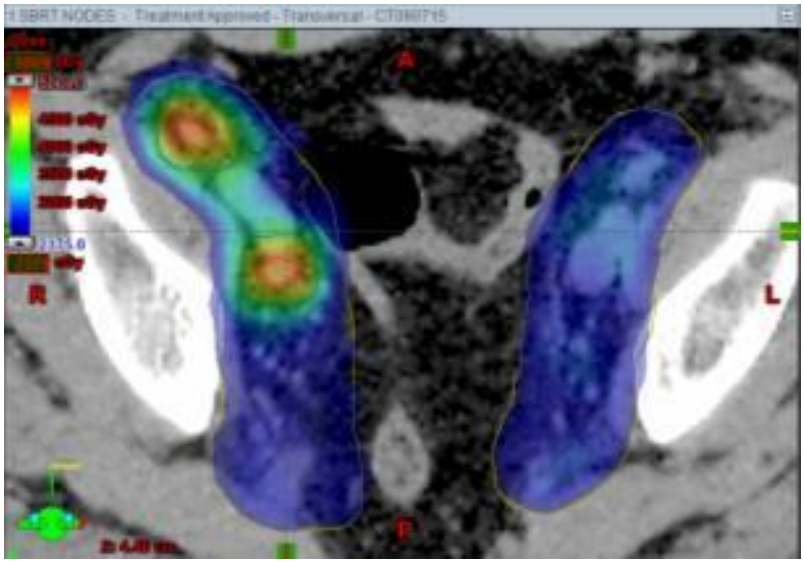

Figure 7.5: Radiotherapy dose distribution from a SABR treatment of oligometastatic lymph nodes in the pelvis. Note the intense dose in Orange to the PSMA PET avid lymph nodes, and much lower dose in blue to the uninvolved nodes.

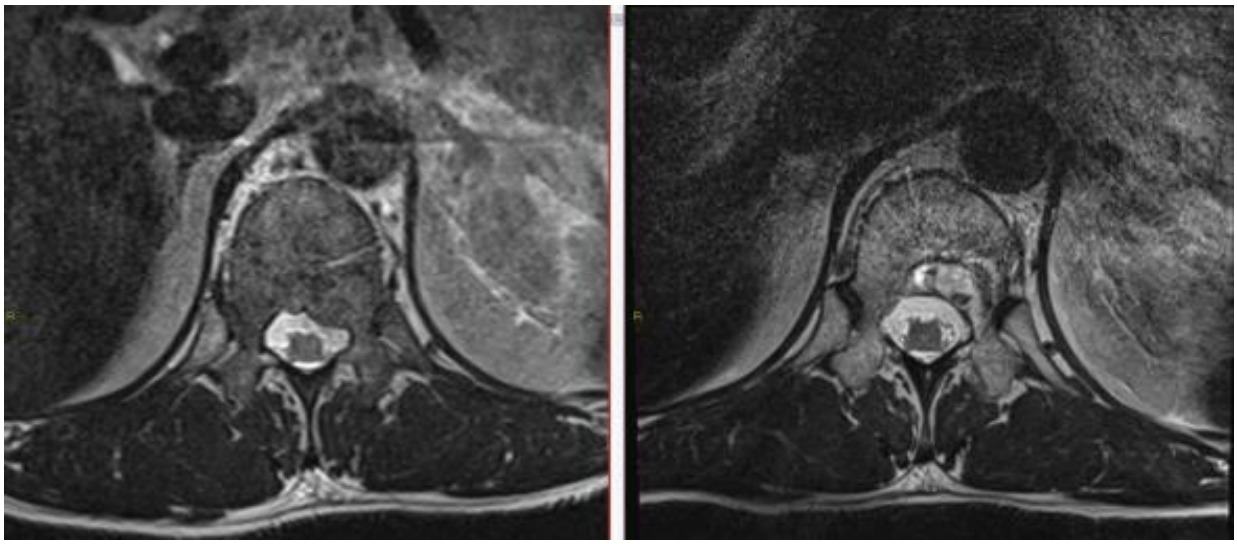

Figure 7.6: Serial T2 axial MRI showing oligometastatic disease to the posterior aspect of the T12 vertebral body pre-SABR (left) and 3 months post SABR (right). Note the normalisation of the spinal canal, and absence of persistent disease in the vertebral body, which correlated with a complete metabolic response on FDG-PET.

Summary

The management of men with high risk prostate cancer will continue to be a challenge for clinicians. Even though this work has touched on several aspects of the treatment of such men, many other areas present additional complexities. Surgery is now often offered as a treatment option for high risk prostate cancer, driven largely by the widespread uptake of robotic radical prostatectomies and increasing aggression of urologists favouring surgical management of locally advanced and even metastatic disease. Hormono-radiotherapy offers a very different toxicity profile and unknown differences in efficacy compared with surgery for such patients, making the initial decision about which treatment course to pursue largely uninformed by high level evidence. Survivorship is another key area, as toxicities as broad as loss of sexual function to cognitive impairment can all lead to impaired quality of life, making the minimisation and management of these an area of need. Numerous more focussed aspects of the technical delivery of EBRT including optimal planning approaches, treatment verification, radiation dose and incorporation of brachytherapy are all active areas of research. At times, it can appear that far more is unknown than known about how best to guide patients through this ever more complex journey.

There are some certainties, generally applicable throughout medicine and science rather than being specific to the management of prostate cancer. The trend towards greater subspecialisation will continue. Despite this, clinicians who build bridges with other clinicians, imaging specialists, technical experts and consumer groups will be better placed to guide patients through their journey than clinicians who function in a more isolated fashion. The volume of evidence and number of options will continue to increase, probably at a faster rate. Skills on how to diffuse innovation appropriately will gain ever more importance. Newer tools such as social media are likely to have an expanded role.[54] In short, a resilience to change informed by a constantly evolving evidence base and in cooperation with professionals both within and outside their field of subspecialisation will be the most valuable tools a clinician can offer their patients.

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Appendix: PROCITT Clinical Trial Protocol

The following is the clinical trial protocol used as a structure for the accrual, investigation and analysis of the men involved in the study which lead to the manuscripts in chapters 3 through 6.

A Phase 2 Clinical Trial Exploring 3-Dimensional Imaging of Androgen Deprivation Induced Osteoporosis, Radiotherapy Hypofractionation and the Prognostic Significance of Micrometastatic disease in men with Prostate Cancer.

Short Title: PROstate Cancer Imaging, Treatment and Toxicity (PROCITT)

Funding Sponsor:	Abbott Australasia Ltd ABN 95 000 180 389 Sir Joseph Banks Corporate Park 32-34 Lord St Botany NSW 2019 (+612) 9384 9800
Protocol Number:	IIS MET-10-0030

Current version: **Version 2:** July 2012 (Newcastle study)

Previous version: **Version 1-3:** June 2011 (Toowoomba pilot study)

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

Trial Management Committee

Principle Investigator:	Dr Jarad Martin (Calvary Mater Hospital, Newcastle [CMN])
Medical Imaging:	Dr Gary Cowin (Centre for Advanced Imaging, University of Queensland [UQ])
	Dr Peter Stanwell (University of Newcastle)
	Mr Jameen Arm (CMN)
Endocrinology:	Prof Geoff Nicholson (School of Medicine, UQ)
Osteoporosis Imaging:	Prof Christian Langton (Queensland University of Technology [QUT])
Urology:	Dr Peter Chong (Hunter New England Health)
Radiation Oncology:	Prof Jim Denham (CMN)
	Dr Colin Tang (CMN)
	Dr Anne Capp (CMN)
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Radiation Therapy:	Ms Leisa Newton (CMN)
Medical Physics:	Dr Trish Ostwald (CMN)
	Dr Peter Greer (CMN)

Molecular Biology:	Prof Colleen Nelson (QUT)
	Dr Lidija Jovanovic (QUT)
Statistics:	Dr Ashley Plank (Toowoomba Cancer Research Centre)
Trial Coordinator:	Ms Sarah Gallagher (CMN)

List of Abbreviations

Abbreviation	Term
AE	Adverse Event
ALP	Alkaline phosphatase
BM	Bone Marrow
BMD	Bone Mineral Density
BMF	Bone Marrow Fat
bNED	Biochemical No Evidence of Disease
bsALP	Bone Specific ALP
CRF	Case Report Form
CT	Computerised Tomogram
CTC	Circulating Tumour Cells
CTV	Clinical Target Volume
CTX	C-telopeptide of type I collagen
DEXA	Dual Energy X-Ray Absorptiometry
Dpd	Deoxypuridinoline
DRE	Digital Rectal Examination
EPI	Electronic Portal Image
FBC	Full Blood Count
FWR	Fat-to-water peak ratio
HAT	Hepatic Adipose Tissue
Hb	Haemoglobin
HREC	Human Research Ethics Committee
HypoRT	Hypofractionated Radiotherapy
IDEAL	T2-weighted iterative decomposition of water and fat with echo asymmetric and least squares estimation.
IGRT	Image Guided Radiotherapy
IMRT	Intensity modulated Radiotherapy
MDCT	Multidetector CT
MRI	Magnetic Resonance Imaging
NHMRC	National Health and Medical Research Council
NHT	Neoadjuvant Hormonal Therapy

NTX	N-telopeptide
P1NP	N-Terminal Pro-peptide of Type 1 Procollagen
PC	Prostate Cancer
PCSS	Prostate Cancer Specific Survival
pHR-QCT	Peripheral High Resolution Quantitative CT
PI	Principal Investigator
PICF	Patient Informed Consent Form
PROCITT	PROstate Cancer Imaging, Treatment and Toxicity
PSA	Prostate Specific Antigen
PTV	Planning Target Volume
QCT	Quantitative CT
RCT	Randomized Controlled Trial
RT	Radiotherapy
SAE	Serious Adverse Event
SAT	Subcutaneous Adipose Tissue
SB PRV	Small Bowel Planning Target at Risk Volume
SHBG	Sex Hormone Binding Globulin
SUSAR	Suspected, unexpected, serious adverse reaction
TRUS	Transrectal Ultrasound
VAT	Visceral Adipose Tissue
WBBS	Whole Body Bone Scan
WPRT	Whole Pelvic Radiotherapy

Study Summary

Title	A Phase 2 Trial Exploring Magnetic Resonance Imaging of Androgen Deprivation Induced Osteopaenia, Radiotherapy Hypofractionation and the Prognostic Significance of Micrometastatic disease.
Short Title	PROstate Cancer Imaging, Treatment and Toxicity (PROCITT)
Protocol Number	IIS MET-10-0030
Phase	Phase 2
Methodology	Prospective observational non-interventional study
Study Duration	5 years (2 years accrual, 3 years minimum follow-up)
Study Center(s)	Single-centre– Calvary Mater Hospital, Newcastle.
Objectives	Primary Objective: That baseline MR imaging of lumbar spine fat fraction combined with clinical factors predicts which men are at greater risk of accelerated Androgen Deprivation Therapy (ADT) induced bone mineral density loss than baseline DEXA scanning alone. Secondary Objectives • Feasibility, toxicity (acute and late) and efficacy (3 year bNED by Phoenix definition) of multimodality therapy with hypofractionated radiotherapy • To correlate marrow changes on MR with changes in blood counts and patient reported fatigue • Prognostic value of circulating tumour cells • Describe changes in marrow fat distribution under the influence of ADT.
Number of Subjects	100

Diagnosis and Main Inclusion Criteria	Men with histological confirmed prostate cancer High risk disease (any one of PSA≥20, Gleason 8-10, Stage T3-4) CT abdomen and pelvis and Whole body bone scan not positive for metastatic disease. Planned for 18 months of Androgen Deprivation Therapy Informed Consent
Statistical Methodology	• Construction of a model predicting annual rate of bone loss based on baseline imaging, clinical and biochemical characteristics. • Correlation of changes in bone marrow with changes in blood counts and patient reported fatigue. • Incidence of Circulating Tumour Cells (CTC) • Final report of efficacy measured by PSA control using Phoenix definition compared with novel parameters including CTC count and haemoglobin drop.

Introduction

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

Investigators and Facilities

1. Study Location

Calvary Mater Hospital
Edith St
Waratah, Newcastle
New South Wales 2298
Australia

2. Study Management

The study will be coordinated by a research team consisting of the Principal Investigator and a study coordinator. Informed consent discussions and clinical assessments will be conducted by the principal investigator. The study coordinator will be delegated responsibility for subject's imaging appointments, follow-up visits, data collection and maintenance of study documentation. Dr Jarad Martin is the Principle Investigator and sponsor for this study. This study is investigator initiated, and funded through a competitive grant from Abbott pharmaceuticals.

Background

1. ADT induced Osteoporosis

Prostate cancer is a common malignancy in Australian men. In men with localized disease at the time of diagnosis, baseline PSA level, tumour stage and Gleason grade can be used to help stratify into risk categories. Men with high risk disease

are defined by an absence of metastatic disease using conventional imaging, and any one of the following: a presenting PSA of >20, Gleason grade 8-10 disease on histology, or stage T3-4 disease.[1] Such men are often treated with a combination of radiotherapy to the prostate and pelvic lymph nodes, in conjunction with a course of adjuvant androgen deprivation therapy (ADT) of between 18-36 months.[2] Recent literature suggests that the greatest benefit from adjuvant ADT comes from the first 4-6 months of treatment, and although there is measurable benefit from prolonging the course of ADT, it follows the law of diminishing returns with progressively smaller benefit per unit of increased treatment time.[3] This is important, in that if cumulative toxicities are being inflicted by prolonging the treatment, there is likely to be a duration where the harm of further treatment will start to outweigh the diminishing disease control benefits.

With greater clinical experience of the use of adjuvant ADT, there has become a better awareness of the toxicities associated with this treatment. Accelerated loss of bone mineral density has long been recognized as a complication of being hypogonadal. There is now good evidence that this leads to an approximately 7% higher risk of fractures for men with prostate cancer managed with ADT.[4] Osteoporotic fractures are associated with increased morbidity and mortality, and a high proportion of patients who suffer them never fully regain their pre-fracture level of functioning.

There are Australian guidelines for the management of osteopaenia / osteoporosis for men managed with ADT.[5, 6] They recommend monitoring of bone mineral density (BMD) using annual DEXA scanning, supplementary Vitamin D with Calcium, and the use of bisphosphanate therapy for men with prevalent minimal trauma fracture or baseline BMD T-Score <-2.0. One point high-lighted is that there is a wide spectrum in the rate of bone mineral loss between patients and techniques of measurement, with figures as high as 8% per year reported. This is far in excess of a normal rate of bone loss amongst males of 0.5% per year.[7]

Although validated nomograms exist for the general population combining DEXA findings with clinical parameters to predict long term fracture risks, no such tool exists for men rendered hypogonadal with the use of ADT.[8] Guidelines for men on ADT are empirical, and largely copy risk factors from the general population.[9]

Beyond baseline BMD, the only clinical factor shown to have any accuracy in predicting bone loss for men on ADT is the change in serum P1NP (N-Terminal Pro-peptide of Type 1 Procollagen, a marker of bone formation).[10] One study showed that men in the highest tertile for P1NP after 6 months of ADT had the greatest loss in BMD at 12 months. This finding has not been verified, and there remains a need to investigate the utility of other clinical parameters either at baseline or early in ADT therapy to find accurate predictors of which patients are at highest risk for accelerated BMD loss.

Osteoporosis Imaging

Currently, the main method to reliably determine which men are more rapid bone losers is to perform serial DEXA imaging. Thus, by the time that rapid bone loss occurs, it is too late to take measures to prevent it by interventions such as curtailing the duration of adjuvant ADT. Furthermore, we have level 2 evidence from a randomized clinical trial, that intervention with a bisphosphonate needs to be instigated at the commencement of ADT and continued throughout the duration of ADT to maximize bone density.[10] This study will aim to define a predictive tool combining baseline imaging and clinical characteristics to help determine which patients are at higher risk of accelerated bone loss prior to the initiation of ADT.

Osteoporosis is a complex condition characterized by loss of both cortical and trabecular bone.[11] The structural basis of bone loss is poorly quantified by DEXA scanning which combines cortical and trabecular bone density in its measurement.[12] However, they can be separately and non-invasively quantified with the use of ultrasound (US), computerized tomography (CT), peripheral high resolution quantitative CT (pHR-QCT) or magnetic resonance imaging (MRI).[13] The last of these methods has the advantages of not being operator dependent, not requiring exposure to ionizing radiation and wide availability. A disadvantage is the relatively poor characterization of cortical and trabecular bone at a field strength of 1.5 T.

There has been some work using CT imaging to separately quantify both cortical and trabecular BMD, as well as other parameters of trabecular bone quality. Much of this work has used pHR-QCT, which has revealed detailed changes in the porosity of

cortical bone for men on ADT which is likely to weaken the bone, and as been termed ‘trabecularization’.[14] Recent studies have compared this technique which has relatively limited accessibility, with more widely available technologies such as Quantitative CT (QCT) and Multidetector CT (MDCT).[15, 16] A very accurate correlation for Trabecular BMD was found between all 3 CT modalities. This raises the possibility that BMD can be estimated from the staging MDCT performed on all prostate cancer patients, without needing to expose them to the extra radiation dose required to perform a QCT.

An advantage of MRI is that it also allows the collection of additional information regarding bone marrow (BM) including fat fraction and perfusion. These measures have previously shown some correlation with BMD measured by DEXA imaging, however the correlation is relatively poor, with a wide degree of unexplained variation.[17-19] BM has intimate proximity with trabecular bone, and paracrine factors such as the RANK-Ligand secreted from the BM plays a key role in recruiting bone resorbing osteoclasts.[20] It might therefore be that some of the variation in BMD measured with DEXA is due to baseline variation in BM composition. There are also possible correlations between BM fat (BMF), and subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and hepatic adipose tissue (HAT), all of which can be separately quantified by MRI.[21] This, is turn, may be linked with the deranged insulin levels and response linked with ADT administration, and may be posited as a cause of increased cardiovascular morbidity.[22]

Other evidence shows that ADT induces a drop of haemoglobin from an average baseline value of 151 g/L down to 135 within 18 months of starting treatment.[23] No haemolytic process is evident, and the circumstantial evidence points to bone marrow suppression as being the mechanism for this. Such mild anaemia may also contribute to the insidious fatigue often seen in men treated with ADT. There is also some evidence from reanalysis of randomized trial data, that men who have the greatest drop in haemoglobin in the 3 months following initiation of ADT have a poorer overall survival in the setting of metastatic disease.[24] As such, measuring BM at baseline may help in predicting which patients are at risk of both losing bone faster, becoming anaemic, and suffering fatigue. It is therefore plausible that measurement of BM will add an important dimension to our knowledge of the bone

as a functional unit as well as better explaining some of the toxicities associated with ADT.

2. Circulating Tumour Cells

For a cancer to metastasize from the primary site of origin to other parts of the body, malignant cells must undergo a series of changes. One crucial step involves being able to use blood vessels to transport tumour cells around the body. Assays are now commercially available to measure these Circulating Tumour Cells (CTCs), including one which has FDA approval with the brand-name 'CellSearch'.[25, 26] This has superceded older approaches using reverse transcriptase polymerase chain reaction to detect CTC in men with prostate cancer.[27]

Work over the last decade in patients with metastatic cancer has shown that the presence of CTCs in men with PC are a bad prognostic factor, with higher levels of CTCs correlating with poorer overall survival.[28] On the other side of the spectrum of tumour burden, work looking at patients undergoing a radical prostatectomy has shown only a very low incidence of CTCs (~20%) prior to surgery, which was no different to that measured in a cohort of healthy controls.[29] One issue with this study is that <5% of the patients involved would be predicted to eventually suffer metastatic failure, hence the chance of finding CTCs was likely to be very low based on the mainly low to intermediate risk patient cohort examined.

Men with high risk PC have a much higher chance of eventual metastatic failure, of the order of 20-30%, or higher depending on their initial risk factors (PSA level, tumour stage and Gleason grade). At the time of diagnosis these men may therefore exhibit CTC levels intermediate between the metastatic and surgical cohorts previously considered. It may be that high risk PC patients with CTCs detected represent a very high risk group, and apart from providing important prognostic information for men, it could therefore warrant treatment intensification with increased duration of adjuvant ADT, or entry into clinical trials.

3. Prostate Radiotherapy Hypofractionation

Radiotherapy (RT) has been shown to independently improve overall survival for men with high risk PC managed with ADT.[30] As such, standard of care for these men remains bimodality treatment with both RT and ADT.[1]

RT has traditionally been given at doses of 1.8-2 Gy per day due to concerns about the potential for larger fraction sizes to cause late toxicity. Over the last 10 years multiple randomized controlled trials (RCTs) have shown that higher doses of RT (of the order of 74-80 Gy) lead to better rates of no biochemical evidence of disease (bNED).[31, 32] Due to the long natural history of PC, bNED is a validated surrogate endpoint looking at PSA control,[33] however the trial with the longest follow-up is now also beginning to show an improvement in Prostate Cancer Specific Survival (PCSS).[31] The use of such regimens leads to treatment durations of 8-10 weeks, which can be inconvenient for patients, consume a large proportion of the capacity of a RT department, and consequently be a significant factor in the existence of waiting lists for radiotherapy.

There is growing data for PC suggesting that hypofractionation (that is, daily fraction sizes of >2 Gy) is particularly effective at maximizing tumour effect. Newer technologies such as image guided RT (IGRT) which ensures more accurate delivery of the RT, and intensity modulated RT (IMRT) which reduces unwanted radiation dose to adjacent normal structures are now in clinical use in Australia. They both have been used in phase 2 trials of Hypofractionated RT (HypoRT), with results for efficacy and late toxicity comparable to those reported in the literature for conventionally fractionated cohorts.[34, 35] There have been two small RCTs recently reported comparing HypoRT and conventionally fractionated populations, both showing no increased toxicity with the HypoRT, and better bNED.[36, 37] One of these focused mainly on high-risk men and included ADT, similar to the patient population eligible for PROCITT.[36]

4. Radiotherapy Volume

When defining the RT treatment volume for a man with PC, traditional thinking has been to treat the prostate alone. However, for a local treatment modality such as RT or surgery, it is important to appreciate the natural patterns of spread of the disease. For instance, there are good consensus guidelines for patients with head and neck

cancer to help radiation oncologists to know who are most likely to benefit from elective treatment of their cervical neck lymph nodes. This is because, despite the neck being negative at the time of diagnosis, surgical neck dissection series have helped to inform decision aids regarding the chance of a clinically normal neck harbouring sub-clinical disease.

Nomograms have been constructed from large surgical PC cohorts to help define the risk of extracapsular extension, seminal vesicle involvement and lymph node involvement based on initial clinical parameters. Trying to treat all patients with the progressively larger treatment volumes required to include these areas would potentially increase toxicity without a high chance of improving efficacy. However, if a threshold risk level of 15-25% were required prior to including each elective target volume, we would aim to apply such treatments to patients most likely to benefit. Such concepts are already beginning to enter into consensus guidelines,[1, 38] and represent a promising avenue of investigation.

Of all of these expanded treatment volumes, only Whole Pelvic Radiotherapy (WPRT) has been investigated in men with PC in RCT.[39] Neither of the contemporary RCTs found a significant benefit for the use of WPRT. However, many practice changing RCTs have used WPRT on all patients.[2, 40-42] One of the reasons for this discrepancy is likely to be that entry criteria for the largest WPRT RCT estimated a 15% risk of pelvic lymph node involvement.[39] Later work has shown that this only corresponded to a 2% pathological risk of nodal involvement. This emphasizes the need to use validated decision tools to select appropriate treatments.

5. Duration of neoadjuvant ADT

Often adjuvant ADT is given prior to commencing RT. This is known as neoadjuvant hormonal therapy (NHT). There is no clear guidance on what duration to give this for, although 3-6 months is a common approach. Results from an Australian randomized trial have shown 6 months of NAT to result in superior survival than 3 months.[43] Intuitively, it would seem that some patients would benefit from a shorter duration of NHT than others depending on their tumour response. There has been some preliminary work looking at an adaptive approach for this, where RT is started

once a maximal PSA response has been achieved.[44] Given the Australian data, this study will apply a 6 month period of NHT for all patients.

Study Objectives

Primary Hypothesis

That baseline MR imaging of lumbar spine bone marrow and fat fraction combined with clinical factors predicts which men are at greater risk of accelerated Androgen Deprivation Therapy (ADT) induced bone loss than baseline DEXA scanning alone.

Secondary Hypotheses

- Determine feasibility, toxicity (acute and late) and efficacy (3 year bNED by Phoenix definition) of multimodality therapy with hypofractionated radiotherapy
- Correlate marrow changes on MR with changes in blood counts and patient reported fatigue
- Determine prevalence of CTCs in men with high risk prostate cancer and the prognostic significance of CTCs
- Implementation of a nomogram based radiotherapy target delineation algorithm.

Study Design

General Design

A Phase 2 Clinical Trial Exploring Advanced Imaging of Androgen Deprivation Induced Osteoporosis, Prognostic Significance of Circulating Tumour Cells and Hypofractionated Radiotherapy in men with Prostate Cancer.

Primary Study Endpoint

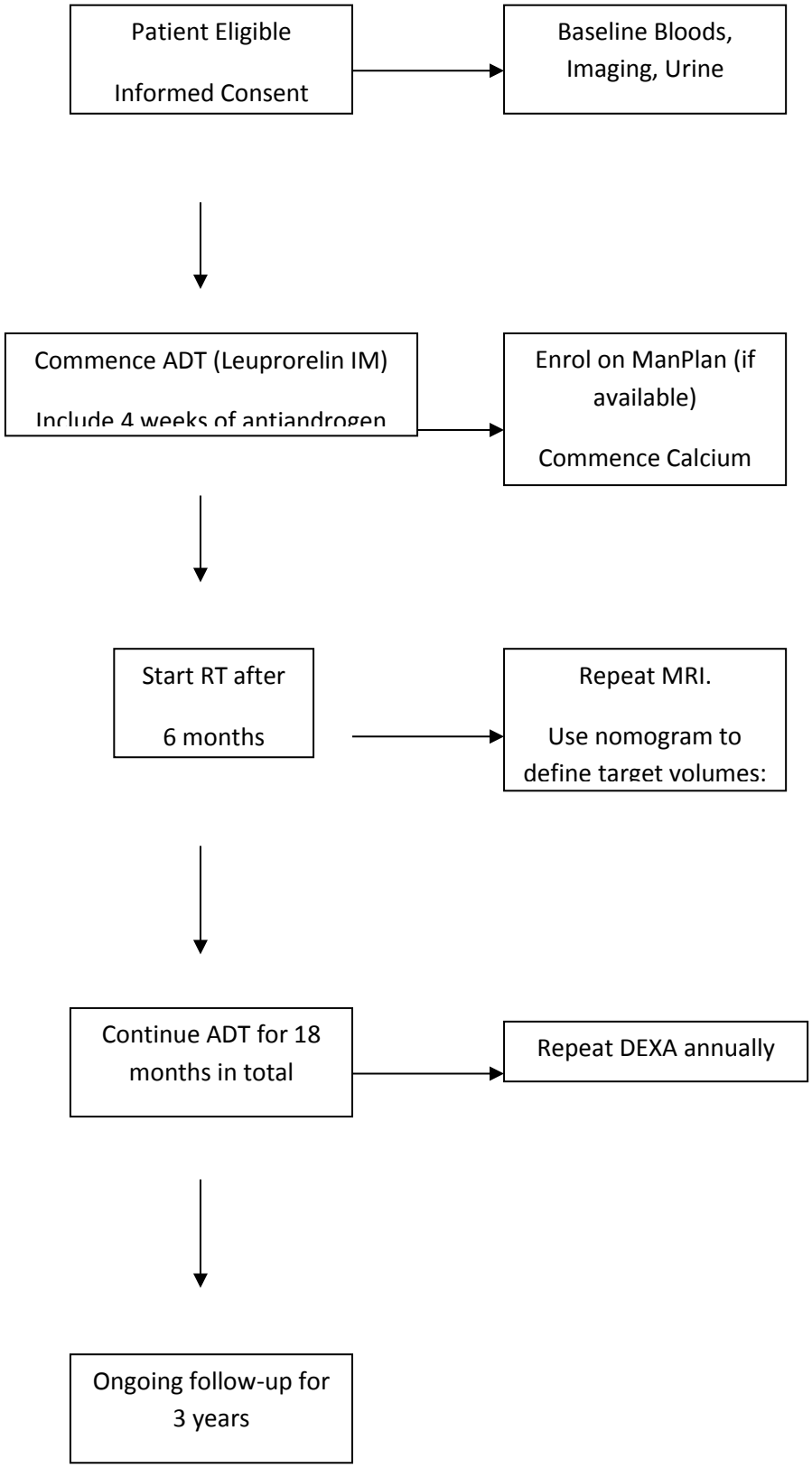
Construction of a predictive model based on pre-treatment imaging and clinical parameters with rate of bone loss measured on serial DEXA scans.

Secondary Study Endpoints

- Feasibility, toxicity (acute and late) and efficacy (3 year bNED by Phoenix definition) of multimodality therapy with hypofractionated radiotherapy
- Correlation of marrow changes on MR with changes in blood counts and patient reported fatigue
- Incidence of CTCs in men with high risk prostate cancer

- Correlation of CTC with efficacy outcomes
- Feasibility of nomogram based radiotherapy target delineation

Study Design



Subject Selection and Withdrawal

Inclusion Criteria

1. Patient capable of giving informed consent
2. Histological diagnosis of prostate cancer
3. High risk disease defined by any one of:
    a. Baseline PSA≥20
    b. Gleason grade 8-10 disease
    c. Clinical stage T3-T4
4. Negative conventional staging in the form of a:
    a. T99m whole body bone scan
    b. CT of the abdomen and pelvis
5. No previous pelvic radiotherapy

Exclusion Criteria

1. History of prior malignancy within the last 5 years with the exception of non-melanomatous skin cancers.
2. ECOG performance status >1
3. Inability to have intraprostatic fiducials inserted.
4. Inability to have a MRI due to:
    a. Implanted magnetic metal eg intraocular metal
    b. Pacemaker / Implantable defibrillator
    c. Extreme claustrophobia

Subject Recruitment and Screening

Patients from the Calvary Mater Newcastle Hospital outpatient Radiation Oncology clinics will be offered recruitment into the study by Radiation Oncologists. Information about the study will be discussed with prospective participants, who will be allowed time to consider the Patient Informed Consent Form (PICF). This information will then be reviewed again with patients interested in study participation to ensure they have fully comprehended the information provided. Patients will be aware that declining to take part in the study will not affect the standard treatment which they will receive. Any agreement will be voluntary, and free from coercion. Consent will be obtained either by a Radiation Oncologist, or a research assistant approved in the investigator log by the Principle Investigator (PI).

Early Withdrawal of Subjects

Reasons for withdrawal

The investigator may withdraw a patient from the study treatment and follow-up procedures if the patient:

- Is in violation of the protocol;
- Experiences a serious or intolerable adverse event
- Develops, during the course of the study, symptoms or conditions listed in the exclusion criteria
- Requests or requires early discontinuation for any reason
- Metastatic disease confirmed prior to commencing pelvic radiotherapy. For such patients, the pelvic radiotherapy component of the study will be suspended.

The investigator will also withdraw all subjects from the study if the study is terminated. Subjects are free to withdraw from the study at any time upon their request or the request of their legally acceptable representative.

Data Collection and Follow-up for Withdrawn Subjects

When a patient withdraws from the study, the reasons for withdrawal shall be recorded by the investigator on the relevant page of the CRF. Whenever possible, all subjects who withdraw from the study prematurely will undergo scheduled visits for study assessments (follow-up). Subjects who fail to return for study assessments will be contacted by the research team in an attempt to have them comply with the protocol via two documented phone calls and one registered letter.

Study Procedures

Study Period	Baseline	Enrolment	Prior to RT	Radiotherapy[b]	Post-RT	18 Months	Follow-Up	Study End
Visit Number	Visit 0	Visit 1	Visit 2a	Visit 3a-3f	Visit 4	Visit 5a, 5b	Visit 6a, 6b…	Visit 7
Week	-2 – 0	0-4	17	RT wk0-6	RT week 14	Every 6 months until end of ADT	Every 12 months	3 years from beginning of study
Informed Consent [a]	X							
Eligibility Check	X							
Demographic Information	X							
Medical History	X							
Physical Examination	X		X		X	X	X	X
Whole Body Bone Scan	X							
CT Abdomen & Pelvis	X							
Blood collection		X	X		X	X	X	X
Bone Markers		X	X					
MRI Lumbar Spine [f]		X		X				
Fatigue Questionnaire [f]		X				X		
DEXA scan [e]		X				X	X	

Thoracic Spine plain films		X						
Circulating Tumour Cells		X						X [d]
RTOG Radiotherapy Toxicity Assessment [c]		X		X	X	X	X	X

[a] See Appendix 9

[b] See Appendices 7 and 8

[c] See Appendix 5

[d] Only for men with an initially positive CTC assay

[e] To be performed annually while on study

[f] To be performed at baseline and after 18 months

Definitions of events:

Informed consent: Patient reads, understands and signs informed consent.
Eligibility check: Inclusion and exclusion criteria reviewed to ensure patient eligibility to be included in the study.
Demographic information: Date of birth, age at enrollment
Medical History: Prostate cancer related parameters (PSA results, prostate biopsy results including overall Gleason grade), Medical comorbidities, medications (including bisphosphonates), ECOG performance status (appendix 1).
Physical Examination: Must include a digital rectal examination (DRE). Also height at baseline, and annual weight.
Whole Body Bone Scan: To be performed by a diagnostic imaging facility able to interpret initial and delayed images of the skeleton for the presence of any areas of increased uptake following the administration of Technetium 99m. Any areas suspicious for metastatic disease need further imaging such as plain films, CT or MRI to exclude metastases.
CT Abdomen/Pelvis: Any lymph nodes greater than 15 mm in size in the pelvis require further imaging or biopsy to exclude metastases to allow eligibility. In the absence of pelvic disease, lymph nodes in the abdomen need to be at least 20 mm in the short axis to be treated as positive.
Blood collection:

- At baseline:
  - PSA
  - Total testosterone, Sex Hormone Binding Globulin (SHBG), calculated free testosterone
  - 17□-oestradiol
  - Follicle Stimulating Hormone, Luteinizing Hormone, Prolactin
  - Full Blood count (FBC)
  - Urea and electrolytes
  - Liver function tests including alkaline phosphatase (ALP), Lactate Dehydrogenase

- 25-Hydroxy Vitamin D
- Calcium/Phosphate
- Circulating Tumour Cells

– All other visits requiring blood collection
  - PSA
  - Testosterone (until back in normal range)
  - 17□-oestradiol (until testosterone normal)
  - FBC

Bone Markers: Serum and urine markers to be collected at baseline, and every 3 months for the first 6 months on ADT:

– Serum: Bone specific ALP (bsALP), osteocalcin, Procollagen Type 1 N-Terminal Pro-peptide (P1NP), C-telopeptide of type I collagen (CTX)
– Urine: N-telopeptide (NTX)/creatinine,

Quality of Life: Collected via the PROMIS fatigue questionnaire (Appendix 4). Perform annually until end of year 3.

MRI Lumbar Spine: As outlined in appendix 3. Performed at baseline and then again only if the patient is having a MRI for prostate radiotherapy planning.

DEXA Scan: Performed as outlined in appendix 3 at baseline, after 1 year, and after 2 years.

Thoracic spine plain films: Performed at baseline to assess for insufficiency fractures.

RTOG Radiotherapy Toxicity assessment: Acute genitourinary (GU) and gastrointestinal (GI) toxicity to be recorded weekly while proceeding through radiotherapy, and then again at visit 4. Late GU and GI toxicity to be recorded at all subsequent visits. See appendix 5.

Statistical Plan

Sample Size Determination

The primary endpoint is the construction of a prognositc model based on early treatment imaging and clinical parameters with rate of bone loss measured on serial DEXA scans. There is no comprehensive data regarding even the

variability of fat fraction on MRI in elderly males, let alone the likely impact this will have on the rate of bone loss (if any). This makes any power calculations very difficult, although clearly a larger number of patients will give greater confidence of being able to detect a relationship, if one exists. As a compromise, the study will remain accruing for 2 years or a maximum of 100 patients. Data from these patients will be used to construct a model to predict the rate at which patients lose BMD based on data obtained from only baseline data and information available within 6 months of commencing ADT.

Statistical Methods

1. BMD Loss

For the primary endpoint, the response variable is the rate of bone loss measured between at least two DEXA scans of the neck of femur performed at least 12 months apart with units grams/cm^2/year. The model will be constructed following the final patient who receives their second analyzable DEXA scan using a discriminant analysis. Potential explanatory variables will include:

Patient (From WHO Fracture Risk Assessment Tool)	Age (Years) Weight (kg) Height (cm) Smoking status (Current, No) Bisphosphonate therapy (Yes/No) Previous fracture (Yes/No) Parent Hip fracture (Yes/No) Steroids (Yes/No) Rheumatoid arthritis (Yes/No) Alcohol ≥30g/day (Yes/No)
Laboratory	Changes after 3 months in: Haemoglobin Bone specific alkaline phosphatase

	Osteocalcin Procollagen Type 1 N-Terminal Pro-peptide Urine N-telopeptide/Creatinine Oestradiol Testosterone
Imaging	Baseline DEXA BMD Neck of femur (g/cm^2) Bone Marrow fat fraction from MRI (%)

Any variable which achieves a significance level of less than 0.1 will be incorporated into the predictive model.

2. Correlation of CTC with efficacy

Patients will be dichotomized from baseline CTC results into those with negative readings (<1 CTC/7.5 mL blood sample) and those with positive readings (≥1 CTC/7.5 mL blood sample). The response variable of interest will be bNED by the Phoenix definition (nadir + 2). The timing to any events or censoring will be measured from the date the first dose of ADT is delivered. A Kaplan-Meier survival function will be constructed to determine if a positive CTC assay has any prognostic significance, and the magnitude of that effect on bNED. For men who had an initially non-zero CTC count, a repeat assay will be performed at the completion of the study to assess any change following treatment.

3. Correlation of changes in Haemoglobin with MRI measured marrow fraction and fatigue

For this component, there are three main endpoints:

- Change in Haemoglobin: This will be the difference between the baseline Hb and the lowest level subsequently measured while the patient has a castrate level of testosterone. Patients with an incurrent illness responsible for the drop in HB (eg gastrointestinal bleed) will be excluded.

- MRI measured marrow fraction: As assessed prior to commencing ADT using the MRS and other imaging sequences on the lumbar spine.
- Fatigue: As recorded by the validated PROMIS short fatigue questionnaire v1. A total score of between 7 to 35 is possible, with a higher score representing greater fatigue. We are interested in the change in this score between baseline, and the 18 month repeat questionnaire.

Scatterplots and correlation coefficients will be performed to see if there is any inter-relationship between these 3 variables.

4. Hypofractionated radiotherapy

Aim is to report the efficacy and toxicity associated with the radiotherapy regimen delivered. Efficacy to be measured by bNED via the Phoenix definition as for CTC, with exploratory analyses based on baseline PSA, Gleason grade (6-8 v 9-10), tumour stage (T1-2 v T3-4), marrow fraction, change in Hb, delivery of whole pelvic radiotherapy and duration of testosterone suppression. The latter quantity is defined as the period of time where the testosterone level is <0.5, presumed to commence 2 weeks after the first ADT dose is delivered.

Late GI and GU toxicity recorded. Maximal late GI and GU toxicity recorded for each patient as well as the timing of this event and the late GI and GU toxicity at last follow-up.

5. Feasibility Outcomes

- *Nomogram based radiotherapy target delineation*: The main interest with this endpoint is the ability for clinicians to adhere with a novel, but potentially complex means of contour delineation. All CTVs as defined in appendix 7 will be scored by a radiation therapist who was not involved in generating those contours. For each patient they will assess the following criteria:

- CTVece Required (Yes / No)
- CTVece Generated as per protocol (Yes / No / NA)
- CTVsv Required (Yes / No)
- CTVsv Generated as per protocol (Yes / No / NA)
- CTVln Required (Yes / No)
- CTVln Generated as per protocol (Yes / No / NA)

For any areas where a potential violation is observed, this will be reviewed by a Radiation Oncologist prior to being recorded as such. The aim is to observe a protocol compliance rate of ≥90%.

Subject Population for Analysis

All patients with data available for the relevant analyses will be included. For example, only patients who receive radiotherapy will be included for the hypofractionated radiotherapy and nomogram based radiotherapy target delineation analyses. For the BMD analysis, as a minimum patients require two DEXA scans at least 12 months apart, as well as a baseline MRI. For the CTC analysis, only patients who had a baseline CTC blood test taken will be included. For the change in Hb analysis, at least 2 Hb levels (including a baseline measure) 3 months apart, and a baseline Lumbar spine MRI are necessary as a minimum.

Safety and Adverse Events

Definitions

Adverse Event (AE):

Any untoward medical occurrence in a patient enrolled into this study regardless of its causal relationship to study treatment (see appendix 10).

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results

of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event (SAE):

Adverse events are classified as serious or non-serious.

An SAE is defined as any event that:

• results in death; or

• is immediately life threatening; or

• requires inpatient hospitalisation; or

• requires prolongation of existing hospitalisation; or

• results in persistent or significant disability/incapacity; or

• is a congenital anomaly/birth defect.

Important medical events may be considered an SAE when, based upon appropriate medical judgement, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Suspected Unexpected Serious Adverse Reaction (SUSAR):

A SUSAR is any SAE that is both suspected to be related to the study and is unexpected (i.e. not consistent with applicable product information).

Eliciting Adverse Event Information

Adverse events will be recorded from the time the patient signs the Informed Consent Form until 30 days after the last visit. At every study visit subjects will be asked “How have you felt since your last visit?” in order to elicit any medically related changes in their well-being. They will also be asked if they

have been hospitalised, had any accidents, used any new medication or changed concomitant medication regimens. In addition, AEs will be documented from physical examinations findings, clinically significant lab results or other documents (including patient diaries and correspondence from their primary care physician) that are relevant to patient safety.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 3 months following the last day of radiotherapy.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related

to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

Assessment and documentation of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

For the purposes of this study the investigator is responsible for recording all Adverse Events, regardless of their relationship with the exposure, with the following exceptions:

• Conditions that are present at screening and do not deteriorate will not be considered adverse events.

• Abnormal laboratory values will not be considered adverse events unless deemed clinically significant by the investigator and documented as such.

The description of each AE on the CRF will include:

- A description of the AE;
- The onset date, duration, date of resolution;
- Severity (mild, moderate or severe);
- Seriousness (i.e. is it a Serious AE?)
- Any action taken (eg treatment, follow-up tests);

- The outcome (eg recovery, death, continuing, worsening);
- The likelihood of the relationship of the AE to the treatment being investigated (e.g. Unrelated, Possible, Probable, Definite).

Changes in the severity of an AE will be reported (eg worsening headache). AEs characterized as intermittent will be document for each episode. All AEs will be followed to adequate resolution.

Reporting of Serious Adverse Events

Any SAE occurring in a study participant will be reported to the local HREC within 24-72 hours of occurrence, in accordance with the safety reporting policy of the HREC. The HREC safety reporting form will be completed, signed and submitted by the investigator

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others

The minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

SUSARs

All SUSARs occurring in a study participant will be reported to the local HREC in an expedited fashion (i.e. within 15 calendar days of first knowledge), or for fatal or life threatening events, an initial or full report within 7 calendar days and a follow-up report if necessary within the 15 calendar day timeframe. An investigator will complete, sign and submit the SUSAR report.

Data Handling and Record Keeping

Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, research staff, and the sponsoring institution and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the principal investigator. All laboratory specimens, evaluation forms, reports and other records that leave the site will be identified only by the Subject Identification Number (SIA) to maintain subject confidentiality. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by HREC or regulatory agencies.

Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”. All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT

ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

Records Retention

It is the investigator's responsibility to retain study essential documents for at least 15 years after the completion of this clinical trial. All information will be stored in the Radiation Oncology research office of the Calvary Mater Newcastle, either on a password protected computer or in files kept in a locked room. Access to this information will be limited to the principal investigator, research assistants and statistician as authorized by the delegation log.

Ethical Considerations

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the HREC. A letter of protocol approval by HREC will be obtained prior to the commencement of the study, as well as approval for other study documents subject to HREC review.

This study will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed consent Form that affects the scientific intent, study design, patient safety, or may affect a participant's willingness to continue participation in the study, is considered an amendment, and therefore will be written and filed as an amendment to this protocol and/or Informed Consent Form. All such amendments will be submitted to the HREC for approval prior to becoming effective.

All protocol deviations must be recorded in the patient record (source document) and on the CRF and must be reported to the PI. Protocol deviations will be assessed for significance by the PI. Those deviations deemed to have a potential impact on the integrity of the study results, subjects safety or the ethical acceptability of the study will be reported to the

HREC within 30 days. Where deviations to the protocol identify issues for protocol review, the protocol will be amended as per this section.

Study Finances

This study is financed through an unrestricted research grant provided by Abbott pharmaceuticals. Abbott was not involved in the development of this protocol, and will not be involved in analyzing or reporting the results. Full disclosure of the funding source will be provided in any publications and presentations relating to this work.

Publication Plan

Primary responsibility for the publication of the study results rests with the principal investigator. Information cannot be passed onto any other party without permission of the principal investigator. Abbott pharmaceuticals will be acknowledged as the funding source in all publications, and will have the opportunity to review all manuscripts prior to submission for publication.

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Appendices

1. Prostate cancer staging and risk stratification
2. ECOG/Zubroid Performance Status
3. Imaging Specifications
4. PROMIS Fatigue Questionnaire
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Appendix 1: Clinical Prostate Cancer Staging (TNM 7th edition 2010)

Stage	Interpretation
TX	Primary tumor cannot be assessed.
T0	No evidence of primary tumor.
T1	Clinically inapparent tumor neither palpable nor visible by imaging.
T1a	Tumor incidental histologic finding in ≤5% of tissue resected.
T1b	Tumor incidental histologic finding in >5% of tissue resected.
T1c	Tumor identified by needle biopsy (e.g., because of elevated PSA).[a]
T2	Tumor identified by needle biopsy (e.g., because of elevated PSA).
T2a	Tumor involves ≤one-half of one lobe.
T2b	Tumor involves >one-half of one lobe but not both lobes.
T2c	Tumor involves both lobes.
T3	Tumor extends through the prostate capsule.[b]
T3a	Extracapsular extension (unilateral or bilateral).
T3b	Tumor invades seminal vesicle(s).
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles such as external sphincter, rectum, bladder, levator muscle and/or pelvic wall.

[a]Tumor found in one or both lobes by needle biopsy, but not palpable or reliably visible by imaging, is classified as T1c.

[b]Invasion into the prostatic apex or into (but not beyond) the prostatic capsule is classified not as T3 but as T2.

Prostate Cancer Risk Group Stratification (NCCN):

Low Risk: All of PSA<10, Gleason Grade 6 AND Stage T1 or T2a

Intermediate Risk: One of PSA 10-20, Gleason Grade 7 OR Stage T2b-c

High Risk: Any one of PSA>20, Gleason Grade 8-10 OR Stage T3-4

Appendix 2: ECOG/Zubrod Performance Status

0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Appendix 3: Imaging Specifications

DEXA Scan

Ensure that calibration procedures have been performed as per published guidelines.[12, 45] DEXA scans to be performed on the hip and lumbar spine with individual vertebrae L1-L5 measured independently. BMD Data to be recorded from all of these sites individually.

MRI Lumbar Spine

3 Tesla MRI
Position with knee rest to reduce lumbar lordosis.
Use lumbar phased-array coil and abdominal flex coils.
Standard clinical magnetic resonance imaging sequences will be used for this study including T1, T2, and Diffusion Weighted Imaging will be performed to allow calculation of an Apparent Diffusion Coefficient.

<u>Lumbar spine fat content</u>

Imaging: Standard in-phase/out phase imaging will used to generate pure fat images and water images of the lumbar vertebrae. Sagittal images will be positioned along the spine. This will allow determination of the fat content for each vertebra contained within the imaging field-of-view.

Total time the patient is required to be in the MRI system is less than 40 min. One initial patient will be request to repeat measurements 2 times to ensure reproducibility of the imaging. Standard phantom calibration will also be recorded.

Appendix 4: PROMIS Fatigue Questionnaire

Please see attached PROMIS Fatigue – Short Form 1
This is summarized below:

Please respond to each question by marking one box per row.

In the past 7 days:

	Never	Rarely	Sometimes	Often	Always
How often did you feel tired?					
How often did you experience extreme exhaustion?					
How often did you run out of energy?					
How often did your fatigue limit you at work (include work at home)?					
How often were you too tired to think clearly?					
How often were you too tired to take a bath or shower?					
How often did you have enough energy to exercise strenuously?					

Appendix 5: RTOG toxicity scales

Acute Genitourinary

Grade 0	1	2	3	4	5
No change	Frequency of urination or nocturia twice pre-treatment habit: dysuria, urgency, not requiring medication	Frequency of urination or nocturia less frequent than every hour: urgency, bladder spasm requiring local anaesthetic eg Ural	Frequency with urgency and nocturia hourly or more frequently. Dysuria, pelvic pain or bladder spasm requiring regular, frequent narcotic. Gross haemturia with or without passage of clot	Haematuria requiring transfusion. Acute bladder obstruction not due to clot passage. Ulceration or necrosis.	Death

Acute Gastrointestinal

Grade 0	1	2	3	4	5
No change	Increased frequency or change in quality of bowel habits not requiring	Diarrhoea requiring parasympatolytic drugs. Mucous discharge not requiring sanitary pads.	Diarrhoea requiring parenteral support. Severe mucous or blood	Acute or subacute obstruction, fistula or perforation. Gi bleeding requiring	Death

	medication. Rectal discomfort not requiring medication.	Rectal or abdominal pain requiring analgesics.	discharge requiring sanitary pads. Abdominal distension with distended bowel loops on plain X-ray.	transfusion, abdominal pain or tenesmus requiring decompression or bowel diversion.	

Late Gastrointestinal

Grade 0	1	2	3	4	5
None	Mild diarrhea. Mild cramping. Bowel movement up to 5 times daily. Slight rectal discharge or bleeding.	Moderate diarrhea and colic. Bowel movement >5 times daily. Excessive rectal mucous or intermittent bleeding.	Obstruction or bleeding requiring surgery.	Necrosis / Perforation. Fistula.	Dead

Late Genitourinary

Grade 0	1	2	3	4	5
None	Slight	Moderate	Severe	Necrosis /	Dead

	epithelial atrophy. Minor telangiectasia (microscopic haematuria).	frequency. Generalized telangiectasia. Macroscopic haematuria.	frequency and dysuria. Severe generalized telangiectasia (often with petechiae). Frequent haematuria. Reduction in bladder capacity (<150 cc).	Contracted bladder (capacity <100 cc). Severe haemorrhagic cystitis.	

Appendix 6: Radiotherapy Contouring Guide

Clinical Target Volumes (CTV)

Prostate: The prostate is defined using CT and/or MRI imaging. Additional information such as the position of the most inferior gold fiducial marker from the apex of the prostate, any extraprostatic disease detected on pre-ADT MRI or clinically and the volume of the prostate as recorded during Trans-rectal Ultrasound (TRUS) should be incorporated into delineation of the prostate CTV. This volume is labeled CTVp

Extracapsular extension (ECE): If the predicted risk of ECE is greater than 15% via the online Sloan Kettering nomogram, a 3 mm margin is added in all directions around the prostate CTV. Any overlap of this volume with the rectum is removed. The remaining volume is labeled CTVece.

Seminal Vesicle Invasion (SVI): If the predicted risk of SVI is greater than 15% via the online Sloan Kettering nomogram, the proximal 20 mm of the seminal vesicles (SV) are contoured as a separate CTV measured along the length axis of the SV. The 20 mm is measured obliquely along the axis of the SV, not in the longitudinal axis. Note that the SV often commences inferior to the most superior visualized prostate, and it is therefore not always necessary to contour the SV for 20 mm above the prostate. If less than 20 mm of SV are visible, contour only what SV can be identified. If the SV are identified as harbouring disease, the whole SV will need to be contoured. The resulting volume is labeled CTVsv.

Pelvic Lymph Nodes (PLN): If the predicted risk of PLN involvement is greater than 15%, they are contoured according to guidelines from the RTOG. Use the Roach formula to calculate this (2/3 x PSA + (Gleason – 6) x 10). This

volume is not attempted for men with bilateral total hip joint replacements due to difficulty in safely delivering treatment to such a large treatment volume. The CTV extends 7 mm around relevant vascular structures bilaterally, not including vessels, muscle, bladder or bone. Some summary points working from the inferior aspect of the volume:

- Superior to the upper limit of the pubic symphsis, the obturator lymph nodes are included.
- Superior to the top of the femoral heads, both internal and external iliac lymph nodes are included.
- Superior to the upper limit of the rectum, the presacral region is included in addition to the internal and external iliac lymph nodes
- Cease contouring 10 mm inferior to the anterior-superior aspect of the S1 vertebral body (the sacral promontory)

The resulting CTV is labeled CTVln.

Planning Target Volume

PTV70 Gy =

- CTVp + 5 mm in all directions

PTV61.6 Gy =

- For patients with a CTVece but no CTVsv: CTVece + 5 mm in all directions
- For patients with both CTVece and CTVsvi: CTVece + 5 mm in all directions and CTVsvi + 5 mm in superior, inferior, left and right directions, and 7 mm in anterior and posterior directions.
- This volume is not required for patients with no CTVece or CTVsv.

PTV50.4 Gy = CTVln + 5 mm in all directions

Critical Structure Contouring

Bladder = Whole bladder (solid organ)
Rectum = From ano-rectal junction (usually around bottom of ischial tuberosities) to recto-sigmoid flexure (solid organ)
Small Bowel = Contour any visible small bowel as well as peritoneal contents within 8 mm of the superior aspect of the PTV and label as small bowel. This is because small bowel can occupy any space within the peritoneal cavity. Extend this volume by 3 mm in all directions to create the Small Bowel Planning Target at Risk volume (SB PRV).

Appendix 7: Radiotherapy Details

Preparation:

- Empty rectum and comfortably full Bladder prior to planning and each day of treatment
- 2-3 Gold fiducial markers placed in prostate region

Position: Supine

Immobilisation: Vac-lock bag or similar custom device

Scanning:

- CT: <3 mm axial slice thickness commencing above L4 vertebra, and extending inferiorly to >3 cm below perineum
- MRI: Prostate MRI is optional to assist with contouring. 2 mm slices with T2 weighting are performed. Fusion to the planning CT is based on the location of the gold fiducial markers.

Planning: Intensity Modulated radiotherapy (IMRT) technique using either static fields or arc therapy (volumetric modulated arc therapy [VMAT]). Planning objectives and field arrangement optimized to achieve best dosimetry.

Treatment: Pre-treatment electronic portal image (EPI) taken using kilovoltage imaging. Any deviation of the gold seed centre of mass from that observed in the planning scan is corrected for in the transverse, sagittal and coronal planes prior to treatment each day ie 0 mm action threshold.

Radiotherapy Dose Constraints

Guidelines taken from ICRU 83 (dose reporting for IMRT)[48], RTOG protocol 04-15 (hypofractionated prostate radiotherapy)[49], and QUANTEC[50]. For all PTV and CTV volumes, doses below that prescribed would be a protocol variation.

PTV70 Gy: Dose prescription - D98 = 70 Gy
PTV70 Gy: Maximum to 1 cc – Major variation if >77 Gy. Minor variation if 74.9-77 Gy.
CTVp, CTVece, CTVsvi: D99 = 70 Gy
PTV61.6 Gy: Dose prescription - D98 = 61.6 Gy
PTV50.4 Gy: Dose prescription - D98 = 50.4 Gy
CTVln: D99 = 50.4 Gy
Rectum (Mandatory): D15% < 74 Gy. D25% < 69 Gy, D35% < 64 Gy, D50% < 59 Gy.
Rectum (Ideal): D15% < 74 Gy. D25% < 60 Gy, D35% < 50 Gy, D50% < 40 Gy.
Small Bowel PRV: D99% < 52 Gy
Neck of Femur: D5% < 44 Gy
Bladder (Mandatory): D15% < 79 Gy. D25% < 74 Gy, D35% < 69 Gy, D50% < 64 Gy.
Bladder (Ideal): D15% < 74 Gy. D25% < 60 Gy, D35% < 50 Gy, D50% < 40 Gy.
Penile Bulb: Mean dose < 51 Gy (Not mandatory if PTV coverage needs to be compromised to achieve).

If the mandatory rectal or small bowel dose constraints cannot be met, the patient can be treated with an alternative radiotherapy regimen giving 74-78 Gy at 2 Gy per day. Scale PTV and CTV objectives accordingly eg if prescribed dose is 76 Gy:

Rename PTV: PTV70 Gy → PTV76 Gy
PTV76 Gy: D98 = 76 Gy

PTV76 Gy: Maximum 81.3 Gy. 81.31 – 83.6 Gy = Minor violation. >83.6 Gy = Major violation.

CTVp, CTVece, CTVsvi: D99 = 76 Gy

Rectum (Mandatory): V75 Gy < 15%, V70 Gy < 20%, V65 Gy < 25%, V60 Gy < 35%, V50 Gy < 50%.

Small Bowel PRV: D99% < 52 Gy

Neck of Femur: D5% < 53 Gy

Bladder (Mandatory): V80 Gy < 15%, V75 Gy < 25%, V70 Gy < 35%, V55 Gy < 50%.

Penile Bulb: Mean dose < 51 Gy (Not mandatory if PTV coverage needs to be compromised to achieve).

Appendix 8: Patient Information Sheet

A Phase 2 Clinical Trial Exploring 3-Dimensional Imaging of Androgen Deprivation Induced Osteopaenia, Radiotherapy Hypofractionation and the Prognostic Significance of Micrometastatic disease in men with Prostate Cancer.

Short Title: PROstate Cancer Imaging, Treatment and Toxicity (PROCITT)

This document is designed to complement information given to you verbally by your doctor.

You are being invited to take part in a clinical research study for men with Prostate Cancer (PC). The doctors at this hospital are trying to develop better methods of treatment for this disease. They are also trying to better understand what causes some of the side effects of treatments. This is called clinical research. In order for you to decide whether you should agree to be part of this study, you should understand enough about its aims, risks and benefits to make an informed decision. This process is known as informed consent.

This Participant Information Sheet contains information about the research trial. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project before you decide whether or not to take part in it.

Please read this Participant Information Sheet carefully. Feel free to ask questions about any information in the Information Sheet. Before deciding whether or not to take part, you may wish to discuss the trial with friends or relatives or your local health worker.

Should you decide not to participate in this trial, your doctor will discuss details of your treatment options with you. Your decision not to participate will not affect any other aspect your treatment.

'What is the main purpose of this trial?'

Your doctor has explained that you require radiotherapy for your PC. You also need a type of hormonal therapy known as Androgen Deprivation Therapy (ADT). Although ADT improves the chance of cure, it can also have side effects. One of these is thinning of the bones. When this is advanced, it is called osteoporosis. Men with osteoporosis have a higher chance of getting fractures of bones such as the hip and spine. To help stop this from happening, your Doctor will recommend you start taking Calcium and Vitamin D tablets every day.

We do not know exactly how ADT speeds up thinning of the bones. We also cannot pick which men have fast or slow bone thinning while on ADT. If we could find the men likely to have faster bone thinning before starting treatment, we might be able to add other treatments to help strengthen the bone, or change the time they remain on ADT.

Currently, the best way to measure for osteoporosis is to do a bone mineral density scan using a DEXA scanner. This technology is widely available, and has a lot of experience about how best to use it. It also has limitations in that it cannot see the different parts of the bone or the bone marrow within the bone. All of these interact to keep bones strong. Doing special scans to look at these different parts of bone more accurately may help us understand better how ADT speeds up bone thinning.

The main purpose of this study is to see if doing extra tests on the bone can help figure out how ADT speeds up bone loss as well as which men loss bone faster. These tests include a type of scan called a Magnetic Resonance Imager (MRI), as well as urine and blood tests. You would not need to have

any more blood samples taken than is normally the case for men receiving this type of treatment, but each time you have blood taken more tests would be done on that blood sample.

'Does the trial have any other purposes?'

Sometimes cancer can travel to other parts of the body. Although scans can be normal before you start treatment, very small amounts of cancer can be impossible to find using current scans such as a CT and bone scan. To get to other parts of the body, small parts of the original cancer need to use blood vessels.

There is a blood test which can sometimes detect cancer cells in the blood. This blood test may help show which men with metastatic prostate cancer will do better with a particular treatment. However, it has not been well looked at for men without metastatic disease. We aim to see if this blood test for circulating tumour cells is useful for men without metastatic prostate cancer.

'How is the radiotherapy in this trial different?'

Radiation Oncologists try to predict where the cancer is most likely to be, and to target the radiotherapy to those areas. There is no standard way to do this, so different Radiation Oncologist will use different methods. This trial aims to use a decision making tool called a nomogram to help tailor the area to treat in a more structured way.

Radiotherapy for prostate cancer can take up to 8 weeks of treatment, 4-5 times per week. In recent year several other studies have shown that by giving a larger dose of radiotherapy every day, treatment can be completed in a shorter time. The largest trial reported so far used a 28 day schedule given in 5 and a half weeks, and reported cure rates and side effects equal to what would be expected from an 8 week program. Although used overseas, this 5

½ regimen has not been used widely in Australia, and we would like to see if we gain similar results here as have been reported from the US.

‘Why have I been invited to participate in this trial?’

You have been invited to participate in this trial because your doctor has recommended radiotherapy and ADT for your prostate cancer.

‘What if I don’t want to take part in this trial or withdraw later?’

Participation in this trial is voluntary. It is completely up to you whether you participate. If you decide not to participate, it will not affect the treatment you receive now or in the future. Whatever your decision, it will not affect your relationship with the staff caring for you. Your doctor will discuss other treatment options that may be available if you do not wish to take part in the trial. Discuss all these options with your doctor before deciding whether to take part in this research trial. If you feel that you are only able to participate in the part of the study looking at the shorter radiotherapy approach, or the aspect of the trial about bone health, this is also possible.

New information about the treatment being studied may become available during the course of the trial. You will be kept informed of any significant new findings that may affect your willingness to continue in the trial.

If you wish to withdraw from the trial once you have started, you can do so at any time without having to give a reason. However, data or samples which have been made anonymous may not be withdrawn. If you join the trial and then decide to withdraw your consent, please notify a member of the research team immediately. This is important so that you can be informed if there are any health risks or special requirements that you need to know.

Your doctor may decide you should stop treatments if they consider it in your best interest, but this does not mean that you have to withdraw from the trial.

Your doctor will advise you if alternative treatment is available or appropriate if you stop the trial treatment or withdraw from the trial.

It is important that you tell your doctor about any treatments or medications you may be taking including non-prescription medications, vitamins or herbal remedies, or other alternative procedures. You should also tell your doctor about any changes to these during your participation in the trial.

'What does this trial involve - What will happen if I take part?'

Trial Procedures:
If you agree to participate in this trial, you will be asked to sign this Participant Consent Form. The timing of the start of your treatment is usually between 2 to 4 weeks following your first meeting with the radiation oncologist.

Prior to starting treatment:

Routine Procedures:
There are some routine assessments that will be done before you start any treatment that would have been completed whether you decide to participate in this study or not. These assessments will be performed to evaluate the extent of your disease. Some of these assessments include blood tests and scans.

CT Scan of the Abdomen and Pelvis:
A CT is a computerised scan that provides a picture of your body using a highly sensitive x-ray beam. Some patients may require an injection of contrast (dye) to improve the quality of their scans. There is a possibility of an allergic reaction (anaphylaxis) to the contrast used in scans which, although rare, can be life threatening.

Bone Scan:

A whole body bone scan is necessary to look specifically at the bones for any signs of cancer which may have spread there. This involves the injection of a weakly radioactive dye and two sets of scans.

DEXA Scan and Bone X-Rays:
A DEXA scanner looks specifically at bone mineral density. Specific x-rays may also be needed to see if there is any sign of bone weakness.

Blood Tests:
Standard blood tests include PSA, testosterone, blood counts, and Vitamin D levels.

Other Tests:
Other relevant tests may be performed at the discretion of your doctor(s), and may, to some extent, be made necessary by the results of other tests. These tests, as well as determining the extent of your cancer, will assess how well your various organs are functioning.

Trial Specific Assessments:

The following assessments will only be performed if you agree to participate in the trial:

Blood Tests:

Extra blood samples will be needed to test for the circulating tumour cells amongst other tests associated with cancer, osteoporosis, and anaemia. These will be performed at the same time as the routine blood testing, so that no extra trips to a pathology lab should be necessary.

Fatigue (QOL) Questionnaire:

Prior to starting treatment you will be asked to fill in a questionnaire about your energy levels. This is known as a Quality of Life Questionnaire. It will usually take less than 5 minutes to complete. You will be asked to fill it out again 18 months after starting the study.

MRI Scan Lumbar Spine

An MRI helps look specifically at the bone marrow in the spine**. It is important you tell your doctor if you have a pacemaker, defibrillator, or have ever had metal in your eye.** An MRI tunnel is similar to a CT, except more narrow. Tell your doctor if you have claustrophobia. The total time for the scan is around 40 minutes.

Urine Tests

A special urine test is needed before starting ADT, and then again while on ADT, to measure the rate your bones are rebuilding.

During treatment:

Radiotherapy

A normal course of radiotherapy extends over 8 weeks, with 39 actual days of treatment given daily, Monday to Friday. For this study, the radiation

treatment will extend over a period of 5 ½ weeks, with 28 days of treatment. The area treated may include not only the prostate, but potentially areas surrounding the prostate such as the glands behind the prostate (the seminal vesicles) or the lymph nodes in your pelvis. This shorter treatment schedule has been used for many years in some American hospitals, but has not being widely used in Australia.

Treatment takes approximately 10-15 minutes each day. The actual areas treated will depend on the type of prostate cancer you have, and your doctor will be able to discuss this with you prior to you starting the radiotherapy.

Post-Treatment Follow-up:

Following the completion of your treatment you will have regular follow-up visits by your doctors, occurring every 6 months for a total of 5 years. You will have complete clinical examinations and will be monitored for the effect of the treatment. A DEXA scan will be repeated 12 months, then again after 2 years to look at the health of your bones.

'Are there risks to me in taking part in the trial?'

All medical procedures involve some risk of injury. In addition, there may be risks associated with this trial that are presently unknown or unforeseeable. In spite of all reasonable precautions, you might develop medical complications from participating in this trial. Blood tests, scans, radiotherapy and hormonal therapy are all part of the usual management of prostate cancer, and it is unlikely you will experience any different risks by taking part in the trial.

This trial aims to further medical knowledge and may improve future treatment of Prostate Cancer; however it may not directly benefit you. Your doctor will discuss with you the benefits for your personal circumstances of the treatments recommended.

Blood tests:

You may experience some mild discomfort and minor bruising or swelling at the site where blood is collected.

Hormonal Therapy:

There are several side effects associated with Hormonal therapy:

- Common (>20%)
  - Sweats and hot flashes
  - Poor sex drive
  - Poor erections
- Occasional (10-20%):
  - Tiredness
  - Weight gain

- Uncommon (1-10%)
    - Low mood

Many of these side effects will persist for several months after the completion of hormonal therapy, and sometimes they never fully go away.

Radiotherapy:

Side effects of radiotherapy are common, they usually occur within days or weeks of starting radiotherapy and most resolve within a month of its completion. These side effects may include:

- A skin reaction like a mild sunburn, which may occur in the lower pelvis (creams can be prescribed by your doctor to help ease any discomfort),
- You may feel tired during the treatment.
- Bladder irritation which can usually be managed with medication, but in around 1 in a hundred men temporarily requires a catheter.
- Bowel irritation such as diarrhoea is uncommon (1-10%), but if it does occur, can usually be treated by reducing the fibre in your diet or starting traveller's diarrhoea type remedies.

After all of the side effects from radiotherapy have gone away, other side effects may occur months, or even years after treatment finishes. Less than 1 in 50 men would be expected to have side effects severe enough to warrant medical intervention. Around 1 in 10 may have a moderate side effect, but choose not to have any direct treatment for it.

- Alternating constipation and diarrhoea needing changes to the diet
- Bladder urgency, where you need to pass water with relatively little warning
- Bleeding from the back passage. In about 1 in 50 men, this needs to be treated, which involves a colonoscopy (camera in the back passage).
- Permanent problems with erections

Each individual is different and the occurrence and severity of these side effects will vary from participant to participant. Any toxicity will be carefully monitored and your treatment may be modified if necessary.

Radiation treatment does not make you radioactive. In other words, you will not expose anyone else to radiation and it is perfectly safe to be in close contact with family or friends including children.

Other potential risks:
There are no known short or long term side effects of a MRI scan.

'Are there benefits to me in taking part in the trial?'
It is not possible to predict if participating in this study will have any personal benefit for you. By Including MRI scanning and other ways of better looking at your bones, it is hoped to improve our understanding of why hormonal therapy causes some men's bones to get weaker faster. If we are able to answer this question, further research will be needed to confirm the results. Your personal benefit cannot be guaranteed, however other patients may benefit in the future from knowledge gained in this trial.

‘What happens if I suffer injury or complications as a result of the trial?’
If you suffer any injuries or complications that may be as a result of your participation in this cancer research trial, you should immediately contact either your radiation oncologist, general practitioner or local hospital emergency department, who will assist you in arranging appropriate medical treatment. In the unlikely event of an injury caused by your participation in this cancer research trial, compensation may be payable to you. The Principal Investigator of this trial, Dr Jarad Martin, maintains a clinical trials insurance policy to protect you in these circumstances.

‘Will taking part in this trial cost me anything, and will I be paid?’
Participation in this trial will not cost you anything more than your usual treatment costs. You will not receive payment for taking part in this research trial.

‘How will my confidentiality be protected and what happens with the results?’
All records including medical history, radiological imaging, laboratory tests and radiotherapy treatment records will be considered “source data” and retained for at least 15 years after the completion of the study. The information collected will be kept in the Calvary Mater Newcastle Research Centre under lock and key and computer password protection. Your medical records may be released in confidence to the regulatory authorities and Human Research Ethics Committee with the understanding that these records will be used only in connection with carrying out our obligations relating to this study.

If you withdraw from the study, the study data collected prior to your withdrawal may still be processed along with other data collected as part of the clinical trial. Should you allow it, your medical information regarding your progress would still be collected.

When the results of the trial are presented at scientific meetings or published in a medical journal no individual participant will be recognisable from the data presented. In any publication, information will be provided in such a way so you cannot be identified. By signing the attached Consent Form, you authorise release of, or access to, this confidential information to the relevant trial personnel and regulatory authorities.

It is desirable that your family doctor be advised of your decision to participate in this research trial. By signing the Consent Form, you agree to your family doctor being notified of your decision to participate in this research trial.

At all times, you have the right to access and to request correction of information held about you by the Calvary Mater Newcastle Research Centre.

If you do not consent to the access to your information described above and how it will be used, you will not be able to join the research trial.

'What should I do if I want to discuss this trial further before I decide?'
When you have read this information, your doctor will discuss it with you and any queries you may have. If you would like to know more at any stage, please do not hesitate to contact your doctor on 02 4921 1211 or the Clinical Trial Coordinator on 02 4014 3947.

'Who should I contact if I have concerns about the conduct of this trial?'
This project will be carried out according to the National Statement on Ethical Conduct in Research Involving Humans (2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to take part in research. The ethical aspects of this research project have been approved by the Hunter Research Ethics Committee. You may contact the Research Ethics Coordinator on 02 4921 4950 if you have concerns about the conduct of this trial.

‘What are my rights?’

a) You may ask questions regarding this trial and can expect clear and understandable answers in return.

b) Participation in this trial is voluntary and you are not obligated to participate if you do not wish to. You may withdraw from this trial at any time you wish without jeopardising further treatment at this hospital. Your doctor may withdraw you from the trial if it is felt that continuing would involve a risk to you.

c) Your medical records will be released in confidence to the trial coordinators, to the regulatory authorities and the Human Research Ethics Committee with the understanding that these records will be used only in connection with carrying out our obligations relating to this trial. You will not be identified as an individual in any of these reports or subsequent publications.

d) If any complications of this disease or of the treatment occur, the oncology centre will provide appropriate treatment for these problems.

e) If any new information becomes available that may influence your decision to continue in this trial, such information will be given to you.

f) Your participation in this trial will not involve any additional costs.

This study has been reviewed and approved by the Human Research Ethics Committee for the Calvary Mater Newcastle. Should you wish to discuss the study with someone not directly involved, in particular in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make an independent complaint, you can contact the coordinator of the Research Ethics Committee of the Calvary Mater Newcastle on 02 4921 4950

“Who do I ask if I have a question?’

Clinical Trial:

The doctor you should contact should any problems arise is Dr Jarad Martin
The contact telephone number is 02 4921 1211.
If after hours, ask for the Radiation Oncologist on call.

Urgent Medical Assistance:

If at any time during your treatment you require urgent medical assistance after-hours, contact your nearest general practicioner or hospital emergency department. You should tell the medical staff if you are participating in this clinical research study.

Ethical Approval:

This study has been reviewed by the Hunter Research Ethics Committee and has been duly approved. You may contact the Research Ethics Coordinator 02 4921 4950 should you have any complaints about the conduct of the research or wish to raise any concerns. The Research Ethics Coordinator may contact specific member of the Research Ethics Committee at their discretion.

CONSENT FORM

A Phase 2 Clinical Trial Exploring 3-Dimensional Imaging of Androgen Deprivation Induced Osteoporosis, Radiotherapy Hypofractionation and the Prognostic Significance of Micrometastatic disease in men with Prostate Cancer.

Short Title: PROstate Cancer Imaging, Treatment and Toxicity (PROCITT)

Dr _____ has discussed this trial with me.
I have:

- Read, understood and kept a copy of the Patient Information Sheet;
- Had the opportunity to ask questions about this trial and have had any questions or queries answered to my satisfaction;
- Been informed of the possible risks or side effects of the tests or procedures being conducted;
- Understood that the project is for the purpose of research and not for treatment;
- Been informed that the confidentiality of the information will be maintained and safeguarded;
- Given permission for access to my medical records, for the purpose of this research;
- Given permission for medical practitioners, other health professionals, hospitals or laboratories outside this hospital, to release information concerning my disease and treatment, which is needed for this study and understand that such information will remain confidential.
- Given permission for my pathology samples to be reviewed and further non genetic tests to be performed on this material to confirm diagnosis.
- Given consent to the publishing of results from the study provided my identity is not revealed.
- Been assured that I am free to withdraw at any time without comment or penalty; and

- Agreed to participate in the study. Please tick boxes which apply:
  - □ Shortened course of radiotherapy including tailoring of target volumes
  - □ Spinal Bone Loss component, including MRI and extra blood and urine testing.

PATIENT'S NAME:

_____

Please Print

PATIENT'S SIGNATURE:_____ DATE:

_____

I, the supervising physician, confirm that I have fully explained the nature, purpose and reasonably foreseeable risks to the patient taking part in the study. I confirm that he/she has read and kept a copy of the Patient Information Sheet and that he/she freely agrees to participate in the study.

PHYSICIAN'S NAME:

_____

Please Print

PHYSICIAN'S SIGNATURE: _____ DATE:

_____

REVOCATION OF CONSENT FORM

A Phase 2 Clinical Trial Exploring 3-Dimensional Imaging of Androgen Deprivation Induced Osteoporosis, Radiotherapy Hypofractionation and the Prognostic Significance of Micrometastatic disease in men with Prostate Cancer.

Short Title: PROstate Cancer Imaging, Treatment and Toxicity (PROCITT)
I hereby wish to;
(Please initial one)

Partially withdraw from the study above.
I do not wish to receive any further treatment prescribed by the study named above however I consent for my information to continue to be collected for the purposes of this study.

Totally withdraw my consent to participate in the study named above.
I do not wish to receive any further treatment or attend study related follow up assessments. I understand that such withdrawal WILL NOT jeopardise the treatment that I receive now or in the future, my relationship with the staff caring for me or my relationship with Radiation Oncology Queensland.

PATIENT'S NAME:

_____

Please Print

PATIENT'S SIGNATURE: _____

DATE:_____

Appendix 9: Causality and assessment of severity – Adverse Events

The severity of an Adverse Event will be assessed as follows:

Mild: Events that require minimal or no treatment and do not interfere with the patient's daily activities

Moderate: Events that cause sufficient discomfort to interfere with daily activity and/or require a simple dose of medication

Severe: Events that prevent usual daily activity or require complex treatment

The relationship of the event to the study drug will be assessed as follows:

Unrelated: There is no association between the exposure and the reported event. AEs in this category do not have a reasonable temporal relationship to exposure, or can be explained by a commonly occurring alternative aetiology.

Possible: The event could have cause or contributed to the AE. AEs in this category follow a reasonable temporal sequence from the time of exposure and/or follow a known response pattern to the test article, but could also have been produced by other factors.

Probable: The association of the event with the exposure seems likely. AEs in this category follow a reasonable temporal sequence from the time of exposure and are consistent with the known action of the exposure, known or previously reported adverse

reactions to the exposure, or judgement based on the investigators clinical experience.

Definite: The AE is a consequence of exposure. AEs in the category cannot be explained by concurrent illness, progression of disease state or concurrent medication reaction. Such events may be widely documented as having an association with the exposure or that they occur after rechallenge.

Appendix 10: Circulating Tumour Cell Assay

Circulating Tumour Cells (CTC) enumeration using the CellSearch® System (Veridex, LLC, Johnson & Johnson).

The CellSearch® System is cleared by US Food and Drug Administration as a diagnostic tool for the detection and enumeration of CTCs in metastatic breast, colorectal and prostate cancer. The CellSearch system can detect a single circulating tumour cell in 7.5 ml of blood.

Description of technique for CTC enumeration

CTC enumeration using the CellSearch® system is based on immunomagnetic capture and immunofluorescent profiling of the cells of epithelial origin from whole blood. The CellSearch® **Epithelial Cell Kit** used for CTC enumeration contains the ferrofluid reagent, which comprise anti-EpCAM antibodies coated magnetic nanoparticles that specifically bind to EpCAM (epithelial cell adhesion molecule) on CTCs. Immunomagnetically captured cells in the sample are afterwards mixed with fluorescently labelled antibodies and DAPI (4', 6-diamidino-2-phenylindole, dihydrochloride) nuclear stain. Phycoerythrin (PK) conjugated anti-CK 8, 18 and/or19 (intracellular cytokeratins 8, 18 and/or 19) antibodies are used to differentiate CTCs from leukocytes, which are specifically stained with allophycocyanin (APC) conjugated anti-CD45 antibodies. Following the staining, sample is transferred to a cartridge inserted into a Magnest® device, where under the influence of the magnetic field, nanoparticle-labelled and fluorescently stained cells are positioned for the scanning on the CellSearch® CellTracks Analyzer II®. Acquired images of differentially stained cells are presented in a gallery format for identification. The image analysis is done by certified users who have been trained by Johnson & Johnson, and whose proficiency is tested at three-monthly intervals. CTCs are identified based on cell

morphology and CK+, DAPI+, CD45- phenotype. Leukocytes are identified with CK-, DAPI+ and CD45+ phenotype.

Appendix 11: Patient Information regarding Calcium and Vitamin D supplementation

GUIDELINES FOR THE PREVENTION OF OSTEOPOROSIS
FOR MEN WITH PROSTATE CANCER ON HORMONE THERAPY

Thinning of the bones is a common problem for men on hormonal therapy for prostate cancer. Left untreated, bones can become weak enough to fracture. It is important to try to reduce the chance of this happening.

The following guidelines are provided to reduce the risk of bone loss during hormonal therapy.

Calcium & Vitamin D:
Calcium and vitamin D are both essential for strong bones. Men over the age of 50 years are recommended to consume **1500 mg of calcium** and **800 IU of vitamin D daily** from all sources, including the amount in the diet and from supplements.

FOOD SOURCES OF CALCIUM:

Food Source	Portion size	Calcium (mg)
Cheese (Swiss)	50 g (2oz)	440
Cheese (Cheddar, Mozzarella)	50 g (2 oz)	390
Milk (skim, 1 or 2% MF or whole)	250 ml (1 cup)	300
Buttermilk or Chocolate Milk	250 ml (1 cup)	300
Yogurt, plain	175 ml (¾ cup)	300
Milk powder, Dry	45 ml (3 Tbsp)	280
Fortified beverages (soy, rice, orange juice)	250 ml (1 cup)	300
Blackstrap molasses	15 ml (1 Tbsp)	180
Parmesan cheese	15 ml (1 Tbsp)	90

Sardines, with edible bones	24 g	90
Cottage cheese, 2% MF	125 ml (1/2 cup)	80
Figs, dried, uncooked	3	80
Orange, raw	1 medium	50
Broccoli, frozen, boiled, drained	250 ml (1 cup)	50

Calcium intake from all sources should not exceed 2500 mg per day.

FOOD SOURCES OF VITAMIN D:

Food Source	Portion size	Vitamin D (IU)
Fish, herring	100 g (3 oz)	900
Fish, mackerel or salmon	100 g (3 oz)	650
Fish, sardines or tuna	100 g (3 oz)	250
Milk or Soy Beverage, fortified	250 ml (1 cup)	90
Margarine, fortified	5 ml (1 tsp)	55
Egg	1 large	25

Adapted from the Manual of Clinical Dietetics, 6th Edition (p. 746-747), by American Dietetic Association et al, 2000.

Vitamin D from all sources should not exceed 2000 IU per day or 50 ug.

Vitamin and Mineral Supplements:

If you can't meet the recommended amounts with food alone, consider a supplement. Some calcium supplements also include vitamin D. A standard multivitamin and mineral supplement provides approximately 200 mg of calcium and 200 IU of vitamin D and other nutrients. It would be common to use 3 tablets containing these quantities every day. It is important to check the label of the preparation you purchase – please review with your pharmacist or doctor if in doubt.

Protein:

Adequate protein is required to maintain bone health. Include one of the following protein rich foods at each meal: meat, fish, poultry, beans, lentils, nuts, eggs, milk, yogurt and cheese.

Caffeine and salt:

Excess caffeine and salt can have a negative effect on bone. Caffeine is found in coffee and also tea, chocolate (cocoa) and some soft drinks. For optimal bone health **limit coffee to less than 4 cups per day**.

Foods high in salt generally include processed foods such as canned soups, snack foods, crackers, packaged pastas and sauces. Check the nutrition label on processed foods and **limit salt to less than 2100 mg per day**.

Physical Activity:

Being physically active maintains optimal bone health and decreases the risk of a bone fracture by improving bone mass and increasing muscular strength, coordination and balance and thereby reducing falls. Physical activity that is weight bearing is best, examples include walking, dancing, stair climbing, aerobics, skating and weight lifting.

Smoking:

Smoking is related to poor bone and general health. If you smoke, ask your doctor for assistance to **stop smoking**.

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For more information visit the following web sites:

Osteoporosis Australia: Booklets to download and other resources
www.osteoporosis.org.au

Find your 30: Information provided by the Queensland government to encourage physical activity.
www.your30.qld.gov.au/